510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY

I Background Information:

A 510(k) Number

K233373

B Applicant

Acon Laboratories, Inc.

C Proprietary and Established Names

Flowflex Plus COVID-19 Home Test

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
QYT	Class II	21 CFR 866.3984 - Over-The-Counter Test To Detect SARS-CoV-2 From Clinical Specimens	Microbiology

II Submission/Device Overview:

A Purpose for Submission:

To obtain 510(k) clearance for the Flowflex plus COVID-19 Home Test.

B Measurand:

Nucleocapsid protein antigen from SARS-Coronavirus 2 (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 Home Test is a visually read lateral flow immunoassay device intended for the rapid, qualitative detection of SARS-CoV-2 virus nucleocapsid protein antigen directly in anterior nasal swab specimens from individuals with signs and symptoms of COVID-19 within the first 6 days of symptom onset.

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.

The Flowflex Plus COVID-19 Home Test does not differentiate between SARS-CoV and SARS-CoV-2.

All negative results are presumptive. Symptomatic individuals with an initial negative test result must be re-tested once between 48 and 72 hours after the first test using either an antigen test or a molecular test for SARS-CoV-2. Negative results do not preclude SARS-CoV-2 infections or other pathogens and should not be used as the sole basis for treatment.

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

This test is not a substitute for visits to a healthcare provider or appropriate follow-up and should not be used to determine any treatments without provider supervision. Individuals who test negative and experience continued or worsening COVID-19 like symptoms, such as fever, cough and/or shortness of breath, should seek follow up care from their healthcare provider.

The performance characteristics for SARS-CoV-2 were established during June 2022 to April 2023 when COVID-19 variant Omicron was dominant. Test accuracy may change as new SARS-CoV-2 viruses emerge. Additional testing with a lab-based molecular test (e.g., PCR) should be considered in situations where a new virus or variant is suspected.

C Special Conditions for Use Statement(s):

OTC - Over The Counter

D Special Instrument Requirements:

Not Applicable

IV Device/System Characteristics:

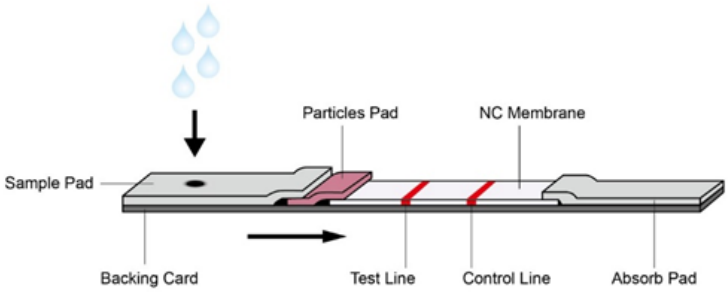
A Device Description:

The Flowflex plus COVID-19 Home Test is a lateral flow immunoassay device intended for the qualitative detection of the SARS-CoV-2 nucleocapsid protein antigen. This device is intended for non-prescription use with self-collected anterior nasal swab samples from individuals 14 years or older or adult collected anterior nasal swab samples from individuals 2 years or older with symptoms of COVID-19 within 6 days of symptom onset.

The test package comprises the following components:

- Test Cassettes
- Extraction Buffer Tubes
- Tube holder
- Disposable Nasal Swabs
- Package Insert

The test cassette is assembled with a test strip in a plastic housing. The test strip consists of: (1) particle pad, containing SARS-CoV-2 antibody conjugated with colored particles (SARS-CoV-2 Antibody conjugates) and biotin-labeled, colored particles for the formation of the control line; (2) a nitrocellulose membrane containing a test line (T line) and a control line (C line). The test line is pre-coated with SARS-CoV-2 antibody, and the control line is pre-coated with mixture of goat anti-mouse antibody and streptavidin. Excess liquid and reagents are absorbed by the absorb pad.



The extraction buffer tube contains extraction buffer comprising of surfactant, salt and buffer solution. Disposable swabs are sterile swabs for specimen collection.

B Principle of Operation:

To perform the test, a nasal swab is collected by self (age ≥14 years) or another lay user (age ≥14 years), the swab is processed in the extraction buffer and then the processed specimen is added to the sample well of the test cassette. When an adequate volume of the processed specimen is added to the sample well (S) of the test cassette, the specimen migrates by capillary action across the test strip. SARS-CoV-2 antigens, if present in the specimen, will react with the colored SARS-CoV-2 antibody-coated particles in the particle pad, the mixture then migrates over the nitrocellulose membrane towards the absorbent pad by capillary action and forms sandwich complexes by binding to the anti-SARS-CoV-2 antibody immobilized on the test line (T) region of the nitrocellulose membrane. A visible colored test line in the test line region (T) appears.

To serve as a procedural control, the colored Biotin particles from the particle pad will bind to streptavidin immobilized at the control line (C) of the nitrocellulose membrane and the colored mouse monoclonal antibody labeled particle will bind to goat anti-mouse antibodies on nitrocellulose membrane to form a colored Control line 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.

Results should be read at 15 minutes after the addition of the processed specimen to the sample well. A false negative or false positive result may occur if the test result is read before 15 minutes or after 30 minutes.

V Substantial Equivalence Information:

A Predicate Device Name(s):

Cue COVID-19 Molecular Test

B Predicate 510(k) Number(s):

DEN220028

C Comparison with Predicate(s):

Device & Predicate Device(s):	K233373	DEN220028
Device Trade Name	Flowflex Plus COVID-19 Home Test	Cue COVID-19 Molecular Test
General Device Characteristic Similarities		
Intended Use/Indications For Use	The Flowflex Plus COVID-19 Home Test is a visually read lateral flow immunoassay device intended for the rapid, qualitative detection of SARS-CoV-2 virus nucleocapsid protein antigen directly in anterior nasal swab specimens from individuals with signs and symptoms of COVID 19 within the first 6 days of symptom onset. 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. The Flowflex Plus COVID-19 Home Test does not differentiate between SARS-CoV and SARS-CoV-2. All negative results are presumptive. Symptomatic individuals with an initial negative test result must be re-tested once between 48 and 72 hours after the first test using either an antigen test or a molecular test for SARS-CoV-	The Cue COVID- 19 Molecular Test is a nucleic acid amplification assay that is used with the Cue Health Monitoring System (Cue Cartridge Reader) for the rapid, qualitative detection of SARS-CoV-2 nucleic acid directly in anterior nasal swab specimens from individuals with signs and symptoms of COVlD-1 9 (i.e., symptomatic). A negative test result is presumptive, and it is recommended these results be confirmed by a lab based molecular SARS-CoV-2 assay if necessary for patient management. Negative results do not preclude SARS-CoV-2 infections and should not be used as the sole basis for treatment. Positive results do not rule out co-infection with other respiratory pathogens. This test is not a substitute for visits to a healthcare provider or appropriate follow-up and should not be used to determine any treatments without provider

	2. Negative results do not preclude SARS-CoV-2 infections or other pathogens and should not be used as the sole basis for treatment. Positive results do not rule out co-infection with other respiratory pathogens. This test is not a substitute for visits to a healthcare provider or appropriate follow-up and should not be used to determine any treatments without provider supervision. Individuals who test negative and experience continued or worsening COVID-19 like symptoms, such as fever, cough and/or shortness of breath, should seek follow up care from their healthcare provider. The performance characteristics for SARS-CoV-2 were established during June 2022 to April 2023 when COVID-19 variant Omicron was dominant. Test accuracy may change as new SARS-CoV-2 viruses emerge. Additional testing with a lab-based molecular test (e.g., PCR) should be considered in situations where a new virus or variant is suspected.	supervision. This test is intended to be sold over-the-counter (OTC) for testing of individuals 18 years of age and older.
Regulation Number	21 CFR 866.3984	Same
Analyte	COVID-19	Same
Intended Use Population	Individuals with symptoms of COVID-19	Same
Intended Use Setting	Over the counter use/self-testing	Same
Usage Type	Single use test	Same
Test Result Type	Qualitative	Same
Format	Test cartridge	Same
Specimen Type	Direct Anterior swab specimen	Same

General Device Characteristic Differences		
Analyte	SARS-CoV-2 nucleocapsid protein antigen	SARS-CoV-2 nucleic acid
Test Principle	Lateral flow Immunoassay	Nucleic acid amplification assay
Antigen Specificity	SARS-CoV-2 nucleocapsid protein antigen	SARS-CoV-2 nucleocapsid (N) region of the gene
Instrument Needed	No	Yes
Development Type	15-30 min	20 min
Result Interpretation	Visually read	Instrument read
Utilizes App to Display results	Yes	No
Storage Temperature	2-30°C	59°F (15°C) to 86°F (30°C)

The Flowflex Plus COVID-19 Home Test employs a lateral flow immunoassay design requiring a visual read of the test strip for manual interpretation of the test results by the lay user. In contrast, the Cue Molecular Test (predicate device) is a molecular nucleic acid amplification test that is intended to detect genetic material from SARS-CoV-2 virus in nasal swab specimen from individuals with signs and symptoms of COVID-19 (i.e., symptomatic). Despite the differences in methodology, both the proposed and predicate devices fall under the generic name, over the counter test to detect SARS-CoV-2 from clinical specimens, according to the Classification order for 21 CFR 866.3984.

An over-the counter test to detect SARS-CoV-2 from clinical specimens is an in vitro diagnostic device for the detection of SARS-CoV-2 in clinical specimens to aid in the diagnosis of SARS-CoV-2 infection. The device is intended to be used by lay users and without required health care provider (HCP) intervention in home settings or similar environments in which lay users perform testing.

The key difference is the methodology used in the Cue COVID-19 Molecular Test which is a nucleic acid amplification assay used with the Cue Monitoring System (Cue Cartridge Reader). A Benefit/Risk analysis provided for ACON's previously cleared device K230828 (similar to the candidate device), was leveraged to address the differences of the candidate device when compared to the predicate. Consistent with the FDA Guidance Document "*Benefit-Risk Factors to Consider When Determining Substantial Equivalence in Premarket Notifications (510(k)) with Different* Technological Characteristics | FDA.", the Agency determined that **these differences of the candidate device compared to the predicate do not affect the overall substantial equivalence of the proposed device to the predicate device in terms of intended use, safety and effectiveness**.

VI Standards/Guidance Documents Referenced:

Document Number	Title	Publishing Organization	Applicable Study
21 CFR 866.3984	DEN220028/ Special controls for Over- the-counter test to detect SARS-CoV-2 from clinical specimens	FDA	All Studies
21 CFR Part 50	Protection of Human Subjects	CFR	Clinical Studies
21 CFR Part 51	Institutional Review Boards	CFR	Clinical Studies
21 CFR Part 812	Investigational Device Exemptions	CFR	Clinical Studies
FDA Guidance	The 510(k) Program: Evaluating Substantial Equivalence in Premarket Notifications [510(k)]	FDA	Substantial Equivalence
21 CFR 809.10	Labeling for in vitro Diagnostic Products	CFR	Labeling

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Multi-lot Precision:

The purpose of this study was to assess lot-lot-lot variability of the Flowflex plus COVID-19 Home Test device using three independently manufactured device lots. Three levels of heat-inactivated SARS-CoV-2 virus were spiked into surrogate matrix as follows:

1. Negative Sample
2. Low positive sample at 1.5x LoD
3. Positive Sample at 5x LoD

Matrix equivalency study was conducted to demonstrate the equivalency of the surrogate matrix and the clinical nasal swab matrix.

On each day, each operator was given 18 samples for a 1st run in the morning (AM) and another 18 testing samples for a 2nd run in the afternoon (PM). Samples were randomized and blinded. The study was conducted for 10 non-consecutive days during the study.

Each operator applied 50 µL of each coded sample to a dry nasal swab first, processed the sample and performed the test following the IFU of the proposed device. Each operator ran 2 replicates per sample level both in the morning and in the afternoon for all three lots for 10 non-consecutive days (i.e., 3 lots x 3 operators x 2 replicates/run x 3 panels x 2 runs x 10 days).

The obtained results were in 100% agreement with expected results across all lots, operators and days. Thus, no variability was observed between three independently manufactured lots.

The results are summarized in **Table 1** below:

Table 1: Summary of Multi Lot Precision Study

Lot #	Negative	Low Positive (1.5x LoD)	Positive (5x LoD)
A21122201N (Lot 1)	120/120	120/120	120/120
23071404 (Lot 2)	120/120	120/120	120/120
23071805 (Lot 3)	120/120	120/120	120/120
% Agreement [95% CI]	100% [99%-100%]	100% [99%-100%]	100% [99%-100%]

2. Linearity:

Not Applicable. This device is a qualitative visually read assay.

3. Analytical Specificity/Interference:

a. Cross-Reactivity/Microbial Interference:

The analytical specificity of the Flowflex Plus COVID-19 Home Test was evaluated by testing various commensal and pathogenic microorganisms in the absence (cross-reactivity) and presence (microbial interference) of SARS-CoV-2 at 3X LoD.

Cross-reactivity and microbial interference were conducted simultaneously, and the samples were tested in a randomized and blinded manner. The results are summarized in **Table 2** below.

Table 2: Summary of Cross-reactivity and Microbial Interference Study

Microorganisms*	Concentration Tested	Cross-reactivity-Positive/Total Tested	Interference-Positive/Total Tested
Adenovirus	2.82×10^6 $TCID_{50}$/mL	0/3	3/3
Bordetella pertussis	1.16×10^9 CFU/mL	0/3	3/3
Candida albicans	1.31×10^7 CFU/mL	0/3	3/3
Chlamydia pneumonia	1.4×10^7 IFU/mL	0/3	3/3
Chlamydia trachomatis	3.52×10^8 IFU/mL	0/3	3/3
Enterovirus	1.0×10^5 $TCID_{50}$/mL	0/3	3/3
Haemophilus influenzae	3.87×10^7 CFU/mL	0/3	3/3
Human coronavirus 229E	2.09×10^5 $TCID_{50}$/mL	0/3	3/3
Human coronavirus HKU1 (specimen # B18378425)	Ct 13.713	0/3	3/3
Human coronavirus HKU1 (specimen # B18431603)	Ct 20.298	0/3	3/3
Human coronavirus HKU1 (specimen # B18427097)	Ct 24.609	0/3	3/3

Microorganisms*	Concentration Tested	Cross-reactivity-Positive/Total Tested	Interference-Positive/Total Tested
Human coronavirus HKU1 (specimen # B18431601)	Ct 21.305	0/3	3/3
Human coronavirus HKU1 (specimen # B18438368)	Ct 16.794	0/3	3/3
Human coronavirus HKU1 (specimen # B18421181)	Ct 19.775	0/3	3/3
Human coronavirus NL63	1.78×10^5 $TCID_{50}$/mL	0/3	3/3
Human coronavirus OC43	2.09×10^5 $TCID_{50}$/mL	0/3	3/3
Human Metapneumovirus	1.78×10^5 $TCID_{50}$/mL	0/3	3/3
Influenza A	1.51×10^5 $TCID_{50}$/mL	0/3	3/3
Influenza B	3.62×10^5 $TCID_{50}$/mL	0/3	3/3
Legionella pneumophila	3.27×10^9 CFU/mL	0/3	3/3
MERS-coronavirus	1.05×10^5 $TCID_{50}$/mL	0/3	3/3
Mycobacterium tuberculosis	1.21×10^7 CFU/mL	0/3	3/3
Mycoplasma pneumoniae	2.70×10^7 CCU/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
Pneumocystis jirovecii -S. cerevisiae recombinant	1.65×10^7 CFU/mL	0/3	3/3
Pooled human nasal cavity wash	N/A	0/3	3/3
Pseudomonas aeruginosa	4.93×10^8 CFU/mL	0/3	3/3
Respiratory Syncytial Virus	2.09×10^5 $TCID_{50}$/mL	0/3	3/3
Rhinovirus	1.0×10^5 $TCID_{50}$/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
Streptococcus pneumoniae	7.22×10^7 CFU/mL	0/3	3/3
Streptococcus pyogenes	4.60×10^8 CFU/mL	0/3	3/3

*All organisms tested were full organism.

Clinically relevant microorganisms, in the absence of SARS-CoV-2 were tested in 3 replicates on the Flowflex Plus COVID-19 Home Test. Based on the test results above, no cross reactivity was observed with these microorganisms when tested at the concentration presented in **Table 2**.

Clinically relevant microorganisms in the presence of SARS-CoV-2 were tested in 3 replicates on the Flowflex Plus COVID-19 Home Test. The study results show that the microorganisms tested at the concentration listed in **Table 2** do not interfere with the results of Flowflex Plus COVID-19 Home Test when SARS-CoV-2 virus is present in a specimen.

In silico analysis was performed to test the cross-reactivity of SARS coronavirus with SARS-CoV-2 as wet testing was not available. Sequence homology between the SARS-CoV-2 nucleocapsid protein and the structural proteins of SARS-CoV was about 91.5%. Thus, there is a high probability of cross-reactivity between the nucleocapsid proteins of SARS-CoV-2 and

SARS-CoV. Therefore, the following statement is added in the "Intended Use" and "Limitations" section of the package insert of Flowflex Plus COVID-19 Home Test.

> *"The Flowflex Plus COVID-19 Home Test does not differentiate between SARS-CoV and SARS-CoV-2."*

Thus, this serves as an adequate risk mitigation to potential cross-reactivity between SARS-CoV and SARS-CoV-2.

b. Interfering Substances Study:

Thirty-one potentially interfering endogenous and exogenous substances were tested in the presence and absence of heat-inactivated SARS-CoV-2 virus at 3x LoD (1.50 x 10^3 $TCID_{50}$/mL) using Flowflex Plus COVID-19 Home Test. All the samples were tested in a randomized and blinded manner. None of the substances either in the presence or absence of SRS-CoV-2 virus interfered with the test results (see **Table 3** below).

Table 3: Summary of Interfering Substances Study

Interfering Substances Tested	Without SARS-CoV-2		With 3x LoD SARS-CoV-2 virus	
	Concentration Tested	Positive/ Tested	Concentration Tested	Positive/ Tested
Beclomethasone	5 mg/mL	0/3	5 mg/mL	3/3
Biotin	3500 ng/mL	0/3	3500 ng/mL	3/3
Dexamethasone	10 mg/mL	0/3	10 mg/mL	3/3
Dyclonine Hydrochloride	2 mg/mL	0/3	2 mg/mL	3/3
Flunisolide	10 mg/mL	0/3	10 mg/mL	3/3
Hand sanitizer	15% v/v	0/3	15% v/v	3/3
Hand Soap	15% v/v	0/3	15% v/v	3/3
Homeopathic allergy relief (Histaminum hydrochloricum)	15% w/v	0/3	15% w/v	3/3
Homeopathic nasal wash	5% v/v	0/3	5% v/v	3/3
Leukocytes	4.8 x 10^6 cells/mL	0/3	4.8 x 10^6 cells/mL	3/3
Molnupiravir	10 mg/mL	0/3	10 mg/mL	3/3
Mucin	2.5 mg/mL	0/3	2.5 mg/mL	3/3
Mupirocin	10 mg/mL	0/3	10 mg/mL	3/3
Nasal corticosteroids (Budesonide)	15% v/v	0/3	15% v/v	3/3
Nasal corticosteroids (Fluticasone furate)	5% v/v	0/3	5% v/v	3/3
Nasal corticosteroids (Fluticasone propionate)	5% v/v	0/3	5% v/v	3/3
Nasal corticosteroids (Mometasone furoate)	15% v/v	0/3	15% v/v	3/3
Nasal corticosteroids (Triamcinolone Acetonide)	15% v/v	0/3	15% v/v	3/3
Nasal decongestant	15% v/v	0/3	15% v/v	3/3

Interfering Substances Tested	Without SARS-CoV-2		With 3x LoD SARS-CoV-2 virus	
	Concentration Tested	Positive/ Tested	Concentration Tested	Positive/ Tested
(Galphimia glauca, Luffa opperculata, sabadilla)				
Nasal gel	5% v/v	0/3	5% v/v	3/3
Nasal spray (Cromolyn sodium nasal solution)	15% v/v	0/3	15% v/v	3/3
Nasal spray (Oxymetazoline HCl)	15% v/v	0/3	15% v/v	3/3
Nasal spray (Phenylephrine HCl)	15% v/v	0/3	15% v/v	3/3
Nasal spray (Sodium Chloride & Preservatives)	15% v/v	0/3	15% v/v	3/3
Oral Anesthetic Cough Lozenge (Menthol)	3 mg/mL	0/3	3 mg/mL	3/3
Oseltamivir Phosphate (Tamiflu)	15% w/v	0/3	15% w/v	3/3
Remdesivir	10 mg/mL	0/3	10 mg/mL	3/3
Sore Throat & Cough Lozenges (Benzocaine, Dextromethorphan HBr)	3 mg/mL	0/3	3 mg/mL	3/3
Sore Throat Spray (Phenol)	5% w/v	0/3	5% w/v	3/3
Tobramycin	50 µg/mL	0/3	50 µg/mL	3/3
Whole Blood	2.5%	0/3	2.5%	3/3

Based on the test results shown in **Table 3**, the endogenous and exogenous substances listed above do no cross-react or interfere with the performance of the Flowflex Plus COVID-19 Home Test at the tested concentration.

4. Assay Reportable Range:

Not applicable, this is a qualitative visually read assay.

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

a. Internal Controls:

The Flowflex Plus COVID-19 Home Test has a built-in internal procedural control. The colored biotin labeled particle will bind to streptavidin on nitrocellulose membrane and the colored mouse monoclonal antibody labeled particle will bind to goat anti-mouse antibody on nitrocellulose membrane to form a colored control line 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.

b. Sample Stability:

Nasal Swab samples (negative clinical matrix or SARS-CoV-2 spiked positive sample at a concentration of 2x LoD) were kept in the swab packaging at either 10°C, 15°C, 30°C, 35°C and ambient temperature (25°C) for 1 hour, 2 hours, 3 hours and 4 hours before the swabs being

placed into the extraction buffer tubes for sample processing. Freshly prepared samples were tested immediately as a control condition.

Nasal swabs stored at each temperature condition for each time point were prepared in 5 replicates and tested with the Flowflex Plus COVID-19 Home Test following the Instructions of Use. The results were read at 15 min as well as at 30 min. The results are summarized in **Table 4** below:

Table 4: Results of Swab Sample Stability Assessment

Temp. Condition	Time Points	Negative Sample Swabs					Positive sample Swabs (2xLoD or 10^3 $TCID_{50}$/mL)				
		1	2	3	4	5	1	2	3	4	5
Freshly Prepared		-	-	-	-	-	+	+	+	+	+
10°C	1 hour	-	-	-	-	-	+	+	+	+	+
	2 hours	-	-	-	-	-	+	+	+	+	+
	3 hours	-	-	-	-	-	+	+	+	+	+
	4 hours	-	-	-	-	-	+	+	+	+	+
15°C	1 hour	-	-	-	-	-	+	+	+	+	+
	2 hours	-	-	-	-	-	+	+	+	+	+
	3 hours	-	-	-	-	-	+	+	+	+	+
	4 hours	-	-	-	-	-	+	+	+	+	+
25°C	1 hour	-	-	-	-	-	+	+	+	+	+
	2 hours	-	-	-	-	-	+	+	+	+	+
	3 hours	-	-	-	-	-	+	+	+	+	+
	4 hours	-	-	-	-	-	+	+	+	+	+
30°C	1 hour	-	-	-	-	-	+	+	+	+	+
	2 hours	-	-	-	-	-	+	+	+	+	+
	3 hours	-	-	-	-	-	+	+	+	+	+
	4 hours	-	-	-	-	-	+	+	+	+	+
35°C	1 hour	-	-	-	-	-	+	+	+	+	+
	2 hours	-	-	-	-	-	+	+	+	+	+
	3 hours	-	-	-	-	-	+	+	+	+	+
	4 hours	-	-	-	-	-	+	+	+	+	+

Throughout the storage times and conditions tested during the study, no false positive results were observed for negative samples and no false negatives results were observed for positive samples. Thus, swab samples are stable at a temperature range from 10°C to 35°C for up to 4 hours.

c. Device Stability/Shelf life:

The stability of the Flowflex Plus COVID-19 Home Test was tested by using three different device lots released within one month of manufacturing. Kits were stored at two different temperature conditions of 30°C and 2-8°C. The test kits were equilibrated to room temperature for 30 minutes prior to testing each lot at replicates of 5. The test kits stored at 30°C were tested side by side with the test kits stored at 2-8°C for 24 months. The following samples were tested at all timepoints.

1. Negative sample
2. Positive sample 3x LoD (1.5 x 10^3 $TCID_{50}$/mL)

Table 5: Real Time Stability Results

Time points	Sample tested	Lot# A21122201N		Lot# A22011402		Lot# A22011903	
		Stored at 2-8℃	Stored at 30℃	Stored at 2-8℃	Stored at 30℃	Stored at 2-8℃	Stored at 30℃
Day 0	Negative sample	- (5/5)	- (5/5)	- (5/5)	- (5/5)	- (5/5)	- (5/5)
	Positive sample	+ (5/5)	+ (5/5)	+ (5/5)	+ (5/5)	+ (5/5)	+ (5/5)
3 months	Negative sample	- (5/5)	- (5/5)	- (5/5)	- (5/5)	- (5/5)	- (5/5)
	Positive sample	+ (5/5)	+ (5/5)	+ (5/5)	+ (5/5)	+ (5/5)	+ (5/5)
6 months	Negative sample	- (5/5)	- (5/5)	- (5/5)	- (5/5)	- (5/5)	- (5/5)
	Positive sample	+ (5/5)	+ (5/5)	+ (5/5)	+ (5/5)	+ (5/5)	+ (5/5)
9 months	Negative sample	- (5/5)	- (5/5)	- (5/5)	- (5/5)	- (5/5)	- (5/5)
	Positive sample	+ (5/5)	+ (5/5)	+ (5/5)	+ (5/5)	+ (5/5)	+ (5/5)
12 months	Negative sample	- (5/5)	- (5/5)	- (5/5)	- (5/5)	- (5/5)	- (5/5)
	Positive sample	+ (5/5)	+ (5/5)	+ (5/5)	+ (5/5)	+ (5/5)	+ (5/5)
15 months	Negative sample	- (5/5)	- (5/5)	- (5/5)	- (5/5)	- (5/5)	- (5/5)
	Positive sample	+ (5/5)	+ (5/5)	+ (5/5)	+ (5/5)	+ (5/5)	+ (5/5)
18 months	Negative sample	- (5/5)	- (5/5)	- (5/5)	- (5/5)	- (5/5)	- (5/5)
	Positive sample	+ (5/5)	+ (5/5)	+ (5/5)	+ (5/5)	+ (5/5)	+ (5/5)
21 months	Negative sample	- (5/5)	- (5/5)	- (5/5)	- (5/5)	- (5/5)	- (5/5)
	Positive sample	+ (5/5)	+ (5/5)	+ (5/5)	+ (5/5)	+ (5/5)	+ (5/5)
24 months	Negative sample	- (5/5)	- (5/5)	- (5/5)	- (5/5)	- (5/5)	- (5/5)
	Positive sample	+ (5/5)	+ (5/5)	+ (5/5)	+ (5/5)	+ (5/5)	+ (5/5)

Throughout the device storage times and conditions tested during the study, no false positive results were observed for negative samples and no false negatives results were observed for

positive samples. Therefore, no reagent degradation occurred during the tested timeframe when devices are stored at the intended storage conditions. The real time stability data is acceptable and supports a device shelf-life of 21-months at the intended storage conditions, 2-30°C.

d. Shipping Stability:

To understand the stability of the test kit under different shipping conditions, one lot of the Flowflex Plus COVID-19 Home Test kits were stored at three different temperature conditions: 60℃ with 85% ±5% relative humidity (RH), RT (around 25℃) and 2~8℃ (as a reference) for 8 days. The stored test kits were taken out daily from each storage condition and tested. Both 3x LoD positive samples (1.50 x 10^3 $TCID_{50}$/mL) and negative samples were tested in 5 replicates following the IFU of the proposed device. As shown in **Table 6** below, all negative samples generated negative results and all positive samples generated positive results for all 8 days for all temperature conditions tested.

Table 6: Stability of Unopened Kit at Different Temperatures

Time Points	Sample tested	Flowflex Plus COVID-19 Home Test		
		Stored at 60℃ with 85% ±5% RH	Stored at 25℃	Stored at 2-8℃ (Reference)
0 day	Negative sample	- (5/5)	- (5/5)	- (5/5)
	Positive sample	+ (5/5)	+ (5/5)	+ (5/5)
1 day	Negative sample	- (5/5)	- (5/5)	- (5/5)
	Positive sample	+ (5/5)	+ (5/5)	+ (5/5)
2 days	Negative sample	- (5/5)	- (5/5)	- (5/5)
	Positive sample	+ (5/5)	+ (5/5)	+ (5/5)
3 days	Negative sample	- (5/5)	- (5/5)	- (5/5)
	Positive sample	+ (5/5)	+ (5/5)	+ (5/5)
4 days	Negative sample	- (5/5)	- (5/5)	- (5/5)
	Positive sample	+ (5/5)	+ (5/5)	+ (5/5)
5 days	Negative sample	- (5/5)	- (5/5)	- (5/5)
	Positive sample	+ (5/5)	+ (5/5)	+ (5/5)
6 days	Negative sample	- (5/5)	- (5/5)	- (5/5)
	Positive sample	+ (5/5)	+ (5/5)	+ (5/5)
7 days	Negative sample	- (5/5)	- (5/5)	- (5/5)
	Positive sample	+ (5/5)	+ (5/5)	+ (5/5)
8 days	Negative sample	- (5/5)	- (5/5)	- (5/5)
	Positive sample	+ (5/5)	+ (5/5)	+ (5/5)

To determine the effect of harsh shipping conditions on the performance of Flowflex Plus COVID-19 Home Test, test kits from three different lots were stored at -20°C for 24 hours and then stored at room temperature for 24 hours. Three freeze/thaw cycles were repeated to mimic harsh shipping conditions. At the last thaw, the products were stored at 55°C for up to 35 days. Both 3x LoD positive samples (1.50 x 10^3 $TCID_{50}$/mL) and negative samples were tested in 5 replicates following the IFU of the proposed device. All negative samples tested negative, and all positive samples tested positive in all storage conditions over the 35 days as shown in **Table 7**.

Table 7: Shipping Stability Results

Time Points	Sample tested	Flowflex Plus COVID-19 Home Test		
		Lot 1	Lot 2	Lot 3
Day 0	Negative sample	- (5/5)	- (5/5)	- (5/5)
	Positive sample	+ (5/5)	+ (5/5)	+ (5/5)
Day 7	Negative sample	- (5/5)	- (5/5)	- (5/5)
	Positive sample	+ (5/5)	+ (5/5)	+ (5/5)
Day 14	Negative sample	- (5/5)	- (5/5)	- (5/5)
	Positive sample	+ (5/5)	+ (5/5)	+ (5/5)
Day 21	Negative sample	- (5/5)	- (5/5)	- (5/5)
	Positive sample	+ (5/5)	+ (5/5)	+ (5/5)
Day 28	Negative sample	- (5/5)	- (5/5)	- (5/5)
	Positive sample	+ (5/5)	+ (5/5)	+ (5/5)
Day 35	Negative sample	- (5/5)	- (5/5)	- (5/5)
	Positive sample	+ (5/5)	+ (5/5)	+ (5/5)

6. Detection Limit:

To determine the analytical sensitivity/limit of detection (LoD) for the Flowflex Plus COVID-19 Home Test, both the SARS-CoV-2 virus (USA-WA1/2020) and the 1st WHO International Standard for SARS-CoV-2 antigen (NIBSC code: 21/368) were used.

a. LoD using SARS-CoV-2 virus (USA-WA1/2020):

The LoD was determined as the lowest virus concentration that was detected ≥ 95% of the time (i.e., concentration at which at least 19 out of 20 replicates tested positive). The LoD was established in two phases.

i. Preliminary LoD Study:

Five ten-fold serial dilutions of heat-inactivated SARS-CoV-2 USA-WA1/2020 virus were prepared in negative clinical nasal swab matrix (NCM). 5 replicates per dilution level were tested using three lots of the test kit. For each replicate, 50 µL of the prepared viral dilution was applied to a dry nasal swab and the swab tested according to the IFU. The lowest concentration with 5/5 positive results from each of the three lots were considered as the preliminary LoD for that viral strain. Results were read at 15 and 30 minutes. The preliminary LoD results are shown in **Table 8**.

Table 8: Preliminary LoD Results

SARS CoV-2 Concentration [$TCID_{50}$/mL] (applied to dry swab)	SARS CoV-2 Concentration [$TCID_{50}$/swab]	Lot A21122201N Positive/Tested	Lot A22011402 Positive/Tested	Lot A22011903 Positive/Tested
5.01×10^4	25×10^2	5/5	5/5	5/5
5.01×10^3	250	5/5	5/5	5/5

SARS CoV-2 Concentration [$TCID_{50}$/mL] (applied to dry swab)	SARS CoV-2 Concentration [$TCID_{50}$/swab]	Lot A21122201N Positive/Tested	Lot A22011402 Positive/Tested	Lot A22011903 Positive/Tested
5.01 x 10^2	**25**	**5/5**	**5/5**	**5/5**
5.01 x 10^1	2.5	0/5	0/5	0/5

Results read at 15 min were the same as results of the same devices read at 30 min. The preliminary LoD for SARS-CoV-2 USA-WA1/2020 (Lot# 326551 from Zeptometrix) was determined to be 5.01 x 10^2 $TCID_{50}$/mL.

ii. Confirmatory LoD Study:

The preliminary LoD concentration and concentrations 3-fold above and 3-fold below the preliminary LoD were diluted into NCM and tested on three test kit lots with 20 individual replicates per lot. The lowest concentration resulting in positive detection of ≥ 95% of the replicates (at least 19 out of 20 replicates) is the final LoD for that viral strain. The confirmatory LoD results are summarized in **Table 9** below.

Table 9: Confirmatory LoD Results

SARS CoV-2 Concentration [$TCID_{50}$/mL] (applied to dry swab)	SARS CoV-2 Concentration [$TCID_{50}$/swab]	Lot A21122201N Positive/Tested	Lot A22011402 Positive/Tested	Lot A22011903 Positive/Tested
1.50 x 10^3 (3-fold LoD)	75	20/20	20/20	20/20
5.01 x 10^2 (Preliminary LoD)	**25**	**20/20**	**20/20**	**20/20**
1.67 x 10^2 (0.3-fold LoD)	8.3	9/20	9/20	8/20

Thus, the limit of detection for Flowflex Plus COVID-19 Home Test was confirmed as 5.01 x 10^2 $TCID_{50}$/mL (25 $TCID_{50}$/swab) using all three tested lots.

b. LoD using WHO International Standard (NIBSC 21/368):

The LoD of the Flowflex Plus COVID-19 Home Test was determined by using different dilutions of the WHO International Standard for SARS-CoV-2 antigen (NIBSC code: 21/368) in NCM. Two ampoules were used for testing 3 lots of the test device that were reconstituted per instructions of the standard (i.e., in 0.25 mL of ultrapure water). The contents of all reconstituted vials were pooled prior to study start. The LoD was determined as the lowest concentration that was detected 95% of the time (i.e., the concentration at which at least 19 out of 20 replicates were tested positive). The LoD study was evaluated in two phases.

i. Preliminary LoD Study:

Three ten-fold serial dilutions were prepared in NCM spiked with the stock SARS-CoV-2 antigen standard. 50 µL of the each WHO SARS-CoV-2 standard prepared concentration was applied to a dry nasal swab and swabs were tested according to the IFU of the test device. Five

replicates per dilution level for each of three device lots were tested. Results were read at 15 and 30 minutes. The preliminary LoD results are tabulated below in **Table 10**.

Table 10: Preliminary WHO Standard LoD Results

Standard Concentration (applied to dry swab)	**Lot A21122201N Positive/Tested**	**Lot A22011402 Positive/Tested**	**Lot A22011903 Positive/Tested**
2 x 10^3 IU/mL	5/5	5/5	5/5
2 x 10^2 IU/mL	**5/5**	**5/5**	**5/5**
2 x 10 IU/mL	0/5	0/5	0/5

Results read at 15 min were the same as results of the devices read at 30 min. The preliminary LoD was determined to be 200 IU/mL.

ii. Confirmatory LoD Study:

The final LoD was confirmed by testing 20 individual replicates with three different concentrations such as the preliminary LoD, 3-fold below the preliminary LoD and 3-fold above the preliminary LoD. The confirmatory LoD results are shown in **Table 11**.

Table 11: Confirmatory WHO Standard LoD Results

Standard Concentration (applied to dry swab)	**Lot 1 Positive/Tested**	**Lot 2 Positive/Tested**	**Lot 3 Positive/Tested**
6 x 10^2 IU/mL	20/20	20/20	20/20
2 x 10^2 IU/mL	**20/20**	**20/20**	**20/20**
6.67 x 10 IU/mL	7/20	6/20	7/20

The lowest concentration resulting in positive detection of ≥95% of the replicates was 200 IU/mL or 10 IU/swab. Therefore, the LoD of the Flowflex Plus COVID-19 Home Test with 1st WHO International standard for SARS-CoV-2 antigen (NIBSC:21/368) is established at 200 IU/mL or 10 IU/swab.

7. <u>High Dose Hook Effect:</u>

To determine if a hook effect is observed, the sponsor tested very high level of SARS-CoV-2 virus on the Flowflex plus COVID-19 Home Test. 50 µL of heat-inactivated SARS-CoV-2 (isolate WA1/2020) at 2.82 x 10^7 $TCID_{50}$/mL was added to each swab and tested in five replicates on the Flowflex Plus COVID-19 Home Test. The testing was conducted in accordance with the IFU of the test. All replicates tested positive as expected.

8. <u>Inclusivity Study:</u>

To determine the analytical reactivity of the Flowflex Plus COVID-19 Home Test, new SARS-COV-2 variants with multiple lineages were tested. Thirteen new omicron variants in addition to alpha, beta, gamma and delta variants were wet tested using contrived samples prepared in pooled negative clinical matrix. Each concentration was tested with 5 replicates. The reactivity of the Flowflex Plus COVID-19 Home Test with the variants is summarized below in **Table 12** with the lowest concentration that generated 100% positive replicates.

Table 12: Inclusivity Study Results for the Flowflex Plus COVID-19 Antigen Home Test

SARS-CoV-2 virus variant	Subtype / Lineage	Lowest test concentration with 100% detection [$TCID_{50}$/mL]
Alpha	B.1.1.7	4.20×10^2
Beta	B.1.351	1.05×10^3
Gamma	P.1 (South Africa)	1.26×10^3
Delta	B.1.617.2	1.05×10^3
Omicron	XBB	1.98×10^4
	BQ.1	1.43×10^3
	BQ.1.3	3.20×10^3
	BQ.1.16	2.19×10^3
	BQ.1.1	2.93×10^2
	BF.7	1.26×10^3
	BF.5	2.93×10^3
	BA.4.6	3.83×10^2
	BA.5.5	1.56×10^2
	B.1.1.529 USA/CO-CDPHE-2102544747/2021	4.17×10^2
	B.1.1.529 USA/MD-HP20874/2021	5.01×10^2
	BA.2.12.1	1.26×10^3
	BA.2.3	7.34×10^2
	BA.5	2.53×10^3
	BA.2.75.5	1.70×10^2

9. Assay Cut-Off:

Not Applicable. This is a qualitative visually read assay.

B Comparison Studies:

1. Method Comparison with Predicate Device:

Not Applicable. See "C. Clinical Studies" for performance comparison with a clinical comparator.

2. Matrix Comparison:

The Flowflex Plus COVID-19 Home test is only intended for the qualitative detection of the nucleocapsid protein antigen from SARS-CoV-2 in direct anterior nasal swab specimens. As no other specimen or sample type is claimed for this device, the matrix comparison study is not applicable.

C Clinical Study:

The performance of the Flowflex Plus COVID-19 Home Test was established in a prospective all-comers clinical study conducted between June 2022 and April 2023 at ten study sites in the U.S. A nasopharyngeal swab (NP) as a reference sample was collected from the patient by healthcare professionals at the study site and put into 3 mL of sterile viral transport medium. These specimens were shipped on dry ice to the central laboratory for testing using a highly sensitive FDA authorized real-time polymerase chain reaction assays as a comparator method.

A total of 758 symptomatic individuals who met the study inclusion and exclusion criteria were enrolled in the clinical study across all ten study sites. 605 patients with age ≥ 14 years old self-collected and self-tested their own anterior nasal swab specimens using the IFU of the proposed device. Approximately 20% (153/758) of the total clinical study population were adults testing individuals older than 2 years of age. Six subjects age ≥ 14 participated in a paired study as a donor but their nasal swab specimens were collected and tested by another lay person above the age of 14. All adults and minors performed the test unassisted and interpreted the result using only the QRI of the proposed device.

The Flowflex Plus COVID-19 Home Test detected SARS-CoV-2 with Positive percent agreement (PPA) of 91.3% and Negative Percent Agreement (NPA) of 99.3% when compared to the results of the SARS-CoV-2 RT-PCR comparator assay. The performance of the candidate device in symptomatic patients are as follows:

Table 13: Performance of Flowflex Plus COVID-19 Home Test in Symptomatic Subjects

Flowflex Plus COVID-19 Home Test	RT-PCR Method		
	Positive	Negative	Total
Positive	178	4	182
Negative	17	559	576
Total	195	563	758
Positive Percent Agreement (PPA)	**91.3% (178/195) (95%CI: 86.5%-94.5%)**		
Negative Percent Agreement (NPA)	**99.3% (559/563) (95%CI: 98.2%-99.7%)**		

Table 14: Days Post Symptom Onset (DPSO) stratified PPA

DPSO	Flowflex Plus COVID-19 Home Test Positive	RT-PCR Positive	PPA
1 day	35	36	97.2%
2 days	65	68	95.6%
3 days	38	40	95.0%
4 days	23	30	76.7%
5 days	10	13	76.9%
6 days	7	8	87.5%
All specimens	**178**	**195**	**91.3%**

Detailed study subject demographics are shown below.

Table 15: Demographics – Age, and Gender of the Clinical study cohort

Age Group	Numbers	Percent Total	# Male	% Male	# Female	% Female
2 -13 years	147	19.4%	78	24.1%	69	15.9%
14 - 24 years	121	16%	52	16.0%	69	15.9%
25 - 64 years	418	55.1%	166	51.2%	252	58.1%
≥ 65 years	72	9.5%	28	8.6%	44	10.1%
Total	**758**	**100%**	**324**	**100%**	**434**	**100%**

Table 16: Demographics - Educational level of the Clinical subjects

Education	Lay Users	Total
At High School	26	3.4%
High School	472	62.3%
College	151	19.9%
Bachelors	74	9.8%
Master	20	2.6%
PhD/Doctorate	4	0.5%
N/A	11	1.5%
Total	**758**	**100%**

D Clinical Cut-Off:

There is no clinical cut-off for this device. This section is therefore not applicable.

E Expected Values/Reference Range:

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

F Other supportive Performance characteristics Data

1. Flex Studies:

To assess the robustness of the Flowflex Plus COVID-19 Home Test, flex studies were conducted that assessed all major aspects of the test procedure (reading time, sample volume, extraction buffer volume, swab elution time and procedure, sample hold times before and during processing) and variability of environmental test conditions that the test may be subjected to when in use (disturbance during run, device orientation, various temperature and humidity stress conditions and light sources impact study). Testing was performed with contrived positive nasal swabs generated by diluting heat-inactivated SARS-CoV-2 virus into negative clinical matrix at 2xLoD. The studies support that the test is robust in the intended use condition with an insignificant risk of erroneous result.

For all conditions, either 50 µL of pooled negative clinical matrix or spiked positive sample with heat inactivated SARS-CoV-2 (USA-WA1/2020, Cat # 0810587CFHI, Lot 326551)) at a concentration of 2x LoD (1.00 x 10^3 $TCID_{50}$/mL) was applied to each dry swab, respectively. Each contrived swab sample was placed in the extraction buffer tube and processed and tested with the Flowflex Plus COVID-19 Home Test following the package insert.

The results of flex testing are summarized in the below table:

Table 17: Flex Testing Results Summary

Condition		Positive Swab Samples (Positive/Total)	Negative Swab Samples (Negative/Total)
Reading Time	Read at 5 min	3/3	3/3
	Read at 10 min	3/3	3/3
	Read at 15 min	3/3	3/3
	Read at 20 min	3/3	3/3
	Read at 30 min	3/3	3/3
	Read at 40 min	3/3	3/3
	Read at 60 min	3/3	3/3
Sample Volume	1 drop	Invalid	Invalid
	2 drops	Invalid	Invalid
	3 drops	3/3	3/3
	4 drops (control)	3/3	3/3
	5 drops	3/3	3/3
	6 drops	3/3	3/3
	7 drops	3/3	3/3
	8 drops	3/3	3/3
Extraction Buffer Volume	50 µL	Invalid	Invalid
	100 µL	Invalid	Invalid
	150 µL	3/3	3/3
	200 µL	3/3	3/3
	250 µL	3/3	3/3
	300 µL (control)	3/3	3/3
	350 µL	3/3	3/3
	380 µL	3/3	3/3
Swab Elution	Condition 1: (Control) 1. Swirl for 30 seconds. 2. Rotate the swab 5 times while squeezing the tube. 3. Remove the swab while squeezing the tube. Dispose the swab in the trash.	3/3	3/3
	Condition 2: 1. Vortex extraction buffer tube for 2 minutes after placing the swab in to generate bubbles. Other steps same as Condition 1.	3/3	3/3
	Condition 3: 1. Swirl for 15 seconds Other steps same as Condition 1.	3/3	3/3
	Condition 4: 1. Swirl for 2 seconds Other steps same as Condition 1.	**0/3**	3/3
	Condition 5: 1. No swirling.	**0/3**	3/3

Condition		Positive Swab Samples (Positive/Total)	Negative Swab Samples (Negative/Total)
	Other steps same as Condition 1.		
	Condition 6: 1. Swirl for 30 seconds 2. Rotate the swab 5 times without squeezing the tube. 3. Remove the swab while squeezing the tube.	3/3	3/3
	Condition 7: 1. Swirl for 30 seconds 2. Rotate the swab 2 times without squeezing the tube. 3. Remove the swab while squeezing the tube.	3/3	3/3
	Condition 8: 1. Swirl for 30 seconds 2. Rotate the swab 5 times while squeezing the tube. 3. Remove the swab without squeezing the tube and without pressing the swab against the tube wall. Dispose the swab in the trash.	3/3	3/3
	Condition 9: 1. Swirl for 30 seconds 2. Rotate the swab 5 times while squeezing the tube. 3. Pressing swab firmly halfway up the extraction buffer tube to release as much liquid as possible. Dispose the swab in the trash.	3/3	3/3
	Condition 10: 1. Swirl for 30 seconds 2. Rotate the swab 5 times while squeezing the tube. 3. Remove the swab while squeezing the tube. Dispose the swab in the trash. 4. Thoroughly mix the vial by swirling the bottom of the tube before adding sample to the test cassette.	3/3	3/3
	Condition 11: 1. Immediately remove the swab without squeezing the extraction	**0/3**	3/3

Condition		Positive Swab Samples (Positive/Total)	Negative Swab Samples (Negative/Total)
	buffer tube once placing the swab sample into the tube. 2. Dispose the swab in the trash.		
Disturbance Effect	Test Cassette on flat surface (Control)	3/3	3/3
	Dropping the test cassette while test was running	3/3	3/3
	Moving test cassette to another flat surface during testing	3/3	3/3
	Placing the test cassette on non-level surface during testing. 1.Tilted up far end of cassette 15° 2. Tilted up near end of cassette 15° 3.Tilted up right side of cassette 15° 4. Tilted up left side of cassette 15°	3/3 (each of the 4 conditions)	3/3 (each of the 4 conditions)
Device Orientation	Test Cassette on flat surface (Control)	3/3	3/3
	Device in upright position (sample well at the bottom)	3/3	3/3
	Device in upright position (sample well at up end)	3/3	3/3
	Turned over device upside down	3/3	3/3
Light Source Impact	Mercury vapor lamp	3/3	3/3
	Sodium vapor lamp	3/3	3/3
	Fluorescent lamp	3/3	3/3
	Incandescent lamp	3/3	3/3
	Cell phone light	3/3	3/3
	Handheld flashlight	3/3	3/3
	Indirect Sunlight	3/3	3/3
	Direct Sunlight	3/3	3/3
	Dome light inside car	3/3	3/3
	Sun at Dawn	Difficult to see ‘C’ & ‘T’ Line	Difficult to see ‘C’ & ‘T’ Line
	Sun at Dusk	Difficult to see ‘C’ & ‘T’ Line	Difficult to see ‘C’ & ‘T’ Line
Environmental Stress Conditions (Open pouch)	20°C with humidity 57% RH (Control)	3/3	3/3
	45°C with humidity 90% RH	3/3	3/3
	35°C with humidity 90% RH	3/3	3/3
	45°C with humidity 10% RH	5/5	5/5
	5°C with humidity 10% RH	3/3	3/3
	5°C with humidity 90% RH	5/5	5/5

Condition			Positive Swab Samples (Positive/Total)	Negative Swab Samples (Negative/Total)
	15°C with humidity 30% RH		3/3	3/3
	15°C with humidity 50% RH		3/3	3/3
	15°C with humidity 80% RH		3/3	3/3
	30°C with humidity 30% RH		3/3	3/3
	30°C with humidity 50% RH		3/3	3/3
	30°C with humidity 80% RH		3/3	3/3
Open Pouch Stability Study for operating at RT[a]	0 minutes (Control)		5/5	5/5
	30 minutes		5/5	5/5
	60 minutes		5/5	5/5
	75 minutes		5/5	5/5
Hold Time between swab collection and swab insertion into Extraction Buffer[b]	Freshly Prepared (Control)			
	15°C	1 hour	3/3	3/3
		2 hours	3/3	3/3
		3 hours	3/3	3/3
		4 hours	3/3	3/3
	25°C	1 hour	3/3	3/3
		2 hours	3/3	3/3
		3 hours	3/3	3/3
		4 hours	3/3	3/3
	30°C	1 hour	3/3	3/3
		2 hours	3/3	3/3
		3 hours	3/3	3/3
		4 hours	3/3	3/3
	35°C	1 hour	3/3	3/3
		2 hours	3/3	3/3
		3 hours	3/3	3/3
		4 hours	3/3	3/3

[a]Open pouch stability was conducted at four different conditions: 1) 15°C and RH 10±3%; 2) 15°C and RH 80±3%; 3) 30°C and RH 10±3% and 4) 30°C and RH 80±3% using three different lots. All the results were identical to those tabulated in the above section.

[b]For all tested temperatures, three scenarios were tested: 1. All steps followed the package insert as a control condition, 2. The contrived swab sample was inserted into extraction buffer without swirl/rotate motion and then stored for the indicated time; 3. The contrived swab sample was inserted into extraction buffer with swirl/rotate motion and then stored for the indicated time. After sample storage the sample was processed through the remaining procedural steps per the IFU. All the results were same as tabulated in the section above.

2. Usability Study:

A usability study was conducted to assess the lay users' ability to understand the instructions for use and to adequately execute the Flowflex Plus COVID-19 Antigen Home Test workflow instructions. A total of 775 subjects were enrolled in the study and were observed during testing. Each lay user completed the questionnaire regarding the ease of operating the test at the end of the clinical study. A total of 99.7% of the lay users found the test to be okay, easy or very easy to operate. No significant failures were observed.

3. Lay User Readability Study:

The purpose of this study was to evaluate whether lay home users (patients or their caregivers) can interpret test results correctly with low positive samples from the Flowflex Plus COVID-19 Home Test.

The readability study was conducted according to the IRB approved protocol in a simulated home environment. A total of 63 lay users with diverse gender, ages and educational background and including those with vision impairment (e.g., glasses, contacts) who met the inclusion criteria were enrolled in this study. Each lay user was asked to interpret four test devices with three different concentrations (Negative, Low positive [1.5x LoD], and positive [5x LoD]) that were provided to the readers in a blinded randomized manner. The results with 3 different sample levels were read by each lay user and results are tabulated below:

Table 18: Age Distribution of Lay User and Result Agreement

Age Group	Number of Lay Users	Percentage	Sample Concentrations			
			Negative Correct/All (Agreement)	Low Positive Correct/All (Agreement)	Positive Correct/All (Agreement)	Overall Correct/Total (Agreement)
14-20 years	12	19%	18/18 (100%)	18/18 (100%)	12/12 (100%)	48/48 (100%)
21-30 years	15	24%	23/23 (100%)	22/22 (100%)	15/15 (100%)	60/60 (100%)
31-55 years	25	40%	39/39 (100%)	36/36 (100%)	25/25 (100%)	100/100 (100%)
> 55 years	11	17%	16/16 (100%)	11/17 (64.7%)	11/11 (100%)	38/44 (86.4%)
Total	**63**	**100%**	**96/96 (100%)**	**87/93 (93.5%)**	**63/63 (100%)**	**246/252 (97.6%)**

A total of 252 test devices were read and recorded in this study. Lay users were able to perceive and interpret all negative results with 100% accuracy (96/96) and positive results at 5xLoD with 100% accuracy (63/63). As the Test Line intensity became fainter with low positive samples at 1.5xLoD, the agreement was 94% (87/93); 4 of 63 lay users (age from 65-86 years) with age related vision impairments found it difficult to determine the positive results for low positive sample at 1.5xLoD. It is therefore recommended that users with conditions affecting their vision such as far-sightedness, glaucoma or color blindness are encouraged to seek assistance to interpret results accurately (e.g., reading glasses, additional light source or another person).

After the Lay User Readability Study was completed, lay users were asked for their overall impressions of the instructional materials they were provided. 98% of participants (62/63) thought the instructions for reading and interpreting the test result were easy to understand and follow.

4. Variant Monitoring Plan:

To determine whether the Flowflex Plus COVID-19 Home Test can detect newly emerging variants, and/or to assess whether new mutations are impacting analytical sensitivity of the test performance, the sponsor provided the variant monitoring plan as described below:

1. The antibodies used for the Flowflex Plus COVID-19 Home Test will be sent to Emory University for analysis of "Escape mutation Profiling (Escape-MaP)" analysis. The resulting data from this analysis should identify all nucleocapsid mutations that negatively affect antibody recognition, including any recognized or potential mutations in SARS-CoV-2 variants and predict how specific mutations on the SARS-CoV-2 N protein will impact the binding efficacy of the antibodies used in the candidate device.
2. Pairwise sequence alignment comparison for emergence of new variants will be provided.
3. Once the newly emergent virus samples are available, ACON will conduct wet-testing to further verify the performance of the Flowflex Plus COVID-19 Home Test in detecting variants.
4. ACON will pay close attention to the customers' complaints regarding false negative results at the time of newly emergent variants of concerns.
5. A mini-clinical study will be conducted at POC site if the test performance may be impacted for SARS-CoV-2 variants. The POC site will collect and send NP swabs to a reference lab for confirming RT-PCR and validating whole genome sequencing for the RT-PCR positive samples.

VIII Proposed Labeling:

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

IX Conclusion:

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