510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY

I Background Information:

A 510(k) Number

K243561

B Applicant

Nano-Ditech Corporation

C Proprietary and Established Names

Nano-Check Influenza+COVID-19 Dual Test

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
SCA	Class II	21 CFR 866.3987 - Multi-Analyte Respiratory Virus Antigen Detection Test	MI - Microbiology

II Submission/Device Overview:

A Purpose for Submission:

To obtain 510(k) clearance for the Nano-Check Influenza+COVID-19 Dual Test.

B Measurand:

Influenza A and B Nucleoprotein antigens and SARS-CoV-2 nucleocapsid protein antigens

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 Influenza+COVID-19 Dual Test is a lateral flow immunochromatographic assay intended for the qualitative detection and differentiation of influenza A, and influenza B nucleoprotein antigens and SARS-CoV-2 nucleocapsid antigen directly in anterior nasal swab (ANS) samples from individuals with signs and symptoms of respiratory tract infection. Clinical signs and symptoms of respiratory viral infection due to SARS-CoV-2 and influenza can be similar.

All negative results are presumptive and should be confirmed with a molecular assay, if necessary, for patient management. Negative results do not rule out infection with influenza or SARS-CoV-2 and should not be used as the sole basis for treatment or patient management decisions.

Positive results do not rule out bacterial infection or co-infection with other viruses.

C Special Conditions for Use Statement(s):

Rx - For Prescription Use Only

IVD - For In Vitro Diagnostic Use Only

D Special Instrument Requirements:

Not Applicable

E Device/System Characteristics:

1. Device Description:

The Nano-Check Influenza+COVID-19 Dual Test is an in vitro lateral flow immunochromatographic assay intended for rapid, simultaneous qualitative detection and differentiation of influenza A, and influenza B nucleoprotein antigens and SARS-CoV-2 nucleocapsid antigens. This device is intended for prescription use only with anterior nasal swab (ANS) samples from individuals with signs and symptoms of respiratory infection within the first four (4) days of symptom onset.

The assay kit consists of 25 test cassette devices, 25 reagent tubes, 25 ampules containing extraction buffer, 25 anterior nasal specimen collection swabs, one positive control swab (contains noninfectious influenza A, influenza B, and SARS-CoV-2 recombinant antigen), one negative control swab (without recombinant antigen), one Instructions for Use (IFU), and one Quick Reference Instruction (QRI).

Test strips are enclosed in a cassette housing comprised of the following components: sample pad, reagent pad, biotin pad, reaction membrane, and absorbent pad. The reagent pad contains colloidal gold conjugated with monoclonal antibodies (mAb) specific for SARS-CoV-2, influenza A, and influenza B target proteins. The biotin pad contains biotin

conjugated with mAb specific for SARS-CoV-2. The reaction membrane contains the secondary antibodies for the proteins of influenza A and influenza B, and streptavidin for the biotinylated SARS-CoV-2 antibody. The whole strip is fixed inside a plastic cassette. The schematic of the test cassette device is shown below.

Fig 1: Test Device of Nano-Check Influenza+COVID-19 Dual Test Strip

2. Principle of Operation:

The Nano-Check Influenza+COVID-19 Dual Test is designed to detect the extracted nucleoprotein antigen specific to influenza (Flu) A, and Flu B and the extracted nucleocapsid antigen specific to SARS-CoV-2 in ANS specimens directly collected from patients exhibiting signs or symptoms of a respiratory infection.

When the specimens are extracted and added to the sample well of the test device, Flu A, Flu B nucleoproteins and/or SARS-CoV-2 nucleocapsid antigen is present in the specimen, a complex form between the anti-Flu A/Flu B/ SARS-CoV-2 conjugate and the viral antigen will be captured by the streptavidin or specific anti-Flu A/Flu B mAb coated on the test line region (C/A/B line). The absence of the test line (C/A/B line) is interpreted as a negative result. To serve as a procedural control, a red line will always appear in the control line region (CON) indicating that proper volume of sample has been added and membrane wicking has occurred. Any result without this control line is invalid.

IV Substantial Equivalence Information:

A Predicate Device Name(s):

Healgen Rapid Check COVID-19/Flu A&B Antigen Test

B Predicate 510(k) Number(s):

DEN240029

C Comparison with Predicate(s):

Device & Predicate Device(s):	**K243561**	**Predicate: DEN240029**
Device Trade Name	Nano-Check Influenza+COVID-19 Dual	Healgen Rapid Check COVID-19/Flu A&B

Device & Predicate Device(s):	K243561	Predicate: DEN240029
	Test	Antigen Test
General Device Characteristic Similarities		
Intended Use/Indications For Use	The Nano-Check Influenza+COVID-19 Dual Test is a lateral flow immunochromatographic assay intended for the qualitative detection and differentiation of influenza A, and influenza B nucleoprotein antigens and SARS-CoV-2 nucleocapsid antigen directly in anterior nasal swab (ANS) samples from individuals with signs and symptoms of respiratory tract infection. Clinical signs and symptoms of respiratory viral infection due to SARS-CoV-2 and influenza can be similar. All negative results are presumptive and should be confirmed with a molecular assay, if necessary, for patient management. Negative results do not rule out infection with influenza or SARS-CoV-2 and should not be used as the sole basis for treatment or patient management decisions. Positive results do not rule out bacterial infection or co-infection with other viruses.	The Healgen Rapid Check COVID-19/Flu A&B Antigen Test is a lateral flow immunochromatographic assay intended for the qualitative detection and differentiation of influenza A, and influenza B nucleoprotein antigens and SARS-CoV-2 nucleocapsid antigen directly in anterior nasal swab samples from individuals with signs and symptoms of respiratory tract infection. Symptoms of respiratory infections due to SARS-CoV-2 and influenza can be similar. This test is for non-prescription home use by individuals aged 14 years or older testing themselves, or adults testing individuals aged 2 years or older. All negative results are presumptive and should be confirmed with an FDA-cleared molecular assay when determined to be appropriate by a healthcare provider. Negative results do not rule out infection with influenza, SARS-CoV-2 or other pathogens. Individuals who test negative and experience continued or worsening respiratory symptoms, such as fever, cough and/or shortness of breath, should seek follow-up

Device & Predicate Device(s):	K243561	Predicate: DEN240029
		care from their healthcare provider. Positive results do not rule out co-infection with other respiratory pathogens, and therefore do not substitute for a visit to a healthcare provider or appropriate follow-up.
Regulation Number	21 CFR 866.3987	Same
Assay Principle (Technology)	Lateral flow immune chromatographic assay	Same
Specimen Type	Anterior nasal swab specimens	Same
Analyte	Influenza A, and influenza B nucleoprotein antigens and SARS-CoV-2 nucleocapsid antigen	Same
Usage	Single use test	Same
Test Result	Qualitative	Same
Result Interpretation	Visually Read	Same
Reading Time	15 – 20 minutes	15 - 20 minutes
Storage Temperature	2°-30°C	Same
General Device Characteristic Differences		
Patient Use Setting	For prescription use	Over the counter use

V Standards/Guidance Documents Referenced:

Document Number	Title	Publishing Organization	Purpose
21 CFR 866.3987	Special controls for multi-analyte respiratory virus antigen detection test, an in vitro diagnostic device intended for the detection and/or differentiation of respiratory viruses directly from respiratory clinical specimens. The device is intended to be performed at the site of sample collection, does not involve sample storage and/or transport.	FDA/CDRH	General Use
EP12-A2	User Protocol for Evaluation of Qualitative Test Performance; Approved Guideline - Second Edition.	CLSI	General Use

EP05-A3 (Reaffirmed: September 2019)	Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline – Third Edition	CLSI	General Use
EP37 1st Edition	Supplemental tables for Interference Testing in Clinical Chemistry	CLSI	General Use
14971 Third Edition 2019-12	Medical Devices – Application of risk management to medical devices	ISO	General Use

VI Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/Reproducibility:

a. Within-lab Precision/Single-lot Repeatability Study:

The repeatability study was conducted by two operators with each operator conducting 24 test runs over a 12-day period, resulting in a total of 96 replicates per concentration and negative samples. The study included blind sample panels comprising of true negative (TN), low positive (LP, 1x LoD) and moderate positive (MP, 3x LoD) samples for each analyte (SARS-CoV-2, Flu A and Flu B). The study results are summarized in **Table 1** below and shows 100% agreement for all samples.

Table 1: Overall Results from Single-lot Repeatability Study

Analyte	**SARS-CoV-2**		**Flu A**		**Flu B**		**TN**
Sample Level	**MP (3× LoD)**	**LP (1× LoD)**	**MP (3× LoD)**	**LP (1× LoD)**	**MP (3× LoD)**	**LP (1× LoD)**	
# Correct/Total	96/96	96/96	96/96	96/96	96/96	96/96	96/96
All operators Total % Agreement	100%	100%	100%	100%	100%	100%	100%
All operators Total 95% CI	96.2 - 100%	96.2 - 100%	96.2 - 100%	96.2 - 100%	96.2 - 100%	96.2 - 100%	96.2 - 100%

b. Lot-to-lot Precision Study:

The lot-to-lot consistency evaluation was conducted over a 12-day period to assess the precision of test results across different production lots using a contrived sample panel consisting of true negative, a low positive (C90) and a moderate positive sample (3x LoD). The study was conducted by two on-site operators for over 12 non-consecutive days with two replicate tests performed per sample per day per device lot, resulting in a total of 24 independent replicates per sample per device lot. The summary of lot-to-lot precision study results are shown in **Table 2** below and are acceptable.

Table 2: Summary of Between-lot and Between-operator Variability

Analyte	Test line	Between lot						Between operator			
		Lot 1		Lot 2		Lot 3		Operator 1		Operator 2	
		Positive	%*	Positive	%*	Positive	%*	Positive	%*	Positive	%*
Negative	COVID-19	0/24	100%	0/24	100%	0/24	100%	0/36	100%	0/36	100%
	Flu A	0/24	100%	0/24	100%	0/24	100%	0/36	100%	0/36	100%
	Flu B	0/24	100%	0/24	100%	0/24	100%	0/36	100%	0/36	100%
C90 COVID-19	COVID-19	20/24	83.3%	23/24	95.8%	21/24	87.5%	31/36	86.1%	33/36	91.7%
	Flu A	0/24	100%	0/24	100%	0/24	100%	0/36	100%	0/36	100%
	Flu B	0/24	100%	0/24	100%	0/24	100%	0/36	100%	0/36	100%
C90 Flu A	COVID-19	0/24	100%	0/24	100%	0/24	100%	0/36	100%	0/36	100%
	Flu A	20/24	83.3%	21/24	87.5%	23/24	95.8%	32/36	88.9%	32/36	88.9%
	Flu B	0/24	100%	0/24	100%	0/24	100%	0/36	100%	0/36	100%
C90 Flu A	COVID-19	0/24	100%	0/24	100%	0/24	100%	0/36	100%	0/36	100%
	Flu A	0/24	100%	0/24	100%	0/24	100%	0/36	100%	0/36	100%
	Flu B	22/24	91.7%	21/24	87.5%	24/24	100%	33/36	88.9%	34/36	94.4%
3X LoD COVID-19	COVID-19	24/24	100%	24/24	100%	24/24	100%	36/36	100%	36/36	100%
	Flu A	0/24	100%	0/24	100%	0/24	100%	0/36	100%	0/36	100%
	Flu B	0/24	100%	0/24	100%	0/24	100%	0/36	100%	0/36	100%
3X LoD Flu A	COVID-19	0/24	100%	0/24	100%	0/24	100%	0/36	100%	0/36	100%
	Flu A	24/24	100%	24/24	100%	24/24	100%	36/36	100%	36/36	100%
	Flu B	0/24	100%	0/24	100%	0/24	100%	0/36	100%	0/36	100%
3X LoD Flu B	COVID-19	0/24	100%	0/24	100%	0/24	100%	0/36	100%	0/36	100%
	Flu A	0/24	100%	0/24	100%	0/24	100%	0/36	100%	0/36	100%
	Flu B	24/24	100%	24/24	100%	24/24	100%	36/36	100%	36/36	100%

*Percent Agreement

c. Multi-site Reproducibility Study:

A multi-site reproducibility study was performed to assess the performance of the candidate device using a contrived sample panel comprised of a true negative (TN), a high negative sample (HN, 0.1x LoD), a low positive (LP, 1x LoD) and a moderate positive (MP, 5x LoD) sample for each analyte. Contrived swab samples were prepared by spiking pooled human nasal wash solution (confirmed SARS-CoV-2, Flu A, and Flu B negative by RT-PCR) with SARS-CoV-2 (Omicron variant), Flu A (H1N1), and Flu B (Victoria Lineage) to concentrations of 5x LoD, 1x LoD or 0.1x LoD. Each diluted sample (50 µL) was directly applied onto the sample collection swab head. True negative swab samples were prepared by applying fifty (50) µL of negative pooled human nasal wash directly onto the sample collection swab head.

The contrived sample swabs were randomized and blinded to each operator at three (3) discrete CLIA-waived sites and an in-house site. Eight (8) operators at the CLIA-waived sites and three (3) additional trained operators at the internal site conducted testing for over five non-consecutive days. Each operator tested 150 encoded samples, consisting of 15 samples each for various analyte concentrations.

The results in **Table 3** below showed complete concordance, with 100% agreement (95% CI: 97.7-100%) among the eleven (11) operators for TN HN for COVID-19 and Flu A, LP for COVID-19 and Flu B, as well as all MP samples. The results between HN-Flu B and LP-Flu A exhibited agreement of 99.4% (95% CI: 96.7-99.9%). These outcomes all met the predefined acceptance criteria and generated no significant difference between sites.

Table 3: Summary Results of Multi-site Reproducibility Study

Sample	No. of Positive Results/No. of Total Tests (Positive Rate %)				All Sites	
	CLIA-Waived Site 1 (2 operators)	CLIA-Waived Site 2 (3 operators)	CLIA-Waived Site 3 (3 operators)	In-house Site 4 (3 operators)	Agreement	95% CI
True Negative NCM	0/30 (0%)	0/45 (0%)	0/45 (0%)	0/45 (0%)	165/165 (100%)	97.7-100.0
0.1x LoD SARS CoV-2	0/30 (0%)	0/45 (0%)	0/45 (0%)	0/45 (0%)	165/165 (100%)	97.7-100.0
0.1x LoD Flu A	0/30 (0%)	0/45 (0%)	0/45 (0%)	0/45 (0%)	165/165 (100%)	97.7-100.0
0.1x LoD Flu B	0/30 (0%)	0/45 (0%)	1/45 (2.2%)	0/45 (0%)	164/165 (99.4%)	96.7-99.9
1x LoD SARS CoV-2	30/30 (100%)	45/45 (100%)	45/45 (100%)	45/45 (100%)	165/165 (100%)	97.7-100.0
1x LoD Flu A	30/30 (100%)	45/45 (100%)	44/45 (97.8%)	45/45 (100%)	164/165 (99.4%)	96.7-99.9
1x LoD Flu B	30/30 (100%)	45/45 (100%)	45/45 (100%)	45/45 (100%)	165/165 (100%)	97.7-100.0
5x LoD SARS CoV-2	30/30 (100%)	45/45 (100%)	45/45 (100%)	45/45 (100%)	165/165 (100%)	97.7-100.0
5x LoD Flu A	30/30 (100%)	45/45 (100%)	45/45 (100%)	45/45 (100%)	165/165 (100%)	97.7-100.0
5x LoD Flu B	30/30 (100%)	45/45 (100%)	45/45 (100%)	45/45 (100%)	165/165 (100%)	97.7-100.0

2. Linearity:

This is a qualitative assay and linearity is not applicable.

3. Analytical Specificity/Interference:

a. Cross-reactivity and Microbial Interference:

Cross-reactivity and microbial interference studies were conducted to determine if other respiratory pathogens/microbial flora that may be present in the nasal swab samples could cause a false positive test result or interfere with a true positive test result. A total of 50 potential cross-reactive or interfering pathogens comprising of bacteria (19), fungus (1), and viruses (28) and negative matrix (2) were tested in three (3) replicates in the absence (cross-reactivity) or presence (microbial interference) of 2x LoD concentrations of SARS-CoV-2, Flu A and Flu B antigens. These pathogens were diluted using the pooled human nasal fluid in 1X PBS to the target concentration, higher than $1x10^6$ CFU/mL or IFU/mL for bacteria and fungi, or higher than $1x10^5$ pfu/mL ($TCID_{50}$/mL, $CEID_{50}$/mL, or cp/mL) for viruses. Samples were tested in randomized and blinded manner by three operators.

All samples containing potential cross-reactive or interfering pathogens yielded the anticipated test results, as described in **Table 4** below. No instances of false negatives or false positives were observed during the study. Consequently, this study affirms that the Nano-Check Influenza +

COVID-19 Dual Test exhibits no reactivity with, nor interference from, the listed pathogens, high prevalence disease agents, and normal or pathogenic flora that are reasonably likely to be encountered in clinical specimens.

Table 4: Results of Cross-reactivity/Microbial Interference

Microorganism (Strain)	Concentration Tested	Positive Sample (# Positive/ # Tested)	Negative Sample (# Positive/ # Tested)	Cross-Reactivity/ Microbial Interference
Bordetella pertussis	1.0×10^6 CFU/mL	3/3	0/3	No
Candida albicans	1.0×10^6 CFU/mL	3/3	0/3	No
Chlamydophila pneumoniae	1.0×10^6 IFU/mL	3/3	0/3	No
Corynebacterium diphtheriae	1.0×10^6 CFU/mL	3/3	0/3	No
Escherichia coli	1.0×10^6 IFU/mL	3/3	0/3	No
Haemophilus influenzae B	1.0×10^6 CFU/mL	3/3	0/3	No
Lactobacillus acidophilus	1.0×10^6 CFU/mL	3/3	0/3	No
Legionella Pneumophila subsp. *Pneumophila*	1.0×10^6 CFU/mL	3/3	0/3	No
Moraxella catarrhalis	1.0×10^6 CFU/mL	3/3	0/3	No
Mycobacterium tuberculosis	1.0×10^6 CFU/mL	3/3	0/3	No
Mycoplasma pneumoniae	1.0×10^6 CFU/mL	3/3	0/3	No
Neisseria meningitidis	1.0×10^6 CFU/mL	3/3	0/3	No
Neisseria mucosa	1.0×10^6 CFU/mL	3/3	0/3	No
Neisseria subflava	1.0×10^6 CFU/mL	3/3	0/3	No
Pneumocystis jirovecii -S. cerevisiae	1.0×10^6 CFU/mL	3/3	0/3	No
Pseudomonas aeruginosa	1.0×10^6 CFU/mL	3/3	0/3	No
Staphylococcus aureus	1.0×10^6 CFU/mL	3/3	0/3	No
Staphylococcus epidermidis	1.0×10^6 CFU/mL	3/3	0/3	No
Streptococcus pneumoniae	1.0×10^6 CFU/mL	3/3	0/3	No
Streptococcus pyogenes	1.0×10^6 CFU/mL	3/3	0/3	No
Streptococcus salivarius	1.0×10^6 CFU/mL	3/3	0/3	No
Epstein-Barr Virus	1.0×10^5 cp/mL	3/3	0/3	No
Enterovirus 71	1.0×10^5 $TCID_{50}$/mL	3/3	0/3	No
Enterovirus D 68	1.0×10^5 $TCID_{50}$/mL	3/3	0/3	No
Human Herpesvirus 5	8.0×10^4 $TCID_{50}$/mL	3/3	0/3	No
Human Adenovirus 1	1.0×10^5 $TCID_{50}$/mL	3/3	0/3	No
Human Adenovirus 2	1.0×10^5 $TCID_{50}$/mL	3/3	0/3	No
Human Adenovirus 7	1.0×10^5 $TCID_{50}$/mL	3/3	0/3	No
Human Coronavirus, 229E	1.0×10^5 $TCID_{50}$/mL	3/3	0/3	No
Human Coronavirus, NL63	7.0×10^4 $TCID_{50}$/mL	3/3	0/3	No
Human Coronavirus, OC43	4.5×10^4 $TCID_{50}$/mL	3/3	0/3	No
Human Metapneumovirus 3, B1, Peru2-2002	1.95×10^4 $TCID_{50}$/mL	3/3	0/3	No
Human Metapneumovirus, TN/83-1211	1.0×10^5 $TCID_{50}$/mL	3/3	0/3	No
Human Parainfluenza Virus 1	1.0×10^5 $TCID_{50}$/mL	3/3	0/3	No
Human Parainfluenza Virus 2	1.0×10^5 $TCID_{50}$/mL	3/3	0/3	No
Human Parainfluenza Virus 3	1.0×10^5 $TCID_{50}$/mL	3/3	0/3	No
Human Parainfluenza Virus 4B	1.0×10^5 $TCID_{50}$/mL	3/3	0/3	No
Human RSV, A Long	1.0×10^5 $TCID_{50}$/mL	3/3	0/3	No
Human RSV, A 9320	1.0×10^5 $TCID_{50}$/mL	3/3	0/3	No
Human RSV, A2	1.0×10^5 $TCID_{50}$/mL	3/3	0/3	No
Human RSV, B 18537	1.0×10^5 $TCID_{50}$/mL	3/3	0/3	No
Human RSV, B WV/14617/85	1.0×10^5 $TCID_{50}$/mL	3/3	0/3	No

Microorganism (Strain)	Concentration Tested	Positive Sample (# Positive/ # Tested)	Negative Sample (# Positive/ # Tested)	Cross-Reactivity/ Microbial Interference
Human RSV, B1	1.0×10^5 $TCID_{50}$/mL	3/3	0/3	No
Human Rhinovirus 1A	1.0×10^5 PFU/mL	3/3	0/3	No
Measles Virus	1.7×10^4 $TCID_{50}$/mL	3/3	0/3	No
MERS-CoV	1.0×10^5 $TCID_{50}$/mL	3/3	0/3	No
Mumps Virus	1.0×10^5 $TCID_{50}$/mL	3/3	0/3	No
Rhinovirus 20, 15-CV19	1.0×10^5 $TCID_{50}$/mL	3/3	0/3	No
SARS-CoV	1.0×10^5 PFU/mL	3/3	0/3	No
Pooled Human Nasal Wash	N/A	3/3	0/3	No
Pooled Human Nasal Fluid	N/A	3/3	0/3	No
Note: Coronavirus HKU1 was not tested for cross-reactivity due to lack of availability. 20 clinical samples that are positive for Coronavirus HKU1 were tested, and all resulted as negative, however, the viral load/concentration of each sample is unknown.				

b. Endogenous/Exogenous Substances Interference Study:

The interference study aimed to evaluate potential endogenous and exogenous interference with the Nano-Check Influenza+COVID-19 Dual Test. Thirty-four (34) substances, including whole blood, various over-the-counter (OTC) products, household items, and common chemicals, were assessed for cross-reactivity or interference with SARS-CoV-2, Flu A, and Flu B detection. Each substance was spiked onto swab heads in the presence or absence of 2x LoD concentrations of the antigens. Samples were tested in randomized and blinded manner by three operators.

All samples, including those containing potentially interfering substances, yielded expected results without false negatives or false positives as shown in **Table 5** below. Therefore, the identified substances are unlikely to interfere with the ability of the Nano-Check Influenza+COVID-19 Dual Test to detect SARS-CoV-2, Flu A, or Flu B.

Table 5: Endogenous/Exogenous Interference Test Results

Endogenous/Exogenous Substance Test concentration	Spiked Virus (Concentration)	No. of Positive Results / No. of Total replicates		
		SARS CoV-2	Flu A	Flu B
Nasal Spray (Flonase) Test conc. = 15% v/v	Negative	0 / 3	0 / 3	0 / 3
	SARS-CoV-2 (2x LoD)	3 / 3	0 / 3	0 / 3
	Influenza A (2x LoD)	0 / 3	3 / 3	0 / 3
	Influenza B (2x LoD)	0 / 3	0 / 3	3 / 3
Nasal Spray (Samilisan) Test conc. = 15% v/v	Negative	0 / 3	0 / 3	0 / 3
	SARS-CoV-2 (2x LoD)	3 / 3	0 / 3	0 / 3
	Influenza A (2x LoD)	0 / 3	3 / 3	0 / 3
	Influenza B (2x LoD)	0 / 3	0 / 3	3 / 3
Nasal Spray (CVS) Test conc. = 15% v/v	Negative	0 / 3	0 / 3	0 / 3
	SARS-CoV-2 (2x LoD)	3 / 3	0 / 3	0 / 3
	Influenza A (2x LoD)	0 / 3	3 / 3	0 / 3
	Influenza B (2x LoD)	0 / 3	0 / 3	3 / 3
Nasal Spray 4 (Afrin) Test conc. = 15% v/v	Negative	0 / 3	0 / 3	0 / 3
	SARS-CoV-2 (2x LoD)	3 / 3	0 / 3	0 / 3
	Influenza A (2x LoD)	0 / 3	3 / 3	0 / 3
	Influenza B (2x LoD)	0 / 3	0 / 3	3 / 3

Endogenous/Exogenous Substance Test concentration	Spiked Virus (Concentration)	No. of Positive Results / No. of Total replicates		
		SARS CoV-2	Flu A	Flu B
Budesonide Nasal Spray Test conc. = 15% v/v	Negative	0 / 3	0 / 3	0 / 3
	SARS-CoV-2 (2x LoD)	3 / 3	0 / 3	0 / 3
	Influenza A (2x LoD)	0 / 3	3 / 3	0 / 3
	Influenza B (2x LoD)	0 / 3	0 / 3	3 / 3
NASONEX 24 hr. Allergy Test conc. = 15% v/v	Negative	0 / 3	0 / 3	0 / 3
	SARS-CoV-2 (2x LoD)	3 / 3	0 / 3	0 / 3
	Influenza A (2x LoD)	0 / 3	3 / 3	0 / 3
	Influenza B (2x LoD)	0 / 3	0 / 3	3 / 3
Nasacort Allergy 24 hr. Test conc. = 15% v/v	Negative	0 / 3	0 / 3	0 / 3
	SARS-CoV-2 (2x LoD)	3 / 3	0 / 3	0 / 3
	Influenza A (2x LoD)	0 / 3	3 / 3	0 / 3
	Influenza B (2x LoD)	0 / 3	0 / 3	3 / 3
Sore Throat (Oral Pain Reliever spay) Test conc. = 15% v/v	Negative	0 / 3	0 / 3	0 / 3
	SARS-CoV-2 (2x LoD)	3 / 3	0 / 3	0 / 3
	Influenza A (2x LoD)	0 / 3	3 / 3	0 / 3
	Influenza B (2x LoD)	0 / 3	0 / 3	3 / 3
ZICAM Oral mist Test conc. = 15% v/v	Negative	0 / 3	0 / 3	0 / 3
	SARS-CoV-2 (2x LoD)	3 / 3	0 / 3	0 / 3
	Influenza A (2x LoD)	0 / 3	3 / 3	0 / 3
	Influenza B (2x LoD)	0 / 3	0 / 3	3 / 3
Sore Throat Lozenges (Cepacol) Test conc. = 15% w/v	Negative	0 / 3	0 / 3	0 / 3
	SARS-CoV-2 (2x LoD)	3 / 3	0 / 3	0 / 3
	Influenza A (2x LoD)	0 / 3	3 / 3	0 / 3
	Influenza B (2x LoD)	0 / 3	0 / 3	3 / 3
Zinc Cold Therapy (Walgreens) Test conc. = 15% w/v	Negative	0 / 3	0 / 3	0 / 3
	SARS-CoV-2 (2x LoD)	3 / 3	0 / 3	0 / 3
	Influenza A (2x LoD)	0 / 3	3 / 3	0 / 3
	Influenza B (2x LoD)	0 / 3	0 / 3	3 / 3
Homeopathic Allergy Nasal Spray (Alkalol) Test conc. = 15% v/v	Negative	0 / 3	0 / 3	0 / 3
	SARS-CoV-2 (2x LoD)	3 / 3	0 / 3	0 / 3
	Influenza A (2x LoD)	0 / 3	3 / 3	0 / 3
	Influenza B (2x LoD)	0 / 3	0 / 3	3 / 3
NasoGEL (Gel Spray) Test conc. = 15% v/v	Negative	0 / 3	0 / 3	0 / 3
	SARS-CoV-2 (2x LoD)	3 / 3	0 / 3	0 / 3
	Influenza A (2x LoD)	0 / 3	3 / 3	0 / 3
	Influenza B (2x LoD)	0 / 3	0 / 3	3 / 3
Nasalcrom Nasal Allergy spray Test conc. = 15% v/v	Negative	0 / 3	0 / 3	0 / 3
	SARS-CoV-2 (2x LoD)	3 / 3	0 / 3	0 / 3
	Influenza A (2x LoD)	0 / 3	3 / 3	0 / 3
	Influenza B (2x LoD)	0 / 3	0 / 3	3 / 3
Histaminum 30C Test conc. = 15% w/v	Negative	0 / 3	0 / 3	0 / 3
	SARS-CoV-2 (2x LoD)	3 / 3	0 / 3	0 / 3
	Influenza A (2x LoD)	0 / 3	3 / 3	0 / 3
	Influenza B (2x LoD)	0 / 3	0 / 3	3 / 3

Endogenous/Exogenous Substance Test concentration	Spiked Virus (Concentration)	No. of Positive Results / No. of Total replicates		
		SARS CoV-2	Flu A	Flu B
Skin relief hand cream (Aveeno) Test conc. = 1% w/v	Negative	0 / 3	0 / 3	0 / 3
	SARS-CoV-2 (2x LoD)	3 / 3	0 / 3	0 / 3
	Influenza A (2x LoD)	0 / 3	3 / 3	0 / 3
	Influenza B (2x LoD)	0 / 3	0 / 3	3 / 3
Hand Soap Fresh Breeze Scent Test conc. = 1% w/v	Negative	0 / 3	0 / 3	0 / 3
	SARS-CoV-2 (2x LoD)	3 / 3	0 / 3	0 / 3
	Influenza A (2x LoD)	0 / 3	3 / 3	0 / 3
	Influenza B (2x LoD)	0 / 3	0 / 3	3 / 3
Antibacterial liquid Hand Soap (Dial Complete) Test conc. = 1%, 5%, 10% and 15% w/v	Negative	0 / 3	0 / 3	0 / 3
	SARS-CoV-2 (2x LoD)	3 / 3	0 / 3	0 / 3
	Influenza A (2x LoD)	0 / 3	3 / 3	0 / 3
	Influenza B (2x LoD)	0 / 3	0 / 3	3 / 3
Hand Sanitizer Gel Test conc. = 1%, 5%, 10% and 15% w/v	Negative	0 / 3	0 / 3	0 / 3
	SARS-CoV-2 (2x LoD)	3 / 3	0 / 3	0 / 3
	Influenza A (2x LoD)	0 / 3	3 / 3	0 / 3
	Influenza B (2x LoD)	0 / 3	0 / 3	3 / 3
Hand Sanitizer Lotion Test conc. = 15% w/v	Negative	0 / 3	0 / 3	0 / 3
	SARS-CoV-2 (2x LoD)	3 / 3	0 / 3	0 / 3
	Influenza A (2x LoD)	0 / 3	3 / 3	0 / 3
	Influenza B (2x LoD)	0 / 3	0 / 3	0 / 3*
Disinfectant Spray (Lysol) Test conc. = 1% v/v	Negative	0 / 3	0 / 3	0 / 3
	SARS-CoV-2 (2x LoD)	3 / 3	0 / 3	0 / 3
	Influenza A (2x LoD)	0 / 3	3 / 3	0 / 3
	Influenza B (2x LoD)	0 / 3	0 / 3	3 / 3
Mucin from bovine submaxillary glands-Type I-S Test conc. = 5 mg/mL	Negative	0 / 3	0 / 3	0 / 3
	SARS-CoV-2 (2x LoD)	3 / 3	0 / 3	0 / 3
	Influenza A (2x LoD)	0 / 3	3 / 3	0 / 3
	Influenza B (2x LoD)	0 / 3	0 / 3	3 / 3
Purified Human Neutrophils Test conc. = 5×10^6 cells/mL	Negative	0 / 3	0 / 3	0 / 3
	SARS-CoV-2 (2x LoD)	3 / 3	0 / 3	0 / 3
	Influenza A (2x LoD)	0 / 3	3 / 3	0 / 3
	Influenza B (2x LoD)	0 / 3	0 / 3	3 / 3
Whole Blood Test conc. = 2.5%	Negative	0 / 3	0 / 3	0 / 3
	SARS-CoV-2 (2x LoD)	3 / 3	0 / 3	0 / 3
	Influenza A (2x LoD)	0 / 3	3 / 3	0 / 3
	Influenza B (2x LoD)	0 / 3	0 / 3	3 / 3
Acetylsalicylic acid Test conc. = 3.00×10^1 µg/mL	Negative	0 / 3	0 / 3	0 / 3
	SARS-CoV-2 (2x LoD)	3 / 3	0 / 3	0 / 3
	Influenza A (2x LoD)	0 / 3	3 / 3	0 / 3
	Influenza B (2x LoD)	0 / 3	0 / 3	3 / 3
Dexamethasone Test conc. = 1.20×10^1 µg/mL	Negative	0 / 3	0 / 3	0 / 3
	SARS-CoV-2 (2x LoD)	3 / 3	0 / 3	0 / 3
	Influenza A (2x LoD)	0 / 3	3 / 3	0 / 3
	Influenza B (2x LoD)	0 / 3	0 / 3	3 / 3
Mometasone furoate	Negative	0 / 3	0 / 3	0 / 3
	SARS-CoV-2 (2x LoD)	3 / 3	0 / 3	0 / 3

Endogenous/Exogenous Substance Test concentration	Spiked Virus (Concentration)	No. of Positive Results / No. of Total replicates		
		SARS CoV-2	Flu A	Flu B
Test conc. = 4.50×10^{-4} µg/mL	Influenza A (2x LoD)	0 / 3	3 / 3	0 / 3
	Influenza B (2x LoD)	0 / 3	0 / 3	3 / 3
Mupirocin Test conc. = 1.50×10^{0} µg/mL	Negative	0 / 3	0 / 3	0 / 3
	SARS-CoV-2 (2x LoD)	3 / 3	0 / 3	0 / 3
	Influenza A (2x LoD)	0 / 3	3 / 3	0 / 3
	Influenza B (2x LoD)	0 / 3	0 / 3	3 / 3
Oseltamivir phosphate (Tamiflu) Test conc. = 3.99×10^{-1} µg/mL	Negative	0 / 3	0 / 3	0 / 3
	SARS-CoV-2 (2x LoD)	3 / 3	0 / 3	0 / 3
	Influenza A (2x LoD)	0 / 3	3 / 3	0 / 3
	Influenza B (2x LoD)	0 / 3	0 / 3	3 / 3
Tobramycin Test conc. = 3.30×10^{1} µg/mL	Negative	0 / 3	0 / 3	0 / 3
	SARS-CoV-2 (2x LoD)	3 / 3	0 / 3	0 / 3
	Influenza A (2x LoD)	0 / 3	3 / 3	0 / 3
	Influenza B (2x LoD)	0 / 3	0 / 3	3 / 3
Beclomethasone dipropionate Test conc. = 5.04 µg/mL	Negative	0 / 3	0 / 3	0 / 3
	SARS-CoV-2 (2x LoD)	3 / 3	0 / 3	0 / 3
	Influenza A (2x LoD)	0 / 3	3 / 3	0 / 3
	Influenza B (2x LoD)	0 / 3	0 / 3	3 / 3
Flunisolide Test conc. = 870 µg/mL	Negative	0 / 3	0 / 3	0 / 3
	SARS-CoV-2 (2x LoD)	3 / 3	0 / 3	0 / 3
	Influenza A (2x LoD)	0 / 3	3 / 3	0 / 3
	Influenza B (2x LoD)	0 / 3	0 / 3	3 / 3
Molnupiravir Test conc. = 3.29 mg/mL (10 mM)	Negative	0 / 3	0 / 3	0 / 3
	SARS-CoV-2 (2x LoD)	3 / 3	0 / 3	0 / 3
	Influenza A (2x LoD)	0 / 3	3 / 3	0 / 3
	Influenza B (2x LoD)	0 / 3	0 / 3	3 / 3
Remdesivir Test conc. = 240 µg/mL	Negative	0 / 3	0 / 3	0 / 3
	SARS-CoV-2 (2x LoD)	3 / 3	0 / 3	0 / 3
	Influenza A (2x LoD)	0 / 3	3 / 3	0 / 3
	Influenza B (2x LoD)	0 / 3	0 / 3	3 / 3
Zanamivir Test conc. = 30 mg/mL	Negative	0 / 3	0 / 3	0 / 3
	SARS-CoV-2 (2x LoD)	3 / 3	0 / 3	0 / 3
	Influenza A (2x LoD)	0 / 3	3 / 3	0 / 3
	Influenza B (2x LoD)	0 / 3	0 / 3	3 / 3

* Hand Sanitizer Lotion tested at diluted concentrations of 1%, 5%, and 10% w/v showed no interference in test performance for Flu B.

c. Biotin Interference:

The Nano-Check Influenza+COVID-19 Dual Test utilizes a sandwich assay format that incorporates a biotin-streptavidin complex. This study was designed to ascertain whether the test is affected by free biotin at levels up to 3500 ng/mL in samples in the presence of 2x LoD concentrations of SARS-CoV-2, Flu A, and Flu B antigens, spiked individually and were tested in triplicate. As summarized in **Table 6** below, the Nano-Check Influenza+COVID-19 Dual Test demonstrated no interference up to 3,500 ng/mL of biotin in test samples.

Table 6: Biotin Interference Study Results

Sample	Biotin Concentration	(No. of Positive results / Total replicates)		
		COVID	Flu A	Flu B
SARS-CoV-2 2x LoD Positive Sample	3,500 ng/mL	3 / 3	0 / 3	0 / 3
	2,400 ng/mL	3 / 3	0 / 3	0 / 3
	1,200 ng/mL	3 / 3	0 / 3	0 / 3
	600 ng/mL	3 / 3	0 / 3	0 / 3
	300 ng/mL	3 / 3	0 / 3	0 / 3
	150 ng/mL	3 / 3	0 / 3	0 / 3
Influenza A 2x LoD Positive Sample	3,500 ng/mL	0 / 3	3 / 3	0 / 3
	2,400 ng/mL	0 / 3	3 / 3	0 / 3
	1,200 ng/mL	0 / 3	3 / 3	0 / 3
	600 ng/mL	0 / 3	3 / 3	0 / 3
	300 ng/mL	0 / 3	3 / 3	0 / 3
	150 ng/mL	0 / 3	3 / 3	0 / 3
Influenza B 2x LoD Positive Sample	3,500 ng/mL	0 / 3	0 / 3	3 / 3
	2,400 ng/mL	0 / 3	0 / 3	3 / 3
	1,200 ng/mL	0 / 3	0 / 3	3 / 3
	600 ng/mL	0 / 3	0 / 3	3 / 3
	300 ng/mL	0 / 3	0 / 3	3 / 3
	150 ng/mL	0 / 3	0 / 3	3 / 3
Negative sample (NCM)	3,500 ng/mL	0 / 3	0 / 3	0 / 3
	2,400 ng/mL	0 / 3	0 / 3	0 / 3
	1,200 ng/mL	0 / 3	0 / 3	0 / 3
	600 ng/mL	0 / 3	0 / 3	0 / 3
	300 ng/mL	0 / 3	0 / 3	0 / 3
	150 ng/mL	0 / 3	0 / 3	0 / 3

d. Competitive Interference:

The competitive study interference was performed to assess the ability of the Nano-Check Influenza+COVID-19 Dual Test to detect multiple analytes simultaneously without inhibiting each other's detection. Strains of SARS-CoV-2 (Omicron lineage BA.5), Flu A (H1N1 and H3N2) and Flu B (Victoria and Yamagata lineage) were utilized. Each analyte was prepared at a high concentration (at least 10^5 $TCID_{50}$/mL or $CEID_{50}$/mL) and low concentration (3x LoD).

Table 7 below summarizes the results of the competitive interference study. For each condition tested, all three replicates tested at the low target analyte condition tested positive in the presence of other target analytes at high concentrations. Thus, this indicates the capability of the Nano-Check Influenza+COVID-19 Dual Test to detect low levels of Flu A and Flu B in the presence of a high level of SARS-CoV-2, as well as low levels of Flu A and SARS-CoV-2 in the presence of a high level of Flu B and vice versa. No false positive results were observed for analytes that are not present in the sample.

Table 7: Competitive Interference Results

SARS-CoV-2		Influenza A (H1N1)		Influenza B (Victoria)	
Concentration	**% Agreement**	**Concentration**	**% Agreement**	**Concentration**	**% Agreement**
Low	100%	High	100%	-	100%
-	100%	High	100%	Low	100%
Low	100%	High	100%	Low	100%
High	100%	Low	100%	Low	100%
High	100%	-	100%	Low	100%
Low	100%	-	100%	High	100%
-	100%	Low	100%	High	100%
Low	100%	Low	100%	High	100%
High	100%	Low	100%	-	100%
SARS-CoV-2		**Influenza A (H3N2)**		**Influenza B (Victoria)**	
Concentration	**% Agreement**	**Concentration**	**% Agreement**	**Concentration**	**% Agreement**
Low	100%	High	100%	-	100%
-	100%	High	100%	Low	100%
-	100%	Low	100%	High	100%
High	100%	Low	100%	-	100%
Low	100%	High	100%	Low	100%
Low	100%	Low	100%	High	100%
High	100%	Low	100%	Low	100%
SARS-CoV-2		**Influenza A (H1N1)**		**Influenza B (Yamagata)**	
Concentration	**% Agreement**	**Concentration**	**% Agreement**	**Concentration**	**% Agreement**
-	100%	High	100%	Low	100%
-	100%	Low	100%	High	100%
High	100%	-	100%	Low	100%
Low	100%	High	100%	Low	100%
Low	100%	Low	100%	High	100%
High	100%	Low	100%	Low	100%
Low	100%	-	100%	High	100%
SARS-CoV-2		**Influenza A (H3N2)**		**Influenza B (Yamagata)**	
Concentration	**% Agreement**	**Concentration**	**Concentration**	**% Agreement**	**Concentration**
-	100%	High	100%	Low	100%
-	100%	Low	100%	High	100%
Low	100%	High	100%	Low	100%
Low	100%	Low	100%	High	100%
High	100%	Low	100%	Low	100%

4. Assay Reportable Range:

Not applicable; the device is a binary qualitative assay that is visually read.

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

a. Internal Control

The Nano-Check Influenza+COVID-19 Dual Test contains built in internal assay control. The appearance of the control line on the test ensures that sufficient flow of the sample occurred during the assay. Any result without this control line is invalid.

b. External Quality Controls

The Nano-Check Influenza+COVID-19 Dual Test contains one positive external control swab and one negative external control swab that allows for monitoring of the performance of the assay. The positive control swab contains recombinant SARS-CoV-2 nucleocapsid, recombinant

Flu A nucleoprotein, and recombinant Flu B nucleoprotein whereas the negative control swab does not contain recombinant antigen.

A precision study was conducted for multiple lots of the Nano-Check Influenza+COVID-19 Dual Test's external controls. The aim was to assess the precision and repeatability of the external control swab in a blinded manner. Ten (10) individual swabs from three (3) different lots of positive and negative external control swabs were randomized and distributed blind to one (1) assigned operator within the laboratory, who tested the control swabs in accordance with the Instructions for Use of the test. The collected data were evaluated by calculating the percent agreement between test results and sample properties.

The results of this study, as detailed in **Table 8** below indicated 100% agreement, meeting the acceptance criteria for the external control precision study.

Table 8: Lot-to-lot Precision Study Results for External Controls

External control	Lot	Test Results (No. of Positive Results / No. of Total Replicates)			
		COVID	Flu A	Flu B	Agreement
Positive External Control	Lot 1	10 / 10	10 / 10	10 / 10	100%
	Lot 2	10 / 10	10 / 10	10 / 10	100%
	Lot 3	10 / 10	10 / 10	10 / 10	100%
Negative External Control	Lot 1	0 / 10	0 / 10	0 / 10	100%
	Lot 2	0 / 10	0 / 10	0 / 10	100%
	Lot 3	0 / 10	0 / 10	0 / 10	100%

c. Stability

i) Specimen Stability

Two test samples were prepared for the specimen stability study: a negative sample (consisting of pooled human nasal fluid with no analyte) and a low positive sample (containing a diluted SARS-CoV-2, Flu A, and Flu B sample at 2x LoD in negative pooled human nasal fluid). Swabs were spiked with 50 µL of each sample and subjected to four different temperature conditions: room temperature (23.5°C), high room temperature (30 °C), 2°C to 8°C, and frozen (-20°C). The samples were stored under each condition for various time intervals including 0 hours (baseline), 1 hour, 2 hours, 4 hours, 8 hours, 24 hours, and 48 hours. Subsequently, the exposed sample swabs were tested using 5 replicates for each exposure time. The results showed that the nasal swab samples were stable for up 48 hours under all temperature conditions, ranging from -20°C to 30°C. Therefore, freshly collected specimen swabs are recommended to be processed no later than one hour after specimen collection when kept at room temperature (15°C - 30°C) or within 24 hours when stored at 2°C to 8°C.

ii) Real Time Stability

Three lots of Nano-Check Influenza+COVID-19 Dual Test were stored at three different temperature conditions: 30°C, ambient room temperature (23.5°C), and 2-8°C. The study involved positive and negative external control swabs, as well as positive and negative contrived samples. Positive samples were prepared by diluting the SARS-CoV-2 (Omicron variant), Flu A (H1N1), and Flu B (Yamagata lineage) strains to a 3x LoD concentration into pooled human nasal fluid,

while negative contrived swab samples were prepared using pooled human nasal fluid confirmed to be negative for SARS-CoV-2, Flu A, and Flu B by RT-PCR. Testing was performed at time 0 (baseline) and monthly thereafter up to 19 months. All study data are 100% concordant with expected results and support a shelf-life of up to 18 months at the time of 510(k) clearance when stored between 2-30°C.

iii) Transport Stability

The transport simulation study was performed to evaluate the robustness of the Nano-Check Influenza+COVID-19 Dual Test kit under fluctuating transport and storage conditions. Positive and negative external control swabs, as well as positive and negative contrived swab samples, were included in the study. Positive contrived swab samples were prepared by diluting the SARS-CoV-2 (Omicron variant), Flu A (HlNl), and Flu B (Yamagata lineage) strains to a 3x LoD concentration into pooled human nasal fluid, while negative contrived swab samples were prepared using pooled human nasal fluid confirmed to be negative for SARS-CoV-2, Flu A, and Flu B by RT-PCR. The study was scheduled over 15-month period, with assessments carried out on Day 0, Day 14, and every three months from Month 3 to Month 12. Results indicated that the Nano-Check Influenza+COVID-19 Dual Test kit demonstrated stability and remained unaffected by temperature excursions for 12 months.

iv) Open Kit Stability Study:

Open kit stability study was performed to assess the stability of the test devices after opening the sealed pouch using ten replicates of positive samples (2x LoD concentration of SARS-CoV-2, Flu A and Flu B viruses spiked individually) and 5 replicates of negative samples (pooled human fluid confirmed to be SARS-CoV-2, Flu A and B negative by RT-PCR). The test devices were exposed to ambient conditions for various durations: 0 min, 30 min, 1 hr, 2 hrs, 3 hrs and 4 hrs. The results showed that all positive samples had positive results for up to 4 hours and negative samples did not produce any false positive results meeting the acceptance criteria.

v) Short-term Stability:

The short-term reagent stability study for the Nano-Check Influenza+COVID-19 Dual Test aimed to assess the stability of the Nano-Check Influenza+COVID-19 Dual Test kit components when all kit components are stored at extremely low temperature (-20°C) and high temperature (45°C). The Nano-Check lnfluenza+COVID-19 Dual Test kit was stable for 16 days when it was stored at low temperature (-20°C) and for 14 days at a high temperature (45°C). However, it is recommended that the test kit should be stored at 2°C - 30°C in the original sealed pouch.

6. Detection Limit:

a. Single-analyte LoD:

The LoD of the device was performed to determine the lowest detectable concentration of SARS-CoV-2, Flu A and Flu B at which at least 95% of all true positive replicates are consistently detected as positive. The LoD was assessed for each analyte in two parts, a preliminary range finding study, followed by a confirmatory LoD study. A preliminary LoD was determined by first testing serial ten-fold dilutions of live Flu A and B, and inactivated SARS-CoV-2 virus stocks diluted into pooled negative swab fluid in 3 replicates per dilution using one device lot. Single analyte virus dilutions (50 µL/swab) were each spiked onto dry sterile swabs and tested per the IFU. The preliminary LoD results for each individual virus strain are shown in below tables.

Table 9: Preliminary LoD - SARS-CoV-2

Virus strain	Sample conc. ($TCID_{50}$/mL)	Sample conc. ($TCID_{50}$/swab)	# Positive/ # Total
USA/MD HP20874/2021 (Omicron, B.1.1.529)	3.89×10^3	1.95×10^2	3 / 3
	3.89×10^2	1.95×10^1	3 / 3
	3.89×10^1	1.95	0 / 3
	1.95×10^2	**9.75**	**3 / 3**
	9.73×10^1	4.87	1 / 3
USA/COR-22-063113/2022 (Omicron, BA.5)	2.53×10^5	1.27×10^4	3 / 3
	2.53×10^4	1.27×10^3	3 / 3
	2.53×10^3	1.27×10^2	0 / 3
	1.27×10^4	**6.35×10^2**	**3 / 3**
	6.35×10^3	3.18×10^2	0 / 3

Table 10: Preliminary LoD - Flu A

Virus strain	Sample conc. ($TCID_{50}$/mL or $CEID_{50}$/mL)	Sample conc. ($TCID_{50}$/swab or $CEID_{50}$/swab)	# Positive/ # Total
A/California/04/2009 (H1N1)	2.8×10^5	1.4×10^4	3 / 3
	2.8×10^4	1.4×10^3	3 / 3
	2.8×10^3	**1.4×10^2**	**3 / 3**
	2.8×10^2	1.4×10^1	0 / 3
	1.4×10^3	7×10^1	0 / 3
A/Victoria/361/2011 (H3N2)	2.8×10^7	1.4×10^6	3 / 3
	2.8×10^6	1.4×10^5	3 / 3
	2.8×10^5	1.4×10^4	3 / 3
	2.8×10^4	1.4×10^3	0 / 3
	1.4×10^5	**7×10^3**	**3 / 3**
	7.0×10^4	3.5×10^3	1 / 3

Table 11: Preliminary LoD - Flu B

Virus strain	Sample conc. ($TCID_{50}$/mL or $CEID_{50}$/mL)	Sample conc. ($TCID_{50}$/swab or $CEID_{50}$/swab)	# Positive/ # Total
B/Hong Kong/330/2001 (Victoria)	1.8×10^6	9×10^4	3 / 3
	1.8×10^5	9×10^3	1 / 3
	9.0×10^5	9×10^2	3 / 3
	4.5×10^5	2.25×10^4	3 / 3
	2.25×10^5	**1.13×10^4**	**3 / 3**
	1.13×10^5	5.6×10^3	0 / 3
B/Phuket/3073/13 (Yamagata)	4.17×10^4	2.09×10^3	3 / 3
	4.17×10^3	2.09×10^2	3 / 3
	4.17×10^2	2.09×10^1	3 / 3

Virus strain	Sample conc. ($TCID_{50}$/mL or $CEID_{50}$/mL)	Sample conc. ($TCID_{50}$/swab or $CEID_{50}$/swab)	# Positive/ # Total
	4.17×10^1	2.09	0 / 3
	2.09×10^2	1.04×10^1	3 / 3
	1.04×10^2	**5.2**	**3 / 3**
	5.20×10^1	2.6	1 / 3

LoD confirmatory testing was performed individually for each virus by testing 20 replicates at the virus's preliminary 1x LoD concentration, as determined above using three device lots. For the LoD to be confirmed, at least 95% of the replicates (≥57/60) needed to be positive. Results of the LoD confirmation testing for each virus are summarized in the table below.

Table 12: Confirmatory LoD

Virus	Isolate (Lineage)	Strain	LoD concentration ($TCID_{50}$/mL or $CEID_{50}$/mL)	LoD concentration ($TCID_{50}$/Swab or $CEID_{50}$/Swab)	# Positive/ # Total
SARS-CoV-2	Omicron (B.1.1.529)	USA/MD-HPA20874/2021 (Heat Inactivated)	1.95×10^2	9.75	59/60
	Omicron (BA.5)	USA/COR-22-063113/2022 (Heat Inactivated)	1.27×10^4	6.35×10^2	60/60
Flu A	H1N1	A/California/04/2009	2.8×10^3	1.4×10^2	59/60
	H3N2	A/Victoria/361/2011	1.4×10^5	7×10^3	59/60
Flu B	Victoria	B/Hong Kong/330/2001	2.25×10^5	1.13×10^4	57/60
	Yamagata	B/Phuket/3073/13	1.04×10^2	5.2	60/60

b. NIBSC 21/368 – International Standard:

The LoD of the candidate device was determined using the NIBSC 21/368 International Standard for SARS-CoV-2 spiked into pooled negative matrix (nasal fluid). A preliminary LoD test was performed by spiking 50 µL of each diluted sample onto the sample collection swab head in three replicates. The preliminary LoD concentration was determined to be 1,333 IU/mL (or 67 IU/swab).

The LoD confirmatory study was performed using additiona17 replicates at the preliminary LoD concentration totaling 20 replicates. All 20 replicates resulted in positive results. Additional dilutions were investigated bracketing the confirmed LoD concentration with 20 replicates at each dilution level. The lowest concentration at which a minimum of 95% of results were positive was confirmed to be 667 IU/mL (or 33.5 IU/swab) as shown in **Table 13** below.

Table 13: Summary Results of LoD with NIBSC 21/368 International Standard

Preliminary LoD				Confirmatory LoD			
Sample dilution	Concentration		# Positive / # total replicates	Sample dilution	Concentration		# Positive / # total replicates
	IU/mL	IU/swab			IU/mL	IU/swab	
1:5 (Master Stock)	4,000	200	3/3	1:7.5	2,666	134	20/20
1:15	**1,333**	**67**	**20/20**	1:22.5	889	45	20/20
1:45	444	22	1/3	**1:30**	**667**	**33.5**	**19/20**
1:135	148	7.4	0/3	1:37.5	533	27	12/20
1:405	49	2.5	0/3				

7. High Dose Hook Effect Study:

A high-dose hook effect study was conducted to determine the concentration at which false negative results might occur when very high levels of the target antigen are present in a testing sample. In this study, 50µL of the highest concentration possible for inactivated SARS-CoV-2 virus stock and for live Flu A and Flu B virus stocks were spiked onto sterile swabs for triplicate measurements, and swabs were tested on the device per IFU of the candidate device. No hook effect for SARS-CoV-2, Flu A, and Flu B were observed up to the concentrations listed in **Table 14** below.

Table 14: Summary of High Dose Hook Effect Study

Virus	Isolate/Lineage	Strain	Virus Concentration		# of Positive/# Total tested		
			$TCID_{50}$/mL or $CEID_{50}$/mL	$TCID_{50}$/swab or $CEID_{50}$/swab	COVID	Flu A	Flu B
SARS-CoV-2	Omicron (B.1.1.529)	USA/MD-HPA20874/2021	3.89×10^4	1.95×10^3	3/3	0/3	0/3
	Omicron (BA.5)	USA/COR-22-063113/2022	2.53×10^6	1.27×10^5	3/3	0/3	0/3
Flu A	H1N1	A/California/04/2009	2.8×10^6	1.4×10^5	0/3	3/3	0/3
	H3N2	A/Victoria/361/2011	2.8×10^8	1.4×10^7	0/3	3/3	0/3
Flu B	Victoria	B/Hong Kong/330/2001	1.8×10^7	9×10^6	0/3	0/3	3/3
	Yamagata	B/Phuket/3073/13	4.17×10^5	2.08×10^4	0/3	0/3	3/3

8. Analytical Reactivity Study:

a. Inclusivity – Selected Strains

An inclusivity study was conducted involving three (3) operators encompassing various variants, lineages and clades of the SARS-CoV-2 virus and Flu viruses. The study involved 14 strains of SARS-CoV-2, thirty (30) strains of Flu A, and sixteen (16) strains of Flu B. The virus stocks were diluted at the predetermined concentration in the pooled negative nasal fluid matrix and 50µL were pipetted onto the swabs. The swabs were tested in 3 replicates in this study. The

lowest concentration of each strain that resulted in 100% detection (3/3) is presented in **Table 15** below.

Table 15: Summary of Inclusivity Results

Virus	Virus Strains	Concentration	Units
SARS-CoV-2 (B.1.1.7, Alpha)	USA/CA/CDC/5574/2020[1)]	6.77E+06	GE /mL
	USA/CA/CDC/5574/2020[2)]	2.39E+04	$TCID_{50}$/mL
	England/204820464/2020	7.19E+03	$TCID_{50}$/mL
SARS-CoV-2 (B.1.1.351, Beta)	USA/MD-HP01542/2021[3)]	7.20E+04	GC/mL
	USA/MD-HP01542/2021[4)]	3.80E+06	GC/mL
	South Africa/KRISP-K0053 25/2020	1.90E+04	$TCID_{50}$/mL
SARS-CoV-2 (B.1.617.2, Delta)	USA/MD-HP05285/2021[5)]	7.20E+07	GC/mL
	USA/MD-HP05285/2021[6)]	5.00E+06	GC/mL
	USA/PHC658/2021	5.21E+02	$TCID_{50}$/mL
SARS-CoV-2 (P.1, Gamma)	Japan/TY7-503/2021	1.58E+04	$TCID_{50}$/mL
	USA/NY-Wadsworth-21033 899-01/ 2021	7.85E+03	$TCID_{50}$/mL
SARS-CoV-2 (B.1.617.1, Kappa)	USA/CA-Stanford-15_S02/ 2021	8.48E+04	$TCID_{50}$/mL
SARS-CoV-2 (B.1.1.529, Omicron)	USA/GA-EHC-2811C/2021	2.11E+07	GC/mL
SARS-CoV-2 (JN.1, Omicron)	USA/New York/PV96109/20 23	3.14E+03	$TCID_{50}$/mL
Flu A H1N1	A/Puerto Rico/8/34	8.00E+06	$CEID_{50}$/mL
	A/Brisbane/59/2007	4.45E+06	$CEID_{50}$/mL
	A/Denver/1/57	4.00E+05	$CEID_{50}$/mL
	A/San Diego/1/2009 pdm09	2.80E+04	$TCID_{50}$/mL
	A/Tijuana/4/09	2.45E+01	$TCID_{50}$/mL
	A/Solomon Islands/3/2006	4.45E+05	$CEID_{50}$/mL
	A/NWS/33	1.23E+05	$CEID_{50}$/mL
	A/FM/1/47	4.25E+05	$CEID_{50}$/mL
	A/New Jersey/8/76	1.70E+04	$CEID_{50}$/mL
	A/New Caledonia/20/1999	4.00E+05	$CEID_{50}$/mL
	A/Hawaii/66/2019	1.28	HA
	A/Hawaii/66/2019 X-345A	1.28	HA
	A/Guangdong-Maonan/1536/ 2019	1.28	HA
	A/Guangdong-Maonan/1536/ 2019 CNIC-1909	0.64	HA
	A/Victoria/4897/2022(pdm09)	1.58E+07	EID_{50}/mL
	A/Victoria/2570/2019 (pdm09)	9.98E+05	EID_{50}/mL
Flu A H1N2	A/Swine/Ohio/09SW1477/2009	2.30E+04	$TCID_{50}$/mL
Flu A H3N2	A/Hong Kong/8/1968	1.40E+05	$CEID_{50}$/mL
	A/Aichi/2/1968	4.00E+05	$CEID_{50}$/mL
	A/Wisconsin/67/2005	7.00E+05	$CEID_{50}$/mL
	A/Hong Kong/4801/2014	9.60E+05	$CEID_{50}$/mL
	A/Netherlands/22/2003	8.00E+02	$TCID_{50}$/mL
	A/Netherlands/823/1992	1.44E+01	$TCID_{50}$/mL

Virus	Virus Strains	Concentration	Units
	A/Brisbane/10/2007	1.38E+05	$CEID_{50}$/mL
	A/Wisconsin/15/2009	5.00E+03	$CEID_{50}$/mL
	A/Sydney/5/97	4.45E+04	$CEID_{50}$/mL
	A/Port Chalmers/1/73	2.00E+05	$CEID_{50}$/mL
	A/Victoria/3/75	4.00E+05	$CEID_{50}$/mL
	A/Perth/16/2009 x A/Puerto Rico/8/19 34, NIB-64	2.80E+06	$CEID_{50}$/mL
	A/Singapore/INFIMH-16-0019 /16	2.51E+03	$TCID_{50}$/mL
Flu A H5N1	A/mallard/Wisconsin/2576/2009	5.25E+05	GE/mL
Flu B (Victoria lineage)	B/Brisbane/60/2008	9.00E+04	$CEID_{50}$/mL
	B/Malaysia/2506/2004	1.12E+06	$CEID_{50}$/mL
	B/New York/1056/2003	3.20E+03	$TCID_{50}$/mL
	B/Brisbane/60/2008	9.00E+04	$CEID_{50}$/mL
Flu B (Yamagata lineage)	B/Florida/78/2015	2.80E+04	$TCID_{50}$/mL
	B/Texas/06/2011	4.45E+08	$CEID_{50}$/mL
	B/New York/1061/2004	8.00E+02	$TCID_{50}$/mL
	B/Christchurch/33/2004	8.00E+02	$TCID_{50}$/mL
	B/Sydney/507/2006	1.60E+05	$TCID_{50}$/mL
	B/Wisconsin/1/2010	1.80E+06	$CEID_{50}$/mL
	B/Florida/4/2006	3.50E+05	$CEID_{50}$/mL
Flu B (Non-Victoria/Yamagata)	B/Colorado/6/17	1.78E+02	$TCID_{50}$/mL
	B/Taiwan/2/1962	4.45E+03	$CEID_{50}$/mL
	B/Lee/1940	9.00E+04	$CEID_{50}$/mL
	B/GL/1739/54	5.00E+04	$CEID_{50}$/mL
	B/Great Lakes/1739/1954	3.20E+04	$CEID_{50}$/mL

1) Source: Bei Resources (Cat. #: NR-55245, Lot#:70043111), 2) Source: ZeptoMetrix (Cat. #: 0810612CFHI, Lot#:328055), 3) Source: Bei Resources (Cat. #: NR-553651, Lot#:70045299), 4) Source: Bei Resources (Cat. #: NR-55350, Lot#:70045608), 5) Source: Bei Resources (Cat. #: NR-56128, Lot#:70048021), 6) Source: ATCC (Cat. #: VR-3342HK, Lot#:70048932

9. Assay Cut-Off:

Not applicable as this is a qualitative visually read assay without numeric data.

B Comparison Studies:

1. Method Comparison with Predicate Device:

Please refer to section VI.C (Clinical Studies) below for the clinical validation regarding the method comparison studies.

2. Matrix Comparison:

The Nano-Check Influenza+COVID-19 Dual Test is intended only for the qualitative detection of nucleocapsid protein antigen from SARS-CoV-2 and the nucleoprotein from Flu A and Flu B in direct anterior nasal swab specimens. As no other sample types are claimed herein, a matrix comparison study is not applicable.

C Clinical Studies:

The clinical performance of the Nano-Check Influenza+COVID-19 Dual Test was evaluated in a multi-center, prospective clinical study in the U.S. between November 2022 and February 2025. The study only enrolled subjects who presented with symptoms of respiratory infection. A total of 2,223 subjects were initially enrolled however, 254 samples were excluded due to subjects having symptoms longer than 4 days, invalid comparator results, or samples lost during shipment. The remaining 1,969 subjects were consecutively enrolled and tested by fourteen (14) operators representative of CLIA-waived users (i.e., not formally trained in a laboratory setting) across six (6) different clinical CLIA-waived sites. Two anterior nasal swabs (ANS) were collected from each study subject during the same visit. The first ANS specimen for the comparator method was collected by the operators from both sides of the nose. The comparator ANS specimens were stored in UVT media and packaged in dry ice for aero-transport, sent to a reference laboratory, and tested with an FDA cleared RT-PCR method as per the cleared instructions for use. The second ANS specimen was collected from both sides of the nose using the provided swab and was tested immediately using the Nano-Check Influenza+COVID-19 Dual Test by each operator using the QRI only and with no training specifically with the candidate test.

The summary of demographics of the clinical subjects are shown in **Table 16** below.

Table 16: Subject Demographics

Age Group	Female		Male		Total	
	No. of Sample	%	No. of Sample	%	No. of Sample	%
≤ 5 years	201	19.8	215	22.5	416	21.1
6 to 21 years	401	39.5	462	48.5	863	43.8
22 to 60 years	313	30.8	204	21.4	517	26.3
≥ 61 years	101	9.9	72	7.6	173	8.8
Total	1016	100	953	100	1969	100
	Female: 51.6% (1016/1969)		Male: 48.4% (953/1969)			

The clinical performance of the Nano-Check Influenza+COVID-19 Dual Test compared to the highly sensitive RT-PCR comparator is shown below.

Table 17: SARS-CoV-2 Clinical Performance

Nano-Check Influenza+ COVID-19 Dual Test	RT-PCR Comparator		Total
	Positive	Negative	
Positive	232	3	275
Negative	33	1701	1734
Total	265	1704	1969
Positive Percent Agreement (PPA) = 87.6% (232/265, 95% CI: 83.0% - 91.0%)			
Negative Percent Agreement (NPA) = 99.8% (1701/1704, 95% CI: 99.5% - 99.9%)			

Results of SARS-CoV-2 were also analyzed stratified by the days post symptom onset (DPSO) and are presented in the table below.

Table 18: Clinical Performance for Detection of SARS-CoV-2 stratified by DPSO

Days Post Onset	Comparator Positive	Candidate Test Positive	PPA %	95%CI
1	82	74	90.2	81.9-95.0
2	98	85	86.7	78.6-92.1
3	64	56	87.5	77.2-93.5
4	21	17	81.0	60.0-92.3
Total	265	232	87.6	83.0-91.0

The clinical performance for the detection of Influenza A and Influenza B are shown in **Table 19** and **Table 20** respectively.

Table 19: Clinical Performance for Detection of Influenza A

Nano-Check Influenza+ COVID-19 Dual Test	RT-PCR Comparator		Total
	Positive	Negative	
Positive	417	6	423
Negative	63	1483	1546
Total	480	1489	1969
Positive Percent Agreement (PPA) = 86.9% (417/480, 95% CI: 83.6% - 89.6%)			
Negative Percent Agreement (NPA) = 99.6% (1483/1489, 95% CI: 99.1% - 99.8%)			

Table 20: Clinical Performance for Detection of Influenza B

Nano-Check Influenza+ COVID-19 Dual Test	RT-PCR Comparator		Total
	Positive	Negative	
Positive	99	5	104
Negative	15	1850	1865
Total	114	1855	1969
Positive Percent Agreement (PPA) = 86.8% (99/114, 95% CI: 79.4% - 91.9%)			
Negative Percent Agreement (NPA) = 99.7% (1850/1855, 95% CI: 99.4% - 99.9%)			

1. Clinical Sensitivity:

Please refer to Section VI.C (clinical Studies) above for the clinical validation. The PPA for the test for each analyte is as follows:

SARS-CoV-2: 87.6% (232/265) – 95% CI: 83.0% - 90.0%
Flu A: 86.9% (417/480) – 95% CI: 83.6% - 89.6%
Flu B: 86.8% (99/114) – 95% CI: 79.4% - 91.9%

2. Clinical Specificity:

Please refer to Section VI.C (Clinical Studies) above for the clinical validation. The NPA for the test for each analyte is as follows:

SARS-CoV-2: 99.8% (1701/1704) – 95% CI: 99.5% - 99.9%
Flu A: 99.6% (1483/1489) – 95% CI: 99.1% - 99.8%
Flu B: 99.7% (1850/1855) – 95% CI: 99.4% - 99.9%

D Clinical Cut-Off:

The test is a qualitative test with a binary positive/negative signal and there is no clinical cut-off for the test.

E Expected Values/Reference Range:

When the test result is valid, it produces binary values, positive or negative for SARS-CoV-2, influenza A and influenza B antigens. Therefore, the reference range is not applicable.

VII Proposed Labeling:

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

VIII Conclusion:

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