510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY

I Background Information:

A 510(k) Number
K250377

B Applicant
ACON Laboratories, Inc.

C Proprietary and Established Names
Flowflex Plus COVID-19 + Flu A/B Home Test

D Regulatory Information

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

II Submission/Device Overview:

A Purpose for Submission:
To obtain 510(k) clearance for the for the Flowflex Plus COVID-19 + Flu A/B Home Test.

B Measurand:
Protein antigen from Influenza A/B and SARS-CoV-2

C Type of Test:
Qualitative lateral flow immunoassay

III Intended Use/Indications for Use:

A Intended Use(s):
See Indications for Use below.

B Indication(s) for Use:

The Flowflex Plus COVID-19 + Flu A/B Home Test is a lateral flow immunoassay intended for the qualitative detection and differentiation of SARS-CoV-2, influenza A, and influenza B protein antigens directly in anterior nasal swab samples from individuals with signs and symptoms of respiratory tract infection. Symptoms of respiratory infections due to SARS-CoV-2 and influenza can be similar.

This test is for non-prescription home use with self-collected anterior nares nasal swab specimens from individuals aged 14 years or older, or with adult-collected anterior nasal swab specimens from individuals two (2) years or older.

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 therefore seek follow-up care from their healthcare provider.

Positive results do not rule out co-infection with other respiratory pathogens and therefore do not substitute for a visit to a healthcare provider or appropriate follow-up.

C Special Conditions for Use Statement(s):

OTC - Over The Counter

D Special Instrument Requirements:

N/A

IV Device/System Characteristics:

A Device Description:

The Flowflex Plus COVID-19 + Flu A/B Home Testis a lateral flow immunoassay in sandwich format intended for the qualitative detection and differentiation of SARS-CoV-2, Influenza A, and Influenza B protein antigens.

This test is for non-prescription home use (OTC) with self-collected anterior nares nasal swab specimens from symptomatic individuals aged 14 years or older, or with adult-collected anterior nasal swab specimens from symptomatic individuals 2 years or older.

The test package is composed of:

- Test Cassettes
- Extraction Buffer Tubes
- Tube Holder
- Disposable Nasal Swabs
- Quick Reference Instructions

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

Test strip structure:

The test strip in the plastic housing contains the following mounted on a card backing: a nitrocellulose membrane with three test lines (T), and one control line (C). The test lines are each pre-coated with one virus specific antibody (SARS-CoV-2, Flu A or Flu B) that binds one of the three analytes detected by the test. The control line is pre-coated with a control IgG antibody.

Attached to the nitrocellulose membrane are the sample pad that absorbs and distributes the sample, the reagent pad with the reagents necessary for binding and detecting the viral antigens, and an absorbent pad that absorbs the remaining liquid sample and reagents. The reagent pad contains virus specific monoclonal antibodies conjugated with colored particles (SARSCoV-2 antibody conjugates, Flu A antibody conjugates, Flu B antibody conjugates) and a labeled control antibody (IgG).

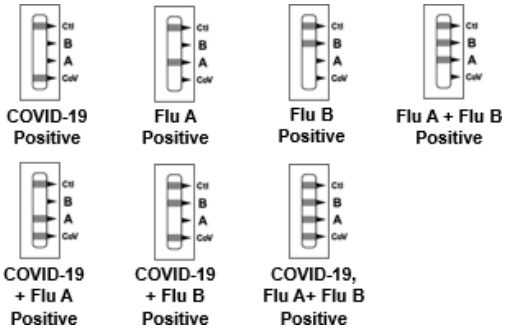
B Principle of Operation:

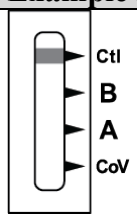
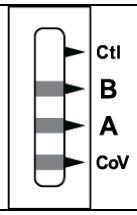
To perform the test, collect a nasal swab by lay user, process the swab in the extraction buffer, add the processed specimen to the sample well of the test cassette. When an adequate volume of the processed specimen is added to the sample well of the test cassette, the specimen migrates by capillary action across the test strip. The antigen of SARS-CoV-2, Flu A or Flu B, if present in the specimen, will react with the specific antibody-coated colored particles, the mixture then migrates toward the membrane by capillary action to form sandwich binding to the specific antibody on the nitrocellulose membrane with a visible colored test line in the related test line region (CoV/A/B).

To serve as procedural control, the colored Rabbit IgG labeled particle will bind to Goat Anti-Rabbit IgG on nitrocellulose membrane to form a colored Control line (Ctl) in the control line region. Formation of the Control line 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.

Result	Interpretation	Example Image
Positive	If the Control line is visible and any other line or multiple lines at "CoV", "A" and/or "B" appear, the test is positive.	

Result	Interpretation	Example Image
Negative	If the Control line is visible, but the Test (CoV/A/B) line is not visible, the test is negative. Repeat testing is needed for all samples that are negative for COVID-19 on the first day of testing, even if they are positive for Flu A and/or B	
Invalid	If the Control line is not visible, the test is not valid. Re-test with a new swab and a new test cassette.	

I Substantial Equivalence Information:

A Predicate Device Name(s):

QuickFinder COVID-19/Flu Antigen Self Test / QuickFinder COVID-19/Flu Antigen Pro Test

B Predicate 510(k) Number(s):

K243262

C Comparison with Predicate(s):

Device & Predicate Device(s):	K250377	K243262
Device Trade Name	Flowflex Plus COVID-19 + Flu A/B Home Test	QuickFinder COVID-19/Flu Antigen Self Test / QuickFinder COVID-19/Flu Antigen Pro Test
General Device Characteristic Similarities		
Intended Use/ Indications For Use	The Flowflex Plus COVID-19 + Flu A/B Home Test is a lateral flow immunoassay intended for the qualitative detection and differentiation of SARS-CoV-2, influenza A, and influenza B protein antigens directly in anterior nasal swab samples from individuals with signs and symptoms of respiratory tract infection. Symptoms of respiratory infections due to SARS-CoV-2 and influenza can be similar. This test is for non-prescription home use with self-collected	***QuickFinder COVID-19/Flu Antigen Self Test****:* QuickFinder COVID-19/Flu Antigen Self 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

	anterior nares nasal swab specimens from individuals aged 14 years or older, or with adult-collected anterior nasal swab specimens from individuals two (2) years or older. 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 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.	influenza can be similar. This test is for non-prescription home use by individuals aged 14 years or older testing themselves, or adults testing individuals aged 2 years or older. All negative results are presumptive and should be confirmed with an FDA-cleared molecular assay when determined to be appropriate by a healthcare provider. Negative results do not rule out infection with influenza, SARS-CoV-2 or other pathogens. Individuals who test negative and experience continued or worsening respiratory symptoms, such as fever, cough and/or shortness of breath, should seek follow-up 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. ***QuickFinder COVID-19/Flu Antigen Pro Test:*** The QuickFinder COVID-19/Flu Antigen Pro 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 protein 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 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 provider.
Regulation	21 CFR 866.3987	Same
General Device Characteristic Differences		
Patient Use	Over the counter use/self-testing	Same
Intended Specimen	Direct anterior nasal swabs	Same
Usage Type	Single-use test	Same
Assay Technique	Immunochromatographic Assay, visual-read	Same
Components of Test Kit	Test Cassettes, Extraction Buffer Tubes, Disposable Nasal Swabs, Quick Reference Instructions	Same
Test Result	Qualitative	Same
Antigen Specificity	SARS-CoV-2, influenza A, and influenza B protein antigens	Same
Sample Collection Method	Nasal swab supplied in the kit	Same
Reading Time	15 - 30 minutes	Same
Storage Temperature	36-86 °F (2- 30°C)	Same

V 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 8663987.pdf	FDA/CDRH	All Studies
ISO11135:2014	Sterilization of health care products - Ethylene oxide - Requirements for development, validation and routine control of a sterilization process for medical devices	ISO	Sterility
ISO 10993-7	Biological Evaluation of Medical Devices – Part 7: Ethylene Oxide Sterilization Residuals	ISO	Sterility

VI Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/Reproducibility:

The precision study for the Flowflex Plus COVID-19 + Flu A/B Home Test was evaluated in two different studies conducted at single internal site each using three lots of test kits and three trained operators.

Study 1 was conducted using a panel of 27 test samples that were freshly prepared in the morning of each testing day. The 27 samples were composed of various combinations of the three virus analytes detected by the test (influenza A Brisbane/02/18 (H1N1), influenza B Washington/02/19 (Victoria), and SARS-CoV-2 (USA-WA1/2020)), including triple negative, single, double, and triple analyte positive combinations. Each sample was tested daily over 10 days with 1 replicate / run × 2 runs/operator × 3 operators. While the operators in the study each tested with three different lots and generated a total of 180 replicate results for each analyte and concentration, the randomization approach described below was targeted to generate the same number of 60 replicates for each operator, but not the same number of replicates for each lot.

Three analyte concentrations, negative, low positive (1.5x LoD) and positive (5x LoD), were tested for each of the three viruses and the concentrations were randomized across the 27 samples with varying analyte combinations. To efficiently blind operators towards the sample results, testing of these samples was further randomized across the two runs per day, the three independent test lots used in the study, and the three operators.

Results for this study are summarized in the Table below. Since the study obtained 100% agreement for all samples, both with and without analytes, the data was not stratified by the individual sources of variation.

Table 1: Summary results of multi-lot precision study

Sample level	Analyte	Obtained results per analyte / Expected results per analyte	Total percent lot-to-lot agreement	95% CI
Negative	SARS-CoV-2	(180/180)	100%	98.0-100%
	Flu A	(180/180)	100%	98.0-100%
	Flu B	(180/180)	100%	98.0-100%
1.5xLoD	SARS-CoV-2	(180/180)	100%	98.0-100%
	Flu A	(180/180)	100%	98.0-100%
	Flu B	(180/180)	100%	98.0-100%
5xLoD	SARS-CoV-2	(180/180)	100%	98.0-100%
	Flu A	(180/180)	100%	98.0-100%
	Flu B	(180/180)	100%	98.0-100%

Study 2 was specifically conducted to further evaluate potential differences 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 a negative sample without any of the analytes, and four 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):

(1) Negative
(2) FluA + FluB + SARS-CoV2
(3) FluA + FluB
(4) Flu B + SARS-CoV2
(5) FluA + SARS-CoV2

Unlike the first study, randomization was targeted towards providing the same number of replicate test results for each analyte of the test and its concentration per lot. Three operators tested the samples above in a randomized and blinded manner each using three different lots of the Flowflex Plus COVID-19 + Flu A/B Home Test in a total of 2 runs/operators and across 2 non-consecutive days (i.e., 1 replicate/run × 2 runs/day × 2 days × 3 operators = 12 sample replicates/lot). Considering the co-spiking of the analytes in the four positive test samples above (i.e., each virus is present in three of the low positive samples but not present in the 4th positive and the negative sample), the testing would at most generate 72 positive and 36 negative test results for each analyte and concentration. Results from this testing are summarized in Table 3 below.

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 significant impact on the precision estimates of a device for very low positive samples below the LoD. At the same time, these concentrations yield results with less than 100% agreement that reveals variability between different, independently manufactured lots.

Overall, the results were in 100% agreement between expected and read result within run, by lot, by operator, by day, and overall, for all concentration tested except for low positive samples (0.75x LoD). The results are summarized below.

Table 2. Summary - supplemental precision study (analyte specific results combined from all samples)

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	12/12	100%	12/12	100%	12/12	100%	36/36	100%	90.4-100%
	Flu A	12/12	100%	12/12	100%	12/12	100%	36/36	100%	90.4-100%
	Flu B	12/12	100%	12/12	100%	12/12	100%	36/36	100%	90.4-100%
0.75X LoD	SARS-CoV-2	18/24	75%	19/24	79%	14/24	58%	51/72	71%	58.9-81.0%
	Flu A	17/24	71%	18/24	75%	19/24	79%	54/72	75%	63.4-84.5%
	Flu B	17/24	71%	17/24	71%	18/24	75%	52/72	72%	60.4-82.1%

[1] n/N = Obtained results per analyte / Expected results per analyte
[2] Agmt = Agreement;

2. Linearity:

N/A (The device is a qualitative assay with binary visually-read 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 viruses, bacteria, fungi, and pooled nasal wash were used for these studies.

For the cross-reactivity study, dilutions of the panel organisms were made in pooled nasal swab matrix (PNSM) and tested in triplicates in the absence of SARS-CoV-2, influenza A, and influenza B. No cross-reactivity was observed with the organisms tested for any of the 3 analytes (Table 3).

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 (USA-WA1/2020), influenza A (H1N1; A/Brisbane/02/18), and influenza B (Victoria; B/Washington/02/19) and tested in triplicate. No microbial interference was observed for any of the 3 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]
Human coronavirus 229E	2.09×10^5	$TCID_{50}$/mL	0/3	3/3
Human coronavirus OC43	2.09×10^5	$TCID_{50}$/mL	0/3	3/3
Human coronavirus NL63	1.75×10^5	$TCID_{50}$/mL	0/3	3/3
Human coronavirus HKU1 Specimen #B18438368	16.794	Ct value	0/3	3/3
Human coronavirus HKU1 Specimen #B18378425	13.713	Ct value	0/3	3/3
Human coronavirus HKU1 Specimen #B18421181	19.775	Ct value	0/3	3/3
MERS-coronavirus	1.05×10^5	$TCID_{50}$/mL	0/3	3/3
Adenovirus 1	2.82×10^6	$TCID_{50}$/mL	0/3	3/3
Adenovirus 7	1.15×10^6	$TCID_{50}$/mL	0/3	3/3
Human metapneumovirus 27	3.80×10^5	$TCID_{50}$/mL	0/3	3/3
Parainfluenza virus 1	3.80×10^5	$TCID_{50}$/mL	0/3	3/3
Parainfluenza virus 2	3.39×10^6	$TCID_{50}$/mL	0/3	3/3
Parainfluenza virus 3	1.15×10^6	$TCID_{50}$/mL	0/3	3/3
Parainfluenza virus 4	9.55×10^5	$TCID_{50}$/mL	0/3	3/3
Enterovirus	1.95×10^4	$TCID_{50}$/mL	0/3	3/3
Respiratory syncytial virus	2.09×10^5	$TCID_{50}$/mL	0/3	3/3
Rhinovirus	1.00×10^5	$TCID_{50}$/mL	0/3	3/3
Haemophilus influenzae	3.87×10^7	CFU/mL	0/3	3/3
Streptococcus pneumoniae	7.22×10^7	CFU/mL	0/3	3/3
Streptococcus pyogenes	4.60×10^8	CFU/mL	0/3	3/3
Candida albicans	1.31×10^7	CFU/mL	0/3	3/3
Bordetella pertussis	1.16×10^9	CFU/mL	0/3	3/3
Mycoplasma pneumoniae	2.70×10^7	CFU/mL	0/3	3/3

Virus/Microorganism	Concentration	Units	# of Positive results / # of Replicates tested	
			Cross-reactivity[1]	Interference[1]
Chlamydia pneumoniae	1.4×10^7	IFU/mL	0/3	3/3
Chlamydia trachomatis	3.52×10^8	IFU/mL	0/3	3/3
Legionella pneumophila	3.27×10^9	CFU/mL	0/3	3/3
Mycobacterium tuberculosis	1.21×10^7	CFU/mL	0/3	3/3
Pneumocystis jiroveci-Saccharomyces cerevisiae recombinant	1.65×10^7	CFU/mL	0/3	3/3
Staphylococcus aureus	9.23×10^8	CFU/mL	0/3	3/3
Staphylococcus epidermidis	6.84×10^8	CFU/mL	0/3	3/3
Pooled Negative Nasal Wash	N/A	N/A	0/3	3/3
Corynebacterium jeikeium	5.18×10^7	CFU/mL	0/3	3/3
Escherichia coli	7.17×10^8	CFU/mL	0/3	3/3
Lactobacillus salivarius	5.15×10^8	CFU/mL	0/3	3/3
Moraxella catarrhalis	5.92×10^7	CFU/mL	0/3	3/3
Neisseria meningitidis	2.04×10^7	CFU/mL	0/3	3/3
Neisseria mucosa	1.49×10^8	CFU/mL	0/3	3/3
Neisseria sicca	9.55×10^8	CFU/mL	0/3	3/3
Pseudomonas aeruginosa	4.93×10^8	CFU/mL	0/3	3/3
Streptococcus salivarius	1.35×10^8	CFU/mL	0/3	3/3
Measles	1.23×10^7	$TCID_{50}$/mL	0/3	3/3
Mumps	4.78×10^6	$TCID_{50}$/mL	0/3	3/3
Epstein Barr Virus	2.94×10^6	CP/mL	0/3	3/3
Cytomegalovirus	1.00×10^5	$TCID_{50}$/mL	0/3	3/3

[1] All analytes in the sample replicates had the same results

b. Competitive Interference:

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 (2x LoD) and high concentrations (66x- 2000x LoD) of SARS-CoV-2, influenza A, and influenza B. The study used inactivated SARS-CoV-2 but live influenza A and B virus strains. There was no competitive interference observed.

Table 4: Competitive inhibition study results

Test combo	Viral targets in sample			Results (# pos / total reps)		
	Influenza A	Influenza B	SARS-CoV-2	Influenza A	Influenza B	SARS- CoV-2
1	High	Low	Negative	3/3	3/3	0/3
2	High	Negative	Low	3/3	0/3	3/3
3	Low	High	Negative	3/3	3/3	0/3
4	Negative	High	Low	0/3	3/3	3/3
5	Low	Negative	High	3/3	0/3	3/3
6	Negative	Low	High	0/3	3/3	3/3
7	High	Low	Low	3/3	3/3	3/3
8	Low	High	Low	3/3	3/3	3/3

Test combo	Viral targets in sample			Results (# pos / total reps)		
	Influenza A	Influenza B	SARS-CoV-2	Influenza A	Influenza B	SARS- CoV-2
9	Low	Low	High	3/3	3/3	3/3

c. Endogenous/Exogenous Substances Interference:

The Flowflex Plus COVID-19 + Flu A/B Home Test 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 PNSM to the recommended concentration. Virus negative PNSM specimens were evaluated in triplicate to confirm that the potentially interfering substances were not cross-reactive with the test. Positive samples were prepared in PNSM at 3x LoD using each analyte individually and in combination together (co-spiked). 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, and influenza B. No interference was observed amongst the substances tested herein.

Table 5. 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	SARS-CoV-2	Flu A	Flu B	Co-Spiked
Beclomethasone	5 mg/mL	0/3	0/3	0/3	3/3	3/3	3/3	3/3
Biotin	3500 ng/mL	0/3	0/3	0/3	3/3	3/3	3/3	3/3
Dexamethasone	5 mg/mL	0/3	0/3	0/3	3/3	3/3	3/3	3/3
Dyclonine Hydrochloride	1.5 mg/mL	0/3	0/3	0/3	3/3	3/3	3/3	3/3
FluMIST (Influenza Vaccine Live, Intranasal)	1.5% ~ 15% v/v	0/3	**3/3**	**3/3**	NT*	NT*	NT*	3/3
	0.75% v/v	0/3	**3/3**	0/3	NT*	NT*	NT*	3/3
	0.375% v/v	0/3	0/3	0/3	NT*	NT*	NT*	3/3
Flunisolide	5 mg/mL	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	3/3	3/3	3/3	3/3
Hand Soap	15% v/v	0/3	0/3	0/3	3/3	3/3	3/3	3/3
Homeopathic Allergy Relief (histaminum hydrochloricum)	15% w/v	0/3	0/3	0/3	3/3	3/3	3/3	3/3
Homeopathic nasal wash	15% v/v	0/3	0/3	0/3	3/3	3/3	3/3	3/3
Leukocytes	4.8×10^6 cells/mL	0/3	0/3	0/3	3/3	3/3	3/3	3/3
Molnupiravir	10 mg/mL	0/3	0/3	0/3	3/3	3/3	3/3	3/3
Mucin	2.5 mg/mL	0/3	0/3	0/3	3/3	3/3	3/3	3/3
Mupirocin	10 mg/mL	0/3	0/3	0/3	3/3	3/3	3/3	3/3
Nasal corticosteroids (Budesonide)	15% v/v	0/3	0/3	0/3	3/3	3/3	3/3	3/3
Nasal corticosteroids (fluticasone furate)	5% v/v	0/3	0/3	0/3	3/3	3/3	3/3	3/3
Nasal corticosteroids (fluticasone propionate)	5% v/v	0/3	0/3	0/3	3/3	3/3	3/3	3/3
Nasal corticosteroids (Mometasone furoate)	15% v/v	0/3	0/3	0/3	3/3	3/3	3/3	3/3
Nasal corticosteroids (Triamcinolone Acetonide)	15% v/v	0/3	3/3	3/3	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	SARS-CoV-2	Flu A	Flu B	Co-Spiked
Nasal gel	15% v/v	0/3	0/3	0/3	3/3	3/3	3/3	3/3
Nasal Spray (Cromolyn Sodium)	15% v/v	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	3/3	3/3	3/3	3/3
Nasal Spray (Phenylephrine HCl)	15% v/v	0/3	0/3	0/3	3/3	3/3	3/3	3/3
Nasal Wash (Saline nasal wash: sodium chloride & preservatives)	15% v/v	0/3	0/3	0/3	3/3	3/3	3/3	3/3
Oral Anesthetic Cough Lozenge (Menthol)	3 mg/mL	3/3	3/3	3/3	3/3	3/3	3/3	3/3
Oseltamivir Phosphate	5% w/v	3/3	3/3	3/3	3/3	3/3	3/3	3/3
Remdesivir	10 mg/mL	3/3	3/3	3/3	3/3	3/3	3/3	3/3
Sore Throat & Cough Lozenges (Benzocaine, Dextromethorphan HBr)	3 mg/mL	3/3	3/3	3/3	3/3	3/3	3/3	3/3
Sore Throat Spray (Phenol)	5% w/v	3/3	3/3	3/3	3/3	3/3	3/3	3/3
Tobramycin	50 µg/mL	3/3	3/3	3/3	3/3	3/3	3/3	3/3
Whole Blood	2.5%	3/3	3/3	3/3	3/3	3/3	3/3	3/3
Zanamivir	5 mg/mL	3/3	3/3	3/3	3/3	3/3	3/3	3/3
ZICAM Nasal Decongestant, Cold Remedy Nasal Spray (Galphimia glauca, Luffa operculata, Sabadilla)	15% v/v	3/3	3/3	3/3	3/3	3/3	3/3	3/3

*NT: not tested

4. Assay Reportable Range:

N/A, 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 needed to indicate the test device is functioning properly and to ensure 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 'C'-Line of the test membrane in the device and captures leftover, unbound IgG complexes to generate a color signal at the 'C-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 is being conducted to evaluate stability and determine the shelf-life of the unopened kit. To validate shelf life for room temperature storage (i.e., 15-30 °C), three unopened Flowflex Plus COVID-19 + Flu A/B Home Test kit lots are stored at 2-8 °C and 30±3 °C. At defined intervals, an assessment of each lot is conducted with the following panel of test samples: negative clinical matrix, and co-spiked positive samples with inactivated SARS-CoV-2, live Flu A or Flu B viruses, each at 3x LoD.

Fifty (50) µL of each sample are applied to the swab and tested according to the Instructions for Use. Five replicates of each sample are tested for each time point.

Baseline testing was performed within one month of each manufactured lot. Subsequent time testing time points are 3, 6, 9, 12, 15, 18, 21, and 24 months. Testing is ongoing and will continue for 27 months to target a final shelf-life claim of 24 months. At the time of clearance, all study data have met the protocol defined acceptance criteria, and support storage of the test kits at room temperature (15-30 °C) for up to 21 months. The shelf life will be updated as additional passing time points will become available.

ii. Shipping Stability:

Transport stability under simulated summer and winter shipping conditions was tested to evaluate worst-case shipping and handling conditions. Performance of unopened test kits was assessed by comparing pre- (T0) and post-distribution (Td) results, using the same sample panel described for the real-time stability study (above). Samples were tested in replicates of five for each of three 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 40 ºC for 13 hours then moved to 21 ºC for 4 hours.
- To mimic winter shipping conditions, test kits were incubated at -10 ºC for 13 hours and then moved to 18 ºC for 4 hours.

All results were as expected for all time points and support shipping at temperatures up to 40 ºC.

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 SARS-CoV-2 (USA-WA1/2020, heat-inactivated), two strains of live influenza A (H1N1 and H3N2), and two strains of influenza B (Victoria and Yamagata) at which at least 95% of all true positive replicates return a positive result. Testing was conducted on three lots of test devices.

A preliminary LoD was first determined by testing serial 10-fold dilutions of virus stocks diluted in PNSM in five replicates per device lot for a total of 15 replicates per dilution. A 50-µL sample of each virus diluted in PNSM was pipetted onto the dry swab. The swab was

then tested per the instructions for use. 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	3.80×10^5	1.90×10^4	15/15
	3.80×10^4	1.90×10^3	15/15
	3.80×10^3	**1.90×10^2**	**15/15**
	3.80×10^2	1.90×10^1	0/15
Influenza A H1N1	1.51×10^5	7.55×10^3	15/15
	1.51×10^4	7.55×10^2	15/15
	1.51×10^3	**7.55×10^1**	**15/15**
	1.51×10^2	7.55	0/15
Influenza A H3N2	**5.62×10^3**	**2.81×10^2**	**15/15**
	5.62×10^2	2.81×10^1	0/15
Influenza B Victoria	1.55×10^3	7.55×10^1	15/15
	1.55×10^2	7.55	15/15
	1.55×10^1	**0.755**	**15/15**
	1.55	0.075	0/15
Influenza B Yamagata	1.86×10^3	9.3×10^1	15/15
	1.86×10^2	9.3	15/15
	1.86×10^1	**0.93**	**15/15**
	1.86	0.093	0/15

* positive replicates per concentration were combined from all lots because all were detected for all lots.

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

Table 7: Confirmatory single analyte LoD results

Virus strain	Virus concentration		Positive replicates
	$TCID_{50}$/mL	**$TCID_{50}$/Swab**	
SARS-CoV-2	1.14×10^4	5.70×10^2	60/60
	3.80×10^3	1.90×10^2	60/60
	1.27×10^3	**6.35×10^1**	**60/60**
Influenza A H1N1	4.53×10^3	2.27×10^2	60/60
	1.51×10^3	**7.55×10^1**	**60/60**
	5.03×10^2	2.52×10^1	0/60
Influenza A H3N2	1.69×10^4	8.45×10^2	60/60
	5.62×10^3	2.81×10^2	60/60
	1.87×10^3	**9.35×10^1**	**60/60**
Influenza B Victoria	4.65×10^1	2.33	60/60
	1.55×10^1	0.755	60/60
	5.17	**0.259**	**60/60**

Virus strain	Virus concentration		Positive replicates
	$TCID_{50}$/mL	$TCID_{50}$/Swab	
Influenza B Yamagata	5.58×10^1	2.79	60/60
	1.86×10^1	**0.93**	**60/60**
	6.20	0.31	14/60

* positive replicates per concentration were combined from all lots because all were detected for all lots.

b. Co-spiked multi-analyte LoD:

After single analyte LoDs were determined, co-spike equivalency testing was conducted to characterize the performance of samples that contained all analytes at their respective 1x LoD concentrations. Based on individual analyte LoDs, 1x co-spiked samples were prepared by mixing viruses (one each of SARS-CoV-2, Flu A, and Flu B) in PNSM.

Overall, 21 replicates were prepared by pipetting 50 µL of co-spiked sample onto the dry swab and testing swabs with the device according to the instructions for use. Equivalency is confirmed separately for each analyte if ≥95% replicates are positive within 2x LoD of the individually tested analyte.

The Flowflex Plus COVID-19 + Flu A/B Home Test demonstrated co-spike equivalency for SARS- CoV-2, Influenza A and Influenza B at 1x single analyte LoD. This study supports the use of co-spiked samples in subsequent analytical studies.

Table 8: Summary of Co-spike equivalency LoD study results

Virus	Fold LoD	Concentration ($TCID_{50}$/mL)	Concentration ($TCID_{50}$/Swab)	# Positive replicates
SARS-CoV-2 (USA-WA1/2020)	1x	1.27×10^3	6.35×10^1	21/21
Flu A H1N1 (A/Brisbane/02/18)	1x	1.51×10^3	7.55×10^1	
Flu B Victoria (B/Washington/02/19)	1x	5.17	0.259	

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

The LoD of the Flowflex Plus COVID-19 + Flu A/B Home Test was also determined by evaluating different dilutions of the International Standard for SARS-CoV-2 antigen (NIBSC code: 32/368) in negative pooled nasal swab matrix. The International Standard for SARS-CoV-2 containing lyophilized SARS-CoV-2 antigen was reconstituted in 0.25 mL of ultra-pure water (for a final concentration of 20,000 IU/mL). The LoD was determined as the lowest virus concentration that was detected ≥95% of the time (i.e., concentration at which at least 19/20 replicates tested positive).

Ten-fold serial dilutions were made from the International Standard for SARS-CoV-2 antigen into negative clinical matrix (pooled nasal swab matrix). Five replicates were tested on three lots of the test device for each dilution to determine the preliminary LoD concentration of the device. The lowest concentration with 15/15 positive results from all three lots 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 for each of the three lots of the test device, for a total of 25 replicates per lot, to confirm the LoD.

Concentrations above and below the preliminary LoD were also tested with 20 replicates per lot of test device to further refine the LoD. Samples were prepared as for the preliminary LoD study above. To confirm the LoD, at least 19 of 20 replicates should be positive per lot. The results are summarized in Table below.

Table 9: Summary of LoD study for the international standard

Testing	Concentration (IU/mL)	Concentration on dry swab (IU/Swab)	# Positive replicates
Preliminary LoD (10-fold dilutions)	2.00×10^3	1.00×10^2	15/15
	2.00×10^2	**1.00×10^1**	**15/15**
	2.00×10^1	1.00	0/15
Confirmatory LoD	6.00×10^2	3.00×10^1	60/60
	2.00×10^2	**1.00×10^1**	**60/60**
	6.67×10^1	3.34	22/60

The LoD for the Flowflex Plus COVID-19 + Flu A/B Home Test using the 1st International Standard for SARS-CoV-2 antigen (NIBSC code: 21/368) in nasal matrix was determined to be 2.00×10^2 IU/mL (10 IU/swab).

7. High Dose Hook Effect:

A high-dose hook effect study was conducted to evaluate whether high levels of any of the target analytes in a sample could result in a false-negative test result. Fifty (50) µL of the highest viral stock concentration possible was spiked onto swabs and swabs processed in accordance with the instructions for use. Analytes were tested individually and co-spiked, and all samples were tested in triplicates. No evidence of a high-dose hook effect was observed with the virus stocks and concentrations tested.

Table 10: High-dose hook effect study results

Sample	Concentration ($TCID_{50}$/mL)	Results (# pos / total reps)		
		SARS- CoV-2	Influenza A	Influenza B
Single analyte samples				
SARS-CoV-2	3.80×10^6	3/3	0/3	0/3
Influenza A (H1N1)	1.51×10^6	0/3	3/3	0/3
Influenza B (Victoria)	1.55×10^4	0/3	0/3	3/3
Co-spiked samples				
SARS-CoV-2	1.27×10^6	3/3	3/3	3/3
Influenza A (H1N1)	5.03×10^5			
Influenza B (Victoria)	5.17×10^3			

8. Inclusivity:

Analytical reactivity testing for the Flowflex Plus COVID-19 + Flu A/B Home Test was performed to ensure that the device can adequately detect a variety of strains for the SARS-CoV-2, influenza A, and influenza B viruses. A selection of temporally, geographically, and genetically diverse SARS-CoV-2 and influenza strains were tested for inclusivity, including 29 Influenza A strains (14 H1N1 and 11 H3N2, 2 H5N1, 1 H5N8, and 1 H7N3), 15 Influenza B strains (10 Yamagata and 5 Victoria lineages), and 26 SARS-CoV-2 strains (22 Omicron, 1 Delta, 1 Alpha, 1 P.1, and 1 B.1.1.7). A series of ten-fold dilutions of each virus strain was spiked into PNSM and tested to determine an approximate LoD of the test for each virus. The

lowest concentration with 100% positive replicates was identified and additional 3-fold dilutions above and below that approximate LoD were tested to demonstrate inclusivity. Based on the dilution series, the minimum detectable concentration was defined as the lowest concentration for which all five replicates were detected. Results are summarized below and demonstrate that the test tests can detect the analytes across a range of viral strains.

Table 11: Inclusivity results for SARS-CoV-2 variants

SARS-CoV-2 Subtype/ Lineage	**Virus strain**	**Lowest concentration 100% positive results ($TCID_{50}$/mL)**
B1.1.529, Omicron	SARS-Cov-2 (B.1.1.529 Omicron) *USA/MD-HP20874/2021*	5.01×10^2
B.1.17, Alpha	SARS-Cov-2 (B.1.1.7 Alpha)	4.20×10^2
B.1.351, South Africa	SARS-Cov-2 (B.1.351 South Africa)	1.05×10^3
B.1.617.2 Delta	SARS-Cov-2 (B.1.617.2 Delta)	1.05×10^3
BA. 2.3, Omicron	SARS-Cov-2 (BA.2.3 Omicron)	7.34×10^2
Brazil, P.1	SARS-Cov-2 (Brazil P.1)	1.26×10^3
B.1.1.529, Omicron	USA/CO-CDPHE- 2102544747/2021	4.17×10^2
BA.2.12.1, Omicron	USA/NY-Wadsworth-22014351- 01/2022	1.26×10^3
BA.2.75.5, Omicron	USA/NY-Wadsworth-22037154- 01/2022	1.70×10^2
BA.4.6, Omicron	USA/MD-HP35538/2022	1.15×10^3
BA.5, Omicron	USA/COR-22-063113/2022	2.53×10^3
BA.5.5, Omicron	USA/NY-Wadsworth-22023478- 01/2022	1.56×10^2
BF.5, Omicron	USA/MD-HP34985/2022	2.93×10^3
BF.7, Omicron	USA/NY-Wadsworth-22042128- 01/2022	1.26×10^3
BQ.1, Omicron	USA/NY-Wadsworth-22050462-01/2022	1.43×10^3
BQ.1.1, Omicron	USA/MD-HP38861/2022	2.93×10^2
BQ.1.16, Omicron	USA/NY-Wadsworth-22050865-01/2022	2.19×10^3
BQ.1.3, Omicron	USA/NY-Wadsworth-22047869-01/2022	3.20×10^3
XBB, Omicron	USA/CA-Stanford-109_S21/2022	1.98×10^4
JN.1, Omicron	USA/New York/PV96109/2023	1.05×10^3
JN.1.4 (Omicron)	USA/NY-Wadsworth-23068107-01/2023	5.43×10^2
EG.5.1 (Omicron)	USA/MD-HP47946/2023	1.22×10^5
JG.3 (Omicron)	USA/NY-Wadsworth-23067147-01/2023	2.63×10^4
XBB.1.5 Omicron	USA/NY-Wadsworth-22061020-01/2022	1.15×10^4
HV.1 Omicron	USA/MD-HP49152/2023	8.73×10^3

Table 12: Inclusivity results for influenza A variants

Influenza A Subtype/ Lineage	**Virus strain**	**Lowest concentration 100% positive results ($TCID_{50}$/mL)**
H1N1	A/California/07/09	1.38×10^4
	A/Guangdong Maonan/ SWL/1536/19	1.39×10^4
	A/Kumamoto/102/02	1.05×10^4
	A/Mexico/4108/09 (pdm)	2.41×10^3
	A/Michigan/45/15	6.20×10^2
	A/New Caledonia/20/99	1.67×10^3
	A/New York/18/09 (pdm)	1.41×10^2
	A/Puerto Rico/8/34	1.67×10^3
	A/Solomon Islands/03/06	5.62×10^1

Influenza A Subtype/ Lineage	Virus strain	Lowest concentration 100% positive results ($TCID_{50}$/mL)
	A/Taiwan/42/06	4.57×10^3
	A/Victoria/4897/22	3.24×10^2
	A/Baltimore/JH-22400/2022	8.9×10^4
	A/Connecticut/11/2023	9.3×10^2
	A/Wisconsin/67/22	1.16×10
H3N2	A/Brisbane/10/07	4.17×10^2
	A/Hong Kong/2671/19	5.67×10^2
	A/Kansas/14/17	3.80×10^3
	A/Norway/466/14	1.23×10^4
	A/Singapore/INFIMH-16-0019/16	1.67×10^3
	A/Texas/50/12	1.18×10^3
	A/Victoria/361/11	1.80×10^3
	A/Wisconsin/67/05	4.70×10^2
	A/Michigan/173/20	5.20×10^4
	A/Cambodia/E0826360/20	1.17×10^3
	A/Tasmania/503/20	1.41×10^4
H5N1	A/Chicken/Liaoning/SD007/2017	1.3×10^4
	A/Vietnam/1194/2004	1.1×10^4
H5N8	Inactivated IAV H5N8	1.1×10^3
H7N3	A/waterfowl/Chenhu/367-1/2021	5.0×10^3

Table 13: Inclusivity results for Influenza B variants

Influenza B Subtype/ Lineage	Virus strain	Lowest concentration 100% positive results ($TCID_{50}$/mL)
Victoria	B/Alabama/2/17	1.39×10^2
	B/Lee/40	1.26×10^3
	B/Malaysia/2506/04	1.27×10^3
	B/Michigan/01/21	3.87×10^4
	Austria/1359417/21	9.40×10^2
Yamagata	B/Panama/45/90	3.80×10^3
	B/Florida/02/06	4.70×10^1
	B/Florida/04/06	3.55×10^1
	B/Massachusetts/2/12	4.20×10^2
	B/Texas/6/11	2.41×10^2
	B/Utah/9/14	1.27×10^3
	B/Victoria/504/00	4.20×10^2
	B/Wisconsin/1/10	1.87×10^1
	B/Yamagata/16/88	4.70×10^1
	B/Brisbane/9/14	4.20×10^2

9. Assay Cut-Off:

N/A 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:

   The Flowflex Plus COVID-19 + Flu A/B Home Test is only intended for use with direct anterior nasal swab specimens. As no other sample types are claimed for use with the Flowflex Plus COVID-19 + Flu A/B Home Test, a matrix comparison study is not applicable.

   However, the sponsor performed a matrix equivalency study between pooled negative nasal swab matrix (PNSM) and the surrogate matrix (1X PBS with 0.5% mucin) that was used in some analytical studies. The data demonstrated equivalent performance of the test with both matrices.

C Clinical Studies:

1. Clinical Performance Study

   A prospective lay person clinical study was conducted from December 2022 to March 2024 to assess the performance of the Flowflex Plus COVID-19 + Flu A/B Home Test when compared to a 510(k)-cleared SARS-CoV-2 RT-PCR assay with an extraction step. The study prospectively enrolled symptomatic subjects at 10 clinical study sites located in the United States. Enrolled subjects were aged 2 years or older who exhibited symptoms of respiratory infection consistent with COVID-19 or influenza at the time of collection and who were within 7 days post symptom onset (DPSO).

   Testing was performed in a simulated home environment. A nasopharyngeal swab was collected first by the study operator, placed into transport tube containing Universal transport media, refrigerated, and shipped to a central lab for testing with a highly sensitive RT-PCR comprator assay. Thereafter, an anterior nasal swab (ANS) was collected per the candidate test's QRI. The ANS were either self-collected by a lay user aged ≥14 years or collected by an adult (parent/ guardian) from individuals aged 2 to <14 years. Samples were then immediately tested with the Flowflex Plus COVID-19 + Flu A/B Home Test according to the test summary instructions.

   A total of 741 subjects were enrolled of which 720 subjects within 5 days post symptom onset were evaluable for analysis. Of these 720 subjects 4 subjects were excluded only from the SARS-CoV-2 analyses because they were invalid (n=2) or inconclusive (n=2) when tested with the comparator method. Hence, for SARS-CoV-2 only 716 study subjects were evaluable. However, samples from these 4 study subjects generated valid comparator results for Flu A and B and were therefore retained in the influenza A/B analysis.

   Results obtained with the Flowflex Plus COVID-19 + Flu A/B Home Test were compared to the results obtained with highly sensitive RT-PCR comparator tests giving rise to the following performance estimates:

a. SARS-CoV-2 Performance:

Table 14: Clinical performance for SARS-CoV-2

	Comparator positive	Comparator negative	Total
Candidate positive	160	4	164
Candidate negative	17	535	552
Total	177	539	716*
Positive Percent Agreement (PPA) = 90.4% (95% CI = 85.1 to 94.3%)			
Negative Percent Agreement (NPA) = 99.3% (95% CI = 98.4 to 99.9%)			

* 4 samples excluded due to invalid/inconclusive results with comparator methods.

Table 15: SARS-CoV-2 performance stratified by date of symptom onset (DPSO)

DPSO	Candidate positive	Comparator positive	PPA
Day 0	4	6	66.7%
Day 1	39	42	92.9%
Day 2	47	52	90.4%
Day 3	50	53	94.3%
Day 4	16	19	84.2%
Day 5	4	5	80%

b. Influenza A Performance:

Table 16: Clinical performance for influenza A

	Comparator positive	Comparator negative	Total
Candidate positive	76	1	77
Candidate negative	7	636	643
Total	83	637	720
Positive Percent Agreement (PPA) = 91.6% (95% CI = 83.4 to 96.5%)			
Negative Percent Agreement (NPA) = 99.8% (95% CI = 99.1 to 100%)			

c. Influenza B Performance:

Table 17: Clinical performance for influenza B

	Comparator positive	Comparator negative	Total
Candidate positive	44	2	46
Candidate negative	3	671	674
Total	47	673	720
Positive Percent Agreement (PPA) = 93.6% (95% CI = 82.5 to 98.7%)			
Negative Percent Agreement (NPA) = 99.7% (95% CI = 98.9 to 99.9%)			

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

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

2. Usability Study:

Concurrent with the clinical study, the sponsor evaluated usability of the Flowflex Plus COVID-19 + Flu A/B Home Test and user comprehension of the test's Quick Reference Instructions (QRI). All 741 individuals, regardless of whether they were evaluable for analysis in the clinical study, were included in the usability and user comprehension study. Each subject filled out a usability questionnaire as part of their visit and interpreted mock test results in the user comprehension (readability) study. Demographics of the enrolled subjects are summarized in table below.

Table 18: Demographics of human factors assessment subjects

	Lay user collecting and testing (N=153)	**Self-collecting and testing (N=588)**	**Overall (N=741)**
Age			
Mean (SD)	7.3 (3.2)	44.6 (17.9)	36.9 (22.0)
Median [Min, Max]	7 [2, 13]	44 [14, 91]	36 [2, 91]
Age Group			
≥2 - <14 years of age	153 (100%)	0 (0%)	153 (20.6%)
14-24 years of age	0 (0%)	88 (15%)	88 (11.9%)
25-64 years of age	0 (0%)	407 (69%)	407 (54.9%)
≥65 years of age	0 (0%)	93 (16%)	93 (12.6%)
Sex at Birth			
Female	68 (44%)	333 (57%)	401 (54%)
Male	85 (56%)	255 (43%)	340 (46%)
Ethnicity			
Hispanic/Latino	53 (35%)	155 (26%)	208 (28%)
Not Hispanic/Latino	100 (65%)	433 (74%)	533 (72%)
Race			
Black or African American	4 (2.6%)	67 (11.4%)	71 (9.6%)
Asian	89 (58%)	197 (34%)	286 (38.6%)
White	3 (2%)	155 (26.4%)	158 (21.3%)
Other	4 (2.6%)	14 (2.4%)	18 (2.4%)
Education Level			
	Lay users (n=741)		
Some High School	32 (4.3%)		
High school diploma	522 (70%)		
College	142 (19.2%)		
Bachelor's Degree	27 (4%)		
Master's Degree	7 (1%)		
Professional Degree	11 (1.5%)		
Total	(100%)		

All subjects in the study received the QRI prior to performing the test. For the human factors assessment, the study personnel or a healthcare provider observed and evaluated the subject/tester's ability to correctly perform the test and interpret the results by evaluating critical and non-critical tasks during the testing process as shown below in table format. The study personnel did not provide any training, or assistance to the subjects/testers. Overall, 100% of all critical and non-critical tasks associated with sample collection and running the Flowflex Plus COVID-19 + Flu A/B Home Test were performed correctly.

Table 19: Critical and non-critical tasks correctly performed

Steps	Tasks performed correctly	Total number of tasks	Percentage of tasks performed correctly
Critical	5928	5928	100%
Non-critical	2964	2964	100%
Sum	8892	8892	100%

Table 20: List of critical and non-critical tasks performed

Task description*	Criticality
Wash hands	Non Critical
Remove the test cassette from the pouch. Locate the Result Window and Sample Well on the cassette	
Remove tube from pouch. Remove the foil from tub	
Punch through perforation on box to form a tube holder. Place tube in hold	
Remove swab from packaging at the stick end	Critical
Swab both nostrils, at least 5 times in each nostril	
Insert swab into the extraction buffer.	
Swirl the swab 5 times while squeezing the tube	
Squeeze the tube while removing the swab	
Attach dropper tip and mix by swirling or flicking tube	
Gently squeeze and put 4 drops into Sample Well. Discard tube	
Set timer and read result after 15 minutes. Do not read after 30 minutes. Discard test cassette	

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

3. User Comprehension / Readability Assessment

A readability study was conducted with a total of 61 subjects. Subjects interpreted a mock panel of investigational test results and thereafter, completed a labeling and comprehension questionnaire. The mock panel contained results for various analyte combinations and analyte concentrations. Mock samples were prepared with analytes at concentrations of 2x LoD and 5x LoD. All 61 lay users interpreted the mock results in a blinded and randomized fashion. Each lay user interpreted four random mock samples to avoid a training effect. After the lay user interpreted the results, the study personnel also interpreted and recorded the test result. Overall, the study demonstrated that lay user participants can accurately read and interpret the test as 98.9% of the 5x LoD samples were accurately read. As expected for a visually read test, false negative results occur more frequently for very low positive samples with faint test lines for which the accuracy dropped to 92.1%.

D Clinical Cut-Off:

N/A. The candidate device is a qualitative assay with a visually read binary result without numeric raw data.

E Expected Values/Reference Range:

A patient sample is expected to be negative for SARS-CoV-2, influenza A and influenza B. This section is therefore not applicable.

F Other Supportive Information:

1. Flex Studies:

   To assess the robustness and risk for false results of the test when deviating from the IFU/QRI test steps, flex studies were conducted that assessed all major aspects of the test procedure (extraction buffer volume, reading time, procedure involving sample port [swab insertion, rotation, and removal], sample hold time before and during processing) and variability of environmental test conditions that the test may be subjected to when in use (non-level surface, lighting, disturbance during use, temperature and humidity stress conditions). In these studies, a test panel comprised of 3-5 negative samples (PNSM only), 3-5 low positive samples (2x co-spike LoD with all analytes in PNSM), and 3-6 low positive individual samples (2x single-analyte LoD in PNSM) were tested under various flex conditions with the candidate device. The strains used for testing were SARS-CoV-2 USA-WA1/2020, Influenza A H1N1, and Influenza B Victoria. Samples were blinded and randomized for testing.

   False results were observed with 5-minute reading time, specifically with less than 110 µL (5-6 drops) of extraction buffer added onto the swab inserted into the sample port and read times of five and fifty minutes. To assess the robustness and risk for false results of the test when deviating from the IFU/QRI test steps, flex studies were conducted that assessed all critical aspects of the test procedure. A test panel comprised of 3-5 negative samples (PNSM only), 3-5 low positive samples (2x co-spike LoD with all analytes in PNSM), and 3-6 low positive individual samples (2x single-analyte LoD in PNSM) were tested upon varying the conditions during testing with the candidate device. The strains used for testing were SARS-CoV-2 USA-WA1/2020, Influenza A H1N1, and Influenza B Victoria. Samples were blinded and randomized for testing.

   Extreme deviations from the instructions, such as sample and extraction buffer, critical operating conditions (temperatures >30 °C) and operational steps (e.g., swirling and removing the swab from the buffer vial), and timed test steps (reading times) can impact the test results of samples, and result in false negative or invalid results. However, the labeling is clear in its instructions and includes adequate warnings at these critical steps that mitigate the risk that such extreme deviations occur.

   In contrast, minor deviations in these conditions do not impact the accuracy of the results. The studies therefore support that the test is robust when used as instructed with an insignificant risk of erroneous result.

2. Variant Monitoring Plan:

   ACON will conduct ongoing monitoring for new and emerging SARS-CoV-2 viral mutations and variants and assess the impact of the mutations and variants that have been identified as prevalent and/or clinically significant to the performance of the Flowflex Plus COVID-19 +Flu A/B Home Test. The variant monitoring plan provided as part of this submission is acceptable.

VII Proposed Labeling:

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

VIII Conclusion:

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