510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY

I Background Information:

A 510(k) Number

K231187

B Applicant

Nano-Ditech Corporation

C Proprietary and Established Names

Nano-Check COVID-19 Antigen Test

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
QVF	Class II	21 CFR 21 CFR 866.3982 - Simple Point-Of-Care Device to Directly Detect Sars-Cov-2 Viral Target From Clinical Specimens In Near-Patient Setting	

II Submission/Device Overview:

A Purpose for Submission:

To obtain a substantial equivalence determination for the Nano-Check COVID-19 Antigen Test

B Measurand:

Nucleocapsid protein antigen from SARS-Coronavirus 2 (SARS-CoV-2)

C Type of Test:

Qualitative lateral flow immunoassay

III Intended Use/Indications for Use:

A Intended Use(s):

See Indications for Use below.

B Indication(s) for Use:

The Nano-Check COVID-19 Antigen Test is a lateral flow immunochromatographic assay for the rapid, qualitative detection of SARS-CoV-2 nucleoprotein protein antigens directly in anterior nasal swab specimens from individuals with signs and symptoms of upper respiratory infection (i.e., symptomatic) when testing is started within 4 days of symptom onset. The test is intended for use as an aid in the diagnosis of SARS-CoV-2 infections (COVID-19) in symptomatic individuals when either: tested at least twice over three days with at least 48 hours between tests; or when tested once, and negative by the Nano-Check COVID-19 Antigen Test and followed with a molecular test.

The test does not differentiate between SARS-CoV or SARS-CoV-2.

A negative test result is presumptive, and it is recommended these results be confirmed by a molecular SARS-CoV-2 assay.

Positive results do not rule out co-infection with other bacteria or viruses and should not be used as the sole basis for diagnosis, treatment, or other patient management decisions.

Performance characteristics for SARS-CoV-2 were established during the 2022 SARS-CoV-2 pandemic when SARS-CoV-2 Omicron was the predominant SARS-CoV-2 variant in circulation. When other SARS-CoV-2 virus variants are emerging, performance characteristics may vary.

C Special Conditions for Use Statement(s):

Rx - For Prescription Use Only
IVD - For In Vitro Diagnostic Use Only

D Special Instrument Requirements:

N/A

IV Device/System Characteristics:

A Device Description:

The Nano-Check COVID-19 Antigen Test is an immunochromatographic lateral flow assay for detection of SARS-CoV-2 nucleoprotein antigens in human anterior nasal swab specimens without transport media from those who are suspected of COVID-19 within the first 5 days of symptom onset.

To initiate testing, a flocked swab is used to collect anterior nasal swab specimens from both nostrils. The patient sample is placed in the Reagent Tube, which is either provided pre-filled with buffer, or that the user pre-filled with buffer provided in the test kit's ampule. The buffer disrupts the virus particles in the sample, exposing internal viral nucleoproteins. After disruption, the sample is dispensed into the Test Cassette sample well.

The Test Cassette is comprised of a plastic cassette with a nitrocellulose membrane and sample pads. Specifically, the test strip contains: (1) the Sample Pad, which receives the buffer and distributes the sample across the test strip; (2) the Biotin Pad, which contains biotinylated monoclonal antibodies specific to the SARS-CoV-2 nucleocapsid antigen; (3) the Dye Pad, which contains colloidal gold particles coupled with monoclonal antibodies specific to the SARS-CoV-2 nucleocapsid antigen; (4) the Test Line, which contains embedded streptavidin to capture the antibody-antigen immunocomplexes; and (5) the Absorbent Pad, which absorbs sample after it has migrated across the nitrocellulose membrane.

The sample will migrate up the test strip via capillary action. If SARS-CoV-2 nucleoprotein antigens are present, they will bind to the biotinylated monoclonal capture antibodies present on the biotin pad. As the sample passes through the dye pad, SARS-CoV-2 nucleoprotein antigen/biotin-antibody complexes will bind to specific monoclonal detection antibodies labelled with colloidal gold particles to form an immunocomplex with the biotinylated antibody, the nucleoprotein antigen, and the colloidal gold labelled antibody. At the test line, the immunocomplexes will be captured through interaction of the biotinylated antibody of the complexes with the streptavidin embedded into the test line, concentrating the colloidal-gold labeled antigen at the test line. This will form a visible pinkish-red line. Sample continues to flow through the test device which also contains a procedural control line to assess for sample presence and adequate sample flow. A visible pinkish-red line at the control region should always appear if the assay is performed correctly to verify proper liquid flow and gold conjugation. If no visible signal appears on C line, the test result is invalid, and this sample should be tested again with another test cassette. Test results are read between 15 and 20 minutes.

External positive control and negative control swabs are provided with each kit of Nano-Check COVID-19 Antigen Tests and should be processed according to the IFU upon receiving a new lot of test kits. The control swabs are intended to be used as quality control samples representative of positive and negative test samples to demonstrate that the reagents are functional, and the assay procedure is performed correctly.

B Principle of Operation:

The Nano-Check COVID-19 Antigen Test employs lateral flow technology in a sandwich design to detect nucleocapsid protein from SARS-CoV-2 in anterior nasal samples. Following sample collection, the swab is placed in the reagent tube containing extraction buffer to lyse the sample and solubilize viral nucleoproteins. After lysis, two drops of the sample are loaded into the test cassette sample well. The sample migrates across a pad containing biotinylated capture antibodies, a pad containing colloidal gold-labelled detection antibodies, and then the test strip including the test and control lines. If SARS-CoV-2 viral antigens are present, they will be captured and bound by the antibodies and the biotinylated immunocomplex will be bound to streptavidin embedded on the Test Line. The test results are visually read. A pink-red line at the Test Line and a pink-red line at the Control Line indicate a positive sample. A pink-red line at

the Control Line only indicates a negative sample. Any test without a visible line at the Control Line should be interpreted as invalid and testing should be repeated with a new device.

V Substantial Equivalence Information:

A Predicate Device Name(s):

Sofia 2 SARS Antigen+ FIA, Sofia 2 SARS Antigen+ FIA Control Swab Set

B Predicate 510(k) Number(s):

DEN220039

C Comparison with Predicate(s):

Device & Predicate Device(s):	Device K231187	Predicate DEN220039
Device Trade Name	Nano-Check COVID-19 Antigen Test	Sofia 2 SARS Antigen+ FIA, Sofia 2 SARS Antigen+ FIA Control Swab Set
General Device Characteristic Similarities		
Intended Use/ Indications For Use	The Nano-Check COVID-19 Antigen Test is a lateral flow immunochromatographic assay for the rapid, qualitative detection of SARS-CoV-2 nucleocapsid protein antigens directly in anterior nasal swab specimens from individuals with signs and symptoms of upper respiratory infection (i.e., symptomatic) when testing is started within 4 days of symptom onset. The test is intended for use as an aid in the diagnosis of SARS-CoV-2 infections (COVID-19) in symptomatic individuals when either: tested at least twice over three days with at least 48 hours between tests; or when tested once, and negative by the Nano-Check COVID-19 Antigen Test and followed up with a molecular test. The test does not differentiate between SARS-CoV and SARS-CoV-2. A negative test result is presumptive, and it is recommended these results be confirmed by a molecular SARS-	The Sofia 2 SARS Antigen+ FIA is a lateral flow immunofluorescent sandwich assay that is used with the Sofia 2 instrument for the rapid, qualitative detection of SARS-CoV-2 nucleocapsid protein antigens directly in anterior nasal swab specimens from individuals with signs and symptoms of upper respiratory infection (i.e., symptomatic) when testing is started within 6 days of symptom onset. The test is intended for use as an aid in the diagnosis of SARS-CoV-2 infections (COVID-19) in symptomatic individuals when tested at least twice over three days with at least 48 hours between tests. The test does not differentiate between SARS-CoV and SARS-CoV-2. A negative test result is presumptive, and it is recommended these results be confirmed by a molecular SARS-CoV-2 assay. Negative results do not preclude SARS-CoV-2 infections and should not be used as the sole basis for treatment or other patient

Device & Predicate Device(s):	**Device** K231187	**Predicate** DEN220039
	CoV-2 assay. Positive results do not rule out co-infection with other bacteria or viruses and should not be used as the sole basis for diagnosis, treatment, or other patient management decisions. Performance characteristics for SARS-CoV-2 were established during the 2022 SARS-CoV-2 pandemic when SARS-CoV-2 Omicron was the predominant SARS-CoV-2 variant in circulation. When other SARS-CoV-2 virus variant are emerging, performance characteristics may vary. This test is intended for prescription use only and can be used in Point-of-Care settings.	management decisions. Positive results do not rule out co-infection with other respiratory pathogens. Performance characteristics for SARS-CoV-2 were established during the 2021-2022 SARS-CoV-2 pandemic when SARS-CoV-2 Omicron was the predominant SARS-CoV-2 variant in circulation. When other SARS-CoV-2 virus variant are emerging, performance characteristics may vary. This test is intended for prescription use only and can be used in Point-of-Care settings.
Regulation Number	21 CFR 866.3982	Same
Indication Type	In vitro diagnostic	Same
Intended Use Population	Individuals with symptoms of COVID-19	Same
Intended Use Setting	Point-of-Care	Same
Specimen Type	Direct anterior nasal swab	Same
Analyte	SARS-CoV-2 nucleocapsid protein	Same
Test Type	Lateral flow immunochromatographic	Same
Test Result Type	Qualitative	Same
Test Time	15 minutes	Same
General Device Characteristic Differences		
Instrumentation	None	Sofia 2 Instrument
Result Interpretation	Visually read - visual interpretation of the presence or absence of colored line(s) on the control and test line(s) of the test strip is used to determine Positive, Negative, or Invalid results.	Instrument read - the Sofia 2 instrument scans the test strip and measures the fluorescent signal by processing the results using method specific algorithms. Sofia 2 displays the test results (Positive, Negative, or Invalid) on the screen.

VI Standards/Guidance Documents Referenced:

Document Number	Title	Publishing Organization	Applicable Study
21 CFR 866.3982	Simple Point-Of-Care Device To Directly Detect Sars-Cov-2 Viral Targets From Clinical Specimens In Near-Patient	FDA	All
OMB:0910-0595	U.S. FDA's SARS-CoV-2 Emergency Use Authorization Antigen Template for Test Developers (Dated 06 Oct 2021)	FDA	All
EP12-A2	User Protocol for Evaluation of Qualitative Test Performance; Approved Guideline-Second Edition (Sections 7.1 Controls, Section 8 Bias and Imprecision Studies (with focus on 8.3), and Appendix: Statistical Reasoning for Precision Experiment Conclusions)	CLSI	Precision/ Repeatability and Reproducibility
EP-37	Supplemental Tables for Interference Testing in Clinical Chemistry	CLSI	Interference
BS EN ISO 23640:2015	In vitro diagnostic medical devices. Evaluation of stability of in vitro diagnostic reagents	BSI Standard	Shelf-life
N/A	Guidance for Industry and FDA Staff, Establishing the Performance Characteristics of In Vitro Diagnostic Devices for the Detection or Detection and Differentiation of Influenza Viruses (15 Jul 2011; Section 9.A.i)	FDA	Limit of Detection
N/A	Recommendations for Clinical Laboratory Improvement Amendments of 1988 (CLIA) Waiver Applications for Manufacturers of In Vitro Diagnostic Devices; Guidance for Industry and Food and Drug Administration Staff (dated 26 Feb 2020; Section IV.A. Tier I: Risk Analysis and Flex Studies)	FDA	Cross-reactivity and Microbial Interference; Development Read Time and Test Result Stability
N/A	Testing for Biotin Interference in In Vitro Diagnostic Devices	FDA	Biotin Interference

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/Reproducibility:

a) Precision and Repeatability

In the precision/repeatability (P/R) study, two blinded operators evaluated negative and contrived positive samples prepared with Gamma-irradiated SARS-CoV-2 in negative clinical matrix (NCM) at four (4) concentrations: true negative ("TN", composed of NCM only), high negative ("HN", 0.1x LoD), low positive ("LP", 1x LoD), and positive ("P", 4x LoD) (**Table 1**). Each operator tested two sample replicates per concentration twice daily for 12 days, resulting in a total of 96 replicates per sample level (2 operators x

2 sample replicates x 2 sample runs daily x 12 days). Precision testing was conducted in accordance with the Package Insert. One lot of test devices was used.

Table 1. Precision and Repeatability Study – Test Sample Panel

Spiking Concentrations			
Level	**Concentration (x LoD)**	**Concentration ($TCID_{50}$/mL)**	**Concentration on Swab ($TCID_{50}$)**
Negative Sample (TN)	NCM Only	N/A	N/A
High Negative (HN)	0.1 x LoD	7.0×10^1	3.5
Low Positive (LP)	1 x LoD	7.0×10^2	35
Positive (P)	4 x LoD	2.8×10^3	140

Data were analyzed by calculating percent agreement between the test results and the expected results for the sample. Results are summarized below and in **Table 2**:

- True negative samples produced 100% expected negative agreement (96/96)
- High negative samples produced 96.9% expected positive agreement (93/96)
- Low positive samples produced 95.8% expected positive agreement (92/96)
- Positive samples produced 100% expected positive agreement (96/96)
- Out of 384 replicates tested, there were zero (0) invalid results

Table 2. Precision and Repeatability Study - Results

Level	**P (4x LoD)**		**LP (1x LoD)**		**HN (0.1x LoD)**		**TN (NCM only)**	
Operator	1	2	1	2	1	2	1	2
Run 1	24/24	24/24	24/24	23/24	24/24	23/24	24/24	24/24
Run 2	24/24	24/24	22/24	23/24	23/24	23/24	24/24	24/24
Total	48/48	48/48	46/48	46/48	47/48	46/48	48/48	48/48
% Agreement	100%	100%	95.8	95.8	97.9	95.8	100%	100%
95% CI	92.6-100%	92.6-100%	86.0-98.9%	86.0-98.9%	89.1-99.6%	86.0-98.9%	92.6-100%	92.6-100%
Total	96/96		92/96		93/96		96/96	
Total % Agreement	100%		95.8%		96.9%		100%	
Total 95% CI	92.6 - 100%		89.8 – 98.4%		91.2 – 98.9%		92.6 - 100%	

b) Reproducibility

The reproducibility study was designed to evaluate lot-to-lot, operator-to-operator, site-to-site, and analyte level-to-level variability to demonstrate that the Nano-Check COVID-19 Antigen Test can be performed consistently and correctly. The study was conducted at three (3) external CLIA-waived point-of-care (PoC) sites with a total of seven (7) untrained operators and at one (1) internal site with a total of three (3) operators. A total of six (6) lots of test devices were used. Blinded operators tested a coded panel of contrived samples at four analyte levels (refer to **Table 1** above), with three (3) replicates per level per operator being tested across five (5) days. Per analyte level, 150 replicates were tested (10 operators x 5 days of testing x 3 replicates per day) for a total of 600 results. The results, stratified by site, are summarized below and in **Table 2**. No significant differences between sites, or between trained and untrained operators were observed.

- Out of 600 samples tested, there were zero (0) invalid test results obtained
- The true negative samples produced an overall 100.0% expected negative agreement (150/150)
- The high negative (0.1x LoD) samples produced an overall 100% expected negative agreement (150/150)
- The low positive (1x LoD) samples produced an overall 98.0% expected positive agreement (147/150)
- The moderate positive (4x LoD) samples produced an overall 100% expected positive agreement (150/150)

Table 3. Reproducibility Study Results

Site	Operator	True Negative	High Negative (0.1x LoD)	Low Positive (1x LoD)	Positive (4x LoD)
Site 1 (External)	n=3 Untrained	45/45	45/45	42/45	45/45
Site 2 (External)	n=2 Untrained	30/30	30/30	30/30	30/30
Site 3 (External)	n=2 Untrained	45/45	45/45	30/30	45/45
Site 4 (Internal)	n=3 Trained	45/45	45/45	45/45	45/45
Total		150/150	150/150	147/150	150/150
% Agreement		100%	100%	98.0%	100%
95% CI		97.5-100%	97.5-100%	94.3-99.3%	97.5-100%

2. Linearity:

This study is not applicable as this test device is a qualitative assay.

3. Analytical Specificity/Interference:

a) Cross-Reactivity and Microbial Interference Study

Cross-reactivity and potential microbial interference were evaluated by testing various bacteria (10), viruses (31), fungus (1), and negative matrix (1) with the Nano-Check COVID-19 Antigen Test. Each organism and virus was tested in three (3) replicates in the presence or absence of SARS-CoV-2 (heat-inactivated, isolate hCoV-19/USA/MD-HP20874/2021, Omicron variant, lineage B.1.1.529). For test kit format A (pre-filled reagent tubes), positive samples were tested at 2x LoD. For test kit format B (buffer ampules and empty reagent tubes), positive samples were tested at 3x LoD. Three blinded operators tested the samples using one lot of each test device. Cross-reactivity and interference were evaluated by the agreement with the expected negative or positive results, respectively. None of the evaluated organisms and viruses demonstrated cross-reactivity or interference with the assay at the tested concentrations. Results are summarized in **Table 4** below.

Table 4. Cross-Reactivity and Microbial Interference Results

Microorganism / Virus	Strain	Conc.	Cross-Reactivity (Neg. agreement: SCV-2 negative reps* / all reps)		Interference (Pos. agreement: SCV-2 positive reps / all reps)	
			Kit Format A	Kit Format B	Kit Format A	Kit Format B
Adenovirus	Type 2 (Species C)	$1.0x10^5$ $TCID_{50}$/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Adenovirus	Type 1 (Adenoid 71)	$1.0x10^5$ $TCID_{50}$/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Mastadenovirus B	Gomen	$1.0x10^5$ $TCID_{50}$/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Coronavirus	229E	$1.0x10^5$ $TCID_{50}$/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Coronavirus	OC43	$4.45x10^4$ $TCID_{50}$/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Coronavirus	NL63	$1.0x10^5$ $TCID_{50}$/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
MERS-CoV	EMC/2012	$1.0x10^5$ $TCID_{50}$/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
SARS-CoV	Urbani	$1.0x10^5$ $TCID_{50}$/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Metapneumovirus	TN/83-1211	$1.0x10^5$ $TCID_{50}$/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Parainfluenza Virus Type 1	HPIVI/FRA/ 29221106/2009	$1.0x10^5$ $TCID_{50}$/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Parainfluenza Virus Type 2	Greer	$1.0x10^5$ $TCID_{50}$/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Parainfluenza Virus Type 3	NIH 47885	$1.0x10^5$ $TCID_{50}$/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Parainfluenza Virus Type 4B	19503	$1.0x10^5$ $TCID_{50}$/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Influenza A H1N1	New Caledonia/ 20/1999	$1.0x10^5$ $TCID_{50}$/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Influenza A H1N1	San Diego/1/2009	$1.0x10^5$ $TCID_{50}$/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Influenza A H3N2	Victoria/361/2011	$1.0x10^5$ $TCID_{50}$/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Influenza A H3N2	Wisconsin/67/ 2005	$1.0x10^5$ $TCID_{50}$/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Influenza B Virus	Brisbane/60/2008 (Victoria)	$1.0x10^5$ $TCID_{50}$/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Influenza B Virus	Texas/06/2011 (Yamagata)	$1.0x10^5$ $TCID_{50}$/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Influenza B Virus	B/GL/1739/54	$1.0x10^5$ $TCID_{50}$/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Enterovirus 71	MP4	$1.0x10^5$ $TCID_{50}$/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Enterovirus Species D	Type 68, USA/2018-23087	$1.0x10^5$ $TCID_{50}$/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Respiratory Syncytial Virus	Strain long	$1.0x10^5$ $TCID_{50}$/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Respiratory Syncytial Virus	Type A (2001/2-20)	$1.0x10^5$ $TCID_{50}$/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Respiratory Syncytial Virus	Type A (1998/12-21)	$1.0x10^5$ $TCID_{50}$/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)

Microorganism / Virus	Strain	Conc.	Cross-Reactivity (Neg. agreement: SCV-2 negative reps* / all reps)		Interference (Pos. agreement: SCV-2 positive reps / all reps)	
			Kit Format A	Kit Format B	Kit Format A	Kit Format B
Respiratory Syncytial Virus	Type B1	$1.0x10^5$ $TCID_{50}$/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Rhinovirus 20	15-CV19	$1.0x10^5$ $TCID_{50}$/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Measles Virus	Edmonston	$1.7x10^4$ $TCID_{50}$/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Mumps Virus	MuV/Iowa.US/ 2006	$1.0x10^5$ $TCID_{50}$/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Coxsackie Virus	B4	$1.0x10^5$ U/mL $TCID_{50}$	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Epstein-Barr Virus	B95-B	$1.0x10^5$ cp/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Haeomophilus influenzae	Type B (Eagan)	$1.0x10^6$ CFU/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Streptococcus pneumoniae, Type 19F	Strain Z022	$1.0x10^6$ CFU/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Streptococcus pyogenes	Bruno	$1.0x10^6$ CFU/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Streptococcus salivarius	N/A	$1.0x10^6$ CFU/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Candida albicans	Z006	$1.0x10^6$ CFU/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Bordetella pertussis	Strain 5	$1.0x10^6$ CFU/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Mycoplasma pneumoniae	N/A	$1.0x10^6$ CFU/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Chlamydia pneumoniae	TW-183	$1.0x10^6$ CFU/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Legionella pneumophila	N/A	$1.0x10^6$ CFU/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Staphylococcus aureus	MRSA, COL	$1.0x10^6$ CFU/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Staphylococcus epidermidis	MRSE, RP62A	$1.0x10^6$ CFU/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Negative clinical matrix	N/A	N/A	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)

* Reps = Replicates
Kit Format A includes pre-filled reagent tubes
Kit Format B includes buffer-filled ampules and empty reagent tubes

The sponsor additionally tested coronavirus HKU1 (HCoV-HKU1) using 20 positive clinical specimens. Clinical samples were diluted with negative clinical matrix and tested in the same manner as the viruses above. No cross-reactivity or interference was observed for any of the tested samples.

b) Endogenous and Exogenous Interfering Substances Study

This study evaluated endogenous or exogenous substances that may potentially interfere with the performance of the Nano-Check COVID-19 Antigen Test. Each potentially interfering substance was tested in triplicate in negative clinical matrix at targeted concentrations in the presence or absence of 3x LoD SARS-CoV-2 (heat-inactivated, isolate hCoV-19/USA/MD-HP20874/2021, Omicron variant, lineage B.1.1.529). Three blinded operators tested the samples using one lot of kit format A (pre-filled reagent tubes) and one lot of kit format B (buffer ampules and empty reagent tubes). The effects of the substances were evaluated by the agreement with the expected positive or negative results. None of the evaluated substances demonstrated interference with the assay at the tested concentrations. Results are summarized in **Table 5** below.

Table 5. Interfering Substances Results

Substance	Active Ingredient of Substance	Interferent Conc.	Negative Agreement (SCV-2 negative reps* / all reps)		Positive Agreement (SCV-2 positive reps / all reps)	
			Kit Format A	Kit Format B	Kit Format A	Kit Format B
Nasal Spray – Afrin	Oxymetazoline	15% (v/v)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Nasal Spray – NasalCrom	Cromolyn sodium	15% (v/v)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Nasal Spray – Flonase	Fluticasone propionate	15% (v/v)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Nasal Spray – CVS	Phenylephrine HCl	15% (v/v)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Budesonide Nasal Spray	Budenoside	15% (v/v)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Oral Pain Reliever Spray (Chloraseptic)	Phenol, Menthol	15% (v/v)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Orrivine Nasal Drops	Xylometazoline	15% (v/v)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Walgreens Zinc Lozenges	Zincum gluconicum	5% (v/v)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Similasan Nasal Allergy Relief	Cardiospermun, Galphimia glauca, Luffa operculate, Sabadilla	15% (v/v)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
NeilMed NasoGEL	Sodium hyaluronate, Allantoin, NaCl, Methylparaben, Propylparaben	15% (v/v)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Cepacol Sore Throat Lozenges	Benzocaine, Menthol	15% (v/v)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Alkalol Homeopathic Allergy Nasal Spray	N/A	15% (v/v)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
NASONEX 24 hr Allergy	Mometasone furoate monohydrate	15% (v/v)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Nasacort Allergy 24 hr	Triamcinolone acetonide	15% (v/v)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)

Substance	Active Ingredient of Substance	Interferent Conc.	Negative Agreement (SCV-2 negative reps* / all reps)		Positive Agreement (SCV-2 positive reps / all reps)	
			Kit Format A	Kit Format B	Kit Format A	Kit Format B
Histaminum 30C	Histaminum hydrochloricum 30C HPUS	5% (v/v)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Zicam Oral Mist	Zincum aceticum, Zincum gluconicum	15% (v/v)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Tobramycin	Tobramycin	33 µg/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Mupirocin	Mupirocin	1.5 µg/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Oseltamivir Phosphate	Oseltamivir Phosphate	0.39 µg/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Beclomethasone diproprionate	Beclomethasone diproprionate	5.04 µg/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Dexamethasone	Dexamethasone	12 µg/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Flunisolide	Flunisolide	87 µg/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Remdesivir	Remdesivir	240 µg/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Molnupiravir	Molnupiravir	32.9 µg/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Softsoap Hand Soap	N/A	10% (v/v)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
CVS Hand Sanitizer Gel	70% Ethyl Alcohol	1% (v/v)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Bovine Mucin	Mucin protein	2.5 mg/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Purified Human Neutrophils	N/A	$5x10^6$ cells/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Human Whole Blood	N/A	2.5% (v/v)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)

* Reps = Replicates
Kit Format A includes pre-filled reagent tubes
Kit Format B includes buffer-filled ampules and empty reagent tubes

c) Biotin Interference Study

The Nano-Check COVID-19 Antigen Test is based on a sandwich assay format that utilizes a biotin-streptavidin complex. The sponsor conducted a biotin interference study to assess if free biotin (vitamin B7) in samples may compete with the biotinylated complex and impact assay results. Biotin was tested at five concentrations in triplicate in negative clinical matrix in the presence of 3x LoD SARS-CoV-2 (heat-inactivated, isolate hCoV-19/USA/MD-HP20874/2021, Omicron variant, lineage B.1.1.529). One lot of kit format A (pre-filled reagent tubes) and one lot of kit format B (buffer ampules and empty reagent tubes) was used for testing. The effects of biotin interference were evaluated by the agreement with the expected positive results. Interference was not observed at any of the concentrations tested. Results are summarized in **Table 6** below.

Table 6 Biotin Interference Results

Concentration of Biotin (ng/mL)	Concentration of SARS-CoV-2	Positive Agreement (SCV-2 positive reps */ all reps)	
		Kit Format A	Kit Format B
3500	3x LoD	100% (5/5)	100% (5/5)
2400	3x LoD	100% (5/5)	100% (5/5)
1200	3x LoD	100% (5/5)	100% (5/5)
600	3x LoD	100% (5/5)	100% (5/5)
300	3x LoD	100% (5/5)	100% (5/5)
150	3x LoD	100% (5/5)	100% (5/5)

* Reps = Replicates
Kit Format A includes pre-filled reagent tubes
Kit Format B includes buffer-filled ampules and empty reagent tube

4. Assay Reportable Range:

This section is not applicable as this test device is a qualitative assay.

5. Traceability, Stability, Expected Values (Controls, Calibrators, or Methods):

a) Internal Controls

The Nano-Check COVID-19 Antigen Test has a built-in internal procedural control. When sample is added, colloidal gold-conjugated chicken antibodies in the dye pad will mix with the sample and migrate through the test strip. The labeled antibody complexes will bind with the goat anti-chicken antibodies immobilized on the control line on the nitrocellulose membrane. This will form a colored control line in the control line region. Formation of the control line serves as an internal control indicating proper sample application procedure and membrane wicking has occurred, and indicates reagents are functioning appropriately.

b) External Controls

The Nano-Check COVID-19 Antigen Test includes two external controls. The external positive control swab contains recombinant SARS-CoV-2 nucleocapsid protein antigen equivalent to 2x LoD on the swab head. The external positive control swab is intended to mimic positive samples to ensure the test device is functioning as expected. The external negative control swab contains blank Universal Transport media and is intended to mimic negative samples to ensure the test device is functioning as expected.

Lot-to-lot reproducibility of the external positive and negative control swabs was evaluated with three lots of each external control and three blinded operators. Ten replicates from each lot were tested on a unique lot of test kit. For each external control lot, all positive and negative controls produced 100% agreement with the expected results, as summarized in the table below.

Table 7. Validation of External Control Materials

External Control	Lot No.	# Neg.	# Pos.	% Expected Agreement
	0804-CN-2B01-P	0/10	10/10	100%
	0804-CN-2D29-P	0/10	10/10	100%

External Control	Lot No.	# Neg.	# Pos.	% Expected Agreement
External Positive Control	0804-CN-2E04-P	0/10	10/10	100%
External Negative Control	0804-CN-2B01-N	10/10	0/10	100%
	0804-CN-2D29-N	10/10	0/10	100%
	0804-CN-2E04-N	10/10	0/10	100%

c) Reagent Stability/Shelf-Life

(1) In order to determine the shelf-life of the Nano-Check COVID-19 Antigen Test in the intended storage conditions (2-30°C), a real-time stability study was conducted using three (3) lots of test kit stored at 2-8°C, room temperature, and 30°C after manufacturing. Single test kits (Format A) were assessed with negative samples and contrived positive samples prepared using inactivated SARS-CoV-2 omicron variant at 3x LoD in NCM. Five replicates per timepoint per storage condition were analyzed. Three lots of external control swabs were also stored in the same conditions and analyzed along with the contrived positive and negative samples. Data collected to date support a test kit and external control shelf-life of 14 months.

(2) Separately, the shelf-life of ampules containing reagent buffer (included in the multi test kit Format B) was evaluated by storing three lots of ampules at 2-8°C, room temperature, and 30°C after manufacturing and testing them with a single lot of test devices stored in the same condition. Ten (10) replicates of negative samples and contrived positive samples prepared using inactivated SARS-CoV-2 omicron variant at 2x LoD were tested per timepoint per storage condition. Data collected to date support an ampule shelf-life of 17 months.

d) Specimen Stability

Specimen stability was assessed using contrived positive samples prepared with nasal fluid and SARS-CoV-2 (omicron variant, isolate hCoV-19/USA/MD-HP20874/2021). Four test samples were prepared: true negative (TN, negative nasal fluid with no analyte), high negative (HN, 0.3x LoD), low positive (LP, 1x LoD), and positive (P, 3x LoD). Samples were stored at ambient temperature (23.5°C), high room temperature (30°C), and refrigerated (2-8°C) and tested at 1, 2, 4, 8, 24, 48, and 72 hours. The ambient temperature condition was used for time 0 testing of all samples. Five replicates of each concentration were tested in accordance with the package insert at each timepoint for each condition. Positive percent agreement summary results are included in the table below.

Table 8. Specimen Stability Study Results

Specimen Storage Parameters	Conc.	% positive (SCV-2 positive reps* / all reps)							
		0 hrs.	1 hr.	2 hrs.	4 hrs.	8 hrs.	24 hrs.	48 hrs.	72 hrs.
Ambient Temp. (23.5°C)	TN	0.0% (0/0)	0.0% (0/0)	0.0% (0/0)	0.0% (0/0)	0.0% (0/0)	0.0% (0/0)	0.0% (0/0)	0.0% (0/0)
	HN	40.0% (2/5)	40.0% (2/5)	40.0% (2/5)	40.0% (2/5)	40.0% (2/5)	20.0% (1/5)	0.0% (0/0)	0.0% (0/0)
	LP	100% (5/5)	100% (5/5)	100% (5/5)	100% (5/5)	100% (5/5)	100% (5/5)	60.0% (3/5)	20.0% (1/5)

Specimen Storage Parameters	Conc.	% positive (SCV-2 positive reps* / all reps)							
		0 hrs.	1 hr.	2 hrs.	4 hrs.	8 hrs.	24 hrs.	48 hrs.	72 hrs.
	P	100% (5/5)	100% (5/5)	100% (5/5)	100% (5/5)	100% (5/5)	100% (5/5)	100% (5/5)	100% (5/5)
High Room Temp. (30°C)	TN	N/A	0.0% (0/0)	0.0% (0/0)	0.0% (0/0)	0.0% (0/0)	0.0% (0/0)	0.0% (0/0)	0.0% (0/0)
	HN	N/A	40.0% (2/5)	40.0% (2/5)	20.0% (1/5)	20.0% (1/5)	0.0% (0/0)	0.0% (0/0)	0.0% (0/0)
	LP	N/A	100% (5/5)	100% (5/5)	100% (5/5)	100% (5/5)	100% (5/5)	20.0% (1/5)	0.0% (0/0)
	P	N/A	100% (5/5)	100% (5/5)	100% (5/5)	100% (5/5)	100% (5/5)	100% (5/5)	100% (5/5)
Refrigerated (2-8°C)	TN	N/A	0.0% (0/0)	0.0% (0/0)	0.0% (0/0)	0.0% (0/0)	0.0% (0/0)	0.0% (0/0)	0.0% (0/0)
	HN	N/A	60.0% (3/5)	40.0% (2/5)	60.0% (3/5)	60.0% (3/5)	40.0% (2/5)	40.0% (2/5)	0.0% (0/0)
	LP	N/A	100% (5/5)	100% (5/5)	100% (5/5)	100% (5/5)	100% (5/5)	100% (5/5)	100% (5/5)
	P	N/A	100% (5/5)	100% (5/5)	100% (5/5)	100% (5/5)	100% (5/5)	100% (5/5)	100% (5/5)

* Reps = Replicates

Based on the study results, the following storage conditions of nasal swabs are supported:

- 8 hours when stored at ambient room temperature (23.5°C)
- 8 hours when stored at high room temperature (30°C)
- 48 hours when stored refrigerated (2-8°C)

6. Detection Limit:

Kit Format A - LoD

The Limit of Detection (LoD) of the Nano-Check COVID-19 Antigen Test kit format A (multi test packaging with pre-filled reagent tube) was determined by evaluating different dilutions of inactivated SARS-CoV-2 stocks in negative clinical matrix. The following SARS-CoV-2 strains were used: Gamma-irradiated SARS-CoV-2 isolate USA-WA1/2020, heat-inactivated SARS-CoV-2 isolate USA/MD-HP20874/2021 (Omicron variant, linage B.1.1.529) and UV-inactivated SARS-CoV-2 isolate USA/PHC658/2021 (Delta variant, lineage B.1.6172.2). Pooled human nasal wash was used as the negative clinical matrix.

For range finding preliminary LoD determination of SARS-CoV-2 USA-WA1/2020, five (5) 10-fold serial dilutions were made from SARS-CoV-2 USA-WA1/2020 stock into negative clinical matrix. Three replicates were tested for each of the five dilutions to determine the preliminary LoD concentration on one lot of the Nano-Check COVID-19 Antigen Test. The preliminary LoD was further refined using three (3) 2-fold serial dilutions with three replicates each. Thereafter, the LoD was confirmed by testing 20 replicates at the preliminary LoD concentration. LoD confirmatory testing was repeated with a second lot of Nano-Check COVID-19 Antigen Test.

For range finding preliminary LoD determination of the other SARS-CoV-2 isolates, three 10-fold serial dilutions and then 2-fold serial dilutions of SARS-CoV-2 were used to determine the preliminary LoD. The LoD was confirmed by testing 20 replicates at the

preliminary LoD concentration. A single lot of Nano-Check COVID-19 Antigen Test was used for LoD testing with the additional virus strains.

Data are summarized in **Table 9**. The Nano-Check COVID-19 Antigen Test LoD for the following SARS-CoV-2 strains was confirmed to be:

- USA-WA1/2020: 7.0 x 10^2 $TCID_{50}$/mL
- USA/MD-HP20874/2021 (Omicron variant, linage B.1.1.529): 1.95 x 10^2 $TCID_{50}$/mL
- USA/PHC658/2021 (Delta variant, lineage B.1.6172.2): 5.21 x 10^2 $TCID_{50}$/mL

Table 9. Limit of Detection Study Results (Kit Format A)

Isolate	Lot	Conc. ($TCID_{50}$/mL)	Conc. ($TCID_{50}$/swab)	Range Finding			Confirmation		
				n	Neg	Pos (% Pos)	n	Neg	Pos (% Pos)
USA-WA1/2020	Lot 1	$2.8x10^5$	$1.4x10^4$	3	0	3 (100%)			
		$2.8x10^4$	$1.4x10^3$	3	0	3 (100%)			
		$2.8x10^3$	$1.4x10^2$	3	0	3 (100%)			
		$2.8x10^2$	$1.4x10^1$	3	3	0 (0%)			
		$2.8x10^1$	1.4	3	3	0 (0%)			
		$1.4x10^3$	70	3	0	3 (100%)			
		$7.0x10^2$	**35**	**3**	**0**	**3 (100%)**	**20**	**0**	**20 (100%)**
		$3.5x10^2$	17.5	3	3	0 (0%)			
	Lot 2	$1.4x10^3$ *	70	0	-	-	20	0	20 (100%)
		$7.0x10^2$ *	**35**	0	**-**	**-**	**20**	**1**	**19 (95%)**
		$3.5x10^2$ *	17.5	0	-	-	20	3	17 (85%)
USA/MD-HP20874/2021 (Omicron)	Lot 2	$3.89x10^3$	$1.9x10^2$	3	0	3 (100%)			
		$3.89x10^2$	$1.9x10^1$	3	0	3 (100%)	20	0	20 (100%)
		$3.89x10^1$	1.9	3	3	0 (0%)			
		$1.95x10^2$	**9.75**	**3**	**0**	**3 (100%)**	**20**	**1**	**19 (95%)**
		$9.75x10^1$	4.9	3	1	2 (66.6%)	20	7	13 (65%)
USA/PHC658/2021 (Delta)	Lot 2	$4.17x10^4$	$2x10^3$	3	0	3 (100%)			
		$4.17x10^3$	$2x10^2$	3	0	3 (100%)			
		$4.17x10^2$	$2x10^1$	3	2	1 (33.3%)			
		$2.09x10^3$	$1.04x10^2$	3	0	3 (100%)			
		$1.04x10^3$	52	3	0	3	20	0	20

Isolate	Lot	Conc. ($TCID_{50}$/mL)	Conc. ($TCID_{50}$/swab)	Range Finding			Confirmation		
				n	Neg	Pos (% Pos)	n	Neg	Pos (% Pos)
						(100%)			(100%)
		$5.21x10^2$	**26**	**3**	**0**	**3 (100%)**	**20**	**1**	**19 (95%)**
		$2.61x10^2$	13	0	-	-	20	9	11 (55%)

Kit Format B LoD

The LoD of the Nano-Check COVID-19 Antigen Test kit format B (buffer ampule and empty reagent tube) was evaluated and compared to the LoD for kit format A. Dilutions of inactivated SARS-CoV-2 (isolate USA/MD-HP20874/2021, Omicron variant, linage B.1.1.529) prepared in negative clinical matrix (pooled human nasal wash) were tested with test kit formats.

An initial range finding study was conducted with kit format B to identify a tentative LoD using four 10-fold serial dilutions of SARS-CoV-2 in negative clinical matrix. Three replicates were tested for each of the five dilutions to determine the preliminary LoD concentration on one lot of the Nano-Check COVID-19 Antigen Test kit format B. The preliminary LoD was further refined using three 2-fold serial dilutions with three replicates each.

Data are summarized in **Table 10** below. The Nano-Check COVID-19 Antigen Test LoD for kit format A and kit format B was confirmed to be $1.95x10^2$ $TCID_{50}$/mL with SARS-CoV-2 USA/MD-HP20874/2021 (Omicron variant, linage B.1.1.529). The test kit formats have equivalent LoD.

Table 10. Limit of Detection Study (Kit Format B)

Isolate	Conc. ($TCID_{50}$/mL)	Conc. ($TCID_{50}$/swab)	Range Finding			Confirmation		
			n	Neg	Pos (% Pos)	n	Neg	Pos (% Pos)
USA/MD-HP20874/2021 (Omicron)	3.89×10^2	$1.9x10^1$	3	0	3 (100%)	20	0	20 (100%)
	1.95×10^2	**9.75**	**3**	**0**	**3 (100%)**	**20**	**1**	**19 (95%)**
	9.73×10^1	4.8	3	1	3 (100%)	20	6	14 (70%)
	4.86×10^1	2.4	3	2	0 (0%)			
	3.89×10^1	1.9	3	3	0 (0%)			
	3.89×10^0	$1.9x10^{-1}$	3	3	3 (100%)			
	3.89×10^{-1}	$1.9x10^{-2}$	3	3	3 (100%)			

WHO International Standard LoD

The LoD of the Nano-Check COVID-19 Antigen Test was also determined by evaluating different dilutions of the WHO International Standard for SARS-CoV-2 antigen (NIBSC

code: 21/368) in negative clinical swab matrix. The WHO International Standard for SARS-CoV-2 containing lyophilized SARS-CoV-2 antigen was reconstituted in 0.25 mL of ultra-pure water (for a final concentration 20,000 IU/mL). The LoD was determined as the lowest virus concentration that was detected ≥ 95% of the time (i.e., concentration at which at least 19 out of 20 replicates tested positive). The limit of detection was established in two phases.

Three-fold serial dilutions were made from the WHO International Standard for SARS-CoV-2 antigen into negative clinical matrix (nasal fluid). Three replicates were tested on one lot of the assay for each dilution to determine the preliminary LoD concentration of the device. The lowest concentration with 3/3 positive results from each lot was considered the preliminary LoD. For each replicate, 50 µL of virus dilution was applied to a swab and the swab was processed according to the IFU. The results are summarized below.

Table 11. WHO International Standard for SARS-CoV-2 Antigen Preliminary LoD Results

Concentration of WHO International Standard for SARS-CoV-2 Antigen (Sample)	Amount of WHO International Standard for SARS-CoV-2 Antigen on Dry Swab	# Positive / # Tested
4,000 IU/mL	200 IU/swab	3/3
1,333 IU/mL	**67 IU/swab**	**3/3**
444 IU/mL	22 IU/swab	1/2
148 IU/mL	7.4 IU/swab	0/3
49 IU/mL	2.5 IU/swab	0/3

The preliminary LoD concentration was tested with an additional 17 replicates, for a total of 20 replicates, to confirm the LoD. Concentrations above and below the preliminary LoD were also tested with 20 replicates to further refine the LoD. Samples were prepared as for the preliminary LoD study above. To confirm the LoD, at least 19 of 20 replicates should be positive. The results are summarized below.

Table 12. WHO International Standard for SARS-CoV-2 Antigen Confirmatory LoD Results

Concentration of WHO International Standard for SARS-CoV-2 Antigen (Sample)	Amount of WHO International Standard for SARS-CoV-2 antigen on Dry Swab	# Positive / # Tested
2,000 IU/mL	100 IU/swab	20/20
1,333 IU/mL	67 IU/swab	19/20
1,000 IU/mL	50 IU/swab	20/20
889 IU/mL	**44 IU/swab**	**19/20**
800 IU/mL	40 IU/swab	16/20

The LoD for the Nano-Check COVID-19 Antigen Test using the 1st WHO International Standard for SARS-CoV-2 antigen (NIBSC code: 21/368) in nasal matrix was determined to be 889 IU/mL (44 IU/swab).

7. High-dose Hook Effect Study

A high-dose hook effect study was conducted to determine if a hook effect would be observed at high analyte concentration (i.e., a false negative at high concentrations of SARS-CoV-2). Five concentrations of SARS-CoV-2 (isolate USA-WA1/2020) were tested with three replicates each using a single lot of test kit format A (pre-filled reagent tube).

Concentrations ranged from 4,000x LoD (the maximum virus concentration possible, undiluted from the viral stock) to 1x LoD ($7.0x10^2$ $TCID_{50}$/mL) diluted in negative clinical matrix. All tested samples demonstrated 100% positivity, as expected (**Table 13**). The Nano-Check COVID-19 Antigen Test did not display a Hook Effect for the tested SARS-CoV-2 concentrations.

Table 13. High-Dose Hook Effect Study Results

Conc. ($TCID_{50}$/mL)	Sample Level	Positive Agreement (SCV-2 positive reps* / all reps)
2.8×10^6	4000x LoD	100% (3/3)
2.8×10^5	400x LoD	100% (3/3)
2.8×10^4	40x LoD	100% (3/3)
2.8×10^3	4x LoD	100% (3/3)
7.0×10^2	1x LoD	100% (3/3)

** Reps = replicates*

8. Inclusivity (Analytical Reactivity)

An inclusivity study was performed to demonstrate that the Nano-Check COVID-19 Antigen Test can detect circulating SARS-CoV-2 strains/isolates. Fifteen (15) stock strains and/or clinical isolates were included. A preliminary study was conducted to determine a near cutoff concentration for each strain/isolate tested in triplicate with one lot of test kit format A. Three-fold dilution series using the preliminary LoD were then prepared, and five replicates were tested to identify the dilution at which assay reactivity decreased for each strain/isolate using a separate lot of test kit format A (results summarized in **Table 14**). All tested isolates were detected.

Table 14. Inclusivity Study Results

Variant / Lineage	Strain	Concentration	Positive Agreement (SCV-2 pos. reps / all reps)
Wild-type	USA-WA1/2020*	4.79×10^4 $TCID_{50}$/mL	100% (5/5)
		1.60×10^4 $TCID_{50}$/mL	80.0% (4/5)
		5.32×10^3 $TCID_{50}$/mL	0% (0/5)
Alpha (B.1.1.7)	USA/CA_CDC_5574/2020	2.03×10^7 genome copies/mL	100% (5/5)
		6.77×10^6 genome copies/mL	100% (5/5)
		2.26×10^6 genome copies/mL	40.0% (2/5)
	England/204820464/2020	7.19×10^3 $TCID_{50}$/mL	100% (5/5)
		2.40×10^3 $TCID_{50}$/mL	20.0% (1/5)
		7.99×10^2 $TCID_{50}$/mL	0% (0/5)
	USA/CA_CDC_5574/2020	2.39×10^4 $TCID_{50}$/mL	100% (5/5)
		7.97×10^3 $TCID_{50}$/mL	80.0% (4/5)
		2.66×10^3 $TCID_{50}$/mL	20.0% (1/5)
Beta (B.1.351)	hCoV-19/USA/MD-HP01542/2021	2.16×10^5 genome copies/mL	100% (5/5)
		7.20×10^4 genome copies/mL	100% (5/5)
		2.40×10^4 genome copies/mL	0% (0/5)
	USA/MD-HP01542/2021	3.80×10^6 genome copies/mL	100% (5/5)
		1.27×10^6 genome copies/mL	80.0% (4/5)
		4.22×10^5 genome copies/mL	20.0% (1/5)

Variant / Lineage	Strain	Concentration	Positive Agreement (SCV-2 pos. reps / all reps)
	South Africa/KRISP-K005325/2020	1.90 x 10^4 $TCID_{50}$/mL	100% (5/5)
		6.33 x 10^3 $TCID_{50}$/mL	60.0% (3/5)
		2.11 x 10^3 $TCID_{50}$/mL	0% (0/5)
Gamma (P.1)	Japan/TY7-503/2021	1.58 x 10^4 $TCID_{50}$/mL	100% (5/5)
		5.27 x 10^3 $TCID_{50}$/mL	40.0% (2/5)
		1.76 x 10^3 $TCID_{50}$/mL	0% (0/5)
	USA/NY-Wadsworth-21033899-01/2021	7.85 x 10^3 $TCID_{50}$/mL	100% (5/5)
		2.62 x 10^3 $TCID_{50}$/mL	60.0% (3/5)
		8.72 x 10^2 $TCID_{50}$/mL	20.0% (1/5)
Delta (B.1.617.2)	hCoV-19/USA/MD-HP05285/2021	2.16 x 10^8 genome copies/mL	100% (5/5)
		7.20 x 10^7 genome copies/mL	100% (5/5)
		2.40 x 10^7 genome copies/mL	20.0% (1/5)
	USA/PHC658/2010	5.21 x 10^2 $TCID_{50}$/mL	100% (5/5)
		1.74 x 10^2 $TCID_{50}$/mL	40.0% (2/5)
		5.79 x 10^1 $TCID_{50}$/mL	0% (0/5)
	USA/MD- HP05285/2021	1.50 x 10^4 genome copies/mL	100% (5/5)
		5.00 x 10^4 genome copies/mL	100% (5/5)
		1.67 x 10^3 genome copies/mL	20.0% (1/5)
Kappa (B.1.617.1)	USA/CA-Stanford-15_S02/2021	8.48 x 10^4 $TCID_{50}$/mL	100% (5/5)
		2.83 x 10^4 $TCID_{50}$/mL	40.0% (2/5)
		9.42 x 10^3 $TCID_{50}$/mL	0% (0/5)
Omicron (B.1.1529)	hCoV-19/USA/MD-HP20874/2021	1.95 x 10^2 $TCID_{50}$/mL	100% (5/5)
		6.50 x 10^1 $TCID_{50}$/mL	60.0% (3/5)
		2.17 x 10^1 $TCID_{50}$/mL	20.0% (1/5)
	USA/GA-EHC-2811C/2021	6.33 x 10^4 genome copies/mL	100% (5/5)
		2.11 x 10^4 genome copies/mL	100% (5/5)
		7.03 x 10^3 genome copies/mL	60.0% (3/5)

**The USA-WA1/2020 strain tested here is from a different lot/supplier than the strain used for LoD testing. The stock/lot used for LoD testing is the same as that used for the remaining analytical studies. Therefore, the LoD concentration differences observed here are deemed to be a result of propagating the virus stocks and its functional testing method.*

9. Assay Cut-Off:

Not applicable.

B Comparison Studies:

1. Method Comparison with Predicate Device:

Refer to Section VII.C (Clinical Studies) below for clinical validation.

2. Matrix Comparison:

The Nano-Check COVID-19 Antigen Test is only intended for qualitative detection of nucleocapsid protein antigen from SARS-CoV-2 in direct anterior nasal swab specimens. As

no other specimen or sample type is claimed for this device, a matrix comparison study to support other sample types for clinical testing with this device was not performed.

However, the sponsor conducted a matrix equivalency study between negative clinical nasal swab matrix (nasal fluid) and representative negative clinical matrix (NCM derived from nasal wash) that was used in some analytical studies (reproducibility/precision, cross-reactivity, interference, inclusivity, and reagent/kit storage). The data demonstrated the equivalent performance of the test with both matrices.

C Clinical Studies:

The performance of the Nano-Check COVID-19 Antigen Test in detecting SARS-CoV-2 viral nucleoprotein antigen from anterior nasal swab samples was evaluated in a multi-center, prospective study conducted in the U.S. from January 2022 to January 2023 when Omicron was the predominantly circulating variant. A total of seven hundred and eighty-six (786) subjects were consecutively enrolled and tested across four (4) different point-of-care (POC) CLIA-waived sites. Nine (9) operators across the study sites conducted the enrollment and testing of the subjects. The study only enrolled subjects with symptoms of respiratory infection consistent with SARS-CoV-2 infection.

Two anterior nasal (AN) swabs were collected from each study subject during same visit. The first swab (for comparator testing) was collected by the study operator from both sides of the nose, placed into a transport tube containing 1mL of Universal Viral Transport (UVT) media, stored on dry ice, and shipped with dry ice to the reference laboratory. Upon receipt by the reference laboratory, the swab was tested with a highly sensitive FDA cleared RT-PCR comparator assay. The second swab was collected by the study operator from both sides of the nose and tested immediately with the Nano-Check COVID-19 Antigen Test at the site. Demographics, symptom information, and health history were also collected from each subject.

There were 670 evaluable subjects with 40.4% (271/670) male and 59.6% (399/670) female, with a mean age of 37 years. A total of one hundred and sixteen (116) subjects of the 786 enrolled were excluded because they did not meet the enrollment criteria. Results obtained with the Nano-Check COVID-19 Antigen Test were compared to the results obtained with the RT-PCR comparator test to determine clinical sensitivity and specificity. The study cohort included 18.37% low positive samples.

The Nano-Check COVID-19 Antigen Test demonstrated a clinical sensitivity as estimated by positive precent agreement of 83.67% (123/147; 95% CI: 76.86% - 88.78%) and specificity as estimated by negative percent agreement of 99.62% (521/523; 95% CI: 98.62% - 99.90%) compared to the comparator method.

Table 15. Clinical performance of the Nano-Check COVID-19 Antigen Test compared to a highly sensitive cleared RT-PCR comparator

		Cleared RT-PCR Comparator		
		Pos	**Neg**	*Total*
Nano-Check COVID-19 Antigen Test	**Pos**	123	2	*125*
	Neg	24	521	*545*
	Total	*147*	*523*	*670*

Table 16. Clinical performance of the Nano-Check COVID-19 Antigen Test stratified by DPSO

Days Post Symptom Onset	PPA	NPA
1	80.95% (17/21)	100.00% (74/74)
2	80.45% (37/46)	99.51% (202/203)
3	84.09% (37/44)	99.32% (145/146)
4	94.12% (32/34)	100.00% (96/96)
Total	**83.67% (123/147)** 95% CI: 76.86% - 88.78%	**99.62% (521/523)** 95% CI: 98.62% - 99.90%

1. Clinical Sensitivity:
   Refer to Section VII.C (Clinical Studies) above for the clinical validation, including test sensitivity/positive percent agreement (PPA). The PPA for the test is 83.67% (123/147; 95% CI: 73.86% - 88.78%).

2. Clinical Specificity:
   Refer to Section VII.C (Clinical Studies) above for the clinical validation, including test specificity/negative percent agreement (NPA). The NPA for the test is 99.62% (521/523; 95% CI: 98.62% - 99.90%).

3. Serial Testing:
   As a mitigation for the low performance of the device at Day 0 of symptom onset, as indicated by the clinical study data and in other studies for test devices of a similar principle and design, the Intended Use for this test device (and associated Instructions for Use) include recommendations for repeat testing (i.e., test at least twice over three days with at least 48 hours between tests). This mitigation is supported by data generated by the National Institutes for Health (NIH) and the University of Massachusetts Chan Medical School (in collaboration with the FDA) demonstrating that repeat testing over multiple days improves test performance and increases the likelihood that a COVID-19 antigen test will accurately detect an infection. These results have informed the FDA's general understanding that repeat testing after a negative result from a COVID-19 antigen test reduces the risk of a false negative result. Please refer to the following studies for additional details:
   - Finding a Needle in the Haystack: Design and Implementation of a Digital Site-less Clinical Study of Serial Rapid Antigen Testing to Identify Asymptomatic SARS-CoV-2 Infection - https://www.medrxiv.org/content/10.1101/2022.08.04.22278274v1
   - Performance of Screening for SARS-CoV-2 using Rapid Antigen Tests to Detect Incidence of Symptomatic and Asymptomatic SARS-CoV-2 Infection: findings from the Test Us at Home prospective cohort study - https://www.medrxiv.org/content/10.1101/2022.08.05.22278466v1

D Clinical Cut-Off:

The Clinical Cut-off study is not applicable, as there is not clinical cutoff related to the presence of SARS-CoV-2 in patient samples.

E Expected Values/Reference Range:

Patient samples are expected to be negative for SARS-CoV-2.

F Other Supportive Performance Characteristics Data:

1. Flex Studies

To assess the robustness of the Nano-Check COVID-19 Antigen Test, flex studies were conducted that assessed all major aspects of the test procedure (sample volume, reading time, extraction buffer volume, bubbles in the reagent tube, swab elution time and procedure, sample hold time before and during processing, use of incorrect specimen type) and variability of environmental test conditions that the test may be subjected to when in use (lighting, disturbance during use, temperature and humidity stress conditions). Testing was performed with contrived positive nasal swabs generated by diluting heat inactivated SARS-CoV-2 virus (Omicron variant) into negative clinical nasal swab matrix at 2xLoD. The studies support that the test is robust in the intended use condition with an insignificant risk of erroneous result.

VIII Proposed Labeling:

The labeling supports the finding of substantial equivalence for this device.

IX Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.