510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY

I Background Information:

A 510(k) Number

K261212

B Applicant

ACON Laboratories, Inc.

C Proprietary and Established Names

Flow*flex* Plus RSV + Flu A/B + COVID Rapid 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	Microbiology

II Submission/Device Overview:

A Purpose for Submission:

To obtain substantial equivalence determination for the Flow*flex* Plus RSV + Flu A/B + COVID Rapid Test.

B Measurand:

Protein antigens from RSV, SARS-CoV-2, Influenza A and Influenza B

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 Flow*flex* Plus RSV + Flu A/B + COVID Rapid 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. Clinical signs and symptoms of respiratory viral infection due to RSV, influenza, and SARS-CoV-2 can be similar.

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

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

C Special Conditions for Use Statement(s):

Rx - For Prescription Use Only

IVD - For In Vitro Diagnostic Use

D Special Instrument Requirements:

Not applicable

IV Device/System Characteristics:

A Device Description:

The Flow*flex* Plus RSV + Flu A/B + COVID Rapid Test is a lateral flow immunoassay in sandwich format intended for the qualitative detection and differentiation of RSV, influenza A, influenza B, and SARS-CoV-2 protein antigens directly in anterior nasal swab specimens from individuals with signs and symptoms of respiratory tract infection. This test is for healthcare professional use.

The test package is composed of:

- 27 Test Cassettes
- 27 Extraction Buffer Tubes
- 25 Sterile Nasal Swabs
- 25 Pediatric Swab Guards
- 1 Tube Holder
- 1 Positive Control Swab (composed of RSV, influenza A, influenza B, and SARS-CoV-2 non-infectious recombinant antigens dried onto a swab with red stick, containing 0.05% Proclin 300 as a preservative).
- 1 Negative Control Swab (composed of negative control buffer dried onto a swab with blue stick, containing 0.05% Proclin 300 as a preservative)
- 1 English Package Insert
- 1 English Quick Reference Instructions

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

Test Strip Structure:

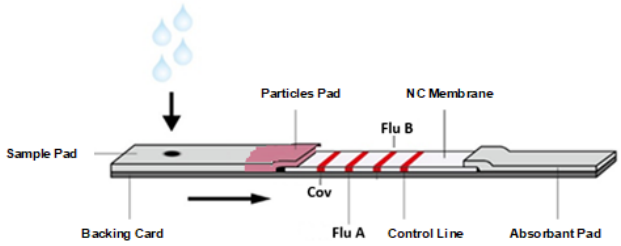
The test strips in the plastic housing include backing card (provides structural support during use), sample receiving and particles pads, nitrocellulose (NC) membrane, test lines for each analyte and absorption pad. The test lines and the control lines are pre-coated with analyte specific antibody on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

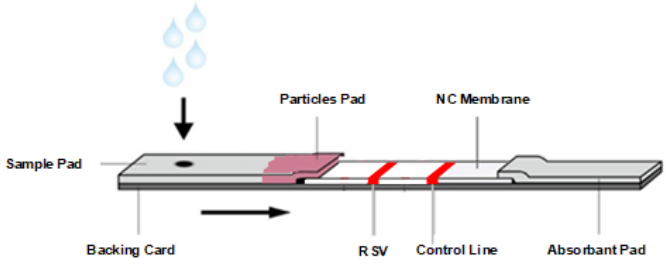
Fig 1: Strip 1



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

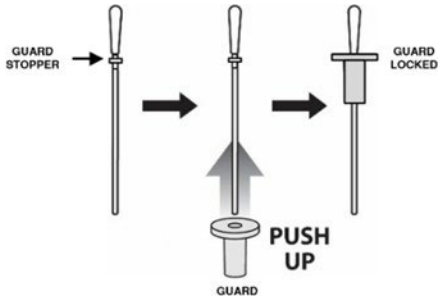


In both strips, attached to the NC membrane are the sample pad (which absorbs and distributes the sample), the reagent pad (containing reagents for antigen binding and detection), and the absorbent pad (which collects the excess liquid sample and reagents).

Swab Guard:

For collecting nasal swabs from children less than 2 years, 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:

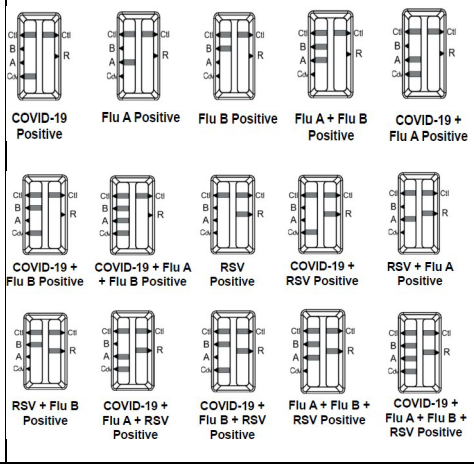
Nasal swabs are collected from individuals with signs and symptoms of respiratory infection. The swab is then processed in extraction buffer and then added to the sample well of the test cassette. When an adequate volume of the processed specimen is assed to the sample well of the test cassette, the 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).

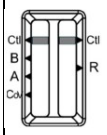
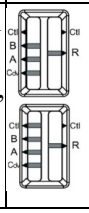
To serve as a procedural control, the colored rabbit IgG labeled particle will bind to goat anti-rabbit IgG on each NC membrane on each test strip to produce a colored line on the control line (Ctl) in the control line region. Formation of the control lines serves as an internal control indicating that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

Result	Interpretation	Example Image
Negative	If both control (Ctl) lines are visible, but the test line(s) (CoV, A, B, R) is/are not visible, the test is negative.	
Invalid	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):

Flow*flex* Plus RSV + Flu A/B + COVID Home Test

B Predicate 510(k) Number(s):

K251749

C Comparison with Predicate(s):

Device & Predicate Device(s):	K261212	Predicate: K251749
Device Trade Name	Flow*flex* Plus RSV + Flu A/B + COVID Rapid Test	Flow*flex* Plus RSV + Flu A/B + COVID Home Test
Intended Use/Indications For Use	The Flow*flex* Plus RSV + Flu A/B + COVID Rapid 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	The Flow*flex* 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

	respiratory tract infection. Clinical signs and symptoms of respiratory viral infection due to RSV, influenza, and SARS-CoV-2 can be similar. All negative results are presumptive and should be confirmed with a molecular assay, if necessary, for patient management. Negative results do not rule out infection with RSV, influenza, or SARS-CoV-2 and should not be used as the sole basis for treatment or patient management decisions. Positive results do not rule out bacterial infection or co-infection with other viruses.	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.
General Device Characteristic Similarities		
Regulation Number	21 CFR 866.3987	Same

Assay Technique	Lateral flow immunochromatographic assay, visually read	Same
Analytes	SARS-CoV-2, influenza A, influenza B and RSV protein antigens	Same
Intended Specimen	Direct anterior nasal swabs	Same
Time to result	15 minutes	Same
Detection Period	Within 5 days of symptom onset	Same
Sample Collection Method	Nasal swab supplied in kit; includes pediatric swab guard for ages less than 2 years.	Nasal swab supplied in kit; includes pediatric swab guard for ages 6-23 months.
Usage	Single use test	Same
Storage Temperature	36-86 °F (2- 30°C)	Same
General Device Characteristic Differences		
Patient Use Setting	For prescription use only by healthcare providers in CLIA-waived settings	Over the counter use

VI Standards/Guidance Documents Referenced:

Document	Title	Publisher	Applicable Study
Special Controls under 21 CFR 866.3987 (multi-analyte respiratory virus antigen detection test)	Reclassification order for DEN240029 and special controls under 21 CFR 866.3987.pdf	FDA/CDRH	All Studies

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/Reproducibility:

a. Lot-to-lot Precision:

The multi-lot precision for the Flow*flex* Plus RSV + Flu A/B + COVID Rapid 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
Negative	SARS-CoV-2	(240/240)	100.0%	98.5-100.0%
	Flu A	(240/240)	100.0%	98.5-100.0%
	Flu B	(240/240)	100.0%	98.5-100.0%
	RSV	(240/240)	100.0%	98.5-100.0%
2X LoD	SARS-CoV-2	(240/240)	100.0%	98.5-100.0%
	Flu A	(240/240)	100.0%	98.5-100.0%
	Flu B	(240/240)	100.0%	98.5-100.0%
	RSV	(240/240)	100.0%	98.5-100.0%
	Flu B + RSV	(480/480)	100.0%	98.5-100.0%
	Flu B + RSV+ SARS-CoV-2	(720/720)	100.0%	98.5-100.0%

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' C_{95} concentration):

i. Negative
ii. 0.75X LoD Flu A & SARS-CoV-2
iii. 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. Seventy two 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[1]	% Agmt[2]	n/N[1]	% Agmt[2]	n/N[1]	% Agmt[2]	n/N[1]	% Agmt[2]	
Negative	SARS-CoV-2		24/24	100.0%	24/24	100.0%	24/24	100.0%	72/72	100.0%	95.0-100.0%
	Flu A		24/24	100.0%	24/24	100.0%	24/24	100.0%	72/72	100.0%	95.0-100.0%
	Flu B		24/24	100.0%	24/24	100.0%	24/24	100.0%	72/72	100.0%	95.0-100.0%
	RSV		24/24	100.0%	24/24	100.0%	24/24	100.0%	72/72	100.0%	95.0-100.0%
Analyte Concentration	**Analyte**		**n/N[3]**	**% Pos**	**n/N[3]**	**% Pos**	**n/N[3]**	**% Pos**	**n/N[3]**	**% 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	75.0%	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	75.0%	14/24	58.3%	49/72	68.1%	56.0-78.6%

[1] *number of agreement with expected results per analyte/total number tested.*

[2] *Agreement.*

[3] *number of positive samples/total number tested.*

b. Multi-Site Reproducibility/Near-Cutoff study:

A multi-site reproducibility study was conducted to assess the performance of the candidate device using a contrived sample panel comprised of a true negative, a high negative pooled (0.3X LoD for FluA/FluB/RSV and 0.1X LoD for SARS-CoV-2), a low positive (1X LoD for each virus), and a moderate positive (3X LoD for each virus) sample for each analyte. The study was conducted by untrained operators in CLIA waived sites for over 5 days.

Contrived samples were prepared by spiking the diluted RSV, influenza A, influenza B or SARS-CoV-2 virus into the negative matrix to the required concentrations. Each diluted sample (50 µL) was directly applied onto the sample collection swab head. True negative swab samples were prepared by applying fifty (50 µL) of negative matrix directly onto the swab head.

Prepared contrived samples were randomized and blinded to each operator at three CLIA-waived sites. Nine (9) operators conducted the testing with each operator testing ten (10) panels with each panel consisting of eight (8) panel members. For each panel member, 90

data points were collected (1 replicate/run x 2 runs/day x 3 operators/site x 3 sites x 5 days = 90 data points).

The results are shown in Table 4 below. All data met the predefined acceptance criteria, and no significant differences were observed among sites, operators, days and runs.

Table 4: Summary of Multi-Site Reproducibility Study

Sample		# Positives / # Total (% Positive Rate)			Total Sample count (% Positive Rate)
		Site 1	Site 2	Site 3	
True Negative	RSV	0/30 (0.0%)	0/30 (0.0%)	0/30 (0.0%)	0/90 (0.0%)
	Flu A	0/30 (0.0%)	0/30 (0.0%)	0/30 (0.0%)	0/90 (0.0%)
	Flu B	0/30 (0.0%)	0/30 (0.0%)	0/30 (0.0%)	0/90 (0.0%)
	SARS-CoV-2	0/30 (0.0%)	0/30 (0.0%)	0/30 (0.0%)	0/90 (0.0%)
Pooled High Negative	RSV	0/30 (0.0%)	0/30 (0.0%)	0/30 (0.0%)	0/90 (0.0%)
	Flu A	0/30 (0.0%)	0/30 (0.0%)	0/30 (0.0%)	0/90 (0.0%)
	Flu B	0/30 (0.0%)	1/30 (3.3%)	0/30 (0.0%)	1/90 (1.1%)
	SARS-CoV-2	0/30 (0.0%)	0/30 (0.0%)	0/30 (0.0%)	0/90 (0.0%)
1X LoD RSV	RSV	30/30 (100.0%)	30/30 (100.0%)	30/30 (100.0%)	90/90 (100.0%)
	Flu A	0/30 (0.0%)	0/30 (0.0%)	0/30 (0.0%)	0/90 (0.0%)
	Flu B	0/30 (0.0%)	0/30 (0.0%)	0/30 (0.0%)	0/90 (0.0%)
	SARS-CoV-2	0/30 (0.0%)	0/30 (0.0%)	0/30 (0.0%)	0/90 (0.0%)
1X LoD Flu A	RSV	0/30 (0.0%)	0/30 (0.0%)	0/30 (0.0%)	0/90 (0.0%)

Sample		# Positives / # Total (% Positive Rate)			Total Sample count (% Positive Rate)
		Site 1	Site 2	Site 3	
	Flu A	30/30 (100.0%)	30/30 (100.0%)	29/30 (96.6%)	89/90 (98.8%)
	Flu B	0/30 (0.0%)	0/30 (0.0%)	0/30 (0.0%)	0/90 (0.0%)
	SARS-CoV-2	0/30 (0.0%)	0/30 (0.0%)	0/30 (0.0%)	0/90 (0.0%)
1X LoD Flu B	RSV	0/30 (0.0%)	0/30 (0.0%)	0/30 (0.0%)	0/90 (0.0%)
	Flu A	0/30 (0.0%)	0/30 (0.0%)	0/30 (0.0%)	0/90 (0.0%)
	Flu B	30/30 (100.0%)	30/30 (100.0%)	30/30 (100.0%)	90/90 (100.0%)
	SARS-CoV-2	0/30 (0.0%)	0/30 (0.0%)	0/30 (0.0%)	0/90 (0.0%)
1X LoD SARS-CoV-2	RSV	0/30 (0.0%)	0/30 (0.0%)	0/30 (0.0%)	0/90 (0.0%)
	Flu A	0/30 (0.0%)	0/30 (0.0%)	0/30 (0.0%)	0/90 (0.0%)
	Flu B	0/30 (0.0%)	0/30 (0.0%)	0/30 (0.0%)	0/90 (0.0%)
	SARS-CoV-2	30/30 (100.0%)	30/30 (100.0%)	30/30 (100.0%)	90/90 (100.0%)
3X LoD RSV& Flu B	RSV	30/30 (100.0%)	30/30 (100.0%)	30/30 (100.0%)	90/90 (100.0%)
	Flu A	0/30 (0.0%)	0/30 (0.0%)	0/30 (0.0%)	0/90 (0.0%)
	Flu B	30/30 (100.0%)	30/30 (100.0%)	30/30 (100.0%)	90/90 (100.0%)
	SARS-CoV-2	0/30 (0.0%)	0/30 (0.0%)	0/30 (0.0%)	0/90 (0.0%)

Sample		# Positives / # Total (% Positive Rate)			Total Sample count (% Positive Rate)
		Site 1	Site 2	Site 3	
3X LoD RSV& Flu A & SARS-CoV-2	RSV	30/30 (100.0%)	30/30 (100.0%)	30/30 (100.0%)	90/90 (100.0%)
	Flu A	30/30 (100.0%)	30/30 (100.0%)	30/30 (100.0%)	90/90 (100.0%)
	Flu B	0/30 (0.0%)	0/30 (0.0%)	0/30 (0.0%)	0/90 (0.0%)
	SARS-CoV-2	30/30 (100.0%)	30/30 (100.0%)	30/30 (100.0%)	90/90 (100.0%)

2. Linearity:

This is a qualitative assay and linearity is not applicable.

3. Analytical Specificity/Interference:

a. Cross-Reactivity and Microbial Interference:

Cross reactivity and microbial interference studies were conducted to determine if other respiratory pathogens/microbial flora that may be present in 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, RSV A and RSV B. 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 5).

Table 5: Cross-Reactivity and Microbial Interference Study Results

Virus/Microorganism	Concentration	Units	# Positive / # Total	
			Cross-Reactivity[1]	Interference[1]
Adenovirus Type 7A	3.16×10^5	$TCID_{50}$/mL	0/3	3/3
Adenovirus 1	1.05×10^5	$TCID_{50}$/mL	0/3	3/3

Virus/Microorganism	Concentration	Units	# Positive / # Total	
			Cross-Reactivity[1]	Interference[1]
Bordetella pertussis	5.04×10^8	CFU/mL	0/3	3/3
Candida albicans	3.32×10^7	CFU/mL	0/3	3/3
Escherichia coli	2.37×10^7	CFU/mL	0/3	3/3
Haemophilus influenzae	9.85×10^7	CFU/mL	0/3	3/3
Neisseria mucosa	6.52×10^8	CFU/mL	0/3	3/3
Corynebacterium jeikeium	3.68×10^8	CFU/mL	0/3	3/3
Legionella pneumophila	5.98×10^9	CFU/mL	0/3	3/3
Moraxella catarrhalis	1.37×10^7	CFU/mL	0/3	3/3
Mumps Virus	4.92×10^6	$TCID_{50}$/mL	0/3	3/3
Mycobacterium tuberculosis	4.60×10^7	CFU/mL	0/3	3/3
Mycoplasma pneumoniae	1.37×10^7	CCU/mL	0/3	3/3
Neisseria meningitidis	2.04×10^7	CFU/mL	0/3	3/3
Neisseria sicca	3.23×10^9	CFU/mL	0/3	3/3
Parainfluenza Virus Type 1	3.80×10^5	$TCID_{50}$/mL	0/3	3/3
Parainfluenza Virus Type 2	3.39×10^6	$TCID_{50}$/mL	0/3	3/3
Parainfluenza Virus Type 3	1.15×10^6	$TCID_{50}$/mL	0/3	3/3
Parainfluenza Virus Type 4	9.55×10^5	$TCID_{50}$/mL	0/3	3/3
Pseudomonas aeruginosa	5.50×10^8	CFU/mL	0/3	3/3
Staphylococcus aureus	5.41×10^8	CFU/mL	0/3	3/3
Staphylococcus epidermidis	3.52×10^8	CFU/mL	0/3	3/3
Streptococcus pyogenes	2.36×10^8	CFU/mL	0/3	3/3
Streptococcus pneumoniae	1.55×10^8	CFU/mL	0/3	3/3
Streptococcus salivarius	4.07×10^7	CFU/mL	0/3	3/3
Corona Virus Strain: 229E	2.09×10^5	$TCID_{50}$/mL	0/3	3/3
Corona Virus Strain: OC43	3.80×10^5	$TCID_{50}$/mL	0/3	3/3
Corona Virus, Strain: NL63	1.78×10^5	$TCID_{50}$/mL	0/3	3/3
Cytomegalovirus (CMV)	2.09×10^5	$TCID_{50}$/mL	0/3	3/3
Human Metapneumovirus	1.02×10^5	$TCID_{50}$/mL	0/3	3/3
Rhinovirus	2.09×10^5	$TCID_{50}$/mL	0/3	3/3
Measles Virus	2.53×10^5	$TCID_{50}$/mL	0/3	3/3
MERS-coronavirus	1.12×10^5	$TCID_{50}$/mL	0/3	3/3
Lactobacillus plantarum	3.13×10^8	CFU/mL	0/3	3/3
Enterovirus	1.05×10^5	$TCID_{50}$/mL	0/3	3/3
Pneumocystis jirovecii	5.18×10^7	CFU/mL	0/3	3/3
Chlamydophila (Chlamydia) pneumoniae	1.40×10^7	IFU/mL	0/3	3/3
Chlamydia trachomatis	1.78×10^7	IFU/mL	0/3	3/3
Epstein Barr Virus	4.02×10^7	CP/mL	0/3	3/3
Corynebacterium diphtheriae	3.45×10^8	CFU/mL	0/3	3/3
Saccharomyces cerevisiae	3.28×10^7	CFU/mL	0/3	3/3
Human Coronavirus HKU1: MINF-1596 [2]	1:10 dilution	N/A	0/3	3/3
Human Coronavirus HKU1: MINF-1597	1:10 dilution	N/A	0/3	3/3
Human Coronavirus HKU1: MINF-1598 [2]	1:10 dilution	N/A	0/3	3/3
Human Coronavirus HKU1: MINF-1601 [2]	1:10 dilution	N/A	0/3	3/3

Virus/Microorganism	Concentration	Units	# Positive / # Total	
			Cross-Reactivity[1]	Interference[1]
Human Coronavirus HKU1: MINF-1602 [2]	1:10 dilution	N/A	0/3	3/3

[1] *All analytes in the sample replicates were in agreement with the expected result.*

[2] *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, influenza A, influenza B, and RSV. The results showed no competitive interference between SARS-CoV-2, influenza A, influenza B, and RSV as listed in Table 6 below.

Table 6: Competitive Inhibition Study Results

Combination #	Viral Targets in Sample				# of Positive Results / # of Replicates Tested			
	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
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***:

Flow*flex* Plus RSV + Flu A/B + COVID Rapid 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 to assess interference. 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 Table 7.

Table 7: 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
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% v/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

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
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	2.5x10^6 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
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. Detection Limit and Assay Reportable Range:

a. Limit of Detection (LoD):

i. Single Analyte Limit of Detection (LoD):

A limit of detection (LoD) study was conducted to determine the lowest detectable concentration of heat-inactivated SARS-CoV-2, live influenza A (H1N1 and H3N2), live influenza B (Victoria and Yamagata) and live RSV (A and B) at which atleast 95% of all true

positive replicates show a positive result. 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 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 LoD confirmation testing for each virus are summarized in Table 8.

Table 8: Limit of Detection of Flow*flex* Plus RSV + Flu A/B + COVID Rapid Test

Virus	Subtype/ Lineage	Strains	Virus concentration		# Positive/ # Total	% Detected
			$TCID_{50}$/mL	$TCID_{50}$/Swab		
SARS-CoV-2	Omicron XBB	USA/CA-Stanford-109_S21/2022	5.95×10^4	2.98×10^3	60/60	100.0%
	Wild Type	USA-WA1/2020	1.27×10^3	6.35×10^1	60/60	100.0%
Flu A	H1N1	A/Guangdong-Maonan/SWL1536/19	3.90×10^3	1.95×10^2	60/60	100.0%
		A/Victoria/4897/22	3.89×10^1	1.95×10^0	60/60	100.0%
	H3N2	A/Darwin/6/2021	1.56×10^2	7.80×10^0	60/60	100.0%
Flu B	Victoria	B/Hong Kong/574/19	5.01×10^2	2.51×10^1	60/60	100.0%
		B/Alabama/02/17	3.90×10^2	1.95×10^1	60/60	100.0%
	Yamagata	B/Phuket/3073/13	1.86×10^1	9.30×10^{-1}	60/60	100.0%
RSV	A	2006 Isolate	1.05×10^3	5.25×10^1	60/60	100.0%
	B	CH93 (18)-18	4.17×10^2	2.09×10^1	59/60	98.3%

ii. 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 Flow*flex* Plus RSV + Flu A/B + COVID Rapid Test for the four combined viruses (SARS-CoV-2, Flu A, Flu B, 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 1X LoD. 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.

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

The LoD of the Flow*flex* Plus RSV + Flu A/B + COVID Rapid 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 9.

Table 9: LoD study Results for the International Standard

Testing	Concentration (IU/mL)	Concentration on dry swab (IU/swab)	# Positive / # Total
Preliminary LoD (10-fold dilutions)	4.0×10^3	2×10^2	5/5
	2.0×10^3	1×10^2	5/5
	1.0×10^3	0.5×10^2	5/5
	5.0×10^2	**2.5×10^1**	**5/5**
	2.5×10^2	1.2×10^1	0/5
	1.2×10^2	0.6×10^1	0/5
Confirmatory LoD	1.0×10^3	0.5×10^2	20/20
	5.0×10^2	**2.5×10^1**	**20/20**
	2.5×10^2	1.2×10^1	0/20

b. 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 Table 10.

Table 10: High-Dose Hook Effect Study Results

Sample	Concentration ($TCID_{50}$/mL)	# Pos/ # Total			
		SARS-CoV-2	Flu A	Flu B	RSV
Negative	N/A	0/3	0/3	0/3	0/3
SARS-CoV-2 (High dose)	5.95×10^6	3/3	0/3	0/3	0/3
SARS-CoV-2 (2X LoD)	1.19×10^5	3/3	0/3	0/3	0/3
Flu A (H1N1) (High dose)	1.17×10^5	0/3	3/3	0/3	0/3
Flu A (H1N1) (2X LoD)	7.80×10^3	0/3	3/3	0/3	0/3

Sample	Concentration ($TCID_{50}$/mL)	# Pos/ # Total			
		SARS-CoV-2	Flu A	Flu B	RSV
Flu B (Victoria) (High dose)	5.01×10^5	0/3	0/3	3/3	0/3
Flu B (Victoria) (2X LoD)	1.00×10^3	0/3	0/3	3/3	0/3
RSV A (High dose)	1.05×10^6	0/3	0/3	0/3	3/3
RSV A (2X LoD)	2.10×10^3	0/3	0/3	0/3	3/3

c. Inclusivity:

Analytical reactivity testing for the Flow*flex* Plus RSV + Flu A/B + COVID Rapid 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. 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 summarized in Table 11, Table 12, Table 13 and Table 14 demonstrate that the test tests can detect analytes across a range of viral strains.

Table 11: Inclusivity Results for SARS-CoV-2 Strains

SARS-CoV-2 Subtypes	Pathogens	Concentration ($TCID_{50}$/mL)	#Pos / # Total
Alpha	B.1.1.7; (USA/CA_CDC_5574/2020)	3.41×10^5	5/5
Beta	B.1.351; (South Africa/KRISP-K005325/2020)	1.51×10^4	5/5
Delta	B.1.617.2; (USA/PHC658/2021)	4.17×10^3	5/5
Gamma	P.1; Gamma (Japan/TY7-503/2021)	8.47×10^4	5/5
Omicron	B.1.1.529; (USA/MDHP20874/2021)	1.29×10^2	5/5
	BA.2.3; (USA/MDHP24556/2022)	3.90×10^3	5/5
	BA.4.6; (USA/MDHP35538/2022)	1.15×10^4	5/5
	BA.5;(USA/COR-22-063113/2022)	2.53×10^4	5/5
	BF.5; (USA/MD-HP34985/2022)	8.80×10^3	5/5
	BF.7; (USA/NY-Wadsworth-22042128-01/2022)	1.26×10^4	5/5
	BQ.1; (USA/NY-Wadsworth-22050462-01/2022)	1.43×10^4	5/5
	BQ.1.1; (USA/MD-HP38861/2022)	2.93×10^3	5/5
	JN.1.4; (USA/NY-Wadsworth-23068107-01/2023)	5.43×10^3	5/5
	EG.5.1; (USA/MD-HP47946/2023)	1.22×10^4	5/5
	JG.3; (USA/NY-Wadsworth-23067147-01/2023)	7.88×10^4	5/5
	HV.1 USA/MD-HP49152/2023	8.73×10^3	5/5

Table 12:Inclusivity Results for Influenza A Strains

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

Table 13: Inclusivity Results for Influenza B Strains

Flu B Subtypes	Pathogens	Concentration ($TCID_{50}$/mL)	#Pos / # Total
Victoria	B/Malaysia/2506/04	3.16×10^3	5/5
	B/Michigan/01/21	1.17×10^4	5/5
	B/Singapore/WUH4618/21	3.90×10^4	6/6
	B/Washington/02/19	6.27×10^3	5/5
Yamagata	B/Florida/04/06	1.17×10^2	5/5
	B/Texas/06/11	3.80×10^3	5/5
	B/Utah/09/14	4.17×10^2	5/5
	B/Wisconsin/01/10	1.41×10^2	5/5

Table 14: Inclusivity Results for RSV Strains

RSV Subtypes	Pathogens	Concentration ($TCID_{50}$/mL)	# Pos / # Total
RSV A	RSV A NY-Wadsworth-22055420-01/2022	4.20×10^3	5/5
	RSV A Long	1.60×10^6	5/5
	RSV A2	1.60×10^6	5/5
	RSV B NY-Wadsworth-22055413-01/2022	3.53×10^3	5/5
	RSV B 18537	1.60×10^3	5/5

RSV Subtypes	Pathogens	Concentration ($TCID_{50}$/mL)	# Pos / # Total
RSV B	RSV B, Isolate: 3/2015	1.69×10^3	5/5

d. 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:

Flow*flex* Plus RSV + Flu A/B + COVID Rapid Test contains positive and negative control swabs. Positive Control Swab with a red stick is composed of a RSV, influenza A, influenza B, and SARS-CoV-2 non-infectious recombinant antigen dried onto a swab, containing 0.05% Proclin 300 as a preservative. The negative Control Swab with a blue stick is composed of negative control buffer dried onto a swab, containing 0.05% Proclin 300 as a preservative.

Reproducibility of the external controls were assessed using three lots of the external control swabs (one lot per site) with the following study design: 1 replicate/operator/day x 3 operators/site x 3 sites x 5 days = 45 replicates. All controls produced expected results for each operator on each day of testing as summarized in Table 15.

Table 15: External Control Swabs Reproducibility Study Results

External Control	Lot No.	# Positive / # Total (% Agreement)			
		RSV	Flu A	Flu B	SARS-CoV-2
Positive Control	Lot 1	15/15 (100%)	15/15 (100%)	15/15 (100%)	15/15 (100%)
	Lot 2	15/15 (100%)	15/15 (100%)	15/15 (100%)	15/15 (100%)
	Lot 3	15/15 (100%)	15/15 (100%)	15/15 (100%)	15/15 (100%)
Negative Control	Lot 1	0/15 (100%)	0/15 (100%)	0/15 (100%)	0/15 (100%)

External Control	Lot No.	# Positive / # Total (% Agreement)			
		RSV	Flu A	Flu B	SARS-CoV-2
	Lot 2	0/15 (100%)	0/15 (100%)	0/15 (100%)	0/15 (100%)
	Lot 3	0/15 (100%)	0/15 (100%)	0/15 (100%)	0/15 (100%)

The real time stability of Flow*flex* Plus RSV + Flu A/B + COVID Positive and negative control swabs were evaluated using three different lots at two different temperature conditions of 2-8°C and 30°C. At each designated timepoint, the control swabs were removed from 2-8°C and 30°C and equilibrated to room temperature prior to testing. Each control swab was processed and tested with the Flow*flex* Plus RSV + Flu A/B + COVID Rapid Test kits per QRI. 5 replicates of the positive control swabs and 5 replicates of the negative control swabs were tested at each timepoint for each lot. At the time of clearance, all study data met the predefined acceptance criteria and showed that the external control swabs are stable for up to 3 months.

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 Flow*flex* Plus RSV + Flu A/B + COVID Rapid 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 met the protocol defined acceptance criteria and support storage of the test kits from 2-30°C for up to 15 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. Assay Cut-Off:

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

B Comparison Studies:

1. Method Comparison with Predicate Device:

   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 clinical study was conducted from October 2024 to April 2025 to assess the performance of the Flow*flex* Plus RSV + Flu A/B + COVID Rapid 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 by lay users. 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 ≥14 years or collected by an adult (parent/ guardian) from individuals aged ≥6 months old. Samples were then immediately tested with the Flow*flex* Plus RSV + Flu A/B + COVID Rapid 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 in Table 16.

Table 16: 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			
≥ 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%)
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%)
Sex at Birth			

Specific Demographic	Lay user collecting and testing	Self-collecting and testing	Overall
	(N=634)	(N=629)	(N=1263)
Female	300 (47.3%)	355 (56.4%)	655 (51.9%)
Male	334 (52.7%)	274 (43.6%)	608 (48.1%)
Ethnicity			
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 Flow*flex* Plus RSV + Flu A/B + COVID Rapid Test when compared to FDA-cleared highly sensitive RT-PCR molecular assays are presented in the tables below.

Table 17: SARS-CoV-2 Clinical Performance

SARS-CoV-2	Comparator Positive	Comparator Negative	Total
Candidate Positive	131	1	132
Candidate Negative	12	1113	1125
Total	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% - 100.0%)		

*6 samples excluded due to invalid results with comparator methods

Table 18: Influenza A Performance

Influenza A	Comparator Positive	Comparator Negative	Total
Candidate Positive	236	2	238
Candidate Negative	18	1005	1023
Total	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% - 100.0%)		

*2 samples excluded due to invalid results with comparator methods

Table 19: Influenza B Performance

Influenza B	Comparator Positive	Comparator Negative	Total
Candidate Positive	91	1	92
Candidate Negative	7	1162	1169
Total	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% - 100.0%)		

*2 samples excluded due to invalid results with comparator methods

Table 20: RSV Performance

RSV	Comparator Positive	Comparator Negative	Total
Candidate Positive	159	2	161
Candidate Negative	10	1090	1100
Total	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% - 100.0%)		

*2 samples excluded due to invalid results with comparator methods

Table 21: RSV Performance Stratified by Age Group

Age Group	Number of Subjects Tested	Candidate Positives	Comparator Positives	PPA (95% CI)
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%)
< 2 years	**114**	**43**	**43**	**100.0% (91.8%, 100.0%)**
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%, 100.0%)
22-59 years	314	8	9	88.9% (56.5%, 98.0%)
>=60 years	238	7	7	100.0% (64.6%, 100.0%)
Total	**1261**	**159**	**169**	**94.1% (89.5%, 96.8%)**

2. Clinical Sensitivity:

   Please refer to Section VI.C (Clinical Studies) above for clinical validation.

3. Clinical Specificity:

   Please refer to Section VI.C (Clinical Studies) above for clinical validation.

4. Clinical Cut-Off:

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

D Expected Values/Reference Range:

A patient sample is expected to be negative for RSV, Influenza A, Influenza B and SARS-CoV-2.

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.
