510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY

I Background Information:

A 510(k) Number:

K251085

B Applicant:

iHealth Labs, Inc.

C Proprietary and Established Names:

iHealth Flu A&B/COVID-19/RSV Rapid Test; iHealth Flu A&B/COVID-19/RSV Rapid Test Pro

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 iHealth Flu A&B/COVID-19/RSV Rapid Test and iHealth Flu A&B/COVID-19/RSV Rapid Test Pro

B Measurand:

SARS-CoV-2 nucleocapsid protein antigens, influenza A nucleoprotein antigens, influenza B nucleoprotein antigens, and RSV protein antigens

C Type of Test:

Qualitative lateral flow immunoassay

III Intended Use/Indications for Use:

A Intended Use(s):

See Indications for Use below.

B Indication(s) for Use:

iHealth Flu A&B/COVID-19 Rapid Test:

The iHealth Flu A&B/COVID-19/RSV Rapid Test is a lateral flow immunochromatographic assay intended for the qualitative detection and differentiation of influenza A, influenza B, SARS-CoV-2, and respiratory syncytial virus (RSV) protein antigens directly in anterior nasal swab samples from individuals with signs and symptoms of respiratory tract infection. Symptoms of respiratory infections due to influenza, SARS-CoV-2, and RSV 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 influenza, SARS-CoV-2, RSV, or other pathogens.

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

iHealth Flu A&B/COVID-19 Rapid Test Pro:

The iHealth Flu A&B/COVID-19/RSV Rapid Test Pro is a lateral flow immunochromatographic assay intended for the qualitative detection and differentiation of influenza A, influenza B, SARS-CoV-2, and respiratory syncytial virus (RSV) protein antigens directly in anterior nasal swab samples from individuals with signs and symptoms of respiratory tract infection. Symptoms of respiratory infections due to influenza, SARS-CoV-2, and RSV can be similar.

This test is for 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 influenza, SARS-CoV-2, RSV, or other pathogens.

Individuals who test negative and/or experience continued or worsening respiratory symptoms, such as fever, cough and/or shortness of breath, should seek follow-up care from their healthcare provider.

Positive results do not rule out co-infection with other respiratory pathogens.

Test results should not be used as the sole basis for treatment or other patient management decisions.

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 iHealth Flu A&B/COVID-19/RSV Rapid Test and Rapid Test Pro (collectively, the candidate device) are lateral flow immunoassays for the qualitative detection and differentiation of influenza (Flu) A, Flu B, SARS-CoV-2, and RSV protein antigens.

Two versions are available for this over-the-counter (OTC) test: one is labeled for lay-user use and one is labeled for professional use, both with identical designs. The candidate device detects antigens in 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 kit for the iHealth Flu A&B/COVID-19/RSV Rapid Test is composed of:

- Test Card(s)
- Extraction Tube(s)
- Swab(s) (sterile)
- Quick Reference Instructions (QRI)

Materials Required but Not Provided

- A clock or timer

Test Card:

The device's test card is composed of several components and materials. The top cover is made of Acrylonitrile Butadiene Styrene (ABS). The strip includes multiple layers: the sample pad is made of glass fiber; the conjugate pad is composed of glass fiber, latex microspheres, and antibody; the front pad is also made of glass fiber; the nitrocellulose (NC) film consists of a NC membrane and antibody; the absorbent pad is made of polyester; and the adhesive pad is composed of polyvinyl chloride (PVC). The bottom cover is made of ABS.

The test card contains two test strips, each with a NC membrane, enclosed in a plastic case. One test strip has four lines: three test lines (Flu A, Flu B, and CoV) and a control line (Ctrl), while the other test strip has two lines: one test line (RSV) and a control line (Ctrl) (Fig 1).

Fig 1: iHealth Flu A&B/COVID-19/RSV Rapid Test and Rapid Test Pro Cassette design

B Principle of Operation:

To begin the test, an anterior nasal swab sample is mixed with an extraction solution in the test tube. The extraction solution in the test tube interacts with the specimen and facilitates exposure of the appropriate viral antigens to the antibodies used in the test. The liquid in the test tube now containing the specimen is added to the sample well of the test card. When an adequate volume of the processed specimen is added to the sample well, the specimen migrates by capillary action across each of the two test strips migrating through the pads and membrane. The pads contain detection antibodies and control antigen conjugated to latex microspheres and the membrane contains immobilized capture antibodies and control antibody.

If influenza A, influenza B, SARS-CoV-2, or RSV antigen is present in the specimen, they will react with the specific antibody labeled with latex microspheres. The mixture then migrates towards the membrane as antigen-antibody-latex microspheres complexes, which then bind to the immobilized capture antibody line(s) on the membrane, producing a visible colored test line in the related test line region (Flu A/Flu B/CoV/RSV).

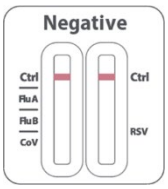
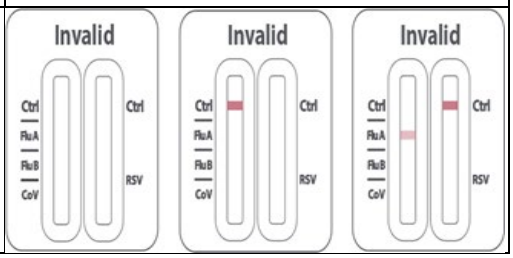
The rest of the sample and unbound/bound latex microspheres complexes continue to migrate to the control line position (Ctrl) in each strip, where immobilized control antibodies capture the control antigen-latex microspheres complexes and form the control line. Formation of the control lines serves as an internal control to demonstrate that test reagents are functional, that the antibody-latex microspheres conjugates in the latex microspheres pad have been hydrated and released, and that sufficient sample has been applied to allow for migration through the test and control lines. If the control lines do not appear within the designated incubation time, the result is invalid, and the test should be repeated using a new test device and specimen.

Interpretation of Results:

After dispensing the test specimen into the sample well, the result should be read between 15-30 minutes.

Table 1: Result Interpretation

Result	Interpretation	Example Image
Positive	If the control (Ctrl) line is visible on both strips and any pink line appear at “Flu A”, “Flu B”, “CoV”, or “RSV” no matter how faint, the test is positive for that virus.	

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

V Substantial Equivalence Information:

A Predicate Device Name(s):

WELLlife COVID-19 / Influenza A&B Home Test; WELLlife COVID-19 / Influenza A&B Antigen Test

B Predicate 510(k) Number(s):

K243256

C Comparison with Predicate(s):

Device & Predicate Device(s):	K251085 (candidate) iHealth Flu A&B/COVID-19/RSV Rapid Test; iHealth Flu A&B/COVID- 19/RSV Rapid Test Pro	K243256 (predicate) WELLlife COVID-19 / Influenza A&B Home Test; WELLlife COVID-19 / Influenza A&B Antigen Test
Intended Use/Indications For Use	**iHealth Flu A&B/COVID-19 Rapid Test** The iHealth Flu A&B/COVID-19/RSV Rapid Test is a lateral flow immunochromatographic assay intended for the qualitative detection and differentiation of influenza A, influenza B, SARS-CoV-2, and respiratory syncytial virus (RSV) protein antigens directly in anterior nasal swab samples from individuals with signs and symptoms of respiratory tract infection. Symptoms of respiratory infections due to influenza, SARS-CoV-2, and RSV can be similar. This test is for non-prescription home use by individuals aged 14 years or	**WELLlife COVID-19 / Influenza A&B Home Test** The WELLlife COVID-19 / Influenza A&B Home Test is a lateral flow immunochromatographic assay intended for the qualitative detection and differentiation of influenza A, and influenza B nucleoprotein antigens and SARS-CoV-2 nucleocapsid antigen directly in anterior nasal swab samples from individuals with signs and symptoms of respiratory tract infection. Symptoms of respiratory infections due to SARS-CoV-2 and influenza can be similar. This test is for non-prescription home use by individuals aged 14 years or older testing

	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 influenza, SARS-CoV-2, RSV, or other pathogens. Individuals who test negative and/or experience continued or worsening symptoms, such as fever, cough and/or shortness of breath, should 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. **<u>iHealth Flu A&B/COVID-19 Rapid Test Pro</u>** The iHealth Flu A&B/COVID-19/RSV Rapid Test Pro is a lateral flow immunochromatographic assay intended for the qualitative detection and differentiation of influenza A, influenza B, SARS-CoV-2, and respiratory syncytial virus (RSV) protein antigens directly in anterior nasal swab samples from individuals with signs and symptoms of respiratory tract infection. Symptoms of respiratory infections due to influenza, SARS-CoV-2, and RSV can be similar. This test is for 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 influenza, SARS-CoV-2, RSV, or other pathogens. Individuals who test negative and/or experience continued or worsening respiratory symptoms, such as fever, cough and/or shortness of breath, should seek follow-up care from their healthcare provider. Positive results do not rule out co-infection	themselves, or adults testing individuals aged 2 years or older. All negative results are presumptive and should be confirmed with an FDA-cleared molecular assay when determined to be appropriate by a healthcare provider. Negative results do not rule out infection with influenza, SARS-CoV-2, or other pathogens. Individuals who test negative and experience continued or worsening respiratory symptoms, such as fever, cough and/or shortness of breath, should seek follow-up care from their healthcare providers. 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. **<u>WELLlife COVID-19／Influenza A&B Antigen Test</u>** The WELLlife COVID-19 / Influenza A&B Antigen Test is a lateral flow immunochromatographic assay intended for the qualitative detection and differentiation of influenza A, and influenza B nucleoprotein antigens and SARS-CoV-2 nucleocapsid antigen directly in anterior nasal swab samples from individuals with signs and symptoms of respiratory tract infection. Symptoms of respiratory infections due to SARS-CoV-2 and influenza can be similar. This test is for use by individuals aged 14 years or older testing themselves, or adults testing aged 2 years or older. All negative results are presumptive and should be confirmed with an FDA-cleared molecular assay when determined to be appropriate by a healthcare provider. Negative results do not rule out infection with influenza, SARS-CoV-2, or other pathogens. Individuals who test negative and experience continued or worsening respiratory symptoms, such as fever, cough and/or shortness of breath, should seek follow-up care from their healthcare providers. Positive results do not rule out co-infection with other respiratory pathogens. Test results should not be used as the sole

	with other respiratory pathogens. Test results should not be used as the sole basis for treatment or other patient management decisions.	basis for treatment or other patient management decisions.
General Device Characteristic Similarities		
Regulation	21 CFR 866.3987	Same
End User	Lay user	Same
Intended Specimen	Direct anterior nasal swabs	Same
Sample Collection Method	Nasal swab supplied in kit	Same
Usage Type	Single -use test	Same
Assay Technique	Immunochromatographic Assay, visual read	Same
Test Result	Qualitative	Same
Storage Temperature	36-86 °F (2- 30°C)	Same
General Device Characteristic Differences		
Analytes Detected	SARS-CoV-2 nucleocapsid protein antigens, Influenza A nucleoprotein antigens, Influenza B nucleoprotein antigens, RSV protein antigens	SARS-CoV-2 nucleocapsid protein antigens, Influenza A nucleoprotein antigens, Influenza B nucleoprotein antigens
Time to Result	15 - 30 min	15 - 20 min

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
11135:2014	Sterilization of health care products - Ethylene oxide - Requirements for development, validation and routine control of a sterilization process for medical devices	ISO	Sterility
10993-7	Biological Evaluation of Medical Devices – Part 7: Ethylene Oxide Sterilization Residuals	ISO	Sterility
10993-1 Fifth edition 2018-08	Biological Evaluation of Medical Devices – Evaluation testing within risk management process	ISO	Biocompatibility
10993-5: Third Edition 2009-06-01	Biological Evaluation of Medical Devices - Tests for in vitro cytotoxicity	ISO	Biocompatibility
10993-10: 2021 Fourth Edition 2021-11	Biological Evaluation of Medical Devices –Tests for irritation and skin sensitization	ISO	Biocompatibility

Document	Title	Publisher	Applicable Study
14971:2019: Third Edition 2019-12	Biological Evaluation of Medical Devices – Application of risk management to medical devices	ISO	Biocompatibility

VII Performance Characteristics (if/when applicable):

A Analytical Performance

1. Lot-to-lot Precision

The multi-lot precision study was conducted at a single site to assess variability between lots, days, runs, and operators.

A panel of nineteen samples was tested, including one negative sample and nine positive sample combinations: single spiked samples (SARS-CoV-2, Flu A, Flu B, RSV), dual co-spiked samples (Flu A + Flu B, SARS-CoV-2 + RSV), triple co-spiked samples (Flu A + Flu B + SARS-CoV-2, Flu A + Flu B + RSV), and quadruple co-spiked samples (Flu A + Flu B + SARS-CoV-2 + RSV) with each combination at two concentrations: 0.8x LoD and 3x LoD.

Two replicates per sample panel were tested per run, per operator, and per lot across 10 days with two test runs per day for a total of 240 results per sample panel (3 lots x 2 operators x 2 replicate x 10 days x 2 runs per day). All samples were prepared in pooled negative nasal fluid (PNF). The study was performed in a randomized and blinded manner.

All negative samples and replicates prepared at 3x LoD demonstrated 100% agreement across the operators, lots, days, and runs tested. The results with concentrations at 0.8x LoD that yielded results with less than 100% showed minor variability between lots and were not deemed significant. The results are summarized below.

Table 2: Summary Results of Multi-lot Precision Study

Analyte	Test line	No. of Positives / No. of Samples tested (%)			Total no. of positives / Total no. of samples (%)
		Lot 1	Lot 2	Lot 3	
Negative	SARS-CoV-2	0/80 (0%)	0/80 (0%)	0/80 (0%)	0/240 (0%)
	Flu A	0/80 (0%)	0/80 (0%)	0/80 (0%)	0/240 (0%)
	Flu B	0/80 (0%)	0/80 (0%)	0/80 (0%)	0/240 (0%)
	RSV	0/80 (0%)	0/80 (0%)	0/80 (0%)	0/240 (0%)
0.8X LoD SARS-CoV-2	SARS-CoV-2	39/80 (48.8%)	46/80 (57.5%)	36/80 (45.0%)	121/240 (50.4%)
	Flu A	0/80 (0%)	0/80 (0%)	0/80 (0%)	0/240 (0%)
	Flu B	0/80 (0%)	0/80 (0%)	0/80 (0%)	0/240 (0%)
	RSV	0/80 (0%)	0/80 (0%)	0/80 (0%)	0/240 (0%)
0.8X LoD Flu A	SARS-CoV-2	0/80 (0%)	0/80 (0%)	0/80 (0%)	0/240 (0%)
	Flu A	56/80 (70.0%)	35/80 (43.8%)	48/80 (60.0%)	139/240 (57.9%)
	Flu B	0/80 (0%)	0/80 (0%)	0/80 (0%)	0/240 (0%)
	RSV	0/80 (0%)	0/80 (0%)	0/80 (0%)	0/240 (0%)
0.8X LoD Flu B	SARS-CoV-2	0/80 (0%)	0/80 (0%)	0/80 (0%)	0/240 (0%)
	Flu A	0/80 (0%)	0/80 (0%)	0/80 (0%)	0/240 (0%)
	Flu B	35/80 (43.8%)	31/80 (38.8%)	35/80 (43.8%)	101/240 (42.0%)
	RSV	0/80 (0%)	0/80 (0%)	0/80 (0%)	0/240 (0%)

Analyte	Test line	No. of Positives / No. of Samples tested (%)			Total no. of positives / Total no. of samples (%)
		Lot 1	Lot 2	Lot 3	
0.8X LoD RSV	SARS-CoV-2	0/80 (0%)	0/80 (0%)	0/80 (0%)	0/240 (0%)
	Flu A	0/80 (0%)	0/80 (0%)	0/80 (0%)	0/240 (0%)
	Flu B	0/80 (0%)	0/80 (0%)	0/80 (0%)	0/240 (0%)
	RSV	53/80 (66.3%)	46/80 (57.5%)	41/80 (51.3%)	140/240 (58.3%)
0.8X LoD Flu A/Flu B	SARS-CoV-2	0/80 (0%)	0/80 (0%)	0/80 (0%)	0/240 (0%)
	Flu A	46/80 (57.5%)	40/80 (50.0%)	48/80 (60.0%)	134/240 (55.8%)
	Flu B	37/80 (46.3%)	39/80 (48.8%)	41/80 (51.3%)	117/240 (48.7%)
	RSV	0/80 (0%)	0/80 (0%)	0/80 (0%)	0/240 (0%)
0.8X LoD SARS-CoV-2/RSV	SARS-CoV-2	41/80 (51.3%)	43/80 (53.8%)	44/80 (55.0%)	128/240 (53.3%)
	Flu A	0/80 (0%)	0/80 (0%)	0/80 (0%)	0/240 (0%)
	Flu B	0/80 (0%)	0/80 (0%)	0/80 (0%)	0/240 (0%)
	RSV	48/80 (60.0%)	39/80 (48.8%)	36/80 (45.0%)	123/240 (51.2%)
0.8X LoD SARS-CoV-2/Flu A/Flu B	SARS-CoV-2	43/80 (53.8%)	44/80 (55.0%)	41/80 (51.3%)	128/240 (53.3%)
	Flu A	50/80 (62.5%)	46/80 (57.5%)	48/80 (60.0%)	144/240 (60.0%)
	Flu B	47/80 (58.8%)	39/80 (48.8%)	42/80 (52.5%)	128/240 (53.3%)
	RSV	0/80 (0%)	0/80 (0%)	0/80 (0%)	0/240 (0%)
0.8X LoD Flu A/Flu B/RSV	SARS-CoV-2	0/80 (0%)	0/80 (0%)	0/80 (0%)	0/240 (0%)
	Flu A	51/80 (63.8%)	52/80 (65.0%)	49/80 (61.3%)	152/240 (63.3%)
	Flu B	42/80 (52.5%)	43/80 (53.8%)	37/80 (46.3%)	122/240 (50.8%)
	RSV	56/80 (70.0%)	47/80 (58.8%)	36/80 (45.0%)	139/240 (57.9%)
0.8X LoD SARS-CoV-2/Flu A/Flu B/RSV	SARS-CoV-2	43/80 (53.8%)	48/80 (60.0%)	43/80 (53.8%)	134/240 (55.8%)
	Flu A	42/80 (52.5%)	50/80 (62.5%)	49/80 (61.3%)	141/240 (58.7%)
	Flu B	42/80 (52.5%)	46/80 (57.5%)	42/80 (52.5%)	130/240 (54.1%)
	RSV	53/80 (66.3%)	52/80 (65.0%)	39/80 (48.8%)	144/240 (60.0%)
3X LoD SARS-CoV-2*	SARS-CoV-2	80/80 (100%)	80/80 (100%)	80/80 (100%)	240/240 (1000%)
	Flu A	0/80 (0%)	0/80 (0%)	0/80 (0%)	0/240 (0%)
	Flu B	0/80 (0%)	0/80 (0%)	0/80 (0%)	0/240 (0%)
	RSV	0/80 (0%)	0/80 (0%)	0/80 (0%)	0/240 (0%)

**The 3x LoD samples were evaluated using the same combination of sample types and test conditions as those used for the 0.8x LoD testing, and all results were concordant, demonstrating 100% agreement.*

2. Linearity

   Not applicable; this candidate 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 PNF and tested in triplicates in the absence of SARS-CoV-2, influenza A, influenza B and RSV B. No cross-reactivity was observed with the organisms tested for any of the 4 analytes.

For the microbial interference study, dilutions of the panel organisms were made in PNF, in the presence of low levels (3x LoD) of SARS-CoV-2 (Omicron Variant, Lineage JN.1: USA/New York/PV96109/202), influenza A (H1N1pdm09: A/Victoria/4897/2022), influenza B (Victoria: B/Austria/1359417/2021), and RSV B (3/2015 Isolate #1) and tested in triplicate. No microbial interference was observed for any of the 4 analytes tested **(Table 3).**

Table 3: Cross-Reactivity and Microbial Interference Study Results

Virus/Microorganism	Concentration	Units	# of Positive results / # of Replicates tested	
			Cross-Reactivity[1]	Interference[1]
SARS-CoV-1	1.25×10^5	PFU/ml	0/3	3/3
MERS-coronavirus	1.58×10^8	GE/mL	0/3	3/3
Human coronavirus OC43	7.00×10^5	$TCID_{50}$/mL	0/3	3/3
Human coronavirus 229E	1.40×10^5	$TCID_{50}$/mL	0/3	3/3
Human coronavirus NL63	8.00×10^4	$TCID_{50}$/mL	0/3	3/3
Adenovirus, Type 1	2.23×10^5	$TCID_{50}$/mL	0/3	3/3
Adenovirus Type 7	1.58×10^5	$TCID_{50}$/mL	0/3	3/3
Cytomegalovirus	1.00×10^5	PFU/mL	0/3	3/3
Epstein Barr Virus	1.83×10^6	CP/mL	0/3	3/3
Human Metapneumovirus	3.50×10^5	$TCID_{50}$/mL	0/3	3/3
Parainfluenza virus 1	2.00×10^5	$TCID_{50}$/mL	0/3	3/3
Parainfluenza virus 2	1.75×10^5	$TCID_{50}$/mL	0/3	3/3
Parainfluenza virus 3	7.00×10^5	$TCID_{50}$/mL	0/3	3/3
Parainfluenza virus 4	2.39×10^5	$TCID_{50}$/mL	0/3	3/3
Enterovirus	2.23×10^5	$TCID_{50}$/mL	0/3	3/3
Rhinovirus	2.23×10^6	$TCID_{50}$/mL	0/3	3/3
Bordetella pertussis	2.50×10^8	CFU/mL	0/3	3/3
Candida albicans	6.03×10^6	CFU/mL	0/3	3/3
Chlamydia pneumoniae	4.33×10^6	IFU/mL	0/3	3/3
Corynebacterium xerosis	2.30×10^7	CFU/mL	0/3	3/3
Escherichia coli	1.18×10^8	CFU/mL	0/3	3/3
Hemophilus influenzae	3.00×10^{10}	CFU/mL	0/3	3/3
Lactobacillus plantarum	8.50×10^6	CFU/mL	0/3	3/3
Legionella pneumophila	6.50×10^6	CFU/mL	0/3	3/3
Moraxella catarrhalis	2.50×10^8	CFU/mL	0/3	3/3
Mycoplasma pneumoniae	2.50×10^7	CFU/mL	0/3	3/3
Mycobacterium tuberculosis	4.15×10^6	CFU/mL	0/3	3/3
Neisseria meningitidis	3.43×10^6	CFU/mL	0/3	3/3
Neisseria elongata	2.68×10^8	CFU/mL	0/3	3/3
Pneumocystis jirovecii	1.30×10^7	CFU/mL	0/3	3/3
Pseudomonas aeruginosa	1.23×10^8	CFU/mL	0/3	3/3
Staphylococcus aureus	2.60×10^8	CFU/mL	0/3	3/3
Staphylococcus epidermidis	9.00×10^7	CFU/mL	0/3	3/3
Streptococcus salivarius	1.01×10^6	CFU/mL	0/3	3/3
Streptococcus pneumoniae	3.88×10^7	CFU/mL	0/3	3/3
Streptococcus pyogenes	7.50×10^7	CFU/mL	0/3	3/3

Virus/Microorganism	Concentration	Units	# of Positive results / # of Replicates tested	
			Cross-Reactivity[1]	Interference[1]
Measles, Strain Edmonston	2.23×10^5	$TCID_{50}$/mL	0/3	3/3
Mumps (Isolate 1)	8.48×10^5	$TCID_{50}$/mL	0/3	3/3
PNF	NA	NA	0/3	3/3
Human coronavirus HKU1 (HKU1/UNC/2/2022)	4.34×10^6	GE/mL	0/3	3/3

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

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 triplicates with different combinations of low (3x LoD for single analyte) and high (either 1000X LoD or the highest achievable) concentrations of SARS-CoV-2, Flu A, Flu B, and RSV B prepared in PNF. For each condition tested, all three replicates at the low target analyte condition tested positive in the presence of a second target analyte at high concentrations. Thus, no competitive interference between SARS-CoV-2, influenza A, influenza B, and RSV B were observed as shown in the table below.

Table 4: Competitive Inhibition Study Results

Combination #	Viral Targets in Sample				Results (# pos/ total reps)			
	Flu A	Flu B	SARS-CoV- 2	RSV B	Flu A	Flu B	SARS-CoV- 2	RSV B
1	Low	Low	Negative	High	3/3	3/3	0/3	3/3
2	Low	Negative	Low	High	3/3	0/3	3/3	3/3
3	Negative	Low	Low	High	0/3	3/3	3/3	3/3
4	Low	Low	Low	High	3/3	3/3	3/3	3/3
5	Low	Negative	Negative	High	3/3	0/3	0/3	3/3
6	Negative	Negative	Low	High	0/3	0/3	3/3	3/3
7	Negative	Low	Negative	High	0/3	3/3	0/3	3/3
8	High	Low	Negative	Low	3/3	3/3	0/3	3/3
9	High	Negative	Low	Low	3/3	0/3	3/3	3/3
10	High	Low	Low	Negative	3/3	3/3	3/3	0/3
11	High	Low	Low	Low	3/3	3/3	3/3	3/3
12	High	Low	Negative	Negative	3/3	3/3	0/3	0/3
13	High	Negative	Low	Negative	3/3	0/3	3/3	0/3
14	High	Negative	Negative	Low	3/3	0/3	0/3	3/3
15	Low	High	Negative	Low	3/3	3/3	0/3	3/3
16	Negative	High	Low	Low	0/3	3/3	3/3	3/3
17	Low	High	Low	Negative	3/3	3/3	3/3	0/3
18	Low	High	Low	Low	3/3	3/3	3/3	3/3
19	Negative	High	Low	Negative	0/3	3/3	3/3	0/3
20	Low	High	Negative	Negative	3/3	3/3	0/3	0/3
21	Negative	High	Negative	Low	0/3	3/3	0/3	3/3
22	Low	Negative	High	Low	3/3	0/3	3/3	3/3

Combination #	Viral Targets in Sample				Results (# pos/ total reps)			
	Flu A	Flu B	SARS-CoV- 2	RSV B	Flu A	Flu B	SARS-CoV- 2	RSV B
23	Negative	Low	High	Low	0/3	3/3	3/3	3/3
24	Low	Low	High	Negative	3/3	3/3	3/3	0/3
25	Low	Low	High	Low	3/3	3/3	3/3	3/3
26	Negative	Low	High	Negative	0/3	3/3	3/3	0/3
27	Low	Negative	High	Negative	3/3	0/3	3/3	0/3
28	Negative	Negative	High	Low	0/3	0/3	3/3	3/3

c. *Endogenous/Exogenous Substances Interference:*

The candidate device was evaluated for performance in the presence of potentially interfering substances that might be present in respiratory specimens. Potentially interfering substances were prepared and diluted in PNF 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, contrived positive samples containing 3x co-spiked analytes for SARS-CoV-2, influenza A, influenza B, and RSV B in diluted in PNF were evaluated in the presence of the interfering substances in triplicate to confirm these substances do not interfere with the detection of the SARS-CoV-2, Influenza A, Influenza B, and RSV B.

In the absence of influenza, SARS-CoV-2, and RSV B, all substances tested showed no interference except the 2024–25 FluMist live intranasal vaccine, which interfered at concentrations ≥ 0.375% v/v but was non-interfering at 0.1875% v/v, and 80% ethanol hand sanitizer, which caused invalid results at 15% v/v but showed no interference at ≤7.5% v/v.

In the presence of the target analytes, 80% ethanol hand sanitizer caused invalid results at 15% v/v. Additionally, 80% ethanol hand sanitizer at >3.75% v/v may interfere with Flu A and Flu B results and fluticasone propionate at >10% v/v may interfere with SARS-CoV-2, Flu B, and RSV results.

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

Table 5: Interfering Substances Study Results

Interfering Substance	Concentration	Cross-reactivity (without analyte) (# pos/ # total)				Interference (with analyte 3X Co-spike LoD) (# pos/ # total)			
		SARS-CoV-2	Flu A	Flu B	RSV	SARS-CoV-2	Flu A	Flu B	RSV
Human Whole Blood (EDTA tube)	4% v/v	0/3	0/3	0/3	0/3	3/3	3/3	3/3	3/3
Mucin	5 mg/mL	0/3	0/3	0/3	0/3	3/3	3/3	3/3	3/3
Chloraseptic (Menthol/Benzocaine)	1.5 mg/mL	0/3	0/3	0/3	0/3	3/3	3/3	3/3	3/3
Chloraseptic (Menthol/Benzocaine)	3 mg/mL	0/3	0/3	0/3	0/3	3/3	3/3	3/3	3/3
Naso GEL (NeilMed)	15% v/v	0/3	0/3	0/3	0/3	3/3	3/3	3/3	3/3
Nasal Drops (Phenylephrine)	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 3X Co-spike LoD) (# pos/ # total)			
		SARS-CoV-2	Flu A	Flu B	RSV	SARS-CoV-2	Flu A	Flu B	RSV
Nasal Spray (Oxymetazoline)	15% v/v	0/3	0/3	0/3	0/3	3/3	3/3	3/3	3/3
Nasal Spray (Cromolyn)	15% v/v	0/3	0/3	0/3	0/3	3/3	3/3	3/3	3/3
Nasal corticosteroid (Dexamethasone)	1 mg/mL	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 allergy relief	15% v/v	0/3	0/3	0/3	0/3	3/3	3/3	3/3	3/3
Zicam Nasal Spray	5% v/v	0/3	0/3	0/3	0/3	3/3	3/3	3/3	3/3
Zicam Nasal Spray	15% v/v	0/3	0/3	0/3	0/3	3/3	3/3	3/3	3/3
Nasal Spray (Alkalol)	15% v/v	0/3	0/3	0/3	0/3	3/3	3/3	3/3	3/3
Sore Throat Phenol Spray	15% v/v	0/3	0/3	0/3	0/3	3/3	3/3	3/3	3/3
Tobramycin	4 µg/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
Anti-viral drug (Remdesivir)	10 mg/mL	0/3	0/3	0/3	0/3	3/3	3/3	3/3	3/3
Nasal corticosteroid (Fluticasone)	15% v/v	0/3	0/3	0/3	0/3	0/3	3/3	0/3	0/3
	10% v/v	N/A	N/A	N/A	N/A	3/3	3/3	3/3	3/3
	5% v/v	0/3	0/3	0/3	0/3	3/3	3/3	3/3	3/3
Tamiflu (Oseltamivir Phosphate)	5 mg/mL	0/3	0/3	0/3	0/3	3/3	3/3	3/3	3/3
FluMist Influenza Vaccine Live intranasal	15% v/v	0/3	3/3	3/3	0/3	3/3	3/3	3/3	3/3
	1.5% v/v	0/3	3/3	0/3	0/3	NT*	NT*	NT*	NT*
	0.75% v/v	0/3	3/3	0/3	0/3	NT*	NT*	NT*	NT*
	0.375% v/v	0/3	3/3	0/3	0/3	NT*	NT*	NT*	NT*
	0.1875% v/v	0/3	0/3	0/3	0/3	NT*	NT*	NT*	NT*
	0.15% v/v	0/3	0/3	0/3	0/3	NT*	NT*	NT*	NT*
Zanamivir	282 ng/mL	0/3	0/3	0/3	0/3	3/3	3/3	3/3	3/3
Biotin	3,500 ng/mL	0/3	0/3	0/3	0/3	3/3	3/3	3/3	3/3
Zinc (TheraZinc Throat Spray)	15% v/v	0/3	0/3	0/3	0/3	3/3	3/3	3/3	3/3
Leukocytes	1.67×10^6 cells/mL	0/3	0/3	0/3	0/3	3/3	3/3	3/3	3/3
Body & Hand Lotion	0.5% w/v	0/3	0/3	0/3	0/3	3/3	3/3	3/3	3/3
Body Lotion, with 1.2% dimethicone	0.5% w/v	0/3	0/3	0/3	0/3	3/3	3/3	3/3	3/3
Hand Lotion	5% w/v	0/3	0/3	0/3	0/3	3/3	3/3	3/3	3/3
Hand Sanitizer with Aloe, 62% ethyl alcohol	5% v/v	0/3	0/3	0/3	0/3	3/3	3/3	3/3	3/3
Hand Sanitizer cream lotion	15% v/v	0/3	0/3	0/3	0/3	3/3	3/3	3/3	3/3
Hand Sanitizer, 80% ethanol, fast drying	15% v/v	Invalid	Invalid	Invalid	Invalid	Invalid	Invalid	Invalid	Invalid
	7.5% v/v	0/3	0/3	0/3	0/3	3/3	0/3	0/3	3/3
	3.75% v/v	0/3	0/3	0/3	0/3	3/3	3/3	3/3	3/3
	1.5% 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 3X Co-spike LoD) (# pos/ # total)			
		SARS-CoV-2	Flu A	Flu B	RSV	SARS-CoV-2	Flu A	Flu B	RSV
Hand soap liquid gel	10% w/v	0/3	0/3	0/3	0/3	3/3	3/3	3/3	3/3
Nasal spray (Saline)	15% v/v	0/3	0/3	0/3	0/3	3/3	3/3	3/3	3/3
Nasal corticosteroid (Triamcinolone)	15% v/v	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 candidate device is a binary qualitative assay that is visually read.

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

a. Controls

i. Internal Controls:

Two built-in internal procedural controls are incorporated into the candidate device to ensure that the test is functioning properly and correct use of the candidate device. Each internal control is part of the test strip membranes and is therefore, automatically run within the development time of each test (i.e., one with SARS-CoV-2 and influenza and one with RSV). The internal procedural control consists of recombinant protein that are immobilized at the "Ctrl"-line of the test membrane in the candidate device and captures leftover, unbound latex microspheres complexes to generate a color signal at the "Ctrl -line".

ii. External Controls:

External quality control materials 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 candidate device lots were stored at 5±3 °C and 30±2 °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 (36-86°F) 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. Performance of unopened test kits was assessed by comparing pre- (T0) and post-distribution (Td) results, using the sample panel as described for the real-time stability study (above) with addition of single analyte (3x LoD) sample panel. Samples were tested in replicates of five (5) for each of three candidate device lots. Candidate test kits were stored at room temperature and the

designated temperature profiles described below and then tested with the test panel to evaluate performance. The following temperature profiles were assessed:

- To mimic summer shipping conditions, test kits were incubated in a chamber set to 45ºC for 7 days then moved to 22 ºC for 4 hours.
- To mimic winter shipping conditions, test kits were incubated at -20ºC for 7 days and then moved to 18ºC for 4 hours.

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 one strain of chemically inactivated SARS-CoV-2 (USA/New York/PV96109/2023), two strains of live influenza A (H1N1 and H3N2), two strains of live influenza B (Victoria and Yamagata) and two strains of live RSV (RSV A and RSV B) at which at least 95% of all true positive replicates tested positive. Testing was conducted on three lots of candidate 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 in PNF in triplicates for three candidate device lots for a total of 9 replicates per dilution. Once the lowest positive ten-fold dilution concentration was established, additional two-fold dilutions were tested in triplicate (n=3). A 50-µL sample of each virus diluted in PNF 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 6: Preliminary Single Analyte LoD Results

Virus Strain	Virus Concentration		Positive Replicates
	$TCID_{50}$/mL	$TCID_{50}$/Swab	
SARS-CoV-2	1.40×10^4	7.0×10^2	9/9
	1.40×10^3	7.0×10^1	9/9
	1.40×10^2	7.0×10^0	9/9
	1.40×10^1	7.0×10^{-1}	9/9
	1.40×10^0	7.0×10^{-2}	0/9
	7.00×10^0	**3.5×10^{-1}**	**9/9**
	3.50×10^0	1.7×10^{-1}	3/9
	1.75×10^0	8.7×10^{-2}	0/9
Influenza A H1N1	2.02×10^4	1.0×10^3	9/9
	2.02×10^3	1.0×10^2	9/9
	2.02×10^2	1.0×10^1	9/9
	2.02×10^1	1.0×10^0	0/9
	1.01×10^2	5.0×10^0	9/9
	5.05×10^1	**2.5×10^0**	**9/9**
	2.53×10^1	1.2×10^0	0/9
Influenza A H3N2	4.17×10^4	2.0×10^3	9/9
	4.17×10^3	2.0×10^2	9/9

Virus Strain	Virus Concentration		Positive Replicates
	$TCID_{50}$/mL	$TCID_{50}$/Swab	
	4.17×10^2	2.0×10^1	9/9
	4.17×10^1	2.0×10^0	0/9
	2.09×10^2	1.0×10^0	9/9
	1.04×10^2	**5.2×10^0**	**9/9**
	5.21×10^1	2.6×10^0	0/9
Influenza B Victoria	2.82×10^5	1.4×10^4	9/9
	2.82×10^4	1.4×10^3	9/9
	2.82×10^3	1.4×10^2	9/9
	2.82×10^2	1.4×10^1	0/9
	1.41×10^3	7.0×10^1	9/9
	7.05×10^2	**3.5×10^1**	**9/9**
	3.53×10^2	1.7×10^1	0/9
Influenza B Yamagata	1.10×10^8	5.5×10^6	9/9
	1.10×10^7	5.5×10^5	9/9
	1.10×10^6	5.5×10^4	9/9
	1.10×10^5	5.5×10^3	0/9
	5.50×10^5	2.7×10^4	9/9
	2.75×10^5	**1.3×10^4**	**9/9**
	1.38×10^5	6.9×10^3	0/9
RSV A	2.80×10^5	1.4×10^4	9/9
	2.80×10^4	1.4×10^3	9/9
	2.80×10^3	1.4×10^2	9/9
	2.80×10^2	1.4×10^1	1/9
	1.40×10^3	7.0×10^1	9/9
	7.00×10^2	**3.5×10^1**	**9/9**
	3.50×10^2	1.7×10^1	1/9
RSV B	2.02×10^5	1.0×10^4	9/9
	2.02×10^4	1.0×10^3	9/9
	2.02×10^3	1.0×10^2	9/9
	2.02×10^2	1.0×10^1	9/9
	2.02×10^1	1.0×10^0	0/9
	1.01×10^2	5.0×10^0	9/9
	5.05×10^1	**2.5×10^0**	**9/9**
	2.53×10^1	1.2×10^0	0/9

LoD confirmation testing was performed by testing twenty (20) replicates at the preliminary (1x) LoD concentration determined above. The acceptance criteria for confirmation of the LoD was that 95% of the replicates (19/20) test positive. Each concentration level was tested in a randomized manner alongside three (3) negative samples (PNF) for validation. LoD testing was conducted on three separate candidate device lots, with each lot generating its own LoD. The final candidate device LoD was determined by selecting the highest LoD from the three lots as the overall LoD for the candidate device. The results of confirmatory LoD testing for 3 lots are summarized below.

Table 7: Confirmatory Single Analyte LoD Results

Strain	Virus Concentration		Positive Replicates
SARS-CoV-2	7.00×10^0 $TCID_{50}$/mL	3.50×10^{-1} $TCID_{50}$/swab	59/60

Strain	Virus Concentration		Positive Replicates
Flu A- H1N1	4.55×10^1 $TCID_{50}$/mL	2.27×10^0 $TCID_{50}$/swab	59/60
Flu A - H3N2	9.38×10^1 $TCID_{50}$/mL	4.69×10^0 $TCID_{50}$/swab	59/60
Flu B - Victoria	6.35×10^2 $TCID_{50}$/mL	3.17×10^1 $TCID_{50}$/swab	58/60
Flu B-Yamagata	2.30×10^5 $CEID_{50}$/mL	1.15×10^4 $CEID_{50}$/swab	57/60
RSV A	1.26×10^3 $TCID_{50}$/mL	6.30×10^1 $TCID_{50}$/swab	59/60
RSV B	4.55×10^1 $TCID_{50}$/mL	2.27×10^0 $TCID_{50}$/swab	58/60

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

After single analyte LoDs were determined, a co-spike equivalency study was conducted to characterize the performance of samples that contained all analytes.

Based on the individual analyte LoDs, 1x LoD co-spiked samples were prepared by mixing the four virus analytes (inactivated SARS-CoV-2, live Flu A H1N1, live Flu B Victoria, and RSV B) into the same sample using PNF as clinical matrix. The viral strains and stocks/lots used in the co-spike study were the same as those used in the single-analyte LoD study. Twenty (20) replicates were prepared by pipetting 50 µL of co-spiked sample onto the test swab and testing swabs with the candidate device according to the IFU. The candidate device demonstrated co-spike equivalency for SARS-CoV-2, Flu A, Flu B, and RSV B at 1x single analyte LoD. 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 candidate device was also determined by evaluating different dilutions of the International Standard for SARS-CoV-2 antigen (NIBSC code: 21/368) in PNF. The International Standard for SARS-CoV-2 containing chemically inactivated Omicron SCV-2 strain B1.1.529, sub-variant BA.1 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.

An initial 5-fold first dilution and subsequent 2-fold serial dilutions were made from the International Standard for SARS-CoV-2 antigen into negative clinical matrix (PNF).

Three (3) replicates were tested for each dilution to determine the preliminary LoD concentration of the candidate device. For each replicate, 50 µL of virus dilution was applied to a swab and was processed according to the IFU.

The preliminary LoD concentration was tested with an additional 20 replicates above and below the preliminary LoD to confirm the LoD. Samples were prepared as for the preliminary LoD study above. To confirm the LoD, at least 19 of 20 replicates (95%) should test positive. Both preliminary LoD and confirmatory LoD studies were determined for one kit lot which generated the highest LoD concentration of SARS-CoV-2 among the 3 lots in the LoD study. The confirmatory LoD for the International SARS CoV-2 standard was determined to be 1.93×10^2 IU/mL (9.6 IU/swab). The results are summarized below with the confirmatory LoD in bold.

Table 8: 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)	4.00×10^3	2.0×10^2	3/3
	2.00×10^3	1.0×10^2	3/3
	1.00×10^3	5.0×10^1	3/3
	5.00×10^2	2.5×10^1	3/3
	2.50×10^2	1.2×10^1	3/3
	1.25×10^2	6.2×10^0	0/3
Confirmatory LoD	2.50×10^2	1.2×10^1	20/20
	1.88×10^2	9.4×10^0	13/20
	2.25×10^2	1.1×10^1	20/20
	1.92×10^2	9.6×10^0	18/20
	1.93×10^2	**9.6×10^0**	**20/20**
	5.80×10^2	2.9×10^1	20/20
	6.44×10^1	3.2×10^0	0/20

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. The study was completed with one strain of chemically inactivated SARS-CoV-2, two strains of live influenza A virus, two strains of live influenza B virus, and two strains of live RSV virus. The study was conducted by spiking 50 µL of the highest viral stock concentration (at least 3 orders of magnitude greater than the candidate device LoD/analyte and/or co-spike LoD) possible onto swabs in triplicate and processing the replicates in accordance with 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 9: High-Dose Hook Effect Study Results

Sample	Concentration	Results (# pos / total reps)			
		SARS-CoV-2	Influenza A	Influenza B	RSV
SARS-CoV-2	1.40×10^5 $TCID_{50}$/mL	3/3	0/3	0/3	0/3
Flu A (H1N1)	2.02×10^5 $TCID_{50}$/mL	0/3	3/3	0/3	0/3
Flu A (H3N2)	4.17×10^5 $TCID_{50}$/mL	0/3	3/3	0/3	0/3
Flu B (Victoria)	2.82×10^6 $TCID_{50}$/mL	0/3	0/3	3/3	0/3
Flu B (Yamagata)	1.10×10^9 $CEID_{50}$/mL	0/3	0/3	3/3	0/3
RSV A	2.80×10^6 $TCID_{50}$/mL	0/3	0/3	0/3	3/3
RSV B	2.02×10^5 $TCID_{50}$/mL	0/3	0/3	0/3	3/3

8. Inclusivity

Analytical reactivity testing for the candidate device was performed to ensure that the device can adequately detect a variety of strains for SARS-CoV-2, influenza A, influenza B, and RSV viruses. A selection of temporally, geographically, and genetically diverse strains were tested for inclusivity, including 4 SARS-CoV-2 strains (1 wild type and 3 Omicron), 25 influenza A strains (11 H1N1, 9 H3N2, 2 H5N1, 1 H5N6, 1 H5N8, and 1 H7N3), 10 influenza B strains (1 non-Victoria, non-Yamagata, 5 Victoria and 4 Yamagata lineages), and 12 RSV strains (6 RSV A and 6 RSV B). A series of ten-fold dilutions of each virus strain was spiked into PNF and tested to determine an approximate LoD of the test for each virus. Once the ten-fold breakpoint was established for each of the strains, an additional series of three (3) two-fold dilutions of the lowest positive ten-fold dilution concentration for each virus were independently tested at triplicates to demonstrate inclusivity.

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

Table 10: Inclusivity Results for SARS-Cov-2 Strains

SARS-CoV-2 Subtypes	Pathogens	Concentration ($TCID_{50}$/mL)	Results (#Pos/Total)
Wild type	USA-WA1/2020	3.16×10^{2}	3/3
Omicron	USA/New York/PV96109/2023	7.00×10^{0}	3/3
	USA/CA-Stanford-109-S21/2022	5.95×10^{3}	3/3
	USA/NY-Wadsworth-23067147-01/2023	7.88×10^{3}	3/3

Table 11: Inclusivity Results for influenza A Strains

Flu A Subtypes	Pathogens	Concentration	Results (#Pos/Total)
H1N1	A/Victoria/4897/22	5.05×10^{1} $TCID_{50}$/mL	3/3
	A/Brisbane/02/2018	3.78×10^{1} $TCID_{50}$/mL	3/3
	A/Macha/O1453/2021	2.80×10^{5} $TCID_{50}$/mL	3/3
	A/NY/03/2009	2.29×10^{4} $TCID_{50}$/mL	3/3
	A/Sydney/5/2021	1.20×10^{3} $TCID_{50}$/mL	3/3
	A/Baltimore/JH-22377/2022	8.00×10^{5} $TCID_{50}$/mL	3/3
	A/Wisconsin/67/2022	2.11×10^{2} $TCID_{50}$/mL	3/3
	A/Hawaii/66/2019	1.85×10^{7} $CEID_{50}$/mL	3/3
	A/Wisconsin/588/2019	7.00×10^{3} FFU/mL	3/3
	A/Indiana/02/2020	4.85×10^{6} $CEID_{50}$/mL	3/3
	A/California/04/2009	7.00×10^{2} $TCID_{50}$/mL	3/3
H3N2	A/Darwin/6/2021	1.04×10^{2} $TCID_{50}$/mL	3/3
	A/Alaska/01/2021	7.50×10^{3} FFU/mL	3/3
	A/New York/21/2020	1.30×10^{5} FFU/mL	3/3
	A/Tasmania/503/2020	3.25×10^{4} FFU/mL	3/3
	A/Montana/08/2023	1.30×10^{5} FFU/mL	3/3
	A/Hong Kong/45/2019	7.50×10^{3} FFU/mL	3/3
	A/Ohio/09/2015 (H1N1) v	3.50×10^{5} $CEID_{50}$/mL	3/3
	A/Minnesota/19/2011 (H1N2) v	4.00×10^{6} $CEID_{50}$/mL	3/3
	A/Indiana/08/2011 (H3N2) v	2.03×10^{2} $TCID_{50}$/mL	3/3
H5N1*	A/mallard/Wisconsin/ 2576/2009 H5N1	1.05×10^{5} GE/mL	3/3
	A/bovine/Ohio/B24OSU-439/2024 H5N1	3.67×10^{5} GE/mL	3/3

Flu A Subtypes	Pathogens	Concentration	Results (#Pos/Total)
H5N6	A/duck/Guangxi/S10888/2024	6.76×10^5 EID_{50}/mL	3/3
H5N8	A/goose/Liaoning/S1266/2021	$3.38x10^5$ EID_{50}/mL	3/3
H7N3	A/northern pintail/Illinois/10OS3959/2010	3.55×10^6 $CEID_{50}$/mL	3/3

** Strains were gamma-irradiated prior to wet-testing*

Table 12: Inclusivity Results for influenza B Strains

Flu B Subtypes	Pathogens	Concentration	Results (#Pos/Total)
non-Victoria non-Yamagata	B/Maryland/1/1959	1.69×10^3 $CEID_{50}$/mL	3/3
Victoria	B/Austria/1359417/2021	7.05×10^2 $TCID_{50}$/mL	3/3
	B/Michigan/01/2021	5.70×10^3 $TCID_{50}$/mL	3/3
	B/New Hampshire/01/2021	6.50×10^2 $TCID_{50}$/mL	3/3
	B/Washington/02/2019	7.90×10^2 $TCID_{50}$/mL	3/3
	B/Texas/02/2013	6.13×10^0 $TCID_{50}$/mL	3/3
Yamagata	B/Phuket/3073/2013	2.75×10^5 $CEID_{50}$/mL	3/3
	B/Florida/04/2006	1.17×10^1 $TCID_{50}$/mL	3/3
	B/Texas/06/2011	8.00×10^5 $CEID_{50}$/mL	3/3
	B/Utah/09/2014	1.26×10^2 $TCID_{50}$/mL	3/3

Table 13: Inclusivity Results for RSV strains

RSV Subtypes	Pathogens	Concentration ($TCID_{50}$/mL)	Results (#Pos/Total)
RSV A	A2023/06-12NSMM	7.00×10^2	3/3
	A1998/3-2	4.00×10^3	3/3
	A2001/2-20	1.40×10^4	3/3
	2013 Isolate	1.41×10^1	3/3
	12/2014 Isolate 2	1.17×10^3	3/3
	3/2015 Isolate #3	2.98×10^2	3/3
RSV B	3/2015 Isolate #1	5.05×10^1	3/3
	B1	8.00×10^2	3/3
	CH93(18)-18	3.15×10^2	3/3
	11/2014 Isolate #2	8.88×10^1	3/3
	12/2014 Isolate #1	1.25×10^2	3/3
	3/2015 Isolate #2	1.26×10^3	3/3

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 candidate 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 between November 2024 and November 2025 to assess the performance of the candidate test when compared to an FDA cleared RT-PCR assay. The study prospectively enrolled symptomatic subjects at 23 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 6 days post symptom onset (DPSO).

Testing was performed in a simulated home environment. Two anterior nasal (AN) swab specimens were collected from each participant. One swab was collected by a healthcare professional, placed into a transport tube containing universal transport media, and shipped on dry ice to a central laboratory for testing using a highly sensitive RT-PCR comparator assay. The other swab was collected according to the candidate test's QRI: either self-collected by a lay user aged ≥14 years or collected by an adult (parent/guardian) from individuals aged ≥6 months. This swab was tested immediately on-site using the iHealth Flu A&B/COVID-19/RSV Rapid Test. The collection order for the investigational and comparator AN swab was randomized.

Out of 1154 enrolled subjects, 35 subjects were excluded resulting in 1119 evaluable subjects. The demographics of the evaluable subjects are shown below:

Table 14: Subject Demographics

Specific Demographic	Lay user collecting and testing	Self-collecting and testing	Overall
	(N=684)	(N=435)	(N=1119)
Age			
Mean (SD)	5.5 (8.4)	49.6 (22.7)	22.5 (26.6)
Median [Min, Max]	3 [0.5, 91]	51 [14, 92]	9 [0.5, 92]
Age Group			
6-11 months	103 (100.0%)	0 (0.0%)	103 (9.2%)
12-17 months	85 (100.0%)	0 (0.0%)	85 (7.6%)
18-24 months	78 (100.0%)	0 (0.0%)	78 (7.0%)
2-5 years	180 (100.0%)	0 (0.0%)	180 (16.1%)
6-14 years	217 (93.5%)	15 (6.5%)	232 (20.7%)
15-59 years	14 (6.0%)	221 (94.0%)	235 (21.0%)
≥60 years	7 (3.4%)	199 (96.6%)	206 (18.4%)
Sex at Birth			
Female	338 (55.9%)	267 (44.1%)	605 (54.1%)
Male	346 (67.3%)	168 (32.7%)	514 (45.9%)

The performance of the candidate test when compared to FDA-cleared highly sensitive RT-PCR molecular assays are presented in the tables below.

Table 15: SARS-CoV-2 Performance

	Comparator Positive	Comparator Negative	Total
Candidate Positive	67	1	68
Candidate Negative	8	1043	1051
Total	75	1044	1119
Positive Percent Agreement (PPA)	89.3% (67/75) (95% C.I.: 80.3%- 94.5%)		
Negative Percent Agreement (NPA)	99.9% (1043/1044) (95% C.I.: 99.5% - 100%)		

Table 16: SARS-CoV-2 Performance by DPSO

DPSO	Candidate Positives	Comparator Positives	PPA
Day 0	1	2	50.0%
Day 1	12	13	92.3%
Day 2	15	17	88.2%
Day 3	22	24	91.7%
Day 4	8	9	88.9%
Day 5	5	5	100%
Day 6	4	5	80.0%
Total	**67**	**75**	**89.3%**

Table 17: Influenza A Performance

	Comparator Positive	Comparator Negative	Total
Candidate Positive	205	4	209
Candidate Negative	29	881	910
Total	234	885	1119
Positive Percent Agreement (PPA)	87.6% (205/234) (95% C.I.: 82.8% - 91.2%)		
Negative Percent Agreement (NPA)	99.5% (881/885) (95% C.I.: 98.8%- 99.8%)		

Table 18: Influenza B Performance

	Comparator Positive	Comparator Negative	Total
Candidate Positive	36	0	36
Candidate Negative	3	1080	1083
Total	39	1080	1119
Positive Percent Agreement (PPA)	92.3% (36/39) (95% C.I.: 79.7% - 97.3%)		
Negative Percent Agreement (NPA)	100% (1080/1080) (95% C.I.: 99.6% - 100.0%)		

Table 19: RSV Performance

	Comparator Positive	Comparator Negative	Total
Candidate Positive	149	12	161
Candidate Negative	21	937	958
Total	170	949	1119
Positive Percent Agreement (PPA)	87.6% (149/170) (95% C.I.: 81.9% - 91.8%)		
Negative Percent Agreement (NPA)	98.7% (937/949) (95% C.I.: 97.8% - 99.3%)		

Table 20: RSV Performance Stratified by Age Group

Age group	Number of Subject samples tested	Candidate Positives	Comparator Positives	PPA (95% CI)
6-24 months	266	66	74	89.1% (80.0%-94.4%)
>2-5 years	180	41	47	87.2% (74.8%-94.0%)
6-14 years	232	19	21	90.4% (71.0%-97.3%)
15-59 years	235	14	19	73.6% (51.2%-88.1%)
>=60 years	206	9	9	100.0% (70.0%-100.0%)
Total	**1119**	**149**	**170**	**87.6% (81.8%, 91.7%)**

2. Usability/ User Comprehension Study

In order to evaluate the overall usability and user comprehension of the candidate 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 candidate test to assess lay user performance and comprehension of the QRI in a simulated home environment. A total of 56 subjects participated. Of these, 29 subjects aged ≥14 years self-collected nasal swab specimens and performed self-testing using the candidate device. In addition, adult-collected swabs were collected from 27 subjects (including 3 subjects 14-24 years of age, 20 subjects >24-64 years of age and 4 subjects ≥ 65 years of age) 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 candidate test, study personnel (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 21: Critical vs Non-Critical Tasks Correctly Performed

Test Procedures (critical or non-critical step) performed by lay users	Tasks performed correctly	Total No. of tasks performed	Percentage of tasks performed correctly
Check test expiration date (critical)	56	56	100%
Swab both nostrils 5 times (critical)	56	56	100%
Insert swab into the extraction buffer (critical)	56	56	100%

Test Procedures (critical or non-critical step) performed by lay users	Tasks performed correctly	Total No. of tasks performed	Percentage of tasks performed correctly
Stir the swab more than 10 times (critical)	56	56	100%
Squeeze both sides of the tube applying pressure on both sides of the tube while removing the swab (critical)	56	56	100%
Seal the tube securely with the nozzle cap (critical)	56	56	100%
Add less than 5 drops to the sample well (critical)	56	56	100%
Read the test between 15 and 30 minutes (critical)	56	56	100%
Wash or sanitize hands prior to testing (non-critical)	56	56	100%
Remove swab from packaging without touching swab head (non-critical)	56	56	100%
Hold the tube upright above the sample well (non-critical)	56	56	100%
Add more than 5 drops to the sample well (non-critical)	56	56	100%

All subjects (100%) followed the QRI and IFU and performed critical tasks correctly, meeting the acceptance criteria. 99.8% of the subjects indicated that the QRI and IFU were clear and easy to use. 96% of subjects answered the knowledge assessment (labeling comprehension test) correctly, meeting the acceptance criteria. These results shows that the labeling effectively conveys critical information necessary for safe and proper candidate device operation.

Study 2: Alongside the additional clinical study, a supplemental usability study was conducted to evaluate users' ability to interpret various results and follow appropriate next steps, identify procedural errors, and assess the safety and difficulty of collecting nasal samples from infants aged 6–24 months—an aspect not evaluated in Study 1. A total of 113 participants were enrolled, including 12 elderly adults and 101 young children aged 6–24 months. For each infant, parents or guardians performed nasal sample collection following the QRI and completed a questionnaire.

Similar to Study 1, in Study 2 the observer documented on an observation form whether each lay user experienced any difficulty using the test kit or performed any step incorrectly. The table below summarizes the assessed steps (both critical and non-critical procedures) based on the observer's evaluations.

Table 22: Critical vs Non-Critical Tasks Correctly Performed

Test Procedures (critical or non-critical step) performed by lay users	Tasks performed correctly	Total No. of tasks performed	Percentage of tasks performed correctly
Check test expiration date (critical)	113	113	100%
Swab both nostrils 5 times (critical)	113	113	100%
Insert swab into the extraction buffer (critical)	113	113	100%
Stir the swab more than 10 times (critical)	113	113	100%

Test Procedures (critical or non-critical step) performed by lay users	Tasks performed correctly	Total No. of tasks performed	Percentage of tasks performed correctly
Squeeze both sides of the tube applying pressure on both sides of the tube while removing the swab (critical)	113	113	100%
Seal the tube securely with the nozzle cap (critical)	113	113	100%
Add less than 5 drops to the sample well (critical)	113	113	100%
Read the test between 15 and 30 minutes (critical)	113	113	100%
Wash or sanitize hands prior to testing (non-critical)	113	113	100%
Remove swab from packaging without touching swab head (non-critical)	113	113	100%
Hold the tube upright above the sample well (non-critical)	113	113	100%
Add more than 5 drops to the sample well (non-critical)	113	113	100%

All subjects (100%) followed the QRI and IFU and performed critical tasks correctly, meeting the acceptance criteria. Nearly all users reported that sample collection and result interpretation were easy and could be performed with confidence. While most comments were very positive, seven users noted suggestions for improvement (e.g., suggestions included adding a depth indicator on the swab, shortening the wait time, offering multiple tests per pack, providing an extra swab, clarifying steps for swab handling, and offering smaller swabs and sampling guidance for infants and toddlers.)

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 6-24 months. The overall evaluation of the lay user experience did not raise any concerns regarding the usability of the candidate device.

3. Lay User Readability Study

A readability study of the candidate test was conducted with 56 lay users from the human factors study, representing varied ages, sexes, and educational backgrounds. Of these, 23 participants (41.07%) reported vision impairments, including 21 with nearsightedness or farsightedness, one with cataracts, and one who used reading glasses. Subjects interpreted a panel of mock devices with various results that reflected the test concentration level at negative, invalid, low positive (1.5x LoD) and moderate positive (5x LoD) in a blinded fashion. Each panel of mock tests included 24 different results with various negative, positive, and invalid results for each analyte. 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. The overall accuracy of the results interpreted by the lay users with and without vision impairment, is 100% (1344/1344).

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 evaluate the robustness of the test and the risk of false results when users deviate from the IFU/QRI instructions, flex studies were performed to assess all major aspects of the test procedure. These included variations in sample volume, reading time, and other procedural deviations (e.g., delays in mixing, uneven mixing, delays in adding the sample to the well, and incubation time). The studies also assessed variability in environmental conditions that the test may encounter during use (e.g., lighting, disturbances during use, temperature and humidity stress, inadequate temperature equilibration, and delayed use after opening the foil pouch), as well as result interpretation by a colorblind user.

A test panel consisting of 5 negative samples (PNF only) and 5 low-positive samples (2x LoD in PNF) for each analyte was evaluated under varying conditions using the candidate device. The strains tested included SARS-CoV-2 Omicron variant JN.1, influenza A H1N1, influenza B Victoria, and RSV B. Samples were blinded and randomized for testing. All results were acceptable except in the following scenarios: false-negative results occurred when the test was read at 3 or 5 minutes, or when the swab was not mixed in the extraction buffer tube. Invalid results were obtained when only one drop of sample was applied to the candidate device. The labeling clearly instructs users on these critical steps and provides adequate warnings to mitigate misuse. Therefore, the flex study results support that the test is robust when used as instructed and demonstrate an insignificant risk of erroneous results.

VIII Proposed Labeling

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

IX Conclusion

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