



**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION  
DECISION SUMMARY  
ASSAY ONLY**

**I Background Information:**

**A 510(k) Number**

K251749

**B Applicant**

ACON Laboratories, Inc.

**C Proprietary and Established Names**

Flowflex Plus RSV + Flu A/B + COVID Home 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 Flowflex Plus RSV + Flu A/B + COVID Home Test

**B Measurand:**

Protein antigen from SARS-CoV-2, Influenza A/B and 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 Flowflex Plus RSV + Flu A/B + COVID Home Test is a lateral flow immunoassay intended for the qualitative detection and differentiation of respiratory syncytial virus (RSV), influenza A, influenza B, and SARS-CoV-2 protein antigens directly in anterior nasal swab samples from individuals with signs and symptoms of respiratory tract infection. Symptoms of respiratory infections due to RSV, influenza, and SARS-CoV-2 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 six (6) months 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 RSV, influenza, SARS-CoV-2 or other pathogens.

Individuals who test negative and/or experience continued or worsening symptoms, such as fever, cough and/or shortness of breath should therefore seek follow-up 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.

#### **C Special Conditions for Use Statement(s):**

OTC - Over The Counter

#### **D Special Instrument Requirements:**

Not applicable.

### **IV Device/System Characteristics:**

#### **A. Device Description:**

The Flowflex Plus RSV + Flu A/B + COVID Home Test is a lateral flow immunoassay in sandwich format intended for the qualitative detection and differentiation of RSV, influenza (Flu) A, Flu B, and SARS-CoV-2 protein antigens.

This test is for non-prescription home use (i.e., OTC) with self-collected anterior nasal swab specimens from symptomatic individuals aged 14 years or older, or with adult-collected anterior nasal swab specimens from symptomatic individuals six (6) months or older.

The test package is composed of:

- Test Cassettes
- Extraction Buffer Tubes
- Sterile Nasal Swabs

- Pediatric (6–23 Months) Swab Guards
- Tube Holder (only for 25 test quantity)
- Quick Reference Instructions

The Test Cassette is assembled with two test strips in a plastic housing.

#### **Test strip structure:**

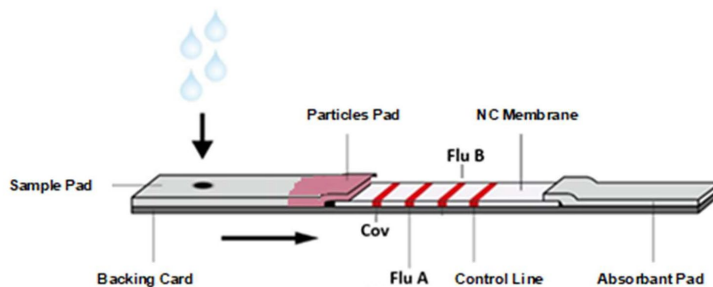
The test strips in the plastic housing include backing card (provides structural support during use), sample receiving and particles pads, nitrocellulose (NC) membrane, test lines for each analyte and absorption pad. The test lines and the control lines are pre-coated with analyte specific antibody on the membrane.

The following two strips are included in the cassette housing:

#### **Strip #1 (SARS-CoV-2 + Flu A + Flu B):**

Includes: 1) particle pad containing monoclonal antibody conjugates with colored particles (SARS-CoV-2, Flu A, and Flu B antibody conjugates) and colored rabbit IgG for the control line; 2) NC membrane containing three test lines that are pre-coated with SARS-CoV-2, Flu A, and Flu B antibodies separately labeled as “CoV”, “A”, and “B” respectively on the cartridge and one control line, pre-coated with goat anti-rabbit IgG antibody (Fig 1) labeled as “Ctl” on the cartridge.

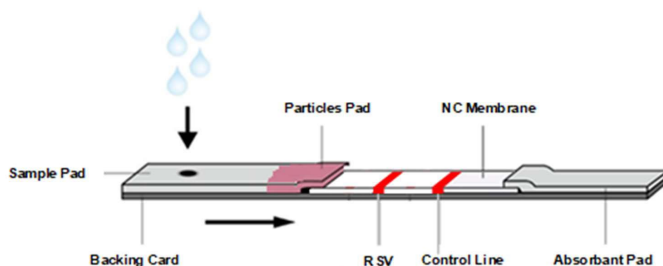
**Fig 1: Strip 1**



#### **Strip #2 (RSV):**

Includes: 1) particle pad containing monoclonal antibody conjugates with colored particles (RSV antibody conjugates) and colored rabbit IgG for the control line; 2) NC membrane containing one test line pre-coated with RSV antibody labeled as “R” on the test cartridge and one control line, pre-coated with goat anti-rabbit IgG antibody (Fig 2) labeled as “Ctl” on the cartridge.

**Fig 2: Strip 2**

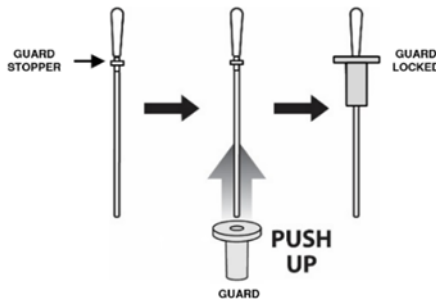


In both strips, attached to the NC membrane are the sample pad (which absorbs and distributes the sample), the reagent pad (containing reagents for antigen binding and detection), and the absorbent pad (which collects the excess liquid sample and reagents).

### **Swab Guard:**

For collecting nasal swabs from children aged 6–23 months, lay users employ a swab guard which serves as an added safety measure, preventing the nasal swab from going deeper than intended, limiting the risk of injury in children. In order to use, lay users slide the guard onto the swab from the stick end through the guard opening, and push it up until it clicks into place at the base of the foam head (Fig 3).

**Fig 3: Infant Swab Guard**



### **B. Principle of Operation:**

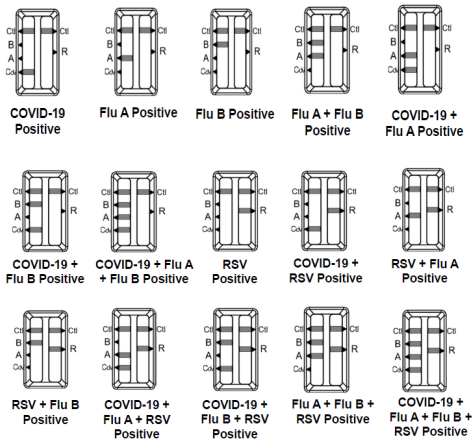
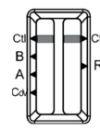
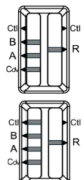
To perform the test, a nasal swab is collected by a user (users 6 – 23 months will use swab guard), the swab is processed in extraction buffer and then added to the sample well of the test cassette. When an adequate volume of the processed specimen is added to the sample well of the test cassette, the specimen migrates by capillary action across each of the two test strips. SARS-CoV-2, Flu A, Flu B, or RSV antigen, if present in the specimen, will react with the specific antibody-coated colored particles. The mixture then migrates towards the membrane by capillary action to bind to the specific antibody on the NC membrane, producing a visible colored test line in the related test line region (CoV/A/B/R).

To serve as a procedural control, the colored rabbit IgG labeled particle will bind to goat anti-rabbit IgG on each NC membrane on each test strip to produce a colored line on the control line (Ctl) in the control line region. Formation of the control lines serves as an internal control indicating that proper volume of specimen has been added, and membrane wicking has occurred.

### **Interpretation of Results:**

After dispensing the test specimen into the sample well, the result should be read at 15 minutes. Results should not be read before 15 minutes and after 30 minutes.

**Table 1: Result interpretation**

Result	Interpretation	Example Image
<b>Positive</b>	If the control (Ctl) line is visible on both strips and any red or pink line appear at “CoV”, “A”, “B”, or “R”, no matter how faint, the test is positive for that virus.	
<b>Negative</b>	If both control (Ctl) lines are visible, but the test line(s) (CoV, A, B, R) is/are not visible, the test is negative.	
<b>Invalid</b>	If the control (Ctl) line is not visible on either or both test strips, even if any test line is visible in the result window, the test is invalid. A new sample should be collected and re-tested with a new cartridge.	

## V Substantial Equivalence Information:

### A. Predicate Device Name(s):

Flowflex Plus COVID-19 + Flu A/B Home Test

### B. Predicate 510(k) Number(s):

K250377

### C. Comparison with Predicate(s):

Device & Predicate Device(s)	<u>K251749</u>	<u>K250377 (predicate)</u>
Device Trade Name	Flowflex Plus RSV + Flu A/B + COVID Home Test	Flowflex Plus COVID-19 + Flu A/B Home Test
Intended	Flowflex Plus RSV + Flu A/B+	The Flowflex Plus COVID-19 + Flu

Device & Predicate Device(s)	<u>K251749</u>	<u>K250377 (predicate)</u>
Use/Indications For Use	<p>COVID Home Test is a lateral flow immunoassay intended for the qualitative detection and differentiation of respiratory syncytial virus (RSV), influenza A, influenza B, and SARS-CoV-2 protein antigens 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, influenza, and RSV can be similar.</p> <p>This test is for non-prescription home use by individuals aged 14 years or older testing themselves, or adults testing individuals aged six (6) months or older.</p> <p>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, RSV, or other pathogens.</p> <p>Individuals who test negative and/or experience continued or worsening symptoms, such as fever, cough and/or shortness of breath should therefore seek follow-up care from their healthcare provider.</p> <p>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.</p>	<p>A/B Home Test is a lateral flow immunoassay intended for the qualitative detection and differentiation of SARS-CoV-2, influenza A, and influenza B protein antigens 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.</p> <p>This test is for non-prescription home use with self-collected anterior nares nasal swab specimens from individuals aged 14 years or older, or with adult-collected anterior nasal swab specimens from individuals two (2) years or older.</p> <p>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.</p> <p>Individuals who test negative and experience continued or worsening respiratory symptoms, such as fever, cough and/or shortness of breath should therefore seek follow-up care from their healthcare provider.</p> <p>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.</p>
<b>General Device Characteristic Similarities</b>		
Regulation	21 CFR 866.3987	Same
Patient Use	Over the counter use/self-testing	Same
Intended Specimen	Direct anterior nasal swabs	Same
Usage Type	Single -use test	Same
Assay Technique	Immunochromatographic Assay, visual read	Same
Test Result	Qualitative	Same

<b>Device &amp; Predicate Device(s)</b>	<b><u>K251749</u></b>	<b><u>K250377 (predicate)</u></b>
Time to Result	15 minutes	Same
Detection Period	within 5 days of symptom onset	Same
Storage Temperature	36-86 °F (2- 30°C)	Same
<b>General Device Characteristic Differences</b>		
Analytes Detected	SARS-CoV-2, influenza A, influenza B and RSV protein antigens	SARS-CoV-2, influenza A, and influenza B protein antigens
Sample Collection Method	Nasal swab supplied in kit; includes pediatric swab guard for ages 6–23 months.	Nasal swab supplied in the kit; no pediatric swab guard is included.

## VI Standards/Guidance Documents Referenced:

<b>Document</b>	<b>Title</b>	<b>Publisher</b>	<b>Applicable Study</b>
Special Controls under 21 CFR 866.3987 (multi-analyte respiratory virus antigen detection test)	<a href="#">Reclassification order for DEN240029 and special controls under 21 CFR 8663987.pdf</a>	FDA/CDRH	All Studies
ISO11135:2014	Sterilization of health care products - Ethylene oxide - Requirements for development, validation and routine control of a sterilization process for medical devices	ISO	Sterility
ISO 10993-7	Biological Evaluation of Medical Devices – Part 7: Ethylene Oxide Sterilization Residuals	ISO	Sterility
ISO 10993-5: 2009	Biological evaluation of medical devices — Part 5: Tests for in vitro cytotoxicity	ISO	Biocompatibility
ISO 10993-10: 2010	Biological evaluation of medical devices- Part 10: Tests for irritation and skin sensitization	ISO	Biocompatibility
ISO 10993-23:2010	Biological evaluation of medical devices Part 23: Tests for irritation	ISO	Biocompatibility

## VII Performance Characteristics (if/when applicable):

## A. Analytical Performance:

### 1. Lot-to-lot Precision:

The multi-lot precision for the Flowflex Plus RSV + Flu A/B + COVID Home Test was evaluated in two different in-house studies to assess variability between reagent lots, days, runs and operators.

**Study 1** was conducted by 2 trained operators each of whom tested seven samples with various analyte concentrations and combinations as described below:

- a. Negative
- b. 2x LoD SARS-CoV-2
- c. 2x LoD Flu A
- d. 2x LoD Flu B
- e. 2x LoD RSV
- f. 2x LoD Flu B & RSV
- g. 2x LoD SARS-CoV-2 & Flu B & RSV

Each operator tested two sample replicates per run across two runs using three lots of devices. Runs were performed in the morning and afternoon over 10 days. This design (2 replicates/run/lot x 2 runs/operator x 2 operators x 3 lots x 10 days) resulted in 240 replicates per sample. All samples were prepared in pooled nasal swab matrix (PNSM). The study was performed in randomized and blinded manner.

Results for this study are shown in table below and were concordant with the expected results. Since the study obtained 100% agreement for all samples, both with and without analytes, the data were not stratified by the individual sources of variation.

**Table 2: Summary results of multi-lot precision study (Study 1)**

Sample level	Analyte	Obtained results per analyte / Expected results per analyte	Total percent lot-to-lot agreement	95% CI
<b>Negative</b>	SARS-CoV-2	(240/240)	100%	98.5-100%
	Flu A	(240/240)	100%	98.5-100%
	Flu B	(240/240)	100%	98.5-100%
	RSV	(240/240)	100%	98.5-100%
<b>2x LoD</b>	SARS-CoV-2	(240/240)	100%	98.5-100%
	Flu A	(240/240)	100%	98.5-100%
	Flu B	(240/240)	100%	98.5-100%
	RSV	(240/240)	100%	98.5-100%
	Flu B + RSV	(480/480)	100%	98.5-100%
	Flu B + RSV+ SARS-CoV-2	(720/720)	100%	98.5-100%

**Study 2** was specifically conducted to assess difference between lots, because study 1 resulted in 100% agreement across all sources of variation and did not allow to conclusively assess lot-to-lot variability of the test. Study 2 was performed using one negative sample without any analyte and two low positive samples with the following analyte combinations, each at 0.75x LoD (i.e., below the concentration tested in study 1 and near the analytes' C95 concentration):



1. Negative
2. 0.75x LoD Flu A & SARS-CoV-2
3. 0.75x LoD Flu B & RSV

All samples were randomized and tested in a blinded manner. The testing was carried out over 3 days only but otherwise followed the same study design as Study 1. 72 data points per sample were collected (2 replicates/run/lot x 2 runs/day x 2 operators x 3 days x 3 lots = 72 data points).

Procedural random errors across various days, combined with the operator's ability to visually read test line intensities of samples with low analyte concentrations are expected to confound variability between lots and to have an impact on the precision estimates of a device for very low positive samples below the LoD. However, the results with concentrations at 0.75x LoD that yielded results with less than 100% showed minor variability between lots and were not deemed significant. The results are summarized below.

**Table 3: Summary of supplemental precision study (Study 2)**

Analyte Concentration	Analyte		Lot 1		Lot 2		Lot 3		Lot-to-Lot Agreement		95% CI
			n/N <sup>1</sup>	% Agmt <sup>2</sup>	n/N <sup>1</sup>	% Agmt <sup>2</sup>	n/N <sup>1</sup>	% Agmt <sup>2</sup>	n/N <sup>1</sup>	% Agmt <sup>2</sup>	
Negative	SARS-CoV-2		24/24	100%	24/24	100%	24/24	100%	72/72	100%	95-100%
	Flu A		24/24	100%	24/24	100%	24/24	100%	72/72	100%	95-100%
	Flu B		24/24	100%	24/24	100%	24/24	100%	72/72	100%	95-100%
	RSV		24/24	100%	24/24	100%	24/24	100%	72/72	100%	95-100%
Analyte Concentration	Analyte		n/N <sup>3</sup>	% Pos	n/N <sup>3</sup>	% Pos	n/N <sup>3</sup>	% Pos	n/N <sup>3</sup>	% Pos	95% CI
0.75x LoD	Flu A+ SARS-CoV-2	Flu A	16/24	66.7%	15/24	62.5%	13/24	54.2%	44/72	61.1%	48.9-72.4%
		SARS-CoV-2	23/24	95.8%	18/24	75%	21/24	87.5%	62/72	86.1%	75.9-93.1%
	Flu B+ RSV	Flu B	19/24	79.2%	22/24	91.7%	15/24	62.5%	56/72	77.8%	66.4-86.7%
		RSV	17/24	70.8%	18/24	75%	14/24	58.3%	49/72	68.1%	56-78.6%

<sup>1</sup> Number of agreement with expected results per analyte/total number tested.

<sup>2</sup> Agreement with expected result.

<sup>3</sup> Number of positive samples/total number tested.

## 2. Linearity:

Not applicable; this test device only produces binary qualitative results.

## 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 nasal swab samples could cause

a false positive test result or interfere with the detection of a true positive result and cause a false negative result. A panel of related viruses, high prevalence disease agents, and normal or pathogenic flora were used for these studies.

For the cross-reactivity study, dilutions of the panel organisms were made in pooled anterior nasal swab matrix (PNSM) and tested in triplicates in the absence of SARS-CoV-2, influenza A, influenza B, RSVA and RSVB. No cross-reactivity was observed with the organisms tested for any of the 4 analytes (Table 4).

For the microbial interference study, dilutions of the panel organisms were made in PNSM, in the presence of low levels (3x LoD) of SARS-CoV-2 (Omicron XBB; USA/CA-Stanford109-S21/2022), influenza A (H1N1; A/Guangdong- Maonan/SWL1536/19), influenza B (Victoria; B/Hong Kong/574/19), RSV A (2006 isolate), and RSV B [CH93(18)-18] and tested in triplicate. No microbial interference was observed for any of the 4 analytes tested (Table 4).

**Table 4: Cross-reactivity and microbial interference study results**

Virus/Microorganism	Concentration	Units	# of Positive results / # of Replicates tested	
			Cross-Reactivity <sup>1</sup>	Interference <sup>1</sup>
Adenovirus Type 7A	3.16 x 10 <sup>5</sup>	TCID <sub>50</sub> /mL	0/3	3/3
Adenovirus 1	1.05 x 10 <sup>5</sup>	TCID <sub>50</sub> /mL	0/3	3/3
<i>Bordetella pertussis</i>	5.04 x 10 <sup>8</sup>	CFU/mL	0/3	3/3
<i>Candida albicans</i>	3.32 x 10 <sup>7</sup>	CFU/mL	0/3	3/3
<i>Escherichia coli</i>	2.37 x 10 <sup>7</sup>	CFU/mL	0/3	3/3
<i>Haemophilus influenzae</i>	9.85 x 10 <sup>7</sup>	CFU/mL	0/3	3/3
<i>Neisseria mucosa</i>	6.52 x 10 <sup>8</sup>	CFU/mL	0/3	3/3
<i>Corynebacterium jeikeium</i>	3.68 x 10 <sup>8</sup>	CFU/mL	0/3	3/3
<i>Legionella pneumophila</i>	5.98 x 10 <sup>9</sup>	CFU/mL	0/3	3/3
<i>Moraxella catarrhalis</i>	1.37 x 10 <sup>7</sup>	CFU/mL	0/3	3/3
Mumps Virus	4.92 x 10 <sup>6</sup>	TCID <sub>50</sub> /mL	0/3	3/3
<i>Mycobacterium tuberculosis</i>	4.60 x 10 <sup>7</sup>	CFU/mL	0/3	3/3
<i>Mycoplasma pneumoniae</i>	1.37 x 10 <sup>7</sup>	CCU/mL	0/3	3/3
<i>Neisseria meningitidis</i>	2.04 x 10 <sup>7</sup>	CFU/mL	0/3	3/3
<i>Neisseria sicca</i>	3.23 x 10 <sup>9</sup>	CFU/mL	0/3	3/3
Parainfluenza Virus Type 1	3.80 x 10 <sup>5</sup>	TCID <sub>50</sub> /mL	0/3	3/3
Parainfluenza Virus Type 2	3.39 x 10 <sup>6</sup>	TCID <sub>50</sub> /mL	0/3	3/3
Parainfluenza Virus Type 3	1.15 x 10 <sup>6</sup>	TCID <sub>50</sub> /mL	0/3	3/3
Parainfluenza Virus Type 4	9.55 x 10 <sup>5</sup>	TCID <sub>50</sub> /mL	0/3	3/3
<i>Pseudomonas aeruginosa</i>	5.50 x 10 <sup>8</sup>	CFU/mL	0/3	3/3
<i>Staphylococcus aureus</i>	5.41 x 10 <sup>8</sup>	CFU/mL	0/3	3/3
<i>Staphylococcus epidermidis</i>	3.52 x 10 <sup>8</sup>	CFU/mL	0/3	3/3
<i>Streptococcus pyogenes</i>	2.36 x 10 <sup>8</sup>	CFU/mL	0/3	3/3
<i>Streptococcus pneumoniae</i>	1.55 x 10 <sup>8</sup>	CFU/mL	0/3	3/3
<i>Streptococcus salivarius</i>	4.07 x 10 <sup>7</sup>	CFU/mL	0/3	3/3
Corona Virus Strain: 229E	2.09 x 10 <sup>5</sup>	TCID <sub>50</sub> /mL	0/3	3/3
Corona Virus Strain: OC43	3.80 x 10 <sup>5</sup>	TCID <sub>50</sub> /mL	0/3	3/3
Corona Virus, Strain: NL63	1.78 x 10 <sup>5</sup>	TCID <sub>50</sub> /mL	0/3	3/3
Cytomegalovirus (CMV)	2.09 x 10 <sup>5</sup>	TCID <sub>50</sub> /mL	0/3	3/3
Human Metapneumovirus	1.02 x 10 <sup>5</sup>	TCID <sub>50</sub> /mL	0/3	3/3

Virus/Microorganism	Concentration	Units	# of Positive results / # of Replicates tested	
			Cross-Reactivity <sup>1</sup>	Interference <sup>1</sup>
Rhinovirus	2.09 x 10 <sup>5</sup>	TCID <sub>50</sub> /mL	0/3	3/3
Measles Virus	2.53 x 10 <sup>5</sup>	TCID <sub>50</sub> /mL	0/3	3/3
MERS-coronavirus	1.12 x 10 <sup>5</sup>	TCID <sub>50</sub> /mL	0/3	3/3
<i>Lactobacillus plantarum</i>	3.13 x 10 <sup>8</sup>	CFU/mL	0/3	3/3
Enterovirus	1.05 x 10 <sup>5</sup>	TCID <sub>50</sub> /mL	0/3	3/3
<i>Pneumocystis jirovecii</i>	5.18 x 10 <sup>7</sup>	CFU/mL	0/3	3/3
<i>Chlamydophila (Chlamydia) pneumoniae</i>	1.40 x 10 <sup>7</sup>	IFU/mL	0/3	3/3
<i>Chlamydia trachomatis</i>	1.78 x 10 <sup>7</sup>	IFU/mL	0/3	3/3
Epstein Barr Virus	4.02 x 10 <sup>7</sup>	CP/mL	0/3	3/3
<i>Corynebacterium diphtheriae</i>	3.45 x 10 <sup>8</sup>	CFU/mL	0/3	3/3
<i>Saccharomyces cerevisiae</i>	3.28 x 10 <sup>7</sup>	CFU/mL	0/3	3/3
Human Coronavirus HKU1: MINF-1596 <sup>2</sup>	1:10 dilution	N/A	0/3	3/3
Human Coronavirus HKU1: MINF-1597 <sup>2</sup>	1:10 dilution	N/A	0/3	3/3
Human Coronavirus HKU1: MINF-1598 <sup>2</sup>	1:10 dilution	N/A	0/3	3/3
Human Coronavirus HKU1: MINF-1601 <sup>2</sup>	1:10 dilution	N/A	0/3	3/3
Human Coronavirus HKU1: MINF-1602 <sup>2</sup>	1:10 dilution	N/A	0/3	3/3

<sup>1</sup> All analytes in the sample replicates were in agreement with the expected result.

<sup>2</sup> Human Coronavirus HKU1 (clinical specimens) were confirmed using a 510(k)-cleared PCR assay.

**b. Competitive inhibition:**

A competitive inhibition study was conducted to evaluate the potential for a high concentration of one target analyte to interfere with the detection of another target analyte at low concentration. Testing was performed in triplicate with different combinations of low (2.5-3x LoD for single analyte) and high (highest achievable) concentrations of SARS-CoV-2, Flu A, Flu B, and RSV. The results showed no competitive interference was observed between SARS-CoV-2, influenza A, influenza B, and RSV as listed in the table below.

**Table 5: Competitive inhibition study results**

Combination #	Viral Targets in Sample				Results (# pos / total reps)			
	Flu A	Flu B	SARS-CoV- 2	RSV	Flu A	Flu B	SARS-CoV- 2	RSV
1	Low	Negative	Negative	High	3/3	0/3	0/3	3/3
2	Negative	Low	Negative	High	0/3	3/3	0/3	3/3
3	Negative	Negative	Low	High	0/3	0/3	3/3	3/3
4	Low	Negative	High	Negative	3/3	0/3	3/3	0/3
5	Negative	Negative	High	Low	0/3	0/3	3/3	3/3
6	Negative	Low	High	Negative	0/3	3/3	3/3	0/3
7	Low	High	Negative	Negative	3/3	3/3	0/3	0/3
8	Negative	High	Low	Negative	0/3	3/3	3/3	0/3

Combination #	Viral Targets in Sample				Results (# pos / total reps)			
	Flu A	Flu B	SARS-CoV-2	RSV	Flu A	Flu B	SARS-CoV-2	RSV
9	Negative	High	Negative	Low	0/3	3/3	0/3	3/3
10	High	Low	Negative	Negative	3/3	3/3	0/3	0/3
11	High	Negative	Low	Negative	3/3	0/3	3/3	0/3
12	High	Negative	Negative	Low	3/3	0/3	0/3	3/3

*c. Endogenous/Exogenous Substances Interference:*

Flowflex Plus RSV + Flu A/B + COVID Home Test was evaluated for performance in the presence of potentially interfering substances that might be present in upper respiratory tract specimens. Substances that are commonly found on the hands were also tested. Potentially interfering substances were prepared and diluted in anterior nasal swab matrix (PNSM) to the recommended concentration. Virus negative specimens were evaluated in triplicate to confirm that the potentially interfering substances were not cross-reactive with the test.

To assess interference, positive samples were prepared in PNSM at 3x LoD using each analyte individually. Positive and negative samples were evaluated in the presence of interfering substances in triplicate to confirm that these substances do not interfere with detection of SARS-CoV-2, influenza A, influenza B and RSV.

False positive results were detected for Flumist (live attenuated influenza vaccine, intranasal) at 15% v/v and 3% v/v for both Flu A and Flu B, and at 1.5% v/v for Flu A only; no cross reactivity was observed at 0.2% v/v.

No interference was observed amongst the rest of the substances tested as shown in the table below.

**Table 6: Interfering substances study results**

Interfering Substance	Concentration	Cross-reactivity (without analyte) (# pos/ # total)				Interference (with analyte) (# pos/ # total)			
		SARS-CoV-2	Flu A	Flu B	RSV	SARS-CoV-2	Flu A	Flu B	RSV
Equate Sore Throat Oral Anesthetic Spray (Phenol)	5% v/v	0/3	0/3	0/3	0/3	3/3	3/3	3/3	3/3
ZICAM Cold Remedy	15% v/v	0/3	0/3	0/3	0/3	3/3	3/3	3/3	3/3
Equate Nasal Four Nasal Spray (Phenylephrine HCl)	15% v/v	0/3	0/3	0/3	0/3	3/3	3/3	3/3	3/3
Nasal spray (Cromolyn sodium nasal solution)	15% v/v	0/3	0/3	0/3	0/3	3/3	3/3	3/3	3/3
Nasal spray (Oxymetazoline HCl)	15% v/v	0/3	0/3	0/3	0/3	3/3	3/3	3/3	3/3
Equate Nasal Spray Premium	15% v/v	0/3	0/3	0/3	0/3	3/3	3/3	3/3	3/3
Equate Nasal Allergy Spray (Triamcinolone Acetonide)	15% v/v	0/3	0/3	0/3	0/3	3/3	3/3	3/3	3/3

Interfering Substance	Concentration	Cross-reactivity (without analyte) (# pos/ # total)				Interference (with analyte) (# pos/ # total)			
		SARS-CoV-2	Flu A	Flu B	RSV	SARS-CoV-2	Flu A	Flu B	RSV
Nasonex 24 HR Allergy (Mometasone furoate)	15% v/v	0/3	0/3	0/3	0/3	3/3	3/3	3/3	3/3
Nasal gel	1.25% v/v	0/3	0/3	0/3	0/3	3/3	3/3	3/3	3/3
Homeopathic nasal wash	15% v/v	0/3	0/3	0/3	0/3	3/3	3/3	3/3	3/3
Homeopathic allergy relief (histaminum hydrochloricum)	15% w/v	0/3	0/3	0/3	0/3	3/3	3/3	3/3	3/3
Ribavirin (RSV antiviral drug)	10 mg/ml	0/3	0/3	0/3	0/3	3/3	3/3	3/3	3/3
Oseltamivir Phosphate (Tamiflu)	5 mg/ml	0/3	0/3	0/3	0/3	3/3	3/3	3/3	3/3
Whole Blood	2.5% v/v	0/3	0/3	0/3	0/3	3/3	3/3	3/3	3/3
Hand sanitizer	15% v/v	0/3	0/3	0/3	0/3	3/3	3/3	3/3	3/3
Hand Soap	10% v/v	0/3	0/3	0/3	0/3	3/3	3/3	3/3	3/3
Equate Budesonide Nasal Spray	15% v/v	0/3	0/3	0/3	0/3	3/3	3/3	3/3	3/3
Flonase sensimist allergy relief spray	15% v/v	0/3	0/3	0/3	0/3	3/3	3/3	3/3	3/3
Fluticasone Propionate Nasal Spray	15% v/v	0/3	0/3	0/3	0/3	3/3	3/3	3/3	3/3
Tobramycin	50 µg/mL	0/3	0/3	0/3	0/3	3/3	3/3	3/3	3/3
Dyclonine Hydrochloride	2 mg/mL	0/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
Mucin (from bovine submaxillary glands)	2.5 mg/mL	0/3	0/3	0/3	0/3	3/3	3/3	3/3	3/3
HALLS (Menthol)	3 mg/mL	0/3	0/3	0/3	0/3	3/3	3/3	3/3	3/3
Sore Throat & Cough Lozenges (Benzocaine, Dextromethorphan HBr)	3 mg/mL	0/3	0/3	0/3	0/3	3/3	3/3	3/3	3/3
Beclomethasone	5 mg/mL	0/3	0/3	0/3	0/3	3/3	3/3	3/3	3/3
Mupirocin	10 mg/mL	0/3	0/3	0/3	0/3	3/3	3/3	3/3	3/3
Flunisolide	5 mg/mL	0/3	0/3	0/3	0/3	3/3	3/3	3/3	3/3
Dexamethasone	5 mg/mL	0/3	0/3	0/3	0/3	3/3	3/3	3/3	3/3
Biotin	3500 ng/mL	0/3	0/3	0/3	0/3	3/3	3/3	3/3	3/3
Leukocytes	2.5x10 <sup>6</sup> cells/mL	0/3	0/3	0/3	0/3	3/3	3/3	3/3	3/3
Flumist (Influenza Vaccine Intranasal)	15% v/v	0/3	3/3	3/3	0/3	3/3	3/3	3/3	3/3
	3% v/v	0/3	3/3	3/3	0/3	NT*	NT*	NT*	NT*
	1.5% v/v	0/3	3/3	0/3	0/3	NT*	NT*	NT*	NT*
	0.2% v/v	0/3	0/3	0/3	0/3	NT*	NT*	NT*	NT*
Molnupiravir	10 mg/ml	0/3	0/3	0/3	0/3	3/3	3/3	3/3	3/3

Interfering Substance	Concentration	Cross-reactivity (without analyte) (# pos/ # total)				Interference (with analyte) (# pos/ # total)			
		SARS-CoV-2	Flu A	Flu B	RSV	SARS-CoV-2	Flu A	Flu B	RSV
Remdesivir (covid-19 antiviral drug)	10 mg/ml	0/3	0/3	0/3	0/3	3/3	3/3	3/3	3/3
Zanamivir	5 mg/ml	0/3	0/3	0/3	0/3	3/3	3/3	3/3	3/3

\*NT: not tested.

#### 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. Controls

##### i. *Internal Controls:*

A built-in internal procedural control is incorporated into the test device to ensure that the test is functioning properly and correct use of the device. The internal control is part of the test strip membrane and is therefore, automatically run within the development time of each test. The internal procedural control consists of IgG antibodies that are immobilized at the “Ctl” line of the test membrane in the device and captures leftover, unbound IgG complexes to generate a color signal.

##### ii. *External Controls:*

Not applicable: external quality controls are not included in the test kit.

##### b. Stability

##### i. *Real Time Stability:*

A real-time stability study was conducted to evaluate stability and determine the shelf-life of the unopened kit. To validate shelf-life, three unopened Flowflex Plus RSV + Flu A/B + COVID Home Test kit lots were stored at 2°C and 30°C. At defined intervals, an assessment of each lot was conducted with the following panel of test samples: negative clinical matrix, and co-spiked positive samples with inactivated SARS-CoV-2, live Flu A, Flu B, or RSV viruses, each at 3x LoD. Fifty (50) µL of each sample was applied directly to the swab and tested according to the instructions for use (IFU). Five replicates of each sample were tested for each time point and baseline testing was performed within one month of each manufactured lot. At the time of clearance, all study data have met the protocol defined acceptance criteria and support storage of the test kits from 2-30°C for up to 9 months.

##### ii. *Shipping Stability:*

The purpose of this study was to evaluate the stability of the unopened kits under the worst-case scenario for anticipated handling and shipping times and temperatures. Three sets of freeze/thaw cycles were conducted to mimic cold shipping conditions. After the final thaw, the kits were stored at 55°C for 35 days. Three lots were tested, and two concentrations of the samples (negative and 3x LoD co-spiked positive samples) were tested in 5 replicates at multiple timepoints (0, 7, 14, 21, 28 and 35

days). Testing was performed after the test kits return to room temperature. Results showed 100% agreement with the expected result across all analytes, lots, and timepoints, with no performance changes observed compared to baseline. These findings demonstrate that the assay remains stable under worst-case temperature conditions.

## 6. Detection Limit:

### a. *Single Analyte Limit of Detection (LoD):*

A limit of detection (LoD) study was conducted to determine the lowest detectable concentration of two strains of heat-inactivated SARS-CoV-2 (Omicron XBB and USA-WA1/2020), two strains of live influenza A (H1N1 and H3N2), two strains of live influenza B (Victoria and Yamagata) and two strains of live RSV (A and B) at which at least 95% of all true positive replicates return a positive result. Testing was conducted on three lots of test devices. The LoD study was determined using a two-step method: a preliminary range finding study, followed by a confirmatory LoD study.

A preliminary LoD was determined by first testing serial ten-fold dilutions of virus stocks diluted into PNSM in 5 replicates for three device lots for a total of 15 replicates per dilution. A 50 µL sample of each virus diluted in PNSM was pipetted onto the dry swab and tested per the IFU. The lowest concentration at which all tested replicates were positive was treated as the preliminary LoD. The results of the preliminary LoD testing are summarized in the table below with the preliminary LoD for each strain in bold.

**Table 7: Preliminary single analyte LoD results**

Virus strain	Virus concentration		Positive replicates*
	TCID <sub>50</sub> /mL	TCID <sub>50</sub> /Swab	
SARS-CoV-2 Omicron XBB (USA/CA-Stanford-109_S21/2022)	5.95 x 10 <sup>5</sup>	2.98 x 10 <sup>4</sup>	15/15
	<b>5.95x 10<sup>4</sup></b>	<b>2.98 x 10<sup>3</sup></b>	<b>15/15</b>
	5.95x 10 <sup>3</sup>	2.98 x 10 <sup>2</sup>	0/15
	5.95x 10 <sup>2</sup>	2.98 x 10 <sup>1</sup>	0/15
	5.95x 10 <sup>1</sup>	2.97 x 10 <sup>0</sup>	0/15
SARS-CoV-2 (USA-WA1/2020)	3.8 x 10 <sup>5</sup>	1.9 x10 <sup>4</sup>	15/15
	3.8 x 10 <sup>4</sup>	1.90 x 10 <sup>3</sup>	15/15
	<b>3.8 x 10<sup>3</sup></b>	<b>1.90 x 10<sup>2</sup></b>	<b>15/15</b>
	3.8 x 10 <sup>2</sup>	1.90 x 10 <sup>1</sup>	0/15
	3.8 x 10 <sup>1</sup>	1.90 x 10 <sup>0</sup>	0/15
FluA H1N1, A/Guangdong-Maonan/SWL 1536/19	<b>1.17 x 10<sup>4</sup></b>	<b>5.85 x 10<sup>2</sup></b>	<b>15/15</b>
	1.17 x 10 <sup>3</sup>	5.85 x 10 <sup>1</sup>	0/15
	1.17 x 10 <sup>2</sup>	5.85 x 10 <sup>0</sup>	0/15
	1.17 x 10 <sup>1</sup>	5.85x10 <sup>-1</sup>	0/15
FluA H1N1, A/Victoria/4897/22	3.89 x 10 <sup>3</sup>	1.94 x 10 <sup>2</sup>	15/15
	3.89 x 10 <sup>2</sup>	1.94 x 10 <sup>1</sup>	15/15
	<b>3.89 x 10<sup>1</sup></b>	<b>1.95 x 10<sup>0</sup></b>	<b>15/15</b>
	3.89 x 10 <sup>0</sup>	1.94 x 10 <sup>-1</sup>	0/15
FluA H3N2, A/Darwin/6/21	1.56 x 10 <sup>3</sup>	7.80 x 10 <sup>1</sup>	15/15
	<b>1.56 x 10<sup>2</sup></b>	<b>7.80 x 10<sup>0</sup></b>	<b>15/15</b>
	1.56 x 10 <sup>1</sup>	7.80 x 10 <sup>-1</sup>	0/15
	1.56 x 10 <sup>0</sup>	7.80 x 10 <sup>-2</sup>	0/15
FluB (Victoria Lineage), B/Hong Kong/574/19	5.01x 10 <sup>4</sup>	2.50 x 10 <sup>3</sup>	15/15
	5.01x 10 <sup>3</sup>	2.50 x 10 <sup>2</sup>	15/15
	<b>5.01x 10<sup>2</sup></b>	<b>2.51 x 10<sup>1</sup></b>	<b>15/15</b>

Virus strain	Virus concentration		Positive replicates*
	TCID <sub>50</sub> /mL	TCID <sub>50</sub> /Swab	
	5.01x 10 <sup>1</sup>	2.51 x 10 <sup>0</sup>	0/15
FluB (Victoria Lineage), B/Alabama/02/17	1.17 x 10 <sup>4</sup>	5.85 x 10 <sup>2</sup>	15/15
	<b>1.17 x 10<sup>3</sup></b>	<b>5.85 x 10<sup>1</sup></b>	<b>15/15</b>
	1.17 x 10 <sup>2</sup>	5.85 x 10 <sup>0</sup>	0/15
	1.17 x 10 <sup>1</sup>	5.85 x 10 <sup>-1</sup>	0/15
FluB (Yamagata Lineage), B/Phuket/3073/13	1.86 x 10 <sup>3</sup>	9.30 x 10 <sup>1</sup>	15/15
	1.86 x 10 <sup>2</sup>	9.30 x 10 <sup>0</sup>	15/15
	<b>1.86 x 10<sup>1</sup></b>	<b>9.30 x 10<sup>-1</sup></b>	<b>15/15</b>
	1.86 x 10 <sup>0</sup>	9.30 x 10 <sup>-2</sup>	0/15
RSV A	1.05 x 10 <sup>5</sup>	5.25 x 10 <sup>3</sup>	15/15
	1.05 x 10 <sup>4</sup>	5.25 x 10 <sup>2</sup>	15/15
	<b>1.05 x 10<sup>3</sup></b>	<b>5.25 x 10<sup>1</sup></b>	<b>15/15</b>
	1.05 x 10 <sup>2</sup>	5.25 x 10 <sup>0</sup>	0/15
	1.05 x 10 <sup>1</sup>	5.25 x 10 <sup>-1</sup>	0/15
RSV B	4.17 x 10 <sup>4</sup>	2.09 x 10 <sup>3</sup>	15/15
	4.17 x 10 <sup>3</sup>	2.09x 10 <sup>2</sup>	15/15
	<b>4.17 x 10<sup>2</sup></b>	<b>2.09 x 10<sup>1</sup></b>	<b>15/15</b>
	4.17 x 10 <sup>1</sup>	2.09 x 10 <sup>0</sup>	0/15
	4.17 x 10 <sup>0</sup>	2.09 x 10 <sup>-1</sup>	0/15

\* Positive replicates per concentration were combined from all lots.

The preliminary LoD of each virus was confirmed by testing an additional twenty samples for each viral stock at the preliminary LoD concentration as well as three-folds above and below the preliminary LoD. Acceptance criteria for confirmation of the LoD were that at least 95% of the replicates test positive for each device lot. The results of confirmatory LoD testing are summarized below with the final stated LoD for each strain bolded.

**Table 8: Confirmatory single analyte LoD results**

Virus strain	Virus concentration		Positive replicates*
	TCID <sub>50</sub> /mL	TCID <sub>50</sub> /Swab	
SARS-CoV-2 Omicron XBB (USA/CA-Stanford-109_S21/2022)	1.79 x 10 <sup>5</sup>	8.95 x 10 <sup>3</sup>	60/60
	<b>5.95x 10<sup>4</sup></b>	<b>2.98 x 10<sup>3</sup></b>	<b>60/60</b>
	1.98x 10 <sup>4</sup>	9.90 x 10 <sup>2</sup>	9/60
SARS-CoV-2 (USA-WA1/2020)	1.14 x 10 <sup>4</sup>	5.70 x 10 <sup>2</sup>	60/60
	3.8 x 10 <sup>3</sup>	1.90 x 10 <sup>2</sup>	60/60
	<b>1.27 x 10<sup>3</sup></b>	<b>6.35 x 10<sup>1</sup></b>	<b>60/60</b>
	4.22 x 10 <sup>2</sup>	2.11 x 10 <sup>1</sup>	0/60
FluA H1N1, A/Guangdong-Maonan/SWL 1536/19	3.51 x 10 <sup>4</sup>	1.75 x 10 <sup>3</sup>	60/60
	1.17 x 10 <sup>4</sup>	5.85 x 10 <sup>2</sup>	60/60
	<b>3.90 x 10<sup>3</sup></b>	<b>1.95 x 10<sup>2</sup></b>	<b>60/60</b>
	1.3 x 10 <sup>3</sup>	6.50 x 10 <sup>1</sup>	0/60
FluA H1N1, A/Victoria/4897/22	1.17 x 10 <sup>2</sup>	5.85 x 10 <sup>0</sup>	60/60
	<b>3.89 x 10<sup>1</sup></b>	<b>1.95 x 10<sup>0</sup></b>	<b>60/60</b>
	1.3 x 10 <sup>1</sup>	6.50 x 10 <sup>-1</sup>	0/60
FluA H3N2, A/Darwin/6/21	4.68 x 10 <sup>2</sup>	2.34 x 10 <sup>1</sup>	60/60
	<b>1.56 x 10<sup>2</sup></b>	<b>7.80 x 10<sup>0</sup></b>	<b>60/60</b>
	5.2 x 10 <sup>1</sup>	2.60 x 10 <sup>0</sup>	0/60



Virus strain	Virus concentration		Positive replicates*
	TCID <sub>50</sub> /mL	TCID <sub>50</sub> /Swab	
FluB (Victoria Lineage), B/Hong Kong/574/19	1.5x 10 <sup>3</sup>	7.50 x 10 <sup>1</sup>	60/60
	<b>5.01x 10<sup>2</sup></b>	<b>2.51 x 10<sup>1</sup></b>	<b>60/60</b>
	1.67x 10 <sup>2</sup>	8.35 x 10 <sup>0</sup>	0/60
FluB (Victoria Lineage), B/Alabama/02/17	3.51 x 10 <sup>3</sup>	1.78 x 10 <sup>2</sup>	60/60
	1.17 x 10 <sup>3</sup>	5.85 x 10 <sup>1</sup>	60/60
	<b>3.90 x 10<sup>2</sup></b>	<b>1.95 x 10<sup>1</sup></b>	<b>60/60</b>
	1.3 x 10 <sup>2</sup>	6.5 x 10 <sup>0</sup>	0/60
FluB (Yamagata Lineage), B/Phuket/3073/13	5.58 x 10 <sup>1</sup>	2.79 x 10 <sup>0</sup>	60/60
	<b>1.86 x 10<sup>1</sup></b>	<b>9.30 x 10<sup>-1</sup></b>	<b>60/60</b>
	6.2 x 10 <sup>0</sup>	3.1 x 10 <sup>-1</sup>	0/60
RSVA	3.15 x 10 <sup>3</sup>	1.58 x 10 <sup>2</sup>	60/60
	<b>1.05 x 10<sup>3</sup></b>	<b>5.25 x 10<sup>1</sup></b>	<b>60/60</b>
	3.5 x 10 <sup>2</sup>	1.75 x 10 <sup>1</sup>	0/60
RSVB	1.25 x 10 <sup>3</sup>	6.25 x 10 <sup>1</sup>	60/60
	<b>4.17 x 10<sup>2</sup></b>	<b>2.09 x 10<sup>1</sup></b>	<b>60/60</b>
	1.39 x 10 <sup>2</sup>	6.95 x 10 <sup>0</sup>	0/60

\* Positive replicates per concentration were combined from all lots.

**b. Co-spiked Multi-Analyte LoD:**

After single analyte LoDs were determined, a co-spike equivalency study was conducted to assess whether the LoD of the Flowflex Plus RSV + Flu A/B + COVID Home Test for the four combined viruses (SARS-CoV-2, FluA, FluB, and RSV) was the same as for each virus tested separately.

The equivalency LoD study was validated by testing 20 replicates with the four pooled viruses at the final concentrations of 1x LoD, 10 replicates at 3x LoD, 10 replicates at 0.3x LoD, and five replicates with negative samples. In parallel, the same conditions and number of replicates were tested with individual viruses. 50 µL of each sample was applied to each dry swab and were tested with the device according to the IFU.

All replicates produced 100% positive results for each analyte at 3x and 1xLoD. At 0.3x LoD, the positive rate dropped as expected. Similar results were obtained when each analyte was tested individually. There was no impact on the sensitivity when all analytes were co-spiked. This study supports the use of co-spiked samples in subsequent analytical studies.

**c. International Standard Material NIBSC code: 21/368 – Limit of Detection:**

The LoD of the Flowflex Plus RSV + Flu A/B + COVID Home Test was also determined by evaluating different dilutions of the International Standard for SARS-CoV-2 antigen (NIBSC code: 32/368) in negative pooled nasal swab matrix. The 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 of 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 19/20 replicates tested positive).

The preliminary LoD concentration was determined by testing a series of 2-fold dilutions with one device lot, starting with a 5-fold pre-dilution from the stock concentration. Each

concentration in the preliminary LoD study was tested in 5 replicates. The lowest concentration with 5/5 positive results 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 preliminary LoD concentration was tested with an additional 20 replicates to confirm the LoD. Concentrations 2-fold higher, and 2-fold lower the preliminary LoD were also tested with 20 replicates to further define 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 per lot. The results are summarized in table below.

**Table 9: Summary of LoD study for the international standard**

Testing	Concentration (IU/mL)	Concentration on dry swab (IU/swab)	# Positive replicates
Preliminary LoD (10-fold dilutions)	$4.0 \times 10^3$	$2 \times 10^2$	5/5
	$2.0 \times 10^3$	$1 \times 10^2$	5/5
	$1.0 \times 10^3$	$0.5 \times 10^2$	5/5
	<b><math>5.0 \times 10^2</math></b>	<b><math>2.5 \times 10^1</math></b>	<b>5/5</b>
	$2.5 \times 10^2$	$1.2 \times 10^1$	0/5
	$1.2 \times 10^2$	$6.0 \times 10^0$	0/5
Confirmatory LoD	$1.0 \times 10^3$	$5.0 \times 10^1$	20/20
	<b><math>5.0 \times 10^2</math></b>	<b><math>2.5 \times 10^1</math></b>	<b>20/20</b>
	$2.5 \times 10^2$	$1.2 \times 10^1$	0/20

The LoD for the Flowflex Plus RSV + Flu A/B + COVID Home Test using the 1st International Standard for SARS-CoV-2 antigen (NIBSC code: 21/368) in nasal matrix was determined to be  $5.0 \times 10^2$  IU/mL (25 IU/swab).

#### 7. High Dose Hook Effect:

A high-dose hook effect study was performed to assess whether very high concentrations of the target analyte could lead to false negative results. One lot of test cassettes was evaluated using three sample concentrations: negative, 2x LoD, and the original stock concentration of the analyte. The analytes were tested individually, and all samples were tested in triplicates. For each test, a dry swab was used to absorb 50 µL of either negative matrix or analyte sample (2x LoD or stock concentration) and were processed according to the IFU. No evidence of a high-dose hook effect was observed for any of the virus stocks or concentrations tested as shown in the table below.

**Table 10: High-dose hook effect study results**

Sample	Concentration (TCID <sub>50</sub> /mL)	Results (# pos/total reps)			RSV
		SARS-CoV-2	Influenza A	Influenza B	
Negative	N/A	0/3	0/3	0/3	0/3
SARS-CoV-2 (High dose)	$5.95 \times 10^6$	3/3	0/3	0/3	0/3
SARS-CoV-2 (2x LoD)	$1.19 \times 10^5$	3/3	0/3	0/3	0/3
Influenza A (H1N1) (High dose)	$1.17 \times 10^5$	0/3	3/3	0/3	0/3
Influenza A (H1N1) (2x LoD)	$7.80 \times 10^3$	0/3	3/3	0/3	0/3
Influenza B (Victoria) (High dose)	$5.01 \times 10^5$	0/3	0/3	3/3	0/3

Sample	Concentration (TCID <sub>50</sub> /mL)	Results (# pos/total reps)			RSV
		SARS-CoV-2	Influenza A	Influenza B	
Influenza B (Victoria) (2x LoD)	1.00 x 10 <sup>3</sup>	0/3	0/3	3/3	0/3
RSV A (High dose)	1.05 x 10 <sup>6</sup>	0/3	0/3	0/3	3/3
RSV A (2x LoD)	2.10 x 10 <sup>3</sup>	0/3	0/3	0/3	3/3

#### 8. Inclusivity:

Analytical reactivity testing for the Flowflex Plus RSV + Flu A/B + COVID Home Test was performed to ensure that the device can adequately detect a variety of strains for the SARS-CoV-2, influenza A, influenza B, and RSV viruses. A selection of temporally, geographically, and genetically diverse SARS-CoV-2 influenza and RSV strains were tested for inclusivity, including 16 SARS-CoV-2 strains (12 Omicron, 1 Alpha, 1 Beta, 1 Gamma, and 1 Delta), 24 influenza A strains (10 H1N1 and 10 H3N2, 2 H5N1, 1 H5N8, and 1 H7N3), 8 influenza B strains (4 Yamagata and 4 Victoria lineages), and 6 RSV strains (3 RSV A and 3 RSV B). A series of ten-fold dilutions of each virus strain was spiked into PNSM and tested to determine an approximate LoD of the test for each virus. The lowest concentration with 100% positive replicates was identified and additional 3-fold dilutions above and below that approximate LoD were tested to demonstrate inclusivity.

Based on the dilution series, the minimum detectable concentration was defined as the lowest concentration for which all five replicates were detected. Results are summarized below and demonstrate that the test tests can detect the analytes across a range of viral strains.

**Table 11: Inclusivity results for SARS-CoV-2 strains**

SARS-CoV-2 Subtypes	Pathogens	Concentration (TCID <sub>50</sub> /mL)	Results (#Pos/Total)
Alpha	B.1.1.7; (USA/CA CDC 5574/2020)	3.41 x 10 <sup>5</sup>	5/5
Beta	B.1.351; (South Africa/KRISP-K005325/2020)	1.51 x 10 <sup>4</sup>	5/5
Delta	B.1.617.2; (USA/PHC658/2021)	4.17 x 10 <sup>3</sup>	5/5
Gamma	P.1; Gamma (Japan/TY7-503/2021)	8.47 x 10 <sup>4</sup>	5/5
Omicron	B.1.1.529; (USA/MDHP20874/2021)	1.29 x 10 <sup>2</sup>	5/5
	BA.2.3; (USA/MDHP24556/2022)	3.90 x 10 <sup>3</sup>	5/5
	BA.4.6; (USA/MDHP35538/2022)	1.15 x 10 <sup>4</sup>	5/5
	BA.5; (USA/COR-22-063113/2022)	2.53 x 10 <sup>4</sup>	5/5
	BF.5; (USA/MD-HP34985/2022)	8.80 x 10 <sup>3</sup>	5/5
	BF.7; (USA/NY-Wadsworth-22042128-01/2022)	1.26 x 10 <sup>4</sup>	5/5
	BQ.1; (USA/NY-Wadsworth-22050462-01/2022)	1.43 x 10 <sup>4</sup>	5/5
	BQ.1.1; (USA/MD-HP38861/2022)	2.93 x 10 <sup>3</sup>	5/5
	JN.1.4; (USA/NY-Wadsworth-23068107-01/2023)	5.43 x 10 <sup>3</sup>	5/5
	EG.5.1; (USA/MD-HP47946/2023)	1.22 x 10 <sup>4</sup>	5/5
	JG.3; (USA/NY-Wadsworth-23067147-01/2023)	7.88 x 10 <sup>4</sup>	5/5
	HV.1 USA/MD-HP49152/2023	8.73 x 10 <sup>3</sup>	5/5

**Table 12: Inclusivity results for influenza A strains**

Flu A Subtypes	Pathogens	Concentration (TCID <sub>50</sub> /mL)	Results (#Pos/Total)
H1N1	A/California/07/09	1.70 x 10 <sup>2</sup>	5/5
	A/Mexico/4108/09	8.10 x 10 <sup>3</sup>	5/5
	A/New York/18/09	1.26 x 10 <sup>4</sup>	5/5
	A/Michigan/45/15	2.70 x 10 <sup>2</sup>	5/5
	A/New Caledonia/20/99	4.20 x 10 <sup>3</sup>	5/5
	A/Solomon Islands/03/06	5.62 x 10 <sup>1</sup>	5/5
	A/PR/8/34	5.01 x 10 <sup>2</sup>	5/5
	A/Wisconsin/67/22	3.87 x 10 <sup>1</sup>	5/5
	A/Connecticut/11/2023	9.33 x 10 <sup>3</sup>	5/5
	A/Baltimore/JH-22400/2022	8.90 x 10 <sup>3</sup>	5/5
H3N2	A/Brisbane/10/07	1.05 x 10 <sup>3</sup>	5/5
	A/Perth/16/09	1.87 x 10 <sup>3</sup>	5/5
	A/Kansas/14/17	1.51 x 10 <sup>5</sup>	5/5
	A/Norway/466/14	4.17 x 10 <sup>4</sup>	5/5
	A/Texas/50/12	1.26 x 10 <sup>3</sup>	5/5
	A/Victoria/361/11	6.20 x 10 <sup>2</sup>	5/5
	A/Wisconsin/67/05	4.70 x 10 <sup>2</sup>	5/5
	A/Michigan/173/20	5.20 x 10 <sup>3</sup>	5/5
	A/Kumamoto/102/02	3.87 x 10 <sup>2</sup>	5/5
	A/Tasmania/503/20	1.41 x 10 <sup>4</sup>	5/5
H5N1	A/Chicken/Liaoning/SD007/2017	1.30 x 10 <sup>4</sup>	5/5
	A/Vietnam/1194/2004	1.10 x 10 <sup>4</sup>	5/5
H5N8	A/H5N8	3.67 x 10 <sup>2</sup>	5/5
H7N3	A/Waterfowl/Chenhu/367-1/2021	5.00 x 10 <sup>3</sup>	5/5

**Table 13: Inclusivity results for influenza B strains**

Flu B Subtypes	Pathogens	Concentration (TCID <sub>50</sub> /mL)	Results (#Pos/Total)
Victoria	B/Malaysia/2506/04	3.16 x 10 <sup>3</sup>	5/5
	B/Michigan/01/21	1.17 x 10 <sup>4</sup>	5/5
	B/Singapore/WUH4618/21	3.90 x 10 <sup>4</sup>	6/6
	B/Washington/02/19	6.27 x 10 <sup>3</sup>	5/5
Yamagata	B/Florida/04/06	1.17 x 10 <sup>2</sup>	5/5
	B/Texas/06/11	3.80 x 10 <sup>3</sup>	5/5
	B/Utah/09/14	4.17 x 10 <sup>2</sup>	5/5
	B/Wisconsin/01/10	1.41 x 10 <sup>2</sup>	5/5

**Table 14: Inclusivity results for RSV strains**

RSV Subtypes	No.	Pathogens	Concentration (TCID <sub>50</sub> /mL)	Results (#Pos/Total)
RSV A	1	RSV A NY-Wadsworth-22055420-01/2022	4.20 x 10 <sup>3</sup>	5/5
	2	RSV A Long	1.60 x 10 <sup>6</sup>	5/5
	3	RSV A2	1.60 x 10 <sup>6</sup>	5/5
RSV B	1	RSV B NY-Wadsworth-22055413-01/2022	3.53 x 10 <sup>3</sup>	5/5
	2	RSV B 18537	1.60 x 10 <sup>3</sup>	5/5
	3	RSV B, Isolate: 3/2015	1.69 x 10 <sup>3</sup>	5/5

9. Assay Cut-Off:

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

**B. Comparison Studies:**

1. Method Comparison with Predicate Device:

See Section C (Clinical Studies) below.

2. Matrix Comparison:

Not Applicable, the device only uses one matrix (i.e., anterior nasal swab).

**C. Clinical Studies:**

1. Clinical Performance Study

A prospective lay person clinical study was conducted from October 2024 to April 2025 to assess the performance of the Flowflex Plus RSV + Flu A/B + COVID Home Test when compared to an FDA cleared RT-PCR assay. The study prospectively enrolled symptomatic subjects at 10 geographically distinct study sites located in the United States. Enrolled subjects were aged 6 months or older who exhibited symptoms of infection consistent with COVID-19, influenza, or RSV at the time of collection and who were within 5 days post symptom onset (DPSO).

Testing was performed in a simulated home environment. A nasopharyngeal swab was collected first by the study operator, placed into transport tube containing viral transport medium, and shipped on dry ice to a central lab for testing with a highly sensitive RT-PCR comparator assay. Thereafter, an anterior nasal (AN) swab was collected per the candidate test's quick reference instructions (QRI). The swabs were either self-collected by a lay user aged  $\geq 14$  years or collected by an adult (parent/ guardian) from individuals aged  $\geq 6$  months old. Samples were then immediately tested with the Flowflex Plus RSV + Flu A/B + COVID Home Test according to the test QRI.

A total of 1263 anterior nasal swab samples were collected from symptomatic individuals within 5 days of respiratory symptom onset and met the inclusion criteria for the analysis, of which 1257 samples were evaluable for SARS-CoV-2 and 1261 samples evaluable for Flu A/B and RSV respectively. The subject demographics are shown below:

**Table 15: Subject Demographics**

Specific Demographic	Lay user collecting and testing	Self-collecting and testing	Overall
	(N=634)	(N=629)	(N=1263)
Age			
Mean (SD)	5.9 (4.0)	48.6 (19.6)	27.2 (25.6)
Median [Min, Max]	5 [0.5, 17]	49 (14, 91)	14 (0.5, 91)
Age Group			
$\geq 6$ months - < 2 years of age	114 (18.0%)	0 (0.0%)	114 (9.0%)
2-5 years of age	212 (33.4%)	0 (0.0%)	212 (16.8%)

Specific Demographic	Lay user collecting and testing	Self-collecting and testing	Overall
	(N=634)	(N=629)	(N=1263)
6-13 years of age	296 (46.7%)	0 (0.0%)	296 (23.4%)
14-21 years of age	12 (1.9%)	75 (11.9%)	87 (6.9%)
22-59 years of age	0 (0.0%)	314 (49.9%)	314 (24.9%)
≥ 60 years of age	0 (0.0%)	240 (38.2%)	240 (19.0%)
<b>Sex at Birth</b>			
Female	300 (47.3%)	355 (56.4%)	655 (51.9%)
Male	334 (52.7%)	274 (43.6%)	608 (48.1%)
<b>Ethnicity</b>			
Hispanic/Latino	140 (22.1%)	75 (11.9%)	215 (17.0%)
Not Hispanic/Latino	494 (77.9%)	554 (88.1%)	1048 (83.0%)

The performance of the Flowflex Plus RSV + Flu A/B + COVID Home Test when compared to FDA-cleared highly sensitive RT-PCR molecular assays are presented in the tables below.

**Table 16: SARS-CoV-2 Performance**

	Comparator Positive	Comparator Negative	Total
<b>Candidate Positive</b>	131	1	132
<b>Candidate Negative</b>	12	1113	1125
<b>Total</b>	143	1114	1257*
Positive Percent Agreement (PPA)	91.6% (131/143) (95% C.I.: 85.9% - 95.1%)		
Negative Percent Agreement (NPA)	99.9% (1113/1114) (95% C.I.: 99.5% - 100%)		

\* 6 samples excluded due to invalid results with comparator methods.

**Table 17: SARS-CoV-2 Performance by DPSO**

DPSO	Candidate Positives	Comparator Positives	PPA
Day 0	2	2	100%
Day 1	40	45	88.9 %
Day 2	42	45	93.3%
Day 3	29	31	93.5%
Day 4	10	12	83.3%
Day 5	8	8	100%
<b>Total</b>	<b>131</b>	<b>143</b>	<b>91.6%</b>

**Table 18: Influenza A Performance**

	Comparator Positive	Comparator Negative	Total
<b>Candidate Positive</b>	236	2	238

<b>Candidate Negative</b>	18	1005	1023
<b>Total</b>	254	1007	1261*
Positive Percent Agreement (PPA)	92.9% (236/254) (95% C.I.: 89.1% - 95.5%)		
Negative Percent Agreement (NPA)	99.8% (1005/1007) (95% C.I.: 99.3% - 100%)		

\* 2 samples excluded due to invalid results with comparator methods.

**Table 19: Influenza B Performance**

	<b>Comparator Positive</b>	<b>Comparator Negative</b>	<b>Total</b>
<b>Candidate Positive</b>	91	1	92
<b>Candidate Negative</b>	7	1162	1169
<b>Total</b>	98	1163	1261*
Positive Percent Agreement (PPA)	92.9% (91/98) (95% C.I.: 86.0% - 96.5%)		
Negative Percent Agreement (NPA)	99.9% (1162/1163) (95% C.I.: 99.5% - 100%)		

\* 2 samples excluded due to invalid results with comparator methods.

**Table 20: RSV Performance**

	<b>Comparator Positive</b>	<b>Comparator Negative</b>	<b>Total</b>
<b>Candidate Positive</b>	159	2	161
<b>Candidate Negative</b>	10	1090	1100
<b>Total</b>	169	1092	1261*
Positive Percent Agreement (PPA)	94.1% (159/169) (95% C.I.: 89.5% - 96.8%)		
Negative Percent Agreement (NPA)	99.8% (1090/1092) (95% C.I.: 99.3% - 100%)		

\* 2 samples excluded due to invalid results with comparator methods.

**Table 21: RSV Performance Stratified by Age Group**

<b>Age group</b>	<b>Number of Subject samples tested</b>	<b>Candidate Positives</b>	<b>Comparator Positives</b>	<b>PPA (95% CI)</b>
6-11 months	51	21	21	100.0% (84.5%, 100.0%)
12-17 months	47	17	17	100.0% (81.6%, 100.0%)
18-23 months	16	5	5	100.0% (56.6%, 100.0%)
<b>&lt; 2 years</b>	<b>114</b>	<b>43</b>	<b>43</b>	<b>100.0% (91.8%, 100.0%)</b>
2-5 years	212	75	83	90.4% (82.1%, 95.0%)
6-13 years	296	20	21	95.2% (77.3%, 99.2%)
14-21 years	87	6	6	100% (60.9%, 100%)
22-59 years	314	8	9	88.9% (56.5%, 98.0%)
>=60 years	238	7	7	100.0% (64.6%, 100.0%)
<b>Total</b>	<b>1261</b>	<b>159</b>	<b>169</b>	<b>94.1% (89.5%, 96.8%)</b>

## 2. Usability/User Comprehension Study:

In order to evaluate the overall usability and user comprehension of the device, a usability study was conducted in two phases. The study was designed to assess whether intended users

could correctly perform the test and interpret the results using the provided labeling materials under simulated home-use conditions.

Study 1: Concurrent with the clinical study, the sponsor conducted a usability evaluation of the Flowflex Plus RSV + Flu A/B + COVID Home Test to assess lay user performance and comprehension of the QRI in a simulated home environment. A total of 1,280 subjects participated. Of these, 645 subjects aged  $\geq 14$  years self-collected nasal swab specimens and performed self-testing using the device. In addition, adult-collected swabs were collected from 635 subjects (including 622 subjects aged 6 months to  $<14$  years and 13 subjects aged  $\geq 14$  years) and tested per the QRI.

To evaluate whether lay users can collect nasal swab samples and perform the test correctly and accurately interpret test results with the Flowflex Plus RSV + Flu A/B + COVID Home Test, an observer was assigned to observe the lay user's operation with the test kit and evaluate critical and non-critical procedures. The observer recorded on the observation form for each procedure if each lay user had any difficulty using the test kit or if a step was performed incorrectly. The table below summarizes each step that was assessed (critical and non-critical test procedures) based on observer's observations.

**Table 22: Critical vs non-critical tasks correctly performed**

<b>Test Procedures (critical or non-critical step) performed by lay users</b>	<b>Tasks performed correctly</b>	<b>Total No. of tasks performed</b>	<b>Percentage of tasks performed correctly</b>
Collect their own nasal swab sample (critical)	1280	1280	100%
Collect another person's nasal swab sample (critical)	1280	1280	100%
Prepare sample solution in the tube (critical)	1280	1280	100%
Dispense the sample solution to the sample well (critical)	1280	1280	100%
Read the test result (critical)	1280	1280	100%
Remove the test cassette from pouch and lay on a clean, flat surface. Locate the result window and sample well on the cassette (non-critical)	1280	1280	100%

All lay users correctly performed each test procedure based on the observer's observations and the lay user questionnaire showed that 95.2 % of the lay users find the test easy or very easy to operate.

Study 2: A supplemental usability study was conducted to assess users' ability to interpret various results and take appropriate follow-up actions, recognize procedural errors, and evaluate the safety and difficulty of sample collection in infants aged 6–24 months, which were not evaluated in Study 1. A total of 38 symptomatic subjects aged 6 to 24 months were enrolled and included in the analysis. Two parents/guardians of each infant performed the collection of nasal samples following the QRI and answered a questionnaire.

Lay users answered all comprehension questions correctly, demonstrating they could follow the QRI to collect nasal samples from 6–23-month infants, interpret results accurately, and determine appropriate follow-up actions. Nearly all users (97–100%) reported that sample collection and result interpretation were easy and could be performed with confidence. While



most comments were very positive, including the use of the swab guard, three users noted minor challenges (e.g., needing more time to read instructions, infant crying), but all indicated these difficulties were manageable.

The results of the usability/user comprehension questionnaire from study 1 and study 2 demonstrated that the instructions were clear and easy to follow, samples could be collected and processed easily from the 6-23 months. The overall evaluation of the lay user experience did not raise any concerns regarding the usability of the investigational device.

### 3. Lay User Readability:

A readability study for the Flowflex Plus RSV + Flu A/B + COVID Home Test was conducted with 61 lay users representing a range of ages, sexes, educational backgrounds, and including individuals with vision impairments (e.g., wearing glasses or contacts). Subjects interpreted a mock panel of investigational test results and thereafter, completed a labeling and comprehension questionnaire. The mock panel contained various combinations of results generated using line intensities equivalent to low positive (2x LoD) and moderate positive (5x LoD) samples for each analyte. Each participant was provided a blinded, randomized panel of five pre-prepared test devices (305 devices total) containing negative, low positive, and moderate positive samples across 17 possible result combinations. Users did not perform sample collection or testing but were asked to interpret results only. The purpose was to assess whether lay users could accurately distinguish negative from positive results and correctly interpret various combinations following the QRI.

Overall, lay users interpreted all negative results with 100% accuracy (744/744), positive results at 5x LoD with 98.9% accuracy (183/185), and low positive results at 2x LoD with 93.8% accuracy (273/291). Reduced agreement for faint test lines at 2x LoD was observed in five participants over age 41 who reported vision impairments, which contributed to a small number of misinterpretations (18 low positive samples and 2 moderate positive samples).

### **D. Clinical Cut-Off:**

Not Applicable. The candidate device is a qualitative assay with a visually read binary result without numeric raw data.

### **E. Expected Values/Reference Range:**

An individual sample is expected to be negative for SARS-CoV-2, influenza A, influenza B, and RSV.

### **F. Other Supportive Information:**

#### 1. Flex Studies

To assess the robustness and risk for false results of the test when deviating from the IFU/QRI test steps, flex studies were conducted that assessed all major aspects of the test procedure (e.g., extraction buffer volume, reading time, procedure involving sample port [swab insertion, rotation, and removal], sample hold time before and during processing) and variability of environmental test conditions that the test may be subjected to when in use (e.g., non-level surface, lighting, disturbance during use, temperature and humidity stress conditions).

A test panel comprised of 5 negative samples (PNSM only) and 5 low positive samples (2x LoD samples in PNSM) for each analyte, were tested upon varying conditions with the candidate device. The strains used for testing were SARS-CoV-2 Omicron XBB, influenza A H1N1, influenza B Victoria, and RSV A. Samples were blinded and randomized for testing. All results were acceptable, except that false negative results were observed when the test was read at 5 minutes, and invalid results were obtained when only one drop of sample was applied to the device. The labeling is clear in its instructions and includes adequate warnings at these critical steps to mitigate the risks of misuse. Therefore, the flex studies support that the test is robust when used as instructed and demonstrate 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.