CLIA Waiver by Application Approval Determination
Decision Summary

I. Document Number

CW260010

II. Parent Document Number

K261212

III. CLIA Waiver Type

Dual 510(k) and CLIA Waiver by Application (Dual Submission)

IV. Applicant

ACON Laboratories, Inc.

V. Proprietary and Established Names

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

VI. Measurand (analyte)

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

VII. Sample Type(s)

Anterior Nasal Swab Samples

VIII. Type of Test

Qualitative lateral flow immunoassay

IX. Test System Description

A Overview

The Flow*flex* Plus RSV + Flu A/B + COVID Rapid Test is a lateral flow immunoassay in sandwich format intended for the qualitative detection and differentiation of RSV, influenza A, influenza B, and SARS-CoV-2 protein antigens directly in anterior nasal swab specimens from

individuals with signs and symptoms of respiratory tract infection. This test is intended for prescription use only by healthcare professionals.

The test cassette is assembled with two strips (Strip 1 for SARS-coV-2, Flu a and Flu B; Strip 2 for RSV) in a plastic housing comprising of the following components: Sample receiving and particles pad, nitrocellulose (NC) membrane, test lines for each analyte and absorption pad. The test lines and the control lines are pre-coated with analyte specific antibodies on the membrane. In both strips, attached to the NC membrane are the sample pad (which absorbs and distributes the sample), the reagent pad (containing reagents for antigen binding and detection), and the absorbent pad (which collects the excess liquid sample and reagents).

For collecting nasal swabs from children less than 2 years, users employ a swab guard which serves as an added safety measure, preventing the nasal swab from going deeper than intended, limiting the risk of injury in children.

Nasal swabs are collected from individuals with signs and symptoms of respiratory infection. The swab is then processed in extraction buffer and then added to the sample well of the test cassette. When an adequate volume of the processed specimen is assed to the sample well of the test cassette, the specimen migrates by capillary action across each of the two test strips. SARS-CoV-2, Flu A, Flu B, or RSV antigen, if present in the specimen, will react with the specific antibody-coated colored particles. The mixture then migrates towards the membrane by capillary action to bind to the specific antibody on the NC membrane, producing a visible colored test line in the related test line region (CoV/A/B/R).

To serve as a procedural control, the colored rabbit IgG labeled particle will bind to goat anti-rabbit IgG on each NC membrane on each test strip to produce a colored line on the control line (Ctl) in the control line region. Formation of the control lines serves as an internal control indicating that proper volume of specimen has been added, and membrane wicking has occurred. After dispensing the test specimen into the sample well, the result should be read at 15 minutes. Results should not be read before 15 minutes and after 30 minutes.

External controls are included with the test kit. It is recommended that positive and negative controls be tested once for each untrained operator and once for each new shipment of kits – provided that each different lot in the shipment is tested. The control swabs are intended to be used as quality control samples representative of positive and negative test samples to demonstrate that the reagents are functional and the assay procedure is performed correctly.

B Test System Components

The composition of the Flow*flex* Plus RSV + Flu A/B + COVID Rapid Test are as follows:

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

- 1 English Package Insert
- 1 English Quick Reference Instructions

X. Specific Contents for CLIA Waiver

A Demonstrating "Simple":

The Flow*flex* Plus RSV + Flu A/B + COVID Rapid Test is designed to be simple and easy to use incorporating the following key features:

- The test is self-contained.
- The test uses direct unprocessed anterior nasal swab samples.
- The test needs only basic, non-technique-dependent specimen manipulation and reagent handling. No extra equipment is needed for sample processing.
- The supplied reagents are premeasured and provided in single-use vials.
- The test does not require any operator intervention during the analysis step.
- The test does not require technical or specialized training for troubleshooting or interpretation of multiple or complex error codes.
- The device is for one-time use and disposable. It does not require any electronic or mechanical maintenance beyond simple tasks.
- The test produces results that do not require operator calibration, interpretation, or calculation.
- The test produces easily determinable results, such as 'positive', 'negative' or 'invalid'.
- The test procedure within the QRI is written at no higher than a 7th-grade reading level.

B Demonstrating "Insignificant Risk of an Erroneous Result"- Failure Alerts and Fail-Safe Mechanisms

1. Risk Analysis:

   A comprehensive risk analysis was conducted in accordance with ISO 14971:2019 which included identification and addressing of potential risks or error sources, analyzing potential causes, effects and the existing measures or mitigation factors related to the Flow*flex* Plus RSV + Flu A/B + COVID Rapid Test. The elements considered included operator error, environmental factors, specimen and reagent handling, storage, and external controls.

   Potential sources of errors that could adversely affect system performance were identified and mitigated first through system design verification and validation studies and then through additional precautions and warnings in the labeling. The identified risks which could result in erroneous test results were evaluated in flex studies that stressed the functional limits of the test system (see Item 3 below).

2. Fail-Safe and Failure Alert Mechanisms:

   The Flow*flex* Plus RSV + Flu A/B + COVID Rapid Test was designed to include numerous features and fail-safe mechanisms built into the system to prevent erroneous results.

Design Features:

- Each test cassette is packaged in a foil pouch with desiccant to maintain the integrity of the test device and reagents.
- The kit is printed with the assay name/type, lot number, and expiration date to ensure clarity and appropriate use.
- Swab guards are provided with the test kit for nasal swab sample collection for patients less than 2 years old.
- Nasal swabs are directly placed into the extraction buffer tube for processing, and the extracted antigen is then applied directly to the sample well of the test cassette eliminating additional specimen-transfer steps.
- The dropper tip of the extraction buffer solution helps prevent sample loss and contamination.
- Each test cassette features distinct position marks within the results window to facilitate clear and accurate result interpretation. The control line is denoted as "Ctl", the SARS-CoV-2 antigen line is denoted as "CoV", the Flu A antigen line is denoted as "A", the Flu B antigen line is denoted as "B", and the RSV antigen line is denoted as "R".
- The test cassette is printed with "RSV + Flu A/B + COVID" to confirm the assay type being tested and to further ensure clarity and accuracy.
- Each test cassette features a four-drop icon above the sample well to ensure the correct amount of sample is added.
- Each positive and negative external control swab is supplied with the test kit and are clearly marked on the labeling with control swab name, lot number, and expiration date to ensure clarity and appropriate use.

Fail-safe Features:

- ***Internal Procedural Control:***

Internal procedural controls are included in the test. A red or pink line appearing in the control line regions (Ctl) is an internal procedural control. The appearance of the procedural control lines indicates that proper volume of specimen has been added and capillary flow occurred. If the procedural control lines do not develop in 15 minutes, the test result is considered invalid, and retesting with a new cassette is recommended.

- ***External Quality Controls:***

Two different external control swabs are provided with the test kit to ensure that the reagents and test cassette are functioning properly, and to demonstrate proper use and performance by the operator:

  - The Positive Control Swab contains RSV, influenza A, influenza B, and SARS-CoV-2 antigen (non-infective recombinant protein).
  - The Negative Control Swab contains negative control buffer (non-infective).

The manufacturer recommends that external control testing be performed once for each untrained operator and once for each new lot of test kits of Flow*flex* Plus RSV + Flu A/B + COVID Rapid Test. This will ensure that the test is working appropriately to generate valid results.

External control swabs are extracted and processed according to the test instructions for use. When the positive control is tested, reddish lines appear at the Ctl as well as RSV, Flu A, Flu B, and CoV positions. When the negative control is tested, a reddish line appears at the Ctl position only. Each control swab is individually packaged in a foil pouch, and the pouch is printed with information such as control swab type, lot number and the expiration date. Users are instructed not to use expired external controls. Upon confirmation of the expected results, the kit is ready for use with patient specimens. If external controls do not perform as expected, users are instructed to not use the test results and to repeat the tests or to contact the manufacturer.

3. Flex Studies:

To assess the robustness and risk of false results of the test, the sponsor conducted a series of flex studies. For all these studies, one device lot was tested with three samples for each study condition: one negative (pooled negative swab matrix [PNSM] only) and two 2X LoD co-spiked positive samples. To mitigate potential biases for visually read multianalyte antigen tests, double and triple co-spiked positive samples were prepared in various analyte combinations and were tested under various flex conditions with the candidate device. Each sample was tested in 5 replicates at each condition, and all test operators were blinded to the status of the test samples. The summary of results is presented in Table 1 below:

Table 1: Summary of Flex Study Results:

Conditions Tested		Samples	# Positive / # Total			
			SARS-CoV-2	Flu A	Flu B	RSV
Reading Time	5 minutes	Negative	0/5	0/5	0/5	0/5
		2X LoD Flu A+ Flu B +RSV	0/5	0/5	0/5	0/5
		2X LoD SARS-CoV-2+ Flu B +RSV	0/5	0/5	0/5	0/5
	10, 13, 15, 30, 45 and 60 minutes	Negative	0/5	0/5	0/5	0/5
		2X LoD Flu A+ Flu B +RSV	0/5	5/5	5/5	5/5
		2X LoD SARS-CoV-2+ Flu B +RSV	5/5	0/5	5/5	5/5
Lighting Flex study	Fluorescent bulb (control condition)	Negative	0/5	0/5	0/5	0/5
		2X LoD SARS-CoV-2 +RSV	5/5	0/5	0/5	5/5
		2X LoD Flu A+ Flu B +RSV	0/5	5/5	5/5	5/5
	Mercury vapor bulb	Negative	0/5	0/5	0/5	0/5
		2X LoD SARS-CoV-2 +RSV	5/5	0/5	0/5	5/5
		2X LoD Flu A+ Flu B +RSV	0/5	5/5	5/5	5/5
		Negative	0/5	0/5	0/5	0/5

Conditions Tested		Samples	# Positive / # Total			
			SARS-CoV-2	Flu A	Flu B	RSV
	Sodium vapor bulb	2X LoD SARS-CoV-2 +RSV	5/5	0/5	0/5	5/5
		2X LoD Flu A+ Flu B +RSV	0/5	5/5	5/5	5/5
	Incandescent bulb	Negative	0/5	0/5	0/5	0/5
		2X LoD SARS-CoV-2 +RSV	5/5	0/5	0/5	5/5
		2X LoD Flu A+ Flu B +RSV	0/5	5/5	5/5	5/5
	LED	Negative	0/5	0/5	0/5	0/5
		2X LoD SARS-CoV-2 +RSV	5/5	0/5	0/5	5/5
		2X LoD Flu A+ Flu B +RSV	0/5	5/5	5/5	5/5
	Dome Light	Negative	0/5	0/5	0/5	0/5
		2X LoD SARS-CoV-2 +RSV	5/5	0/5	0/5	5/5
		2X LoD Flu A+ Flu B +RSV	0/5	5/5	5/5	5/5
	Dusk	Negative	0/5	0/5	0/5	0/5
		2X LoD SARS-CoV-2 +RSV	5/5	0/5	0/5	5/5
		2X LoD Flu A+ Flu B +RSV	0/5	5/5	5/5	5/5
	Direct Sunlight	Negative	0/5	0/5	0/5	0/5
		2X LoD SARS-CoV-2 +RSV	5/5	0/5	0/5	5/5
		2X LoD Flu A+ Flu B +RSV	0/5	5/5	5/5	5/5
Bubbles in Reagent Tube Flex Study	Shake extraction buffer tube up and down vigorously for 60 seconds	Negative	0/5	0/5	0/5	0/5
		2X LoD Flu B+RSV	0/5	0/5	5/5	5/5
		2X LoD SARS-CoV-2+ Flu A+RSV	5/5	5/5	0/5	5/5
	Shake extraction buffer tube up and down vigorously for 30 seconds	Negative	0/5	0/5	0/5	0/5
		2X LoD Flu B+RSV	0/5	0/5	5/5	5/5
		2X LoD SARS-CoV-2+ Flu A+RSV	5/5	5/5	0/5	5/5
	200	Negative	0/5	0/5	0/5	0/5

Conditions Tested		Samples	# Positive / # Total			
			SARS-CoV-2	Flu A	Flu B	RSV
Extraction Buffer Volume Variability Flex Study		2X LoD Flu A+RSV	0/5	5/5	0/5	5/5
		2X LoD SARS-CoV-2+ Flu B +RSV	5/5	0/5	5/5	5/5
	250	Negative	0/5	0/5	0/5	0/5
		2X LoD Flu A+RSV	0/5	5/5	0/5	5/5
		2X LoD SARS-CoV-2+ Flu B +RSV	5/5	0/5	5/5	5/5
	300	Negative	0/5	0/5	0/5	0/5
		2X LoD Flu A+RSV	0/5	5/5	0/5	5/5
		2X LoD SARS-CoV-2+ Flu B +RSV	5/5	0/5	5/5	5/5
	350	Negative	0/5	0/5	0/5	0/5
		2X LoD Flu A+RSV	0/5	5/5	0/5	5/5
		2X LoD SARS-CoV-2+ Flu B +RSV	5/5	0/5	5/5	5/5
	400	Negative	0/5	0/5	0/5	0/5
		2X LoD Flu A+RSV	0/5	5/5	0/5	5/5
		2X LoD SARS-CoV-2+ Flu B +RSV	5/5	0/5	5/5	5/5
Sample Volume Variability Flex Study	1 drop	Negative	Invalid	Invalid	Invalid	Invalid
		2X LoD Flu A+RSV	Invalid	Invalid	Invalid	Invalid
		2X LoD SARS-CoV-2+ Flu B+RSV	Invalid	Invalid	Invalid	Invalid
	2 drops	Negative	0/5	0/5	0/5	0/5
		2X LoD Flu A+RSV	0/5	5/5	0/5	5/5
		2X LoD SARS-CoV-2+ Flu B+RSV	5/5	0/5	5/5	5/5
	3 drops	Negative	0/5	0/5	0/5	0/5
		2X LoD Flu A+RSV	0/5	5/5	0/5	5/5
		2X LoD SARS-CoV-2+ Flu B+RSV	5/5	0/5	5/5	5/5
	4 drops	Negative	0/5	0/5	0/5	0/5
		2X LoD Flu A+RSV	0/5	5/5	0/5	5/5
		2X LoD SARS-CoV-2+ Flu B+RSV	5/5	0/5	5/5	5/5

Conditions Tested		Samples	# Positive / # Total			
			SARS-CoV-2	Flu A	Flu B	RSV
	5	Negative	0/5	0/5	0/5	0/5
		2X LoD Flu A+RSV	0/5	5/5	0/5	5/5
		2X LoD SARS-CoV-2+ Flu B+RSV	5/5	0/5	5/5	5/5
	6	Negative	0/5	0/5	0/5	0/5
		2X LoD Flu A+RSV	0/5	5/5	0/5	5/5
		2X LoD SARS-CoV-2+ Flu B+RSV	5/5	0/5	5/5	5/5
Temperature and Humidity Extremes Flex Study	Condition 1 (5°C & RH 10±1%)	Negative	0/5	0/5	0/5	0/5
		2X LoD SARS-CoV-2 +RSV	5/5	0/5	0/5	5/5
		2X LoD Flu A+Flu B+RSV	0/5	5/5	5/5	5/5
	Condition 2 (5°C & RH 90±1%)	Negative	0/5	0/5	0/5	0/5
		2X LoD SARS-CoV-2 +RSV	5/5	0/5	0/5	5/5
		2X LoD Flu A+Flu B+RSV	0/5	5/5	5/5	5/5
	Condition 3 (45°C & RH 10±1%)	Negative	0/5	0/5	0/5	0/5
		2X LoD SARS-CoV-2 +RSV	5/5	0/5	0/5	5/5
		2X LoD Flu A+Flu B+RSV	0/5	5/5	5/5	5/5
	Condition 4 (45°C & RH 90±1%)	Negative	0/5	0/5	0/5	0/5
		2X LoD SARS-CoV-2 +RSV	5/5	0/5	0/5	5/5
		2X LoD Flu A+Flu B+RSV	0/5	5/5	5/5	5/5
	Condition 5 (Control)	Negative	0/5	0/5	0/5	0/5
		2X LoD SARS-CoV-2 +RSV	5/5	0/5	0/5	5/5
		2X LoD Flu A+Flu B+RSV	0/5	5/5	5/5	5/5
Disturbances while testing	Control (Following IFU)	Negative	0/5	0/5	0/5	0/5
		2X LoD SARS-CoV-2+RSV	5/5	0/5	0/5	5/5
		2X LoD Flu A+Flu B+RSV	0/5	5/5	5/5	5/5
	Dropping the device from the bench to the floor	Negative	0/5	0/5	0/5	0/5
		2X LoD SARS-CoV-2+RSV	5/5	0/5	0/5	5/5

Conditions Tested		Samples	# Positive / # Total			
			SARS-CoV-2	Flu A	Flu B	RSV
	before adding samples	2X LoD Flu A+Flu B+RSV	0/5	5/5	5/5	5/5
	Dropping the device 30-60 seconds after sample addition	Negative	0/5	0/5	0/5	0/5
		2X LoD SARS-CoV-2+RSV	5/5	0/5	0/5	5/5
		2X LoD Flu A+Flu B+RSV	0/5	5/5	5/5	5/5
	Flipping the device 30-60 seconds after adding the samples	Negative	0/5	0/5	0/5	0/5
		2X LoD SARS-CoV-2+RSV	5/5	0/5	0/5	5/5
		2X LoD Flu A+Flu B+RSV	0/5	5/5	5/5	5/5
Inadequate Temperature Equilibration Study	Control (RT)	Negative	0/5	0/5	0/5	0/5
		2X LoD SARS-CoV-2 +RSV	5/5	0/5	0/5	5/5
		2X LoD Flu A+Flu B+RSV	0/5	5/5	5/5	5/5
	Non-equilibrated sample	Negative	0/5	0/5	0/5	0/5
		2X LoD SARS-CoV-2 +RSV	5/5	0/5	0/5	5/5
		2X LoD Flu A+Flu B+RSV	0/5	5/5	5/5	5/5
Swab Elution Variability Flex Study	Condition 1 (Control)	Negative	0/5	0/5	0/5	0/5
		2X LoD SARS-CoV-2 +RSV	5/5	0/5	0/5	5/5
		2X LoD Flu A+Flu B+RSV	0/5	5/5	5/5	5/5
	Condition 2 (Swirl swabs for 60 secs)	Negative	0/5	0/5	0/5	0/5
		2X LoD SARS-CoV-2 +RSV	5/5	0/5	0/5	5/5
		2X LoD Flu A+Flu B+RSV	0/5	5/5	5/5	5/5
	Condition 3 (Swirl swabs for 15 secs)	Negative	0/5	0/5	0/5	0/5
		2X LoD SARS-CoV-2 +RSV	5/5	0/5	0/5	5/5
		2X LoD Flu A+Flu B+RSV	0/5	5/5	5/5	5/5
	Condition 4 (Swirl swabs for 2 secs)	Negative	0/5	0/5	0/5	0/5
		2X LoD SARS-CoV-2 +RSV	5/5	0/5	0/5	5/5
		2X LoD Flu A+Flu B+RSV	0/5	5/5	5/5	5/5
	Condition 5	Negative	0/5	0/5	0/5	0/5

Conditions Tested		Samples	# Positive / # Total			
			SARS-CoV-2	Flu A	Flu B	RSV
	(No swirling swab)	2X LoD SARS-CoV-2 +RSV	5/5	0/5	0/5	5/5
		2X LoD Flu A+Flu B+RSV	0/5	5/5	5/5	5/5
	Condition 6 (Rotating swabs 10 times while squeezing tube)	Negative	0/5	0/5	0/5	0/5
		2X LoD SARS-CoV-2 +RSV	5/5	0/5	0/5	5/5
		2X LoD Flu A+Flu B+RSV	0/5	5/5	5/5	5/5
	Condition 7 (Rotating swabs 2 times while squeezing tube)	Negative	0/5	0/5	0/5	0/5
		2X LoD SARS-CoV-2 +RSV	5/5	0/5	0/5	5/5
		2X LoD Flu A+Flu B+RSV	0/5	5/5	5/5	5/5
	Condition 8 (Rotating swabs 10 times without squeezing tube)	Negative	0/5	0/5	0/5	0/5
		2X LoD SARS-CoV-2 +RSV	5/5	0/5	0/5	5/5
		2X LoD Flu A+Flu B+RSV	0/5	5/5	5/5	5/5
	Condition 9 (Rotating swabs 5 times without squeezing tube)	Negative	0/5	0/5	0/5	0/5
		2X LoD SARS-CoV-2 +RSV	5/5	0/5	0/5	5/5
		2X LoD Flu A+Flu B+RSV	0/5	5/5	5/5	5/5
	Condition 10 (Rotating swabs 2 times without squeezing tube)	Negative	0/5	0/5	0/5	0/5
		2X LoD SARS-CoV-2 +RSV	5/5	0/5	0/5	5/5
		2X LoD Flu A+Flu B+RSV	0/5	5/5	5/5	5/5
	Condition 11 (No Rotating swabs)	Negative	0/5	0/5	0/5	0/5
		2X LoD SARS-CoV-2 +RSV	5/5	0/5	0/5	5/5
		2X LoD Flu A+Flu B+RSV	0/5	5/5	5/5	5/5
	Condition 12 (Remove swab without squeezing tube)	Negative	0/5	0/5	0/5	0/5
		2X LoD SARS-CoV-2 +RSV	5/5	0/5	0/5	5/5
		2X LoD Flu A+Flu B+RSV	0/5	5/5	5/5	5/5
	Condition 13 (No mixing tube after removing the swab)	Negative	0/5	0/5	0/5	0/5
		2X LoD SARS-CoV-2 +RSV	5/5	0/5	0/5	5/5
		2X LoD Flu A+Flu B+RSV	0/5	5/5	5/5	5/5

Conditions Tested		Samples	# Positive / # Total			
			SARS-CoV-2	Flu A	Flu B	RSV
	Condition 14 (No swirling, no rotating, no squeezing, no mixing)	Negative	0/5	0/5	0/5	0/5
		2X LoD SARS-CoV-2 +RSV	2/5	0/5	0/5	2/5
		2X LoD Flu A+Flu B+RSV	0/5	1/5	1/5	2/5
Non-level Surface Flex Study	Flat	Negative	0/5	0/5	0/5	0/5
		2X LoD Flu B+RSV	0/5	0/5	5/5	5/5
		2X LoD SARS-CoV-2+Flu A+Flu B	5/5	5/5	5/5	0/5
	Upright	Negative	0/5	0/5	0/5	0/5
		2X LoD Flu B+RSV	0/5	0/5	5/5	5/5
		2X LoD SARS-CoV-2+Flu A+Flu B	5/5	5/5	5/5	0/5
	Side-laying	Negative	0/5	0/5	0/5	0/5
		2X LoD Flu B+RSV	0/5	0/5	5/5	5/5
		2X LoD SARS-CoV-2+Flu A+Flu B	5/5	5/5	5/5	0/5
	Upside-down	Negative	0/5	0/5	0/5	0/5
		2X LoD Flu B+RSV	0/5	0/5	5/5	5/5
		2X LoD SARS-CoV-2+Flu A+Flu B	5/5	5/5	5/5	0/5
Sample Stability in Extraction Buffer Flex Study	Storing swabs in the extraction buffer before swirling, following storage at 30°C (0, 1, 2, 3 and 4 hrs)	Negative	0/5	0/5	0/5	0/5
		2X LoD SARS-CoV-2+Flu B+RSV	5/5	0/5	5/5	5/5
		2X LoD Flu A+Flu B+RSV	0/5	5/5	5/5	5/5
	Storing swabs in the extraction buffer after swirling, following storage at 30°C (0, 1, 2, 3 and 4 hrs)	Negative	0/5	0/5	0/5	0/5
		2X LoD SARS-CoV-2+Flu B+RSV	5/5	0/5	5/5	5/5
		2X LoD Flu A+Flu B+RSV	0/5	5/5	5/5	5/5

Thus, the flex studies demonstrate that the test is robust in the claimed intended use condition with an insignificant risk of erroneous results.

C Demonstrating "Insignificant Risk of an Erroneous Result" – Accuracy

1. Comparison Study:

A prospective clinical study was conducted from October 2024 to April 2025 to assess the performance of the Flow*flex* Plus RSV + Flu A/B + COVID Rapid Test when compared to an FDA cleared RT-PCR assay. The study prospectively enrolled symptomatic subjects at 10 geographically distinct study sites located in the United States. Enrolled subjects were aged 6 months or older who exhibited symptoms of infection consistent with COVID-19, influenza, or RSV at the time of collection and who were within 5 days post symptom onset (DPSO).

Testing was performed in a simulated home environment by lay users. A nasopharyngeal swab was collected first by the study operator, placed into transport tube containing viral transport medium, and shipped on dry ice to a central lab for testing with a highly sensitive RT-PCR comparator assay. Thereafter, an anterior nasal (AN) swab was collected per the candidate test's quick reference instructions (QRI). The swabs were either self-collected by a lay user aged ≥14 years or collected by an adult (parent/ guardian) from individuals aged ≥6 months old. Samples were then immediately tested with the Flow*flex* Plus RSV + Flu A/B + COVID Rapid Test according to the test QRI.

A total of 1263 anterior nasal swab samples were collected from symptomatic individuals within 5 days of respiratory symptom onset and met the inclusion criteria for the analysis, of which 1257 samples were evaluable for SARS-CoV-2 and 1261 samples evaluable for Flu A/B and RSV respectively. The subject demographics are shown in Table 2 below.

Table 2: Subject Demographics:

Specific Demographic	Lay user collecting and testing	Self-collecting and testing	Overall
	(N=634)	(N=629)	(N=1263)
Age			
Mean (SD)	5.9 (4.0)	48.6 (19.6)	27.2 (25.6)
Median [Min, Max]	5 [0.5, 17]	49 (14, 91)	14 (0.5, 91)
Age Group			
≥ 6 months - < 2 years of age	114 (18.0%)	0 (0.0%)	114 (9.0%)
2-5 years of age	212 (33.4%)	0 (0.0%)	212 (16.8%)
6-13 years of age	296 (46.7%)	0 (0.0%)	296 (23.4%)
14-21 years of age	12 (1.9%)	75 (11.9%)	87 (6.9%)
22-59 years of age	0 (0.0%)	314 (49.9%)	314 (24.9%)
≥ 60 years of age	0 (0.0%)	240 (38.2%)	240 (19.0%)
Sex at Birth			
Female	300 (47.3%)	355 (56.4%)	655 (51.9%)
Male	334 (52.7%)	274 (43.6%)	608 (48.1%)
Ethnicity			
Hispanic/Latino	140 (22.1%)	75 (11.9%)	215 (17.0%)
Not Hispanic/Latino	494 (77.9%)	554 (88.1%)	1048 (83.0%)

The performance of the Flow*flex* Plus RSV + Flu A/B + COVID Rapid Test when compared to FDA-cleared highly sensitive RT-PCR molecular assays are presented in the tables below.

Table 3: SARS-CoV-2 Clinical Performance:

SARS-CoV-2	Comparator Positive	Comparator Negative	Total
Candidate Positive	131	1	132
Candidate Negative	12	1113	1125
Total	143	1114	1257*
Positive Percent Agreement (PPA)	91.6% (131/143) (95% C.I.: 85.9% - 95.1%)		
Negative Percent Agreement (NPA)	99.9% (1113/1114) (95% C.I.: 99.5% - 100.0%)		

*6 samples excluded due to invalid results with comparator methods

Table 4: Influenza A Performance:

Influenza A	Comparator Positive	Comparator Negative	Total
Candidate Positive	236	2	238
Candidate Negative	18	1005	1023
Total	254	1007	1261*
Positive Percent Agreement (PPA)	92.9% (236/254) (95% C.I.: 89.1% - 95.5%)		
Negative Percent Agreement (NPA)	99.8% (1005/1007) (95% C.I.: 99.3% - 100.0%)		

*2 samples excluded due to invalid results with comparator methods

Table 5: Influenza B Performance:

Influenza B	Comparator Positive	Comparator Negative	Total
Candidate Positive	91	1	92
Candidate Negative	7	1162	1169
Total	98	1163	1261*
Positive Percent Agreement (PPA)	92.9% (91/98) (95% C.I.: 86.0% - 96.5%)		
Negative Percent Agreement (NPA)	99.9% (1162/1163) (95% C.I.: 99.5% - 100.0%)		

*2 samples excluded due to invalid results with comparator methods

Table 6: RSV Performance:

RSV	Comparator Positive	Comparator Negative	Total
Candidate Positive	159	2	161
Candidate Negative	10	1090	1100
Total	169	1092	1261*
Positive Percent Agreement (PPA)	94.1% (159/169) (95% C.I.: 89.5% - 96.8%)		

RSV	Comparator Positive	Comparator Negative	Total
Negative Percent Agreement (NPA)	99.8% (1090/1092) (95% C.I.: 99.3% - 100.0%)		

*2 samples excluded due to invalid results with comparator methods

Table 7: RSV Performance Stratified by Age Group:

Age Group	Number of Subjects Tested	Candidate Positives	Comparator Positives	PPA (95% CI)
6-11 months	51	21	21	100.0% (84.5%, 100.0%)
12-17 months	47	17	17	100.0% (81.6%, 100.0%)
18-23 months	16	5	5	100.0% (56.6%, 100.0%)
< 2 years	**114**	**43**	**43**	**100.0% (91.8%, 100.0%)**
2-5 years	212	75	83	90.4% (82.1%, 95.0%)
6-13 years	296	20	21	95.2% (77.3%, 99.2%)
14-21 years	87	6	6	100% (60.9%, 100.0%)
22-59 years	314	8	9	88.9% (56.5%, 98.0%)
>=60 years	238	7	7	100.0% (64.6%, 100.0%)
Total	**1261**	**159**	**169**	**94.1% (89.5%, 96.8%)**

2. Device performance with analyte Concentrations Near the Cutoff:

a. Lot-to-lot Precision:

The multi-lot precision for the Flow*flex* Plus RSV + Flu A/B + COVID Rapid Test was evaluated in two different in-house studies to assess variability between reagent lots, days, runs and operators.

Study 1 was conducted by 2 trained operators each of whom tested seven samples with various analyte concentrations and combinations as described below:

a. Negative
b. 2X LoD SARS-CoV-2
c. 2X LoD Flu A
d. 2X LoD Flu B
e. 2X LoD RSV
f. 2X LoD Flu B & RSV
g. 2X LoD SARS-CoV-2 & Flu B & RSV

Each operator tested two sample replicates per run across two runs using three lots of devices. Runs were performed in the morning and afternoon over 10 days. This design (2 replicates/run/lot x 2 runs/operator x 2 operators x 3 lots x 10 days) resulted in 240 replicates per sample. All samples were prepared in pooled nasal swab matrix (PNSM). The study was performed in randomized and blinded manner.

Results for this study are shown in Table 8 below and were concordant with the expected results. Since the study obtained 100% agreement for all samples, both with and without analytes, the data were not stratified by the individual sources of variation.

Table 8: Summary Results of Multi-lot Precision Study (Study 1):

Sample level	Analyte	Obtained results per analyte / Expected results per analyte	Total percent lot-to-lot agreement	95% CI
Negative	SARS-CoV-2	(240/240)	100.0%	98.5-100.0%
	Flu A	(240/240)	100.0%	98.5-100.0%
	Flu B	(240/240)	100.0%	98.5-100.0%
	RSV	(240/240)	100.0%	98.5-100.0%
2X LoD	SARS-CoV-2	(240/240)	100.0%	98.5-100.0%
	Flu A	(240/240)	100.0%	98.5-100.0%
	Flu B	(240/240)	100.0%	98.5-100.0%
	RSV	(240/240)	100.0%	98.5-100.0%
	Flu B + RSV	(480/480)	100.0%	98.5-100.0%
	Flu B + RSV+ SARS-CoV-2	(720/720)	100.0%	98.5-100.0%

Study 2 was specifically conducted to assess difference between lots, because study 1 resulted in 100% agreement across all sources of variation and did not allow to conclusively assess lot-to-lot variability of the test. Study 2 was performed using one negative sample without any analyte and two low positive samples with the following analyte combinations, each at 0.75X LoD (i.e., below the concentration tested in study 1 and near the analytes' C_{95} concentration):

i. Negative
ii. 0.75X LoD Flu A & SARS-CoV-2
iii. 0.75X LoD Flu B & RSV

All samples were randomized and tested in a blinded manner. The testing was carried out over 3 days only but otherwise followed the same study design as Study 1. Seventy two data points per sample were collected (2 replicates/run/lot x 2 runs/day x 2 operators x 3 days x 3 lots = 72 data points).

Procedural random errors across various days, combined with the operator's ability to visually read test line intensities of samples with low analyte concentrations are expected to confound variability between lots and to have an impact on the precision estimates of a device for very low positive samples below the LoD. However, the results with concentrations at 0.75X LoD that yielded results with less than 100% showed minor variability between lots and were not deemed significant. The results are summarized below.

Table 9: Summary of Supplemental Precision Study (Study 2):

Analyte Concentration	Analyte	Lot 1		Lot 2		Lot 3		Lot-to-Lot Agreement		95% CI
		n/N[1]	% Agmt[2]	n/N[1]	% Agmt[2]	n/N[1]	% Agmt[2]	n/N[1]	% Agmt[2]	
Negative	**SARS-CoV-2**	24/24	100.0%	24/24	100.0%	24/24	100.0%	72/72	100.0%	95.0-100.0%
	Flu A	24/24	100.0%	24/24	100.0%	24/24	100.0%	72/72	100.0%	95.0-100.0%
	Flu B	24/24	100.0%	24/24	100.0%	24/24	100.0%	72/72	100.0%	95.0-100.0%
	RSV	24/24	100.0%	24/24	100.0%	24/24	100.0%	72/72	100.0%	95.0-100.0%

Analyte Concentration	Analyte		n/N[3]	% Pos	n/N[3]	% Pos	n/N[3]	% Pos	n/N[3]	% Pos	95% CI
0.75X LoD	Flu A+ SARS -CoV-2	Flu A	16/24	66.7%	15/24	62.5%	13/24	54.2%	44/72	61.1%	48.9-72.4%
		SARS -CoV-2	23/24	95.8%	18/24	75.0%	21/24	87.5%	62/72	86.1%	75.9-93.1%
	Flu B+ RSV	Flu B	19/24	79.2%	22/24	91.7%	15/24	62.5%	56/72	77.8%	66.4-86.7%
		RSV	17/24	70.8%	18/24	75.0%	14/24	58.3%	49/72	68.1%	56.0-78.6%

[1] *number of agreement with expected results per analyte/total number tested.*
[2] *Agreement.*
[3] *number of positive samples/total number tested.*

b. Multi-Site Reproducibility Study:

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

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

Prepared contrived samples were randomized and blinded to each operator at three CLIA-waived sites. Nine (9) operators conducted the testing with each operator testing ten (10) panels with each panel consisting of eight (8) panel members. For each panel member, 90 data points were collected (1 replicate/run x 2 runs/day x 3 operators/site x 3 sites x 5 days = 90 data points).

The results are shown in Table 10 below. All data met the predefined acceptance criteria, and no significant differences were observed among sites, operators, days and runs.

Table 10: Summary of Multi-Site Reproducibility Study:

Sample		# Positives / # Total (% Positive Rate)			Total Sample count (% Positive Rate)
		Site 1	Site 2	Site 3	
True Negative	RSV	0/30 (0.0%)	0/30 (0.0%)	0/30 (0.0%)	0/90 (0.0%)
	Flu A	0/30 (0.0%)	0/30 (0.0%)	0/30 (0.0%)	0/90 (0.0%)

Sample		# Positives / # Total (% Positive Rate)			Total Sample count (% Positive Rate)
		Site 1	Site 2	Site 3	
	Flu B	0/30 (0.0%)	0/30 (0.0%)	0/30 (0.0%)	0/90 (0.0%)
	SARS-CoV-2	0/30 (0.0%)	0/30 (0.0%)	0/30 (0.0%)	0/90 (0.0%)
Pooled High Negative	RSV	0/30 (0.0%)	0/30 (0.0%)	0/30 (0.0%)	0/90 (0.0%)
	Flu A	0/30 (0.0%)	0/30 (0.0%)	0/30 (0.0%)	0/90 (0.0%)
	Flu B	0/30 (0.0%)	1/30 (3.3%)	0/30 (0.0%)	1/90 (1.1%)
	SARS-CoV-2	0/30 (0.0%)	0/30 (0.0%)	0/30 (0.0%)	0/90 (0.0%)
1X LoD RSV	RSV	30/30 (100.0%)	30/30 (100.0%)	30/30 (100.0%)	90/90 (100.0%)
	Flu A	0/30 (0.0%)	0/30 (0.0%)	0/30 (0.0%)	0/90 (0.0%)
	Flu B	0/30 (0.0%)	0/30 (0.0%)	0/30 (0.0%)	0/90 (0.0%)
	SARS-CoV-2	0/30 (0.0%)	0/30 (0.0%)	0/30 (0.0%)	0/90 (0.0%)
1X LoD Flu A	RSV	0/30 (0.0%)	0/30 (0.0%)	0/30 (0.0%)	0/90 (0.0%)
	Flu A	30/30 (100.0%)	30/30 (100.0%)	29/30 (96.6%)	89/90 (98.8%)
	Flu B	0/30 (0.0%)	0/30 (0.0%)	0/30 (0.0%)	0/90 (0.0%)
	SARS-CoV-2	0/30 (0.0%)	0/30 (0.0%)	0/30 (0.0%)	0/90 (0.0%)
1X LoD Flu B	RSV	0/30 (0.0%)	0/30 (0.0%)	0/30 (0.0%)	0/90 (0.0%)

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

3. Operator Questionnaire:

At the end of the reproducibility study, each of the 9 untrained operators (3 from each of the 3 CLIA waived sites) was given a questionnaire to provide feedback on the ease of use of the Flow*flex* Plus RSV + Flu A/B + COVID Rapid Test. The questionnaire had 13 questions that evaluated the following general topics:

- Ease of use of the test
- Ability to follow the test instructions
- Ability to properly interpret test results
- Ability to test the control materials

The operators performing the testing at each site also filled out a questionnaire about their professional training and background. Based on the operators' feedback, the overall Flow*flex* Plus RSV + Flu A/B + COVID Rapid Test was found to be easy to use without the help of an expert. All operators (100%) reported that the test was user-friendly, and the instructions were clear, easy to follow with understandable instructions and result interpretations.

D Labeling for Waived Devices

The labeling submitted for the Flow*flex* Plus RSV + Flu A/B + COVID Rapid Test consists of the following:

1. Quick Reference Instructions (QRI)
2. Instructions for Use (IFU)
3. Package Labeling – Kit box labels

The following elements are appropriately present:

- The QRI is written at no higher than a 7th grade reading level.
- The QRI and the IFU identify the test as CLIA waived.
- The IFU contains a statement that a Certificate of Waiver is required to perform the test in a waived setting.
- The QRI and the IFU contain a statement that laboratories with a Certificate of Waiver must follow the manufacturer's instructions for performing the test.
- The IFU contains a statement that any modification to the test or the manufacturer's instructions will result in the test being classified as high complexity.
- The IFU and QRI provide instructions for conducting quality control procedures, interpretation of results, including diagrams on how to read and assess validity of test results and control results.
- The IFU includes a warning addressing color blindness when waived tests use color-coded reagents and/or endpoints.
- The IFU and QRI includes a Telephone number to contact manufacturer for technical assistance or troubleshooting the test system and to direct the user to call for assistance when the device or the control materials do not work as specified by the manufacturer.
- The labeling is sufficient and satisfies the requirements of 21 CFR Part 809.10.

XI. Benefit-Risk Considerations

After implementation of all risk control measures identified through the Design, Manufacturing, and Usability FMEA, the overall residual risks are acceptable.

The device design incorporates multiple features that reduce the likelihood of user error, including:

- Simple anterior nasal specimen collection
- Minimal procedural steps
- Internal procedural control to verify proper test execution
- Clearly illustrated Instructions for Use
- Straightforward visual interpretation of test results.

All hazards have been adequately mitigated through a combination of design features, fail-safe mechanisms, manufacturing controls, analytical verification, usability validation, and detailed labeling instructions. Importantly, no new risks were introduced as a result of implementing these risk controls, and additional controls did not create any unintended hazardous situations.

Analytical performance studies demonstrated that the Flow*flex* Plus RSV + Flu A/B + COVID Rapid Test reliably detects SARS-CoV-2, Flu A, Flu B and RSV antigens and distinguishes it from other common respiratory pathogens, including seasonal coronaviruses. Clinical validation performed in simulated home environments showed that users can correctly perform the test and interpret results using only the provided labeling.

Furthermore, internal fail-safe mechanisms (e.g., mandatory control line) ensure that device malfunction or major procedural errors result in INVALID outcomes rather than false negatives or false positives, supporting FDA's requirement for insignificant risk of erroneous result in CLIA-waived settings.

Overall, given the rapid availability of clinically actionable results, the ability to detect and differentiate four respiratory pathogens (RSV/Flu A/Flu B/SARS-CoV-2) from a single specimen, and the implementation of appropriate risk control measures, the probable benefits of using the Flow*flex* Plus RSV + Flu A/B + COVID Rapid Test outweigh the residual risks when used as intended in CLIA waived settings.

XII. Conclusion

The submitted information in this CLIA waiver application supports a CLIA waiver approval decision.