510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY

I Background Information:

A 510(k) Number

K230828

B Applicant

ACON Laboratories, Inc.

C Proprietary and Established Names

Flowflex COVID-19 Antigen Home Test

D Regulatory Information

Table 1: 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 COVID-19 Antigen Home Test. The identical test kit is currently marketed as *Flowflex COVID-19 Antigen Home Test* under Emergency Use Authorization, EUA210494.

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 COVID-19 Antigen 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 COVID-19 Antigen 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 from December 2022 to March 2023 when SARS-CoV-2 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 Flow*flex* COVID-19 Antigen Home Test is a lateral flow immunoassay device intended for the qualitative detection of the nucleocapsid protein antigen from SARS-CoV-2 virus. This test does not differentiate between SARS-CoV and SARS-CoV-2.

The test package is composed of 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, that contains a nitrocellulose membrane with two test lines: a test line (T line) and a control line (C line).

B Principle of Operation:

To perform the test, a nasal swab is collected by the lay user. Swabs can be self-collected by the user (age ≥14 years) or collected from another individual by an adult (age ≥2 years). For sample processing, the swab is first inserted into the extraction buffer during which the extraction buffer disrupts the virus particles in the specimen, exposing internal viral nucleocapsid antigens. The lysed specimen is then added to the sample well of the test cassette. When an adequate volume of the specimen is added to the sample well (S) of the test cassette, the specimen migrates by capillary action from the sample well over the reagent label pad and across the nitrocellulose membrane test strip. During that migration the reagents in the label pad are solubilized. If SARS-CoV-2 antigens are present in the sample, the antigens bind to the specific anti-SARS-CoV-2 antibody conjugates on the label pad and the antigen/antibody complexes then form sandwich complexes at the test line region (T) of the test strip generating a visible purple colored test line. Unbound conjugate continues to migrate across the nitrocellulose membrane and are captured at the control line region where the complexes will result in a purple control line that indicates adequate operations and sample flow during the test. If no SARS-CoV-2 antigens are present in the sample, the conjugate will only be captured at the control line of the test.

Results are interpreted between 15 and 30 minutes after adding the swab solution into 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.

C Instrument Description Information:

1. Instrument Name:
   This is a manually performed visually read Lateral Flow test without an instrument.

2. Specimen Identification:
   N/A

3. Specimen Sampling and Handling:
   N/A

4. Calibration:
   N/A

5. Quality Control:
   N/A

I 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):

Table 2: Comparison with the predicate device

Device & Predicate Device(s):	**DEN220028**	**K230828**
Device Trade Name	Cue COVID-19 Molecular Test	Flowflex COVID-19 Antigen Home Test
Similarities		
Indications For Use	Cue COVID-19 Molecular Test is a qualitative in vitro diagnostic device for the detection of SARS-CoV-2 directly in anterior nasal swab specimens from individuals with signs and symptoms of COVID-19 (i.e., symptomatic). 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 Flowflex COVID-19 Antigen Home Test is a qualitative in vitro diagnostic device for the detection of SARS-CoV-2 in directly in anterior nasal swab specimens from individuals with symptoms of COVID-19 (i.e., symptomatic). 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.
Intended Use	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	The Flowflex COVID-19 Antigen 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

	from individuals with signs and symptoms of COVID-19 (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 supervision. This test is intended to be sold over-the-counter (OTC) for testing of individuals 18 years of age and older.	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 COVID-19 Antigen 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. Performance characteristics for SARS-CoV-2 were established from December 2022 to March 2023 of the SARS-CoV-2 pandemic when SARS-CoV-2 Omicron was the predominant SARS-CoV-2 variant in circulation. When other SARS-CoV-2 virus variant are emerging, performance characteristics may vary. 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.
Regulation number	21 CFR 866.3984	Same

Disease	COVID-19	Same
Intended Use Population	Individuals with symptoms of COVID-19	Same
Patient Use	Over the counter use/self-testing	Same
Usage	Single use test	Same
Test Result	Qualitative	Same
Format	Test cartridge	Same
Sample Type	Direct anterior swab specimen	Same
Differences		
Analyte	SARS-CoV-2 nucleocapsid nucleic acid	SARS-CoV-2 nucleocapsid protein
Test Principle	Nucleic acid amplification assay	Lateral flow immunoassay
Instrument	Yes	No
Development Time	20 min	15-30 min
Result Interpretation	Instrument read	Visually read
Utilizes App to Display Test Results	Yes	No

The Flowflex COVID-19 Antigen Home Test (proposed device) employs a lateral flow immunoassay design requiring a visual read of test strip for manual interpretation of the test results by the user. In contrast, the Cue COVID-19 Molecular Test (predicate device) is a molecular nucleic acid amplification test. Despite the technological differences, both the proposed candidate device and the predicate device are both over-the-counter test for the detection of SARS-CoV-2 in clinical specimens to aid in the diagnosis of SARS-CoV-2 infection. Accordingly they are grouped into the same Classification under 21 CFR 866.3984. The devices are intended to be used by lay users and do not require a health care provider (HCP) intervention. A Benefit/Risk analysis was provided to address the technological differences of the investigational device when compared to the predicates.

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 investigations 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.

II Standards/Guidance Documents Referenced:

Document	Title	Publisher	Applicable Study
Nonbinding recommendations for premarket authorization of SARS CoV-2 antigen tests	Premarket validation recommendations for developers of in vitro diagnostic tests for SARS-CoV-2 antigen	FDA/CDRH/ OHT7/DMD	All Studies
DEN220028/ Special controls for Over- the-counter test to detect SARS-CoV-2 from clinical specimens 21 CFR866.3984	Reclassification order for DEN220028 and special controls under 21 CFR 866.3984	FDA/CDRH	All Studies
CLIA Waiver FDA Guidance	Recommendations for Clinical Laboratory Improvement Amendments of 1988 (CLIA) Waiver Applications for Manufacturers of In Vitro Diagnostic Devices - Guidance for Industry andFood and Drug Administration Staff (fda.gov)	FDA/CDRH	Flex 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
FDA Guidance	Submission and Review of Sterility Information in Premarket Notification (510(k)) Submissions for Devices Labeled as Sterile | FDA.	FDA/CDRH	Sterility
ISO 10993-5	Biological evaluation of medical devices Part 5: Tests for in vitro cytotoxicity	ISO	Biocompatibility
ISO 10993-10	Biological evaluation of medical devices Part 10: Tests for irritation and skin sensitization	ISO	Biocompatibility

III Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Multi-lot Precision:

The purpose of the study was to assess lot-to-lot variability of three different lots of the Flowflex COVID-19 Antigen Home Test. Three (3) levels of heat-inactivated SARS-CoV-2 were spiked in surrogate clinical matrix (1xPBS with 1% mucin) as follows:

A. Negative, Surrogate Negative Matrix
B. Low positive (1.5x LoD)
C. Positive (5x LoD)

A matrix comparison study was conducted in support of the surrogate matrix and demonstrated the equivalency of the surrogate matrix to negative clinical nasal swab matrix.

Each operator applied 50 µL of each coded sample to each dry nasal swab. Then, the operator processed the sample per the IFU of the proposed device. The sample panel was tested in a blinded manner by three operators for 10 non-consecutive days. All three lots were tested by each operator on each testing day. Each sample level was tested in duplicate in each of two runs per operator per day (i.e., 3 lots x 3 operators x 2 replicates/run x 2 runs X 10 days). A total of 360 test were run per panel member.

The agreement of obtained results with expected results was 100% across all lots, operators, and days. Variability in results was not observed between the three independently manufactured lots.

The results are summarized below:

Table 2: Multi-lot precision study results performed by 3 operators for 10 days

Lot #	Negative*	Low positive** (1xLoD)	Positive** (5xLoD)
COV3030007 (lot 1)	120/120	120/120	120/120
COV3030009 (lot 2)	120/120	120/120	120/120
MPS2100029 (lot 3)	120/120	120/120	120/120
% Agreement (95% CI***)	100% [99%-100%]	100% [99%-100%]	100% [99%-100%]

*Virus not detected/total; **Virus detected/total; ***CI: Confidence Interval

2. Linearity:

Not applicable, the device is a binary qualitative assay that is visually read.

3. Analytical Specificity/Interference:

a) Cross Reactivity/Microbial Interference:

The analytical specificity of the Flowflex COVID-19 Antigen Home Test was evaluated by testing various microorganisms (13), and viruses (17) diluted into negative clinical nasal swab matrix (see LoD study section below for generation of the negative clinical nasal swab matrix). Nasal wash was tested as representative of normal respiratory microbial flora. Each organism and virus were tested in three replicates in the absence (cross reactivity) or presence (microbial interference) of 4.57 x 10^5 $TCID_{50}$/mL (3x LoD) of heat-inactivated SARS-CoV-2 (isolate USA-WA1/2020). The microbial interference and the cross-reactivity study were conducted simultaneously with samples tested in a randomized and blinded manner.

To demonstrate that the Flowflex COVID-19 Antigen Home Test does not react with related viruses, high prevalence disease agents, and normal or pathogenic flora that are reasonably likely to be encountered in nasal swab specimens were tested. Viruses were tested at concentrations of ≥10^5 $TCID_{50}$/mL (PFU/mL or copies/mL), Bacteria and fungi were tested at concentrations of ≥10^6 CFU/mL. The results are summarized in the table below:

Table 3: Summary of cross-reactivity and microbial interference study results

Microorganisms	Without SARS-CoV-2 (Cross-Reactivity)		With SARS-CoV-2 (Microbial Interference)	
	Concentration Tested*	Positive/ Tested	Concentration Tested*	Positive/ Tested
Adenovirus	2.82×10^6 $TCID_{50}$/mL	0/3	2.82×10^6 $TCID_{50}$/mL	3/3
Bordetella pertussis	1.16×10^9 CFU/mL	0/3	1.16×10^9 CFU/mL	3/3
Candida albicans	1.31×10^7 CFU/mL	0/3	1.31×10^7 CFU/mL	3/3
Chlamydia pneumonia	1.4×10^7 IFU/mL	0/3	1.4×10^7 IFU/mL	3/3
Chlamydia trachomatis	3.52×10^8 IFU/mL	0/3	3.52×10^8 IFU/mL	3/3
Enterovirus	1.0×10^5 $TCID_{50}$/mL	0/3	1.0×10^5 $TCID_{50}$/mL	3/3
Haemophilus influenzae	3.87×10^7 CFU/mL	0/3	3.87×10^7 CFU/mL	3/3
Human coronavirus 229E	1.0×10^5 $TCID_{50}$/mL	0/3	1.0×10^5 $TCID_{50}$/mL	3/3
Human coronavirus HKU1 (specimen # B18378425)	Ct 13.7	0/3	Ct 13.7	3/3
Human coronavirus HKU1 (specimen # B18431603)	Ct 20.3	0/3	Ct 20.3	3/3
Human coronavirus HKU1 (specimen # B18427097)	Ct 24.6	0/3	Ct 24.6	3/3
Human coronavirus HKU1 (specimen # B18431601)	Ct 21.3	0/3	Ct 21.3	3/3
Human coronavirus HKU1 (specimen # B18438368)	Ct 16.8	0/3	Ct 16.8	3/3
Human coronavirus HKU1 (specimen # B18421181)	Ct 19.8	0/3	Ct 19.8	3/3
Human coronavirus NL63	1.0×10^5 $TCID_{50}$/mL	0/3	1.0×10^5 $TCID_{50}$/mL	3/3
Human coronavirus OC43	1.0×10^5 $TCID_{50}$/mL	0/3	1.0×10^5 $TCID_{50}$/mL	3/3
Human Metapneumovirus	1.0×10^5 $TCID_{50}$/mL	0/3	1.0×10^5 $TCID_{50}$/mL	3/3
Influenza A	1.51×10^5 $TCID_{50}$/mL	0/3	1.51×10^5 $TCID_{50}$/mL	3/3
Influenza B	1.0×10^5 $TCID_{50}$/mL	0/3	1.0×10^5 $TCID_{50}$/mL	3/3
Legionella pneumophila	3.27×10^9 CFU/mL	0/3	3.27×10^9 CFU/mL	3/3
MERS-coronavirus	1.05×10^5 $TCID_{50}$/mL	0/3	1.05×10^5 $TCID_{50}$/mL	3/3

Mycobacterium tuberculosis	1.21 x 10^7 CFU/mL	0/3	1.21 x 10^7 CFU/mL	3/3
Mycoplasma pneumoniae	2.70 x 10^7 CCU/mL	0/3	2.70 x 10^7 CCU/mL	3/3
Parainfluenza virus 1	3.80 x 10^5 $TCID_{50}$/mL	0/3	3.80 x 10^5 $TCID_{50}$/mL	3/3
Parainfluenza virus 2	3.39 x 10^6 $TCID_{50}$/mL	0/3	3.39 x 10^6 $TCID_{50}$/mL	3/3
Parainfluenza virus 3	1.15 x 10^6 $TCID_{50}$/mL	0/3	1.15 x 10^6 $TCID_{50}$/mL	3/3
Parainfluenza virus 4	9.55 x 10^5 $TCID_{50}$/mL	0/3	9.55 x 10^5 $TCID_{50}$/mL	3/3
Pooled human nasal cavity wash	n/a	0/3	n/a	3/3
Pseudomonas aeruginosa	4.93 x 10^8 CFU/mL	0/3	4.93 x 10^8 CFU/mL	3/3
Respiratory syncytial virus	1.0 x 10^5 $TCID_{50}$/mL	0/3	1.0 x 10^5 $TCID_{50}$/mL	3/3
Rhinovirus	1.0 x 10^5 $TCID_{50}$/mL	0/3	1.0 x 10^5 $TCID_{50}$/mL	3/3
Staphylococcus aureus	9.23 x 10^7 CFU/mL	0/3	9.23 x 10^7 CFU/mL	3/3
Staphylococcus epidermidis	6.84 x 10^8 CFU/mL	0/3	6.84 x 10^8 CFU/mL	3/3
Streptococcus pneumoniae	7.22 x 10^7 CFU/mL	0/3	7.22 x 10^7 CFU/mL	3/3
Streptococcus pyogenes	4.60 x 10^7 CFU/mL	0/3	4.60 x 10^7 CFU/mL	3/3

None of the organisms and viruses listed above showed cross-reactivity and interference in the assay at the concentrations listed.

The Sponsor performed *in silico* analysis for SARS-Coronavirus instead of the recommended wet-testing as the virus was not available. Cross reactivity of the test with SARS-Coronavirus could not be excluded from the in silico analysis. Accordingly, the intended use of the proposed device states that it cannot distinguish SARS-CoV-2 and SARS-CoV, the in silico analysis is acceptable.

The *in silico* analysis of *Pneumocystis jirovecii* identified two regions on the nucleocapsid protein of SARS-CoV-2 (P0DTC9), between amino acids 4-47 and 109-171. Both regions showed very low similarity compared to the translated amino acid sequences of *Pneumocystis jirovecii,* 31.8% and 28.1% percent identity, respectively. The longest consecutive matches had only 3 amino acids. Due to the low homology between the SARS-CoV-2 nucleocapsid protein and the amino acid sequences of *Pneumocystis jirovecii*, cross-reactivity or microbial interference of *Pneumocystis jirovecii with* the Flowflex COVID-19 Antigen Home Test is highly unlikely.

b) Interfering Substances:

Thirty-one (31) potentially interfering substances, diluted in negative clinical nasal swab matrix (see LoD study section below for generation of the negative clinical nasal swab matrix), were evaluated with the Flowflex COVID-19 Antigen Home Test. Each substance was tested in three (3) replicates in the absence or presence of heat-inactivated SARS-CoV-2 (isolate USA-WA1/2020) at 3x LoD (4.57 x 10^3 $TCID_{50}$/mL). Based on the test results, the endogenous and exogenous interfering substances tested at a concentration listed in Table 3 do not cross-react or interfere with the performance of the Flowflex COVID-19 Antigen Home Test at the concentrations tested.

Table 4: Interfering Substances Study Results

Interfering Substances Tested	**No SARS-CoV-2**		**SARS-CoV-2 virus (3x LoD)**	
	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	3,500 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 (alkalol)	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	15% v/v	0/3	15% v/v	3/3

Interfering Substances Tested	No SARS-CoV-2		SARS-CoV-2 virus (3x LoD)	
	Concentration Tested	Positive/ Tested	Concentration Tested	Positive/ Tested
(Triamcinolone Acetonide)				
Nasal decongestant (Galphimia glauca, Luffa opperculata, sabadilla)	15% v/v	0/3	15% v/v	3/3
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% v/v	0/3	5% v/v	3/3
Tobramycin	50 ug/mL	0/3	50 ug/mL	3/3
Whole Blood	2.5% v/v	0/3	2.5%	3/3

4. Assay Reportable Range:

Not applicable, the device is a binary qualitative assay that is visually read.

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

a) 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 (C) 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:

Positive nasal swab samples were generated by spiking heat inactivated SARS-CoV-2 WA1/2020 at 3x LoD (4.57 x 10^5 $TCID_{50}$/mL) into negative clinical nasal swab matrix and pipetting 50 µL on each swab. Negative swabs (50 µL negative clinical nasal sab matrix) and moderate positive swabs were then stored at 10°C, 15°C, 30°C, 35°C, and ambient temperature (25°C) for 1 hour, 2 hours, 3 hours and 4 hours before swabs were placed into the extraction buffer tubes for sample processing. Freshly prepared swabs were tested immediately as a control condition. The nasal swabs stored at each temperature condition for each time point were prepared in 5 replicates and tested with Flowflex COVID-19 Antigen Home Test per its IFU. The results are summarized in table below:

Table 5: Test results for sample stability study.

Temperature Condition	Time points	Negative sample					Positive sample at 3xLoD (4.57x10^3 $TCID_{50}$/mL)				
		Swab-1	Swab-2	Swab-3	Swab-4	Swab-5	Swab-1	Swab-2	Swab-3	Swab-4	Swab-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	-	-	-	-	-	+	+	+	+	+

The results read at 15 min were the same results as read at 30 min.

c) Device Stability:

In order to determine the stability of the Flowflex Plus COVID-19 Home Test three (3) test kits were stored at 2-8°C and at 30°C for 27 months. The shelf-life assessment started within one month of test kit manufacturing. Testing supports storage of the test at 2- 30°C for 24 months.

d) Shipping Stability:

In order to understand the stability of the test kit under different shipping conditions, Flowflex COVID-19 Antigen Home Test kits from a single lot were stored at three different temperature conditions: 60°C with 85% ±5% relative humidity, room temperature (25°C) and 2~8°C (as a

reference) for 8 days. The stored test kits were tested daily with five (5) replicates. All negative samples tested negative, all positive samples (3xLoD, $4.57x10^3$ $TCID_{50}$/mL) tested positive for all storage conditions.

In addition, the impact of harsh shipping conditions on the performance of Flowflex COVID-19 Antigen Home Test was assessed for three (3) different test lots that were stored at -20°C for 24 hours and then stored at room temperature for 24 hours. Three (3) freeze/thaw cycles were repeated after which the products were stored at 55°C for 35 days. All negative samples tested negative, all positive samples (3xLoD, $4.57x10^3$ $TCID_{50}$/mL) tested positive in all storage conditions over the 35 days.

6. Detection Limit:

a) Limit of Detection with heat-inactivated SARS-CoV-2 isolate USA-WA1/2020

The Limit of Detection (LoD) of the Flowflex COVID-19 Antigen Home Test was determined by evaluating different dilutions of heat-inactivated SARS-CoV-2 isolate USA-WA1/2020 into pooled negative clinical nasal swab matrix collected from multiple COVID-19 negative individuals. Each nostril was swabbed with a new swab, and each swab was eluted in 0.3 mL of PBS. The swab eluates were combined and mixed thoroughly. The pooled negative clinical nasal swab matrix was confirmed as negative for SARS-CoV-2 by RT-PCR. 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 limit of detection was established in two phases.

Range Finding LoD Study

Five serial dilutions were made from heat inactivated SARS-CoV-2 WA1/2020 virus into negative clinical nasal swab matrix. Five replicates were tested on two kit lots of the assay for each of five 1:10 dilutions to determine the preliminary LoD concentration of the device. The lowest concentration with 5/5 positive results from each lot was considered the preliminary LoD for each virus strain. For each replicate 50 µL of virus dilution was applied to a swab and the swab was processed according to the IFU. The results are summarized below.

Table 6: Preliminary LoD Study Summary for SARS-CoV-2 WA1/2020

Concentration of SARS CoV-2 WA1/2020 applied to dry swab	**Kit Lot MPS2100029 Positive/ Tested**	**Kit Lot MPS3020028 Positive/ Tested**
4.57×10^5 $TCID_{50}$/mL	5/5	5/5
4.57×10^4 $TCID_{50}$/mL	5/5	5/5
4.57×10^3 $TCID_{50}$/mL	**5/5**	**5/5**
4.57×10^2 $TCID_{50}$/mL	3/5	3/5

The results read at 15 min were the same as results read at 30 min.

Confirmatory LoD Study

The Preliminary LoD concentration and the concentrations above and below the LoD were tested with a total of twenty (n = 20) replicates of the same inactivated SARS-CoV-2 material for each of two test kit lots. To confirm the LoD for each lot, at least 19 of the 20 replicates should be positive. Per lot, the final confirmation data set included the confirmed LoD level with at least one additional level tested above and below to demonstrate that levels above LoD were 100% positive and levels below LoD were <95% positive. The results are summarized below.

Table 7: Confirmatory LoD Study Summary for SARS-CoV-2 WA1/2020

Concentration of SARS CoV-2 WA1/2020 applied to dry swab	Lot MPS2100029 Positive/ Tested	Lot MPS3020028 Positive/ Tested
1.37×10^4 $TCID_{50}$/mL (3-fold above the preliminary LoD)	20/20	20/20
4.57×10^3 $TCID_{50}$/mL (Preliminary LoD)	20/20	20/20
1.52×10^3 $TCID_{50}$/mL (3-fold below the preliminary LoD)	20/20	20/20
4.57×10^2 $TCID_{50}$/mL	2/5	2/5

The limit of detection for the Flowflex COVID-19 Antigen Home Test using SARS-CoV-2 strain WA1/2020, was confirmed to be 1.52×10^3 $TCID_{50}$/mL (7.6 x 10 $TCID_{50}$/swab) with both tested lots. In addition, 3 lots had previously been tested with this device under EUA 210494 using a different lot of SARS-CoV-2 WA1/2020 and resulted in a similar LoD of 2.5×10^3 $TCID_{50}$/mL (1.3×10^2 $TCID_{50}$/swab).

b) 1st WHO International Standard for SARS-CoV-2 antigen (NIBSC code: 21/368)

The LoD of the Flowflex COVID-19 Antigen HomeTest was also determined by evaluating different dilutions of WHO International Standard for SARS-CoV-2 antigen (NIBSC code: 21/368) in negative clinical nasal swab matrix (generated as described in the LoD study section above). Three ampoules of WHO International Standard for SARS-CoV-2 antigen were used for testing two lots of the Flowflex COVID-19 Antigen Home Test. Each ampoule of the lyophilized SARS-CoV-2 antigen standard was reconstituted in 0.25 mL of ultra-pure water and pooled together (concentration 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 out of 20 replicates tested positive). The limit of detection was established in two phases.

Range Finding LoD Study

Five serial dilutions were made from the WHO International Standard for SARS-CoV-2 antigen into negative clinical matrix. Three replicates were tested on two kit lots of the assay for each of five 1:2 dilutions to determine the preliminary LoD concentration of the device. The lowest concentration with 3/3 positive results from each lot 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 results are summarized below.

Table 8: Preliminary LoD Study Summary for WHO International Standard for SARS-CoV-2 antigen

Concentration of WHO International Standard for SARS-CoV-2 antigen applied to dry swab	Kit Lot MPS2100029 Positive/ Tested	Kit Lot MPS3020028 Positive/ Tested
10,000 IU/mL	3/3	3/3
5,000 IU/mL	3/3	3/3
2,500 IU/mL	3/3	3/3
1,250 IU/mL	3/3	3/3
625 IU/mL	**3/3**	**3/3**
312.5 IU/mL	2/3	1/3

The results read at 15 min were the same as results read at 30 min.

Confirmatory LoD Study

The Preliminary LoD concentration and the concentrations above and below the LoD were tested with a total of twenty (n = 20) replicates for each of two test kit lots. Sponsor tested a total of twenty (n = 20) replicates for the confirmatory dilutions for WHO International Standard for SARS-CoV-2 antigen. To confirm the LoD for each lot, at least 19 of the 20 replicates should be positive. The results are summarized below.

Table 9: Confirmatory LoD Study Summary for WHO International Standard for SARS-CoV-2 antigen

Concentration of WHO International Standard for SARS-CoV-2 antigen applied to dry swab	IU/Swab	Test concentration relative to LoD	Lot MPS2100029 Positive/ Tested	Lot MPS3020028 Positive/ Tested
1875 IU/mL	(94 IU/swab)	3x	20/20	20/20
625 IU/mL	**(31 IU/swab)**	**1x**	**20/20**	**20/20**
208 IU/mL	(10 IU/swab)	0.3x	8/20	7/20

The LoD for the Flowflex COVID-19 Antigen Home Test using 1st WHO International Standard for SARS-CoV-2 antigen (NIBSC code: 21/368) in nasal matrix was confirmed to be 625 IU/mL (31 IU/swab).

7. High Dose Hook Effect

The purpose of this study was to evaluate the effect of a high concentration of SARS antigen on the performance of the Flowflex COVID-19 Antigen Home Test. Five replicates were tested for each lot. 50 µl of heat-inactivated SARS-CoV-2 (isolate WA1/2020) diluted into negative clinical matrix at 2.82 x 10^7 $TCID_{50}$/mL, were added onto each swab and tested on the Flowflex COVID-19 Antigen Home Test. The testing was conducted according to the Instructions for Use for the test. The results are summarized below.

Table 10: Summary of High Dose Hook Effect Study

Concentration of SARS CoV-2 virus applied to dry swab	Lot#COV3030007 (Positive/Tested)	Lot#COV3030009 (Positive/Tested)	Lot#MPS2100029 (Positive/Tested)
2.82 x 10^7 $TCID_{50}$/mL	5/5	5/5	5/5
A very strong Test Line was observed 10-15 seconds after the addition of sample solution on each test cassete.			

High-dose hook effect was not observed when tested with concentrations up to 2.82 x 10^7 $TCID_{50}$/mL of heat-inactivated SARS-CoV-2 virus.

8. Inclusivity (Analytical Reactivity):

Analytical reactivity for Flowflex COVID-19 Antigen Home Test was demonstrated using 6 additional strains/isolates of SARS CoV-2 virus. Heat-inactivated SARS-CoV-2 isolates were each diluted into negative clinical nasal swab matrix at different concentrations (see LoD study section above for generation of the negative clinical nasal swab matrix). Each concentration was tested with 5 replicates until two consecutive dilutions produced one or more negative replicates out of 5. The reactivity of the Flowflex COVID-19 Antigen Home Test with the variants is

summarized below with the lowest concentration that returned 100% positive replicates (i.e., 5/5):

Table 11. Summary of the Flowflex COVID-19 test's reactivity with SARS-CoV-2 variants

SARS-CoV-2 Variants	Lowest Variant Concentration with 5/5 Positive Replicates [$TCID_{50}$/mL]
SARS-CoV-2 virus (USA-WA1/2020)	1.52×10^3
B.1.1.7 (Alpha)	4.20×10^2
B.1.351 (Beta)	1.05×10^3
P.1 (Gamma)	4.20×10^2
B.1.617.2 (Delta)	1.05×10^3
B.1.1.529 (Omicron)	5.01×10^2
BA.2.3 (Omicron)	2.45×10^3

A plan has been established by the sponsor to closely monitor for the emergence of any circulating variants of concern. The plan includes the monitoring of publicly available databases and information from public health authorities, and a multi-tier approach that determines reactivity of the test with emerging variants (including silico analysis, use of antibody escape mutational profile, and wet testing).

9. Assay Cut-Off:

Not applicable, the device is a qualitative assay with a binary result.

A Comparison Studies:

1. Method Comparison with Predicate Device:

Not applicable. See "C. Clinical Studies." for clinical performance.

2. Matrix Comparison:

The Flowflex COVID-19 Antigen 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. A Matrix Comparison study to support other sample types for clinical testing with this test was therefore not performed.

However, a matrix equivalency study between negative clinical nasal swab matrix and the surrogate matrix composed of 1% mucin in PBS, that was used in the precision study was conducted and demonstrated equivalent performance of the test with both matrices.

B Clinical Studies:

1. Clinical Sensitivity and Specificity:

The performance of the Flowflex COVID-19 Antigen Home Test was compared to the sample results as generated by a highly sensitive EUA authorized molecular SARS-CoV-2 RT-PCR assay with an extraction step. A prospective clinical study was conducted from December 2022 to February 2023. Nine clinical sites (Sites A- I) participated in the study.

A nasopharyngeal (NP) swab was collected first from symptomatic patients by a healthcare professional at the clinical study sites who inserted the swab into a tube containing 3 mL of Viral Transport Medium for comparator testing.

Thereafter, anterior nasal swab samples for the investigational device were either self-collected by a lay user aged ≥14 years or collected by an adult (parent/guardian) from individuals aged 2 - <14 years. Anterior nasal swabs were collected and processed by the lay users per the IFU of the proposed device. A total of 804 subjects were enrolled in this study, 699 subjects (age ≥14 years old) and 99 subjects ages 2 - 13 years. Six (6) subjects age ≥ 14 participated in paired-study as a donor and their nasal swab specimens were collected and tested by another person with age >14. Detailed study subject demographics are given below:

Table 12: Demographics - Age distribution and education of the clinical study cohort

Age group	Percent total	Total
2-13 years*	12%	99
14-24 years	10%	81
25-64 years	64%	514
≥65 years	13%	105
Total	**NA**	**799**

Education	Lay user	Total
Some High School	24**	3%
High School	508	63.6%
Some College	190	23.8%
Bachelors	53	6.6%
Master	18	2.2%
Doctorate	6	0.8%
Total	**799**	**100%**

*Tested by adult >14 years old per the intended use statement.
**24 users age 14 to <18 did not finish high school yet

The clinical study included about 22% low positive samples. The Flowflex COVID-19 Antigen Home Test detected SARS-CoV-2 with Positive Percent Agreement (PPA) of 89.8% and Negative Percent Agreement (NPA) of 99.3% when compared to the result of the SARS-CoV-2 RT-PCR comparator assay. Results are listed in the table below.

Table 13: Performance of Flowflex COVID-19 Test compared to high sensitivity RT-PCR COVID-19.

Flowflex COVID-19 Antigen Home Test	EUA RT-PCR Comparator		
	Positive	**Negative**	**Total**
Positive	212	4	216
Negative	24	559	583
Total	236	563	799
Positive Percent Agreement (PPA)	**89.8% (212/236)** (95%CI: 85.2% - 93.4%)		

Negative Percent Agreement (NPA)	**99.3% (559/563)** (95%CI: 98.2% - 99.8%)

Table 14: Cumulative PPA results by DPSO.

Days Since Symptom Onset	# Specimens Tested	# Cumulative Positive by Flowflex COVID-19 Antigen Home Test	# Cumulative Positive by RT-PCR	Cumulative PPA
0 to 1 day	203	47	53	88.7%
0 to 2 days	496	137	146	93.8%
0 to 3 days	643	169	184	91.9%
0 to 4 days	727	190	209	90.9%
0 to 5 days	774	204	227	89.9%
0 to 6 days	799	212	236	89.8%

C Clinical Cut-Off:

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

D Expected Values/Reference Range:

A patient sample is expected to be negative for SARS-CoV-2.

E Other Supportive Performance Characteristics Data:

1. Flex Studies

To assess the robustness of the Flowflex COVID-19 Antigen Home Test, flex studies were conducted that assessed all major aspects of the test procedure (sample volume, reading time, extraction buffer volume, bubbles in reagent tube, 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, lighting, various temperature and humidity stress conditions). Testing was performed with contrived positive nasal swabs generated by diluting heat inactivated SARS-CoV-2 virus into negative clinical nasal swab matrix at 2xLoD. The studies support that the test is robust in the intended use condition with an insignificant risk of erroneous result.

2. Usability Study:

A usability study was conducted to assess the lay user's ability to understand the instructions for use and to adequately execute the Flowflex COVID-19 Antigen Home Test workflow accordingly. A total of 431 subjects, ages 18 years and older, were enrolled in the study and were observed during testing. A total of 98.8% successfully completed testing by receiving a Negative or Positive result.

Following the usability study, all subjects were issued a questionnaire to assess users' comprehension of the test. The questionnaire was completed by 431 subjects enrolled in the EUA clinical study (one user missed one question). The questionnaire assessed users' understanding of concepts such as the test purpose and interpretation of results.

3. Readability Study:

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

The Readability Study was conducted according to an IRB approved protocol in a simulated home environment. A total of 61 lay users with diverse gender, ages and educational background who met the study inclusion criteria, were enrolled for the Readability Study. Each lay user was asked to interpret four test devices with three different concentrations that were arranged in blinded test panels with the following sample results; the order of the results within the panel was randomized:

- Negative, 1.5xLoD, 1.5xLoD, 5xLoD
- Negative, Negative, 1.5xLoD, 5xLoD

52% of the study participants were male and 48% of the study participants were female. 50% of individuals were vision impaired, and about 55% of individuals were either still at high school or had a high school degree as their highest level of education.

Table 15 Demographics - Age distribution and education of the clinical study cohort

Age group	Number of lay users	Percentage
14-20 years	13	21%
21-30 years	9	15%
31-55 years	25	41%
> 55 years	14	23%
Total	**61**	**100%**

Education	Lay user	Total
Still at High School	9	15%
High School or Equivalent	23	38%
College	13	21%
Bachelors	14	23%
Masters	2	3%
Doctorate	0	0%
Total	**61**	**100%**

*Tested by adult >14 years old per the intended use statement.
**24 users age 14 to <18 did not finish high school yet

Table 16: Vision impairment of lay user in readability study

Type of vision impairment	Yes	Percentage of vision impairment
Near sightedness only (with lens prescriptions)	17	28% (17/61 lay users)
Far sightedness only (with lens prescriptions)	8	13% (8/61 lay users)
Near sightedness and Far sightedness (with lens prescriptions)	5	8% (5/61 lay users)

Far sightedness and Glaucoma (with lens prescriptions)	1	2% (1/61 lay users)
Total lay users with vision impairment	**31**	**51% (31/61 lay users)**

Negative samples and samples with concentrations at 5xLoD were all correctly interpreted. Only samples with low concentration were occasionally interpreted incorrectly as negative. All of these false negative results were determined by the age group of >55 years of age, and were mostly (4/5) generated by individuals with vision impairment. 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).

Table 17: Readability study summary stratified by age and overall

Age group	**Sample Concentrations**			**Correct/ Total**
	Correct/ Negative	**Correct/ Low Positive (1.5xLoD)**	**Correct/ Positive (5xLoD)**	
14-20 years	22/22	17/17	13/13	52/52
21-30 years	13/13	14/14	9/9	36/36
31-55 years	36/36	39/39	25/25	100/100
> 55 years	21/21	16/21	14/14	51/56
Total	**92/92**	**86/91**	**61/61**	**239/244**
Negative Agreement	**100% (92/92)**	**n/a**	**n/a**	**100% (92/92)** (95%CI: 96%-100%)
Low Positive (1.5xLoD) Agreement	**n/a**	**95% (86/91)**	**n/a**	**95% (86/91)** (95%CI: 88%-98%)
Positive (5xLoD) Agreement	**n/a**	**n/a**	**100% (61/61)**	**100% (61/61)** (95% CI: 94%-100%)

IV Proposed Labeling:

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

V Conclusion:

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