510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

I Background Information:

A 510(k) Number

K240280

B Applicant

Nano-Ditech Corporation

C Proprietary and Established Names

Nano-Check RSV Test

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
GQG	Class I	21 CFR 866.3480 - Respiratory Syncytial Virus Serological Reagents	MI - Microbiology

II Submission/Device Overview:

A Purpose for Submission:

To obtain market clearance for the Nano-Check RSV Test.

B Measurand:

Nucleoprotein antigen from Respiratory Syncytial Virus (RSV)

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 RSV Test is a rapid immunochromatographic assay for the qualitative detection of respiratory syncytial virus (RSV) nucleoprotein antigen in anterior nasal swab specimens from patients with signs and symptoms of respiratory infections. This test is intended for in vitro diagnostic use to aid in the diagnosis of RSV infections in infants and pediatric patients aged 6 months to 6 years old, and adults over 60 years of age.

Negative results do not preclude RSV infection and should not be used as the sole basis for treatment or other management decisions. A negative test is presumptive. It is recommended that negative test results be confirmed by viral cell culture or an alternative method, such as an FDA-cleared molecular assay.

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 RSV Test is an immunochromatographic lateral flow assay for detection of extracted RSV nucleoprotein antigens in human anterior nasal swab specimens. To initiate testing, a flocked swab is used to collect anterior nasal swab specimens from both nostrils. The patient swab sample is placed into a Reagent Tube containing extraction buffer. The buffer disrupts the virus particles present in the sample, exposing the internal viral nucleoproteins. The test device is comprised of a plastic cassette containing the test strip and the sample well (Figure 1). After the extraction step, the sample is dispensed into the sample well of the test device.

Figure 1: Test Device of Nano-Check RSV Test

The sample will migrate up the test strip via capillary action. If RSV nucleoprotein antigens are present, they will bind to the colloidal gold particles coupled with monoclonal antibodies specifically targeting the RSV nucleoprotein antigen. The antigen-conjugate immunocomplexes migrate across the test strip and are captured at the test line (T) of the nitrocellulose membrane forming a visible pinkish-red line. Sample continues to flow through the test device which also contains a procedural control line (C) to assess adequate sample flow and functioning of the test device. A visible pinkish-red line at the control line (C) should always appear if the assay is performed correctly. 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 15 minutes after the sample is added to the cassette.

The Nano-Check RSV Test kit contains reagents sufficient to run 20 tests and includes:

- 20 Test devices in sealed aluminum foil pouch with desiccant
- 20 Reagent tubes prefilled with 350 µL extraction buffer containing buffer with detergents, antibiotics and sodium azide
- 20 Dropper tips
- 20 Individually packed sterile nasal swabs
- One Positive Control swab
- One Negative Control swab
- One Instructions for Use (IFU)
- One Quick Reference Instructions (QRI)

External positive control and negative control swabs are provided with each kit of Nano-Check RSV Antigen Tests and are intended to be used as quality control samples to confirm that the reagents are functional and the assay procedure is performed correctly.

B Principle of Operation:

The Nano-Check RSV Test employs lateral flow technology in a sandwich design to detect nucleoprotein antigens from RSV in anterior nasal samples. Following sample collection, the swab is placed in the reagent tube containing extraction buffer to lyse the sample and release viral nucleoproteins. After lysis, two drops of the sample are loaded into the test cassette sample well. The sample migrates across a pad containing colloidal gold-labelled monoclonal antibodies to RSV nucleoprotein antigen. If RSV nucleoprotein antigens are present, they will bind to the colloidal gold particles coupled with monoclonal antibodies specifically targeting the RSV nucleoprotein antigen. The antigen-conjugate immunocomplexes migrate across the test strip and are captured at the Test Line (T) of the nitrocellulose membrane forming a visible pinkish-red line. Sample continues to flow through the test device which also contains a procedural Control Line (C). A visible pinkish-red line at the Control Line should always appear if the assay is performed correctly to verify proper liquid flow and capturing of gold particles in the test device.

Test results are read 15 minutes after the sample is added to the cassette. 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):

BD Veritor System for Rapid Detection of RSV

B Predicate 510(k) Number(s):

K132456

C Comparison with Predicate(s):

Device & Predicate Device(s):	K240280	K132456
Device Trade Name	Nano-Check RSV Test	BD Veritor System for Rapid Detection of RSV
General Device Characteristic Similarities		
Intended Use/Indications For Use	The Nano-Check RSV Test is a rapid immunochromatographic assay for the qualitative detection of respiratory syncytial virus (RSV) nucleoprotein antigen in anterior nasal swab specimens from patients with signs and symptoms of respiratory infections. This test is intended for *in vitro* diagnostic use to aid in the diagnosis of RSV infections in infants and pediatric patients aged 6 months to 6 years old, and adults over 60 years of age. Negative results do not preclude RSV infection and should not be used as the sole basis for treatment or other management decisions. A negative test is presumptive. It is	The BD Veritor System for Rapid Detection of Respiratory Syncytial Virus (RSV) is a chromatographic immunoassay with an instrumented read for the direct and qualitative detection of RSV fusion protein from a direct nasopharyngeal swab from patients suspected of having a viral respiratory infection. This test is intended for in vitro diagnostic use to aid in the diagnosis of RSV infections in infants and pediatric patients under the age of 6 years. Negative results do not preclude RSV infection and should not be used as the sole basis for treatment or for other

	recommended that negative test results be confirmed by viral cell culture or an alternative method, such as an FDA-cleared molecular assay.	management decisions. A negative test is presumptive. It is recommended that negative test results be confirmed by viral cell culture or an alternative method, such as a FDA-cleared molecular assay. The test is intended for professional and laboratory use. It is to be used in conjunction with the BD Veritor System Instrument.
Regulation Number	21 CFR 866.3480	Same
Indication Type	Prescription Use Only	Same
Assay Principle (Technology)	Immunochromatographic	Same
Test Result Type	Qualitative	Same
Test Control	• Kit RSV positive and RSV negative dry swab procedural control • Internal control line	• Kit RSV positive and RSV negative dry swab procedural control • Internal positive control/Internal negative control
General Device Characteristic Differences		
Specimen Type	Anterior nasal swab	Nasopharyngeal swab in transport media, Nasopharyngeal wash/aspirate
Test Target	Nucleoprotein of RSV	Fusion protein of RSV
Instrumentation	None	BD Veritor System Instrument
Result Interpretation	Visual determination of the presence or absence of colored line at the Test line and a colored line at the Control line indicate the presence or absence of RSV	An opto-electronic reader determines the line intensity at each of the spatially- defined test and control line positions, interprets the results using the scoring algorithm, and reports a positive, negative, or invalid result on the LCD screen based on pre-set thresholds.

Reading Time	15 minutes	Approximately 10 minutes

VI Standards/Guidance Documents Referenced:

Document Number	Title	Publishing Organization	Applicable Study
21 CFR 866.3480	Respiratory syncytial virus serological reagents	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
EP05-A3	Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline - Third Edition	CLSI	Precision
EP-37	Supplemental Tables for Interference Testing in Clinical Chemistry	CLSI	Interference
ISO 14971:2019	Application of risk management to medical devices	ISO Standard	Risk Management
N/A	Recommendations for Clinical Laboratory Improvement Amendments of 1988 (CLIA) Waiver Applications for Manufacturers of In Vitro Diagnostic Devices	FDA	CLIA Waiver: Clinical and Analytical Studies
N/A	Recommendations for Dual 510(k) and CLIA Waiver by Application Studies	FDA	CLIA Waiver: Clinical, Near-the-Cutoff and Flex Studies

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/Reproducibility:

a) Within Laboratory Precision

A precision study was conducted at one internal site, with two operators testing samples twice daily over 12 days. Test samples were contrived in negative nasal clinical matrix (NCM), spiked with RSV A Long strain to target concentrations. Four panel members were prepared: true negative (NCM, unspiked), high negative (0.1x LoD), low positive (1x LoD), and moderate positive (3x LoD). The test samples were prepared by coating the viral solutions onto the swabs. The samples were randomized and blind-coded. Each operator tested two replicates of each sample in each run (two runs per day), for a total of 96 replicates per panel member. Results are summarized below and in Table 1:

Table 1. Precision Study Results

Level	Expected Result/Total Tested							
	Mod. Positive (3x LoD)		Low Positive (1x LoD)		High Negative (0.1x LoD)		True Negative (NCM only)	
Operator	1	2	1	2	1	2	1	2
Run 1	24/24	24/24	24/24	24/24	24/24	24/24	24/24	24/24
Run 2	24/24	24/24	22/24	24/24	24/24	24/24	24/24	24/24
Expected Result/Total Tested	96/96		96/96		96/96		96/96	
% Agreement with Expected Results	100%		100%		100%		100%	
95% CI	92.6 - 100%		92.6 - 100%		92.6 - 100%		92.6 - 100%	

b) Reproducibility

Another study was conducted to evaluate the reproducibility of the Nano-Check RSV Test at three external CLIA-waived sites with a total of seven untrained operators. Samples were also tested at one internal site with three trained operators. A total of three lots of test devices were used. Operators tested a randomized blind-coded panel of contrived samples at four concentration levels (prepared in the same manner as above for the precision study), with three replicates per level tested by each operator over five days. Per concentration level, 150 replicates were tested (10 operators x 5 days of testing x 3 replicates per day) for a total of 600 results. No invalid test results were obtained during the study. The results, stratified by site, are summarized below in Table 2. No significant differences between sites, or between trained and untrained operators were observed.

Table 2. Reproducibility Study Results

Site	Operator	Expected Result/Total Tested			
		True Negative (NCM only)	High Negative (0.1x LoD)	Low Positive (1x LoD)*	Mod. Positive (3x LoD)
Site 1 (External)	n=2 Untrained	30/30	30/30	30/30	30/30
Site 2 (External)	n=3 Untrained	45/45	45/45	45/45	45/45
Site 3 (External)	n=2 Untrained	30/30	30/30	30/30	30/30
Site 4 (Internal)	n=3 Trained	45/45	45/45	45/45	45/45
Expected Result/Total Tested		150/150	150/150	150/150	150/150
% Agreement with Expected Results		100%	100%	100%	100%
95% CI		97.5-100%	97.5-100%	97.5-100%	97.5-100%

*While the Low Positive sample used in the study, at an LoD concentration 1.6×10^3 $TCID_{50}$/mL, generated 100% positivity, the subsequent 1:2 dilution of this sample to 8.0×10^2 $TCID_{50}$/mL demonstrated 30% positivity.

2. Linearity:

Not Applicable

3. Analytical Specificity/Interference:

a) Cross-Reactivity and Microbial Interference Study

Cross-reactivity and potential microbial interference were evaluated by testing various bacteria, viruses and fungi with the Nano-Check RSV Test. Each organism was tested in three replicates in the absence and presence of 3x LoD RSV in pooled negative clinical nasal matrix using one lot of the kit reagents. The testing concentrations for potentially interfering microorganisms were $\geq 10^5$ units/mL for viruses and $\geq 10^6$ units/mL for other microorganisms, unless otherwise noted in the table below. None of the evaluated organisms demonstrated cross-reactivity or interference with the assay at the tested concentrations. Results are summarized in Table 3 below.

Table 3. Microbial Cross-Reactivity and Interference Results

Microorganism, Strain	Concentration Tested	Positive Sample with 3x LoD RSV (Positive/ Total Tested)	Negative Sample (Positive/ Total Tested)	Cross-Reactivity/ Microbial Interference
Bordetella pertussis, Strain 5	1 x 10^6 cfu/mL	3/3	0/3	No
Candida albicans, ZMC	1 x 10^6 cfu/mL	3/3	0/3	No
Chlamydophila pneumoniae, AR-39	1 x 10^6 IFU/mL	3/3	0/3	No
Corynebacterium diphtheriae	1 x 10^6 cfu/mL	3/3	0/3	No
Escherichia coli	1 x 10^6 cfu/mL	3/3	0/3	No
Haemophilus influenzae, Type B	1 x 10^6 cfu/mL	3/3	0/3	No
Lactobacillus acidophilus	1 x 10^6 cfu/mL	3/3	0/3	No
Legionella pneumophila	1 x 10^6 cfu/mL	3/3	0/3	No
Moraxella catarrhalis	1 x 10^6 cfu/mL	3/3	0/3	No
Mycobacterium tuberculosis, Strain H37Ra-1	1 x 10^6 cfu/mL	3/3	0/3	No
Mycoplasma pneumoniae	1 x 10^6 cfu/mL	3/3	0/3	No
*Neisseria meningitidis, S*erogroup A	1 x 10^6 cfu/mL	3/3	0/3	No
Neisseria mucosa, Strain AmMs 138	1 x 10^6 cfu/mL	3/3	0/3	No
Neisseria subflava biovar flava	1 x 10^6 cfu/mL	3/3	0/3	No
Pneumocystis jiroveci – S. cerevisiae	1 x 10^6 cfu/mL	3/3	0/3	No
Pseudomonas aeruginosa Strain Boston 41501	1 x 10^6 cfu/mL	3/3	0/3	No
Staphylococcus aureus, MRSA; COL	1 x 10^6 cfu/mL	3/3	0/3	No
Staphylococcus epidermidis, MRSE; RP62A	1 x 10^6 cfu/mL	3/3	0/3	No
Streptococcus mutans	1 x 10^6 cfu/mL	3/3	0/3	No
Streptococcus pneumoniae, 19F	1 x 10^6 cfu/mL	3/3	0/3	No
Streptococcus pyogenes, Bruno	1 x 10^6 cfu/mL	3/3	0/3	No
Streptococcus salivarius	1 x 10^6 cfu/mL	3/3	0/3	No

Adenovirus, Type 2	8.5×10^5 $TCID_{50}$/mL	3/3	0/3	No
Coronavirus, OC43	3.8×10^5 $TCID_{50}$/mL	3/3	0/3	No
Coxsackievirus, B4	1.0×10^5 $TCID_{50}$/mL	3/3	0/3	No
Enterovirus 71, MP4	1.6×10^5 $TCID_{50}$/mL	3/3	0/3	No
Epstein-Barr Virus, Strain B95-8	2.9×10^5 $TCID_{50}$/mL	3/3	0/3	No
Human Coronavirus, 229E	1.6×10^5 $TCID_{50}$/mL	3/3	0/3	No
Human Coronavirus, NL63	8.0×10^3 $TCID_{50}$/mL	3/3	0/3	No
Human herpesvirus 5 (Human Cytomegalovirus), Merlin	8.0×10^4 $TCID_{50}$/mL	3/3	0/3	No
Human metapneumovirus, TN/83-1211	2.8×10^5 $TCID_{50}$/mL	3/3	0/3	No
Human Parainfluenza Virus 1, 1/FRA/29221106/2009	8.9×10^5 $TCID_{50}$/mL	3/3	0/3	No
Human Parainfluenza Virus 2, Greer	1.0×10^5 $TCID_{50}$/mL	3/3	0/3	No
Human Parainfluenza Virus 3, NIH 47885	1.6×10^5 $TCID_{50}$/mL	3/3	0/3	No
Human Parainfluenza Virus 4B, 19503	5.0×10^5 $TCID_{50}$/mL	3/3	0/3	No
Influenza A (H1N1), A/PR/8/34	1.6×10^5 $CEID_{50}$/mL	3/3	0/3	No
Influenza A (H3N2), A/Singapore/INFIMH-16-0019/16	1.0×10^5 $TCID_{50}$/mL	3/3	0/3	No
Influenza A (H3N2), A/California/ 2/2014	1.0×10^5 $TCID_{50}$/mL	3/3	0/3	No
Influenza A (H3N2), A/Hong Kong /4801/2014	9.6×10^5 $CEID_{50}$/mL	3/3	0/3	No
Influenza A (H3N2), A/Switzerland /9715293/2013	1.6×10^5 $CEID_{50}$/mL	3/3	0/3	No
Influenza B (Yamagata Lineage), B/Christchurch/33/2004	1.6×10^5 $TCID_{50}$/mL	3/3	0/3	No
Influenza B, B/Florida/4/2006	2.8×10^5 $CEID_{50}$/mL	3/3	0/3	No
Influenza B, B/Hong Kong/330/2001	1.8×10^5 $CEID_{50}$/mL	3/3	0/3	No
Influenza B, B/Malaysia/2506/04	2.8×10^5 $CEID_{50}$/mL	3/3	0/3	No
Influenza B, B/Sydney/507/2006 (Yamagata Lineage)	1.6×10^5 $TCID_{50}$/mL	3/3	0/3	No
Measles Virus, Edmonston	1.7×10^4 $TCID_{50}$/mL	3/3	0/3	No
MERS-CoV, EMC/2012	1.0×10^5 $TCID_{50}$/mL	3/3	0/3	No

Mumps Virus, MuV/Iowa.US/2006	1.0 x 10^5 $TCID_{50}$/mL	3/3	0/3	No
Rhinovirus 20, 15-CV19	1.0 x 10^5 $TCID_{50}$/mL	3/3	0/3	No
SARS-CoV, Urbani strain	1.0 x 10^5 pfu/mL	3/3	0/3	No
SARS-CoV-2 (Omicron), hCoV-19/ USA/MD-HP20874/2021	1.95 x 10^4 $TCID_{50}$/mL	3/3	0/3	No

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 RSV Test. Each potentially interfering substance was tested in triplicate in negative clinical matrix at targeted concentrations in the presence or absence of 3x LoD RSV. None of the evaluated substances demonstrated interference with the assay at the tested concentrations. Results are summarized in Table 4 below.

Table 4. Interfering Substances Results

Substances	Active Ingredient of the Substance	Concentration Tested	Positive Sample with 3x LoD RSV (Positive/ Total Tested)	Negative Sample (Positive/ Total Tested)	Interference
Nasal Spray 1- Flonase	Fluticasone propionate	15% v/v	3/3	0/3	No
Nasal Spray 2 - Similasan	Cardiospermum Galphimia glauca Luffa operculata Sabadilla	15% v/v	3/3	0/3	No
Nasal Spray 3 - CVS	Phenylephrine HCl	15% v/v	3/3	0/3	No
Nasal Spray 4 - Afrin	Oxymetazoline	15% v/v	3/3	0/3	No
Budesonide Nasal Spray	Budenoside	15% v/v	3/3	0/3	No
NASONEX 24 hr Allergy	Mometasone furoate monohydrate	15% v/v	3/3	0/3	No
Nasacort Allergy 24HR	Triamcinolone acetonide	15% v/v	3/3	0/3	No
Sore Throat (Oral Pain Reliver spay)	Phenol, Menthol	15% v/v	3/3	0/3	No
ZICAM Oral mist	Zincum aceticum, Zincum gluconicum	15% v/v	3/3	0/3	No
Sore Throat Lozenges	Benzocaine, Menthol	15% w/v	3/3	0/3	No
Zinc Cold Therapy	Zincum gluconicum	15% w/v	3/3	0/3	No
Homeopathic Allergy Nasal Spray	N/A	15% v/v	3/3	0/3	No

NasoGEL (Gel Spray)	Sodium Hyaluronate Benzalkonium Chloride Allantoin Sodium Chloride Sodium Bicarbonate Glycerin Propylene Glycol	15% v/v	3/3	0/3	No
Nasalcrom Nasal Allergy Spray	Mometasone furoate monohydrate	15% v/v	3/3	0/3	No
Histaminum 30C	Histaminum hydrochloricum	15% w/v	3/3	0/3	No
Skin Relief Hand Cream	Dimethicone	1% w/v	3/3	0/3	No
Hand Soap Fresh Breeze Scent	N/A	1% w/v	3/3	0/3	No
Antibacterial liquid Hand Soap	Benzalkonium Chloride	1% w/v	3/3	0/3	No
Hand Sanitizer Gel - CVS	70% Ethyl alcohol	1% w/v	3/3	0/3	No
Disinfectant Spray - Lysol	Alkyl (50% Cl4, 40% Cl2, 10% Cl6)-dimethyl benzyl ammonium saccharinate 0.1% Ethanol 58%	1% v/v	3/3	0/3	No
Acetylsalicylic acid	Acetylsalicylic acid	30 µg/mL	3/3	0/3	No
Beclomethasone Dipropionate	Beclomethasone Dipropionate	5.04 µg/mL	3/3	0/3	No
Dexametasone	Dexametasone	12 µg/mL	3/3	0/3	No
Flunisolide	Flunisolide	870 µg/mL	3/3	0/3	No
Molnupiravir	Molnupiravir	3.29 mg/mL	3/3	0/3	No
Mometasone furoate	Mometasone furoate	0.45 ng/mL	3/3	0/3	No
Mupirocin	Mupirocin	1.5 µg/mL	3/3	0/3	No
Oseltamivir phosphate	Oseltamivir phosphate	0.399 µg/mL	3/3	0/3	No
Remdesivir	Remdesivir	240 µg/mL	3/3	0/3	No
Tobramycin	Tobramycin	33 µg/mL	3/3	0/3	No
Zanamivir	Zanamivir	30 mg/mL	3/3	0/3	No
Mucin-bovine submaxillary glands (Type I-S)	Mucin	5 mg/mL	3/3	0/3	No
Purified Human Neutrophils	N/A	5 x 10^6 cells/mL	3/3	0/3	No
Whole Blood	N/A	2.5% (v/v)	3/3	0/3	No

4. Assay Reportable Range:

Not Applicable

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

a) Internal Controls

The Nano-Check RSV Test has a built-in internal procedural control which monitors that sufficient volume of the sample is added and the reagent flow across the membrane occurred. Formation of a pinkish-red line in the control region on the strip confirms proper sample application and that the reagents are functioning appropriately. If no visible signal appears on the control line, the test result is invalid, and the operator is instructed in the labelling to test the sample again with another test device.

b) External Controls

The Nano-Check RSV Test kit 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 noninfectious RSV antigen and the negative control swab contains noninfectious nasal clinical matrix.

Lot-to-lot precision of the external positive and negative control swabs was evaluated by testing ten replicates each of three lots of the external controls. For each external control lot, all positive and negative controls produced 100% agreement with the expected results, as summarized in Table 5 below.

Table 5. Validation of External Control Materials

External Control	Control Lot No.	Test Result/Total Tests		% Agreement to Expected Result
		# Negative	# Positive	
External Positive Control	1	0/10	10/10	100%
	2	0/10	10/10	100%
	3	0/10	10/10	100%
External Negative Control	1	10/10	0/10	100%
	2	10/10	0/10	100%
	3	10/10	0/10	100%

c) Specimen Stability

Specimen stability was assessed using a negative sample (consisting of pooled negative human nasal fluid) and a contrived low positive sample (containing RSV A Long strain at 2x LoD in the pooled negative human nasal fluid). Contrived samples on the swabs were stored at ambient temperature (23.5°C), high room temperature (30°C), refrigerated (2-8°C) and frozen conditions (-20°C) before extraction and tested at 1, 2, 4, 8, 24 and 48 hours. Five replicates of each concentration were tested in accordance with the package insert at each timepoint for each condition. The results obtained in this study are summarized below.

Table 6. Specimen Stability Study Results

Specimen Storage Temperature	Tested Sample	No. of Positive Test Results/No. of Total Test						
		0 hrs.	1 hr.	2 hrs.	4 hrs.	8 hrs.	24 hrs.	48 hrs.
Ambient Temp. (23.5°C)	2x LoD	5/5	5/5	5/5	5/5	5/5	5/5	5/5
	Negative	0/5	0/5	0/5	0/5	0/5	0/5	0/5
	2x LoD	5/5	5/5	5/5	5/5	5/5	5/5	5/5

High Room Temp. (30°C)	Negative	0/5	0/5	0/5	0/5	0/5	0/5	0/5
Refrigerated (2-8°C)	2x LoD	5/5	5/5	5/5	5/5	5/5	5/5	5/5
	Negative	0/5	0/5	0/5	0/5	0/5	0/5	0/5
Frozen (-20°C)	2x LoD	5/5	5/5	5/5	5/5	5/5	5/5	5/5
	Negative	0/5	0/5	0/5	0/5	0/5	0/5	0/5

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. These results support the specimen stability claim of one hour after collection of the nasal swab when kept at room temperature (15°C to 30°C), or within 24 hours when stored refrigerated (2°C to 8°C).

6. Detection Limit:

The Limit of Detection (LoD) of Nano-Check RSV Test was established for four RSV strains. Contrived samples were prepared by spiking the two RSV A and two RSV B strains into pooled negative nasal fluid confirmed negative for RSV. Initially 10-fold serial dilutions of RSV strains in pooled negative nasal fluid were prepared and tested in triplicate to find the lowest level detectable by the Nano-Check RSV Test. Further characterization was performed using closely spaced 2-fold dilutions of RSV strains. The final LoD, a concentration producing 95% positivity, was confirmed by testing thirty replicates of RSV strains at the selected preliminary LoD. The results from these studies are summarized below in Table 7.

Table 7. Nano-Check RSV Test Limit of Detection

RSV subtype	Strain	LoD	No. Positive/Total Tested	% Positive
A	Long*	1.6 x 10^3 $TCID_{50}$/mL	30/30	100.0%
	A-2	2.0 x 10^4 $TCID_{50}$/mL	29/30	96.7%
B	18537**	4.0 x 10^3 $TCID_{50}$/mL	30/30	100.0%
	B1***	3.5 x 10^3 $TCID_{50}$/mL	30/30	100.0%

*2-fold dilution (8 x 10^2 $TCID_{50}$/mL) showed 33.3% detection.
**2-fold dilution (2 x 10^3 $TCID_{50}$/mL) showed 66.7% detection.
***2-fold dilution (1.8 x 10^3 $TCID_{50}$/mL) showed 33.3% detection.

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 RSV). Four different RSV strains (RSV A Long, RSV A2, RSV B 18537 and RSV B1) were diluted to four or five different concentrations (see Table 8 below) in pooled negative nasal fluid matrix and tested in three replicates on Nano-Check RSV Test. Concentrations ranged from 10,000x LoD (the maximum virus concentration possible, undiluted from the viral stock) to 1x LoD diluted in negative clinical matrix. All tested samples demonstrated 100% positivity, as expected (Table 8). The Nano-Check RSV Test did not display a Hook Effect for the tested RSV concentrations.

Table 8. High-Dose Hook Effect Study Results

RSV Strain	Concentration ($TCID_{50}$/mL)	Sample Level	Positive Agreement (RSV positive/Total tested)
RSV A Long	1.6×10^7	10000x LoD	100% (3/3)
	1.6×10^6	1000x LoD	100% (3/3)
	1.6×10^5	100x LoD	100% (3/3)
	1.6×10^4	10x LoD	100% (3/3)
	1.6×10^3	1x LoD	100% (3/3)
RSV A2	1.6×10^8	8000x LoD	100% (3/3)
	1.6×10^7	800x LoD	100% (3/3)
	1.6×10^6	80x LoD	100% (3/3)
	1.6×10^5	8x LoD	100% (3/3)
	2.0×10^4	1x LoD	100% (3/3)
RSV B 18537	1.6×10^7	4000x LoD	100% (3/3)
	1.6×10^6	400x LoD	100% (3/3)
	1.6×10^5	40x LoD	100% (3/3)
	1.6×10^4	4x LoD	100% (3/3)
	4.0×10^3	1x LoD	100% (3/3)
RSV B1	2.8×10^6	800x LoD	100% (3/3)
	2.8×10^5	80x LoD	100% (3/3)
	2.8×10^4	8x LoD	100% (3/3)
	3.5×10^3	1x LoD	100% (3/3)

8. Inclusivity (Analytical Reactivity)

An inclusivity study was performed to demonstrate that the Nano-Check RSV Test can detect a broad range of RSV strains. In addition to the strains evaluated in the LoD study, seven strains of RSV A and two strains of RSV B were included in the study. Ten-fold dilutions of virus stocks were prepared in pooled negative nasal fluid matrix and 50 tested in five replicates in this study. The lowest concentration of each strain that resulted in 100% detection (5/5) is presented in the table below.

Table 9. Inclusivity Study Results

RSV Subtype	Strain	Concentration Detected	Positive Agreement (# Positive/Total Tested)
A	1998/3-2	1.6×10^4 $TCID_{50}$/mL	100% (5/5)
	1998/12-21	2.8×10^1 $TCID_{50}$/mL	100% (5/5)
	2000/3-4	3.2 $TCID_{50}$/mL	100% (5/5)
	2001/3-12	1.4×10^3 $TCID_{50}$/mL	100% (5/5)
	ARG/177/2006	8.9×10^2 $TCID_{50}$/mL	100% (5/5)
	2001/2-20	8.9×10^3 $TCID_{50}$/mL	100% (5/5)
	1997/12-35	1.3×10^3 $TCID_{50}$/mL	100% (5/5)
B	9320	3.5×10^3 $TCID_{50}$/mL	100% (5/5)
	WV/14617/85	1.1×10^3 $TCID_{50}$/mL	100% (5/5)

B Comparison Studies:

1. Method Comparison with Predicate Device:

Refer to Clinical Studies section below.

2. Matrix Comparison:

The sponsor conducted a matrix equivalency study between clinical anterior nasal fluid and a representative negative clinical matrix (nasal wash). Clinical nasal wash was used in some analytical studies (reproducibility/precision, cross-reactivity, interference, and reagent/kit storage). Equivalence was evaluated using one strain each for RSV A (Long strain) and RSV B (B1 strain). Virus cultures were diluted into the matrices at three concentrations: 0.1x LoD, 1x LoD and 3x LoD and tested with 10 replicates for 3x LoD and 0.3x LoD and 20 replicates for 1x LoD. Negativity of each of the matrices was determined by testing five replicates of each of the matrices. The results obtained in this study are summarized below. The data demonstrated equivalent performance of the test with all the three matrices.

Table 10. Matrix Equivalency Study for Nano-Check RSV Test

Sample	**#Positive /Total Tested**		
RSV A Long strain	**3x LoD**	**1x LoD**	**0.3x LoD**
Nasal Wash	10/10	20/20	2/8
Nasal Fluid	10/10	20/20	3/7
RSV B1 strain	**3x LoD**	**1x LoD**	**0.3x LoD**
Nasal Wash	10/10	20/20	3/7
Nasal Fluid	10/10	19/20	4/6
Negative Matrix	**No analyte**		
Nasal Wash	0/5		
Nasal Fluid	0/5		

C Clinical Studies:

1. Clinical Sensitivity:

The performance of the Nano-Check RSV Test in detecting RSV nucleoprotein antigen was evaluated in a multi-center, prospective study across six geographically diverse CLIA-waived sites testing sites using anterior nasal (AN) swab samples collected from patients with signs and symptoms consistent with respiratory tract infection. The study was conducted between November 2023 to March 2024. Informed consent was obtained for all patients prior to testing. Two AN swabs were collected sequentially from each subject. The first swab (for comparator testing) was collected by the study operator from both sides of the nose and placed into transport media for comparator testing. The second swab was collected by the study operator from both sides of the nose and tested immediately with the Nano-Check RSV Test at the site. The performance of the Nano-Check RSV Test with AN swabs was estimated based on the comparison with results obtained with an FDA cleared molecular test for RSV (positive percent agreement (PPA) and negative percent agreement (NPA)).

A total of 886 patients were enrolled in the clinical study of which 68 were excluded due to protocol deviations, leaving 818 specimens to be included in the calculations of estimates of assay performance. Specimens were collected from pediatric patients between 6 months to 6 years of age and adult patients 60 years or older. Of those, 29% (237/818) were from subjects 6 months to 2 years old, 36.9% (302/818) were from patients 2 to 6 years of age and 34.1%

(279/818) were from patients 60 years or older. There were 52.4% (429/818) females and 47.6% (389/818) males.

The clinical performance of the Nano-Check RSV Test, expressed as PPA and NPA with the comparator result, testing AN swab specimens from patients with symptoms of respiratory tract infection, is shown in Table 11 below.

Table 11: Clinical Performance of Nano-Check RSV Test with AN Swab Specimens

Nano-Check RSV Test	Comparator RT-PCR		Total
	Positive	Negative	
Positive	93	3	96
Negative	18	704	722
Total	111	707	818
Positive Percent Agreement (PPA) = 83.8% (95% CI: 75.8% - 89.5)			
Negative Percent Agreement (NPA) = 99.6% (95% CI: 98.8% - 99.9%)			

2. Clinical Specificity:

See section "Clinical sensitivity" above, including test specificity/negative percent agreement (NPA). The NPA for the test is 99.6% (704/707; 95% CI: 98.8% - 99.9%).

D Clinical Cut-Off:

The Clinical Cut-off study is not applicable, as there is no clinical cutoff related to the presence of RSV in patient samples.

E Expected Values/Reference Range:

The rate of positivity as determined by the Nano-Check RSV Test during the 2023-2024 clinical study was 11.7% (96/818).

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.