



CLIA Waiver by Application Approval Determination Decision Summary

I. Document Number

CW250007

II. Parent Document Number

K251742

III. CLIA Waiver Type

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

IV. Applicant

LEX Diagnostics Limited

V. Proprietary and Established Names

VELO Respiratory Test

VI. Measurand (analyte)

Influenza A RNA

Influenza B RNA

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) RNA

VII. Sample Type(s)

Anterior nasal swabs

VIII. Type of Test

Qualitative reverse transcriptase polymerase chain reaction (RT-PCR)

IX. Test System Description

A Overview

The VELO Respiratory Test is an automated rapid multiplex real-time, reverse transcriptase polymerase chain reaction (RT-PCR) test performed on the VELO Instrument and is intended for the simultaneous qualitative detection and differentiation of SARS-CoV-2, influenza A, and

influenza B virus RNA in anterior nasal swabs (ANS) specimens from individuals with signs and symptoms of respiratory tract infection. The VELO System is comprised of a single-use VELO Respiratory Test, and a reusable VELO Instrument. To perform the test, an ANS specimen is collected using the provided ANS swab and the swab is inserted directly into the VELO Respiratory Test Cartridge sample port. The Test Cartridge contains all necessary reagents for the detection of influenza A, influenza B and SARS-CoV-2 viral RNA and the endogenous sample and process control (SPC). The Test Cartridge is then capped and inserted into the VELO Instrument to initiate the test, and all subsequent test steps, including sample processing, target amplification by real-time RT-PCR, fluorescence detection, result interpretation and result reporting are performed automatically by the VELO Instrument.

Sample processing includes elution and a thermal lysis step that then directly rehydrates the lyophilized RT-PCR reagents, with each target RT-PCR amplification reaction proceeding in an independent PCR chamber. In the event of amplification, a fluorescence signal is generated through the degradation of oligonucleotide probes modified with 5' fluorophores and 3' quenchers. Fluorescence is monitored by the VELO Instrument with every thermal cycle and reports as "Detected" once meeting pre-determined criteria. Test outcomes are reported to the operator in real-time via the Instrument view screen with 'Not Detected' results available in under 10 minutes with the completion of all cycles. When the test ends, all results can be viewed via the instrument view screen, and the Test Cartridge may be removed for disposal.

B Test System Components

Each VELO Respiratory Test kit includes sufficient materials and reagents to process fifty ANS specimens and consists of the following:

- Fifty (50) individually pouched VELO Respiratory Test Cartridges per kit
- Fifty-two (52) sterile anterior nasal swabs individually packaged in peel pouches per kit.
- One Quick Reference Guide for VELO Respiratory Test
- One Quality Control Procedure for VELO Respiratory Test

The VELO Instrument is provided separately and is not included within the VELO Respiratory Test kit. The VELO Instrument is packaged with a VELO Power Supply, the VELO Instrument Manual and the Quick Start Guide for the VELO Instrument.

X. Specific Contents for CLIA Waiver

A Demonstrating "Simple":

The VELO Respiratory Test performed on the VELO Instrument was designed to be simple and easy to use. **Table 1** summarizes features that are built into the device design to make it simple to use with minimal risk of erroneous results.

Table 1: Demonstration of Simplicity for the VELO Respiratory Test performed on the VELO Instrument

‘Simple’ Criteria	Device Characteristics
Is a fully automated instrument or a unitized or self-contained test.	The device has a fully automated system and only requires sample collection and insertion into the Test Cartridge. The Test Cartridge, which contains all the reagents required for sample processing and analysis, is then inserted into the instrument, where all test steps are completed by the instrument without further user intervention.
Uses direct unprocessed specimens, such as capillary blood (fingerstick), venous whole blood, nasal swabs, throat swabs, or urine.	The test uses direct anterior nasal swab specimens that are placed directly into the sample chamber port of the Test Cartridge.
Needs only basic, non- technique-dependent specimen manipulation, including any for decontamination.	<ul style="list-style-type: none"> • An untrained operator can conduct the test by performing simple steps without specimen manipulation: 1) collects the anterior nasal swab from the patient using the provided swab, 2) inserts the swab tip directly into the Test Cartridge and closes the Test Cartridge lid, then 3) inserts the Test Cartridge into the VELO Instrument. After insertion, all test steps are completed by the instrument without further user intervention. • Each Test Cartridge is fluidically sealed once the lid is closed. This prevents leakage and contamination of the VELO Instrument by ensuring there is no direct contact between patient samples or test fluids and the instrument. After the run is completed, the single-use Test Cartridge remains sealed to prevent release of the sample or reagents, and the entire cartridge can be disposed without further manipulation in accordance with any applicable local, state, and national regulations.
Needs only basic, non- technique-dependent reagent manipulation, such as “mix reagent A and reagent B.”	The device consists of a single use, ready-to-use Test Cartridge that contains all the reagents required for sample processing and analysis. No manipulation of the reagents by the user is needed. No pipetting, temperature monitoring, or timing of steps are involved in the Test workflow.
Needs no operator intervention during the analysis steps.	<ul style="list-style-type: none"> • The device has a fully automated workflow and results output and does not require any operator intervention during the analysis step. • The device performs automated analysis of test results and reports results as one of 5 potential outcomes: Detected, Not Detected, Invalid, Error or Cancelled.
Needs no technical or specialized training with respect to troubleshooting or interpretation of multiple or complex error codes.	Technical or specialized training is not required for operating the device. If an error code is shown, user follows easy-to-interpret troubleshooting instructions provided in the VELO Instrument manual or can contact technical support.

Needs no electronic or mechanical maintenance beyond simple tasks, e.g., changing a battery or power cord.	The VELO Instrument is maintenance-free and has no serviceable parts.
Produces results that require no operator calibration, interpretation, or calculation.	Interpretation of results is automated. Results are displayed on the VELO Instrument screen and no additional interpretation or calculations are required. Users use a simple touchscreen to operate the VELO Instrument, and all test results are displayed on the instrument view screen. The user has the option to print the tests using an optional USB connected printer.
Produces results that are easy to determine, such as ‘positive’ or ‘negative,’ a direct readout of numerical values, the clear presence or absence of a line, or obvious color gradations.	Interpretation of results is automated. Qualitative test results “Detected”, “Not Detected”, “Invalid”, “Error” or “Cancelled”) are automatically displayed on the integrated view screen of the VELO Instrument and no additional interpretation or calculations are required.
Includes quick reference instructions (QRI, Operator’s Instrument Manual (if applicable), etc.) that are written at no higher than a 7th grade reading level.	A Quick Reference Guide (QRG) for the VELO Respiratory Test, a Quick Start Guide (QSG) for the VELO Instrument, and a Quality Control Procedure (QCP) are provided to the user. The documents are written in simple language at a 7th grade reading level and include clear diagrams to guide the user.

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

1. Risk Analysis:

A comprehensive risk analysis of the VELO Respiratory Test and the VELO Instrument (together, the VELO System) was performed in accordance with ISO 14971 to identify potential hazards, hazardous situations and the associated harms. The detailed device hazard analysis was included in the submission and used to assess the risk of failure (false positive, false negative or delayed test results) that may occur during use or misuse of the device. Severity and probability of occurrence for each potential hazardous situation were evaluated to generate an overall assessment of the risk of the VELO System. The considered risks included operator errors (human factors); sample handling and storage; device handling and storage; and environmental factors.

Based on the hazard analysis, potential sources of errors that could adversely affect system performance were identified and mitigated first through system design and then through additional cautions 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 below).

2. Fail-Safe and Failure Alert Mechanisms:

The VELO Respiratory Test was designed to include various features and fail-safe mechanisms built into the system to prevent erroneous results, summarized below:

Endogenous (RNaseP) Sample Process Control

The sample process control (SPC) built into each VELO Respiratory Test Cartridge controls for all test processes from sample collection to result. This ensures a given test run is controlled for sample quality, inhibition and sample loading without end user interaction.

External Controls

Commercially available external controls, such as the Swab Control Kit NATtrol Flu/RSV/SARS-CoV-2 from ZeptoMetrix (Buffalo, NY), are recommended for use when receiving new device shipments, training new operators, when problems with testing are suspected or identified, to conform with internal quality control procedures, and in accordance with local, state and federal regulations.

VELO Respiratory Test Cartridge

- **Packaging:** Each Test Cartridge is individually packaged in a protective sealed foil pouch, ensuring the cartridge is only removed from the protective environment immediately prior to use.
- **Snap-closed lid:** Once closed, the Test Cartridge lid remains latched closed which prevents attempted re-use.
- **No external fluidic interfaces:** The Test Cartridge is sealed without the need for fluid exchange with the VELO Instrument or environment, this prevents leaks during and after performing a test run, preventing user exposure to reagents.
- **Reagents:** All necessary reagents are pre-loaded into the Test Cartridge removing any need for the user to add additional reagents during the test run. Lyophilized reagents are employed where applicable to extend stability and prevent user exposure.
- **Direct swab loading:** The swab specimen is loaded directly into the Test Cartridge's sample port, minimizing additional sample preparation steps and reducing the risk of user errors.
- **Near field Communication (NFC) tag:** Each Test Cartridge is identified by a NFC tag read by the VELO Instrument, preventing use of cartridges beyond their expiration date. The VELO Instrument also flags the Test Cartridge as used once inserted, preventing re-use on any other VELO Instrument.

VELO Instrument

- **Test cartridge detection:** A test cannot be started until a Test Cartridge is loaded into the VELO Instrument.
- **Lid close detection and latching:** The VELO Instrument detects when the lid has been shut and then latches it closed, preventing access to the internal parts of the VELO instrument and the Test Cartridge while a test is in progress. A test cannot be started while the VELO Instrument lid is open.
- **On-screen guidance:** Graphical workflow instructions are provided on the VELO Instrument touchscreen informing the user of the next necessary step to complete a test.
- **Test run:** The fluidic progression through the Test cartridge and mixing steps are monitored during a test run. If liquid or mixing is not detected as expected, the instrument will display an error message.
- **Thermal monitoring:** Instrument temperatures are monitored during use. If a heater does not respond as expected, or the internal temperature moves out of range, then the instrument will display an error message.

3. Flex Studies:

Flex Studies were performed to evaluate the robustness of the VELO Respiratory Test when challenged outside the operational limits of the system and with variations in workflow and operating environment that may reasonably be expected to occur with untrained operators in the intended use CLIA Waived setting. Contrived samples used for flex study testing were prepared in negative nasal matrix (NNM) and consisted of:

- Negative sample: un-spiked NNM
- Low positive sample: NNM co-spiked at 2x Limit of Detection (LoD) with SARS-CoV-2, influenza A and influenza B.

Each condition was evaluated testing the low positive and negative samples, each in five replicates (unless otherwise specified). Samples were blinded to the operators performing flex testing and used at least two VELO Instruments and two operators. Samples were tested according to the instructions for use protocol, except for the noted deviations dictated by the flex parameter under evaluation. The effect of the following conditions on the performance of the test was evaluated, organized by the potential type of source error:

Environmental conditions

- *Temperature and Humidity:*
This study evaluated the impact of variations in environmental temperature and humidity on the performance of the VELO Respiratory Test. All pouched Test cartridges and VELO Instruments were placed in environmental chambers with the specified conditions (see table below) and allowed to equilibrate for at least two hours prior to testing. The results from the study are summarized in the **Table 2**.

Table 2: Results of Flex Studies Evaluating the Impact of Environmental Temperature and Humidity Conditions

Study Condition	Study Parameters	Agreement (n/N) ^[1]		Invalid Result	Channel Errors	VELO Instrument Error	Erroneous Results Due to Study Condition
		Negative	Positive				
1	Ambient	5/5	5/5	0	0	0	None
2	10°C, 10% RH	0/5	0/5	0	0	10	None
3	12°C, 10% RH	2/5	3/5	0	0	5	None
4	15°C, 10% RH	5/5	5/5	0	0	0	None
5	30°C, 10% RH	5/5	5/5	0	0	0	None
6	33°C, 10% RH	5/5	5/5	0	0	0	None
7	35°C, 10% RH	5/5	5/5	0	0	0	None
8	40°C, 10% RH	0/5	0/5	0	0	10	None
9	10°C, 95% RH	2/5	4/5	0	1	3	None
10	12°C, 95% RH	1/5	4/5	0	0	5	None
11	15°C, 95% RH	5/5	5/5	0	0	0	None
12	30°C, 95% RH	5/5	5/5	0	0	0	None

13	33°C, 95% RH	4/5	3/5	0	2	1	None
14	35°C, 95% RH	0/5	0/5	0	0	10	None
15	40°C, 95% RH	0/5	0/5	0	0	10	None

^[1]n is number of tests with expected results. N is the total number of tests run.

The labeling specifies optimum conditions for storage and performing the test on the VELO Instrument to be room temperature (15°C to 30°C) and relative humidity (10 - 90% (non-condensing)). All samples with valid results yielded expected results. For conditions 2, 3, 8-10, 13-15, VELO Instruments displayed either a channel error or instrument error as fail-safe measures. There were no erroneous results generated, demonstrating that the risk of erroneous results due to extremes of temperature and humidity outside the specified conditions is minimal. The risk of instrument errors due to extreme temperature and humidity conditions are mitigated by clear labeling which instructs the user to operate the VELO Respiratory Test at 15°C to 30°C and relative humidity of 10 - 90% (non-condensing).

- *Environmental Tolerance – Barometric Pressure:*

The VELO Instrument is specified for use at an altitude up to 2,000 meters (~6,500 feet) above sea level. This study examined the effect of altitude on the VELO Respiratory Test performance. Testing was conducted by running the VELO Respiratory Test on a VELO Instrument at either 25 m above sea level (control) or 3116 m (10,200 feet) above sea level. Five replicates of each sample (negative and low positive) were tested in the study according to the test procedure. All samples generated expected results demonstrating that risks of erroneous results due to operating the VELO Instrument outside of the specified altitude are low. As a precaution, the VELO Instrument manual includes clear labeling which informs the user that the maximum operating altitude is 2000 m above sea level.

- *Incorrect Placement of Device: Non-Level Surfaces:*

The purpose of this study was to evaluate the effect of placing the VELO Instrument on a non-leveled benchtop or work surface on the VELO Respiratory Test performance. Five replicates of each sample (negative and low positive) were tested in the study according to the test procedure. Testing was carried out on VELO Instruments inclined at 20° in the forward, backward, right or left direction, inclinations above 20° were not evaluated as the instrument is not mechanically stable and begins to tip. A level (control) condition was also evaluated. All samples generated expected results demonstrating that risks of erroneous results due to operating the VELO Instrument on non-level surface are low. As a precaution, the VELO Instrument manual includes clear labeling instructing the user to operate the VELO Instrument on a clean, flat, level and stable surface.

- *Effect of Sunlight Exposure:*

The purpose of this study was to evaluate the effect of sunlight exposure on the test performance of the VELO Respiratory Test. The study was conducted by testing five replicates of each sample (negative and low positive) according to the test procedure. Testing was carried out by exposing the Test Cartridges (in primary packaging) and the VELO Instrument to direct light from full spectrum light bulbs for a period of one hour. The conditioned Test Cartridges were then used for Test Runs using positive and negative samples while the VELO Instrument was still being illuminated. A non-light

exposed (control) condition was also evaluated. All samples generated expected results demonstrating that the risks of erroneous results due to sunlight exposure during testing are minimal. As a precaution, the labeling instructs the user to operate the VELO Instrument out of direct sunlight.

- *Effect of Poor Ventilation:*

The purpose of this study was to evaluate the effect of poor ventilation on the test performance of the VELO Respiratory Test. The study was conducted by testing five replicates of each sample (negative and low positive) according to the test procedure. Ventilation conditions were simulated by carrying out testing with the VELO Instrument positioned either, >30 cm from a wall (control), positioned as close as possible against a wall, as well as completely sealing the vents on the instrument. The study results are summarized in **Table 3**.

Table 3: Results of Flex Studies Evaluating the Impact of Poor Ventilation

Study Condition	Study Parameters	Agreement (n/N) ^[1]		Invalid Result	Channel Errors	VELO Instrument Error	Erroneous Results Due to Study Condition
		Negative	Positive				
1	>30 cm from wall	5/5	5/5	0	0	0	None
2	Instrument against wall	5/5	5/5	0	0	0	None
3	Instrument with vents sealed	0/5	0/5	0	0	10	None

^[1]n is number of tests with expected results. N is the total number of tests run.

All samples with valid results yielded expected results. For condition 3 in which the VELO Instrument vents were completely sealed the VELO Instruments displayed an instrument error as a fail-safe measure. There were no erroneous results generated, demonstrating that the risk of erroneous results due to poor ventilation outside the specified conditions is minimal. These instrument errors are mitigated by clear labeling which instructs the user to ensure ventilation openings located on the back and base of the VELO Instrument are free of obstruction, and air can flow freely around the instrument during use.

- *Effect of Drafty Conditions:*

The purpose of this study was to evaluate the effect of drafty conditions on the performance of the VELO Respiratory Test. The study was conducted by testing five replicates of each sample (negative and low positive) according to the test procedure. To simulate drafty conditions, testing was carried out in the presence of a fan directed towards either the back or side of the VELO Instrument, where the air vents are located, during testing. A non-drafty control condition was also evaluated. All samples generated expected results demonstrating that the risks of erroneous results due to drafty conditions during testing are minimal.

Operator Errors in Performing the Test

- *Incorrect Specimen Type:*

The VELO Respiratory Test kit includes a sterile anterior nasal swab packaged individually in peel pouches to facilitate anterior nasal swab specimen collection and the instructions clearly describe how to collect an anterior nasal swab specimen. This study evaluated the effect of use of an incorrect specimen type on the performance of the VELO Respiratory Test. The study was conducted by testing five replicates of each sample (negative and low positive) according to the test procedure. The study evaluated anterior nasal swab specimens (control), throat specimens, and throat/nasal specimens. The study results are summarized in **Table 4**.

Table 4: Results of Flex Studies Evaluating the Impact of Incorrect Specimen Type

Study Condition	Study Parameters	Agreement (n/N) ^[1]		Invalid Result	Channel Errors	VELO Instrument Error	Erroneous Results Due to Study Condition
		Negative	Positive				
1	Control: Anterior nasal specimen	5/5	5/5	0	0	0	None
2	Throat specimen	5/5	3/5	2	0	0	None
3	Throat/nose specimen	4/5	0/5	0	0	0	1 ^[2]

^[1] n is number of tests with expected results. N is the total number of tests run.

^[2] One replicate produced a false negative result for SARS-CoV-2. Expected Detected results were obtained for Influenza A and B.

One erroneous result was obtained from a positive nose/throat swab which showed a false negative for SARS-CoV-2. No other erroneous results were obtained in the study. The risk of erroneous results due to an incorrect specimen type being collected and used is mitigated through clear labeling which instructs the user to collect an anterior nasal swab and warns that erroneous results may occur if specimens other than anterior nasal specimens are used in the VELO Respiratory Test.

- *Incorrect Specimen Handling – Manual Elution:*

The VELO Respiratory Test instructions clearly describe how to directly load the collected anterior nasal swab specimen into the Test Cartridge. This study evaluated the effect of using a swab specimen in which the end user manually elutes the nasal specimen into VTM (Viral Transport Medium) or UTM (Universal Transport Medium) and then loads either the resulting eluate or the eluted swab into the Test Cartridge. The study was conducted by testing five replicates of each sample (negative and low positive) according to the test procedure. The study evaluated anterior nasal swab specimens correctly handled (control), eNAT and UTM-RT eluates, and eNAT and UTM-RT eluted swabs. The study results are summarized in **Table 5**.

Table 5: Results of Flex Studies Evaluating the Impact of Manual Elution of the Swab Specimen

Study Condition	Study Parameters	Agreement (n/N) ^[1]		Invalid Result	Channel Errors	VELO Instrument Error	Erroneous Results Due to Study Condition
		Negative	Positive				
1	Control: Anterior nasal specimen	5/5	5/5	0	0	0	None
2	eNAT Eluate	0/5	0/5	9	1 ^[2]	0	None
3	UTM-RT Eluate	0/5	5/5	5	0	0	None

4	eNAT Eluted Swab	0/5	0/5	10	0	0	None
5	UTM-RT Eluted Swab	0/5	5/5	3	0	2	None

^[1] n is number of tests with expected results. N is the total number of tests run.

^[2] One replicate produced a channel error for SARS-CoV-2 and invalid for Influenza A and B.

No erroneous results were obtained, however, substantial rates of failure were observed in the form of Invalid/Error Test Results and Instrument Errors. The risk of Invalid/Error Test results and/or Instrument Errors due to an incorrect handling of the anterior nasal swab specimen is mitigated through labeling which clearly instructs the user to directly load the collected anterior nasal swab specimen into the Test Cartridge.

- *Incorrect Swab Type:*

The VELO Respiratory Test kit includes a sterile anterior nasal swab packaged individually in peel pouches to facilitate nasal swab specimen collection and the instructions clearly describe how to collect an anterior nasal swab specimen. This study evaluated the effect of use of non-supplied swabs on the performance of the VELO Respiratory Test. The study was conducted by testing five replicates of each sample (negative and low positive) according to the test procedure. The study evaluated the supplied swab (control), and various other swabs. The study results are summarized in **Table 6**.

Table 6: Results of Flex Studies Evaluating the Impact of Incorrect Swab Type

Study Condition	Study Parameters	Agreement (n/N) ^[1]		Invalid Result	Channel Errors	VELO Instrument Error	Erroneous Results Due to Study Condition
		Negative	Positive				
1	Control: Anterior nares swab, 30mm break point	5/5	5/5	0	0	0	None
2	Nasopharyngeal swab, longer breakpoint (100mm)	0/5 ^[2]	0/5 ^[2]	0	0	0	None
3	Anterior nares swab with a longer break point (80mm)	0/5	1/5 ^[3]	0	0	6	None
4	Anterior nares swab with a shorter break point (20mm)	5/5	2/5 ^[3]	0	0	0	None
5	Anterior nares swab, 30mm break point, different manufacturer	5/5	5/5	0	0	0	None

^[1] n is number of tests with expected results. N is the total number of tests run.

^[2] All tests unable to be performed as cartridge lid would not close.

^[3] Three tests not performed as cartridge lid damaged or would not close.

No erroneous results were obtained, however, Instrument Errors or the inability to run the test were observed, the risks of which are mitigated through providing the correct anterior nasal swab with the kit and instructing the user to use the provided swab when collecting the anterior nasal swab specimen.

Reagent and Hardware Integrity

- *Environmental Contaminants:*

This study was conducted to determine the effect of potential residuals (environmental contaminants) from reagents used to clean the VELO Instrument after use on the performance of the VELO Respiratory Test. The study was conducted by testing five replicates of each sample (negative and low positive) according to the test procedure, unless otherwise noted. The environmental contaminant was introduced into the Test Cartridge along with the spiked swab sample. The study evaluated no contaminant (control), 70% isopropanol solution, Clinell universal disinfectant spray and 1% w/v Actichlor plus chlorine solution. The study results are summarized in **Table 7**.

Table 7: Results of Flex Studies Evaluating the Impact of Environmental Contaminants

Study Condition	Study Parameters	Agreement (n/N) ^[1]		Invalid Result	Channel Errors	VELO Instrument Error	Erroneous Results Due to Study Condition
		Negative	Positive				
1	Control: No contaminant	10/10 ^[2]	10/10 ^[2]	0	0	0	None
2	70% Isopropanol (3.5% v/v final)	5/5	5/5	0	0	0	None
3	Clinell universal disinfectant spray (5% v/v final)	5/5	5/5	0	0	6	None
4	1% w/v Actichlor plus chlorine solution (0.04% w/v final)	5/5	5/5	0	0	0	None
5	1% w/v Actichlor plus chlorine solution (0.05% w/v final)	1/5	5/5	0	0	0	4 ^[3]

^[1] n is number of tests with expected results. N is the total number of tests run.

^[2] Controls for conditions 2,3,5 and 4 were performed separately due to the test schedule producing a total of ten replicates in the standard condition.

^[3] Four tests returned false negative “Not Detected” results. 4/4 SARS-CoV-2, 3/4Influenza A and 2/4 Influenza B.

Four erroneous results were obtained from positive swabs exposed to 1% w/v Actichlor plus chlorine solution (0.05% w/v final) which showed false negatives for 4/4 SARS-CoV-2, 3/4Influenza A and 2/4 Influenza B. No other erroneous results were obtained in the study, including 1% w/v Actichlor plus chlorine solution (0.04% w/v final). The risk of erroneous results due to potential environmental contamination from a residual cleaning reagent is mitigated through clear labeling which instructs the user to change gloves following cleaning procedures prior to handling tests and/or specimens and warns that erroneous results may occur from contamination of the specimen, such as the recommended cleaning 1% bleach solution, during swab handling.

- Effect of Dropping the Test Cartridge:*

This study was conducted to determine the effect of dropping the Test Cartridge on the performance of the VELO Respiratory Test. The study was conducted by testing five replicates of each sample (negative and low positive) according to the test procedure. In the study, the Test Cartridge was dropped from a height of 1 meter (~3.3-feet) before use. A Test Cartridge that had not been dropped (control) was also evaluated. All samples generated expected results demonstrating that the risks of erroneous results due to cartridge dropping is minimal. The labeling provides clear instructions to the users for cartridge unpacking and preparation for testing. Additionally, users are warned to not use the Test Cartridge if it has been dropped.
- Effect of Using Test Cartridge with Pre-burst Blister:*

This study was conducted to determine the effect of using a Test Cartridge where the blister pouch (containing the elution buffer) has burst prior to use on the performance of the VELO Respiratory Test. The study was conducted by testing five replicates of each sample (negative and low positive) according to the test procedure. The study evaluated intact blister pouch Test Cartridges (control) and Test Cartridges in which the blister pouch had been pre-burst resulting in liquid in the sample port prior to insertion of the swab sample. No erroneous results were obtained, however, four negative samples resulted in an instrument error. While no erroneous results were encountered labelling mitigations include informing the user that the *“Test Cartridges contain a sample elution buffer in a blister pouch on the back side. Do not touch or puncture the pouch. If there is any evidence of leakage or damage, discard the Test Cartridge and obtain a new Test Cartridge.”*
- Disturbance of the VELO Instrument During Use:*

This study was conducted to determine the effect of moving the VELO Instrument during operation on performance of the VELO Respiratory Test. The study was conducted by testing five replicates of each sample (negative and low positive) according to the test procedure. In the study, the VELO Instrument was moved every 30 seconds to a new location during the test run. An undisturbed VELO Instrument (control) was also evaluated. All samples generated expected results demonstrating that the risks of erroneous results due to instrument disturbance are minimal. As a precaution, the instrument labeling instructs the user to *“Never move the VELO Instrument while a test is in progress.”*
- Incorrect Test Kit Storage Temperature:*

Labeling for the VELO Respiratory Test instructs the user to store Test Cartridges and anterior nasal swabs at ambient temperatures (15-30°C). This study was conducted to determine the effect of storing both the Test Cartridge and swabs outside of temperatures defined in the labeling. Test Cartridges and swabs were stored for 24 hours at either ambient (control), refrigerated (2-8°C), frozen (-20°C), and elevated temperature conditions of 38-60°C. For cartridge evaluation, five replicates of low positive or negative samples were tested for each condition, unless otherwise noted. The study results are summarized in **Table 8**.

Table 8: Results of Flex Studies Evaluating the Impact of Test Kit Storage Temperatures

Study Condition	Study Parameters	Agreement (n/N) ^[1]		Invalid Result	Channel Errors	VELO Instrument Error	Erroneous Results Due to Study Condition
		Negative	Positive				
1	Control: Ambient temperature	5/5	5/5	0	0	0	None
2	Refrigerator storage 24 hours	5/5	5/5	0	0	0	None
3	Freezer storage for 24 hours	5/5	5/5	0	0	6	None
4	38°C storage for 24 hours	5/5	5/5	0	0	0	None
5	50°C storage for 24 hours	5/5	9/10	0	0	0	1 ^[2]
6	55°C storage for 24 hours	5/5	5/5	0	0	0	None
7	60°C storage for 24 hours	4/5	4/5	0	0	1	1 ^[3]

^[1] n is number of tests with expected results. N is the total number of tests run.

^[2] One out of five negative samples from cartridge stored at 50°C for 24 hours produced a false positive result for SARS-CoV-2. This was due to suspected environmental contamination. An additional five replicates were performed, and all gave the expected negative (Not Detected) results.

^[3] One out of five positive samples from cartridge stored at 60°C for 24 hours produced a false negative result for SARS-CoV-2.

Two erroneous results were obtained during the study, one false positive result at 50°C and one false negative result at 60°C. No other erroneous results were obtained in the study. The risk of erroneous results due to incorrect VELO Respiratory Test kit storage is mitigated through clear and extensive labeling which instructs the user of the appropriate storage conditions and warns about the risk of erroneous results due to incorrect storage.

Conclusion from Flex Studies

Overall, the Flex Studies demonstrated that the VELO Respiratory Test is robust to foreseeable user-dependent variations in the assay workflow and that the fail-safe features built into the device design, the failure alert mechanisms, along with clear test instructions, which include relevant cautions, are effective in preventing the generation of erroneous results due to operator error and/or use of the VELO Respiratory Test when performed on the VELO Instrument outside the specified operating environmental conditions.

C Demonstrating “Insignificant Risk of an Erroneous Result” - Accuracy

1. Comparison Study:

a. Study Design:

i. Study Sites and Duration:

The performance of the VELO Respiratory Test in the hands of untrained users was evaluated in a multi-site prospective clinical study from December 2024 to March 2025, during the 2024-2025 respiratory season within the United States. Of the nine clinical

sites that participated in the study, seven where geographically diverse in distinct U.S. states, and all nine considered representative of CLIA-waived intended use sites, including physician offices, primary care/outpatient clinics, and urgent care centers.

ii. Operators:

A total of fifteen (15) untrained operators, selected from a larger pool representative of CLIA-waived users, participated in the study, with one to three operators per site. The participants consisted of medical staff personnel providing patient care and included medical and research assistants, clinical research coordinators and study managers. Information on the operators' current job title, education level and any laboratory or relevant work experience was provided. The operators were representative of operators conducting testing at a CLIA Waived setting, did not receive any training on the use of the VELO Respiratory Test and had no hands-on experience with conducting diagnostic testing in a clinical laboratory. The participating operators conducted the test by following the instructions in the Quick Reference Guide (QRG).

iii. Subjects:

A total of 1,815 subjects were initially enrolled for the prospective clinical study. Of these, 97 specimens were excluded from the performance analysis for major protocol deviations. Of the remaining 1,718 study participants the mean age was 41 years, with a range between < 1 to 92 years of age. Specimens for the prospective clinical study were collected under informed consent and according to the following inclusion or exclusion criteria summarized below:

Inclusion Criteria:

- Subject had complete and valid informed consent prior to enrollment, and;
- Subjects presented to the site with signs and symptoms suggestive of respiratory infection through the abrupt onset and symptomatic presentation of one (1) or more of the following signs and symptoms: fever ($\geq 100.0^{\circ}\text{F}$ / 37.8°C), chills, muscle aches and pain (myalgia), chest discomfort/cough, shortness of breath/difficulty breathing, joint pain, headache, sore throat, runny or stuffy nose (congestion), sneezing, nausea/vomiting and/or diarrhea, fatigue/weakness (tiredness), and change in, or loss of, taste or smell, and;
- The subjects' accurate relevant medical history, including signs and symptoms, known or possible exposure (if any), and vaccine history (as applicable) was provided, and;
- Subject agreed to provide two (2) ANS for testing.

Exclusion Criteria:

- Subject was participating in another clinical study requiring the collection of nasal, and/or nasopharyngeal, and/or oropharyngeal swab samples on the day of the study visit, prior to enrollment in this study.
- Subject required or received a nasal wash aspirate on the day of the study visit.
- Subject receiving a positive influenza A, influenza B and/or SARS-CoV-2 test result within the past sixty (60) days.
- Subject was undergoing any of the following treatments within the past sixty (60) days:

- Any “flu antiviral drug” prescription medication to treat Influenza, experimental or otherwise, OR
 - Any “COVID-19 antiviral drug” prescription medication to treat SARS-CoV-2, experimental or otherwise, OR
 - Any convalescent plasma or monoclonal antibody treatment, for any reason.
 - Subject has previously been enrolled in this study.
- iv. **Samples:**
Two anterior nasal swab (ANS) specimens were collected sequentially from each eligible subject by the test operator with a minimum of 15-minute waiting time between specimen collections. One of the ANS specimens was placed into transport media for comparator testing at the site which could be completed by other qualified staff. The other ANS specimen was tested using the VELO Respiratory Test according to the instructions in the Quick Reference Guide (QRG). The order of swab collection was randomly assigned between the comparator swab and the VELO Respiratory Test.
- v. **Comparator Method:**
A multi-analyte FDA-cleared nucleic acid amplification test (NAAT) for SARS-CoV-2, influenza A and influenza B targets was used as the comparator method in the prospective clinical study to establish the performance of the VELO Respiratory Test with anterior nasal swab specimens.

b. Results:

i. Prospective Study:

A total of 1,815 subjects were initially enrolled for the prospective clinical study. Of these, 97 specimens were excluded from the performance analysis for major protocol deviations. Of the remaining 1,718 study participants a further 2 specimens were excluded because the patient subsequently withdrew from the study. For the 1,716 VELO Respiratory Tests performed for these subjects, there were a total of 90 (5.24%, 95% CI: 4.29 - 6.40%) invalid results obtained on initial testing with the VELO Respiratory Test. Of these, 34 were invalid upon retesting, for a final invalid rate of 1.98% (34/1716) with 95% CI: 1.42 – 2.76%.

Of the remaining 1,716 study participants a further 4 specimens were excluded due to invalid comparator tests results for all three target analytes. Of the remaining 1,712 evaluable specimens from the prospective clinical study, additional exclusions due to invalid VELO Respiratory Test results upon retest resulted in the following final sample sizes for the performance evaluation, influenza A 1,677 (35 excluded), influenza B 1,665 (47 excluded), and SARS-CoV-2 1,670 (42 excluded). All remaining specimens had valid VELO Respiratory Test and comparator test results for their respective targets.

The clinical performance of the VELO Respiratory Test with prospectively collected ANS specimens, when used by untrained operators in CLIA-waived settings, is shown in **Table 9**.

Table 9. Prospective, Paired ANS Specimen Results For All Targets

Analyte	Positive Percent Agreement (PPA)			Negative Percent Agreement (NPA)		
	TP/(TP+FN)	%	95% CI	TN/(TN+FP)	%	95% CI
SARS-CoV-2	150/156 ^{a, b}	96.2	91.9 – 98.2	1507/1514	99.5	99.1 – 99.8
Influenza A	342/370 ^{a, c}	92.4	89.3 – 94.7	1295/1307 ^d	99.1	98.4 – 99.5
Influenza B	14/15 ^e	93.3	70.2 – 98.8	1646/1650	99.8	99.4 – 99.9

a Includes three (3) Influenza A / SARS-CoV-2 coinfections.

b One (1) discrepant specimen tested negative on secondary reference testing.

c Two (2) discrepant specimens tested negative on secondary reference testing.

d Three (3) discrepant specimens tested positive on secondary reference testing.

e One (1) discrepant specimen tested negative on secondary reference testing.

ii. Retrospective/Archived Study:

To supplement the prospective data for influenza B, retrospective frozen clinical ANS specimens collected from individuals with signs and symptoms of influenza infection during the 2023-2024 North American respiratory season were evaluated. Frozen paired positive and negative ANS (n=110) specimens prospectively obtained during the 2023-2024 influenza season were distributed to a single CLIA-waived site and tested during their daily workflow over a period of 5 days. Of the 110 retrospective specimens with valid comparator results 12 were excluded due to invalid VELO Respiratory Test results. Retesting was not applicable in this case. All remaining specimens had valid VELO Respiratory Test and comparator RT-PCR assay results for influenza B and the performance when used by untrained operators in CLIA-waived settings is summarized in **Table 10**.

Table 10. Retrospective, Paired ANS Specimen Results For Influenza B Results

Analyte	Positive Percent Agreement (PPA)			Negative Percent Agreement (NPA)		
	TP/(TP+FN)	%	95% CI	TN/(TN+FP)	%	95% CI
Influenza B	15/16	93.8	71.1 – 98.9	82/82	100	95.7 – 100

2. Device Performance with Analyte Concentrations Near the Assay LoD:

The performance of the VELO Respiratory Test was evaluated with low positive samples to demonstrate that untrained operators can reproducibly generate accurate results testing specimens containing low concentrations of the target organisms. Testing was performed at three distinct external CLIA-waived sites with a total of nine operators. A 3-member panel of contrived nasal swabs was evaluated consisting of a true negative (no analyte), low positive (2x LoD of all three targets), and a moderate positive (4x LoD of all three targets). The negative swab samples were contrived using simulated respiratory matrix and the positive swab samples were contrived using simulated respiratory matrix co-spiked with SARS-CoV-2, influenza A and influenza B viruses. The test swab samples were randomized and blinded to the operator running the VELO Instrument. The study was conducted by testing the 3-member panel of contrived nasal swabs using three lots of VELO Respiratory Test cartridges, tested by 9 operators over three sites (3 operators per site), each performing 1 replicate/run and 2 runs per day, over a total of 5 days, resulting in a total of 90 replicates per panel member. Six VELO Instruments (2 instruments per site) were utilized during this study. The participating operators conducted the testing by following the instructions in the QRG. Results stratified by site are summarized in **Table 11**. No significant differences between sites, instruments, lots or operators were observed,

demonstrating that untrained users could perform the test accurately when testing samples with organism concentrations near the assay LoD.

Table 11. Summary of % Agreement with expected results (95% CI) by Site

Analyte	Panel member	Site n/N ^[1]			Overall % Agreement with Expected Results (n/N ^[1])	95% CI
		A	B	C		
SARS-CoV-2	Negative	30/30	30/30	30/30	100% (90/90)	95.91-100%
	Low positive 2x LoD	30/30	29/30	29/30	97.78% (88/90)	92.26-99.39%
	Moderate positive 4x LoD	30/30	30/30	30/30	100% (90/90)	95.91-100%
Influenza A	Negative	30/30	30/30	30/30	100% (90/90)	95.91-100%
	Low positive 2x LoD	30/30	30/30	30/30	100% (90/90)	95.91-100%
	Moderate positive 4x LoD	30/30	30/30	30/30	100% (90/90)	95.91-100%
Influenza B	Negative	30/30	30/30	30/30	100% (90/90)	95.91-100%
	Low positive 2x LoD	30/30	30/30	30/30	100% (90/90)	95.91-100%
	Moderate positive 4x LoD	30/30	30/30	30/30	100% (90/90)	95.91-100%

^[1] n is number of tests with expected results. N is the total number of valid tests

3. Operator Questionnaire:

Following completion of the study, operators at each CLIA-waived site were asked to complete an operator usability questionnaire (post study) to help assess whether participants understood how to use the VELO Respiratory Test correctly. The questionnaire consisted of a series of nine statements pertaining to the ease of use of the test, experience with processing the specimens, adequacy of the labeling, need for assistance while running the test, and if problems were encountered during testing. In addition, their overall opinion of the VELO Respiratory Test was assessed. Each statement had five possible answers rated on a scale from strongly agree to strongly disagree and where applicable specific comments could be added in addition to the rated response. All fifteen operators provided responses to the questionnaire. Based on the study operator responses, the VELO Respiratory Test was easy to set up and use by following the instructions provided in the QRG.

D Labeling for Waived Devices

1. The labeling consists of:
 - a) Quick Reference Guide for VELO Respiratory Test

- b) VELO Respiratory Test Instructions for Use
- c) Quick Start Guide for the VELO Instrument
- d) VELO Instrument Manual
- e) Quality Control Procedure for VELO Respiratory Test
- f) VELO Respiratory Test, for use with the VELO Instrument FluA/Flu B/SARS-CoV-2 Cybersecurity Instructions for Use

2. The following elements are appropriately present:

- The Velo Instrument Manual and Quick Start Guide for the VELO Instrument specifies the environmental operating conditions under which testing may be performed.
- The Quick Reference Guide for VELO Respiratory Test, VELO Respiratory Test Instructions for Use, Velo Instrument Manual and Quick Start Guide for the VELO Instrument are clear and easy to understand.
- The Quick Reference Guide for VELO Respiratory Test and the VELO Respiratory Test Instructions for Use identify the test as CLIA Waived.
- The VELO Respiratory Test Instructions for Use:
 - Indicate that laboratories with a Certificate of Waiver must follow the manufacturer's instructions for performing the test.
 - Include step-by-step instructions for performing the test.
 - Include safety considerations applicable for untrained users.
 - Specify the actions to be taken if an invalid test result is obtained.
 - Include a summary of the studies performed to support CLIA Waiver.
 - Include appropriate warnings and/or limitations pertaining to clinical interpretation of test results.

XI. Conclusion

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