510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY AND INSTRUMENT

I Background Information:

A 510(k) Number

K251742

B Applicant

LEX Diagnostics Limited

C Proprietary and Established Names

VELO Respiratory Test

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
QOF	Class II	21 CFR 866.3981 - Device To Detect And Identify Nucleic Acid Targets In Respiratory Specimens From Microbial Agents That Cause The Sars-Cov-2 Respiratory Infection And Other Microbial Agents When In A Multi-Target Test	MI - Microbiology

II Submission/Device Overview:

A Purpose for Submission:

To demonstrate that the performance of the VELO Respiratory Test is substantially equivalent to the cobas SARS-CoV-2 & Influenza A/B for use on the cobas Liat System (K223591) and to obtain clearance for the VELO Respiratory Test.

B Measurand:

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

C Type of Test:

Qualitative reverse transcriptase polymerase chain reaction (RT-PCR)

III Intended Use/Indications for Use:

A Intended Use(s):

See Indications for Use below.

B Indication(s) for Use:

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 severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), influenza A, and influenza B virus nucleic acid in anterior nasal swab (ANS) specimens from individuals with signs and symptoms of respiratory tract infection. Clinical signs and symptoms of respiratory tract infection due to SARS-CoV-2 and influenza can be similar.

The VELO Respiratory Test is intended for use as an aid in the differential diagnosis of SARS-CoV-2, influenza A, and/or influenza B infection if used in conjunction with other clinical and epidemiological information, and laboratory findings. SARS-CoV-2, influenza A and influenza B viral nucleic acid are generally detectable in ANS specimens during the acute phase of infection.

Positive results do not rule out co-infection with other organisms. The agent(s) detected by the VELO Respiratory Test may not be the definite cause of disease.

Negative results do not preclude SARS-CoV-2, influenza A, and/or influenza B infection. The results of this test should not be used as the sole basis for diagnosis, treatment, or other patient management decisions.

C Special Conditions for Use Statement(s):

Rx - For Prescription Use Only

For In Vitro Diagnostic Use Only

D Special Instrument Requirements:

For use with the VELO Instrument

IV Device/System Characteristics:

A Device Description:

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 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.

B Principle of Operation:

The VELO Respiratory Test is performed on the VELO Instrument which automates and integrates sample processing, target amplification by real-time RT-PCR, fluorescence detection, result interpretation and result reporting. Each VELO Respiratory Test Cartridge contains prepackaged reagents for the detection of influenza A, influenza B and SARS-CoV-2 viral RNA from anterior nasal swab specimens. RT-PCR is used with specific primers and probes within the Test Cartridge to amplify and detect sequences unique to each target pathogen. Specifically, the Matrix protein gene of influenza A, the Non-structural gene of influenza B and the ORF 1a/b non-structural region and Membrane gene of SARS-CoV-2. Each Test Cartridge also contains an endogenous SPC that serves as an Internal Control (IC) to ensure adequate sample collection and processing. The SPC monitors the overall performance of the RT-PCR reaction for potential sample mediated inhibition or failure of the reagents. Additionally, the SPC verifies that the RT-PCR reaction conditions (temperature and duration) are optimal for the amplification process.

Once collected, the anterior nasal swab specimen is directly inserted into the Test Cartridge sample port with the swab shaft then removed at a pre-specified breakpoint. All further operational steps are then automatically executed 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.

C Instrument Description Information:

1. Instrument Name:

   VELO Instrument, software version v7.2.1 or higher.

2. Specimen Identification:

   Specimen identification is either entered manually or scanned via a barcode scanner.

3. Specimen Sampling and Handling:

   Direct anterior nasal swabs. The anterior nasal swab specimen is collected and added directly to the VELO Respiratory Test Cartridge sample port. No collection media is used to store the swab. Each specimen is processed individually.

4. Calibration:

   Not applicable.

5. Quality Control:

   Internal Controls

   The assay contains an endogenous sample and process control (SPC) that serves as an Internal Control (IC) to ensure adequate sample collection and processing. The SPC monitors the overall performance of the RT-PCR reaction for potential sample mediated inhibition or failure of the reagents. Additionally, the SPC verifies that the RT-PCR reaction conditions (temperature and duration) are optimal for the amplification process. Detection of the internal control is not required for a valid result when target RNA is detected.

   External Controls

   External Positive and Negative controls are not provided, but commercially available, inactivated virus controls such as the NATtrol Flu/RSV/SARS-CoV-2 from ZeptoMetrix are recommended. External Controls (EC) may be performed to conform with internal Quality Control (QC) procedures, or with local, state, or federal regulations. The use of external controls may be appropriate when receiving new device shipments, training new operators, or when problems with testing are suspected or identified. The NATtrol Flu/RSV/SARS-CoV-2 (ZeptoMetrix) external controls were evaluated in the analytical and clinical studies.

V Substantial Equivalence Information:

A Predicate Device Name(s):

cobas SARS-CoV-2 & Influenza A/B Nucleic acid test for use on the cobas Liat System

B Predicate 510(k) Number(s):

K223591

C Comparison with Predicate(s):

Device & Predicate Device(s):	K251742	K223591
Device Trade Name	VELO Respiratory Test	Cobas SARS-CoV-2 & Influenza A/B for use on the cobas Liat System
General Device Characteristic Similarities		
Regulation Number and Name	Same	21 CFR 866.3981; Multi-Target Respiratory Specimen Nucleic Acid Test Including SARS-CoV-2 And Other Microbial Agents
Product Code	Same	QOF
Prescription Use Only	Same	Yes
Intended Use/Indications For Use	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 severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), influenza A, and influenza B virus nucleic acid in anterior nasal swab (ANS) specimens from individuals with signs and symptoms of respiratory tract infection. Clinical signs and symptoms of respiratory tract infection due to SARS-CoV-2 and influenza can be similar. The VELO Respiratory Test is intended for use as an aid	The cobas SARS-CoV-2 & Influenza A/B nucleic acid test for use on the cobas Liat System (cobas SARS-CoV-2 & Influenza A/B) is an automated rapid multiplex real-time, reverse transcriptase polymerase chain reaction (RT-PCR) test intended for the simultaneous qualitative detection and differentiation of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), influenza A, and/or influenza B virus nucleic acid in nasopharyngeal swab (NPS) and anterior nasal swab (ANS) specimens from individuals with signs and symptoms of respiratory tract infection. Clinical signs and symptoms of respiratory tract infection due to SARS-CoV-2 and influenza can be similar. cobas SARS-CoV-2 & Influenza A/B is intended for

	in the differential diagnosis of SARS-CoV-2, influenza A, and/or influenza B infection if used in conjunction with other clinical and epidemiological information, and laboratory findings. SARS-CoV-2, influenza A and influenza B viral nucleic acid are generally detectable in ANS specimens during the acute phase of infection. Positive results do not rule out co-infection with other organisms. The agent(s) detected by the VELO Respiratory Test may not be the definite cause of disease. Negative results do not preclude SARS-CoV-2, influenza A, and/or influenza B infection. The results of this test should not be used as the sole basis for diagnosis, treatment, or other patient management decisions.	use as an aid in the differential diagnosis of SARS-CoV-2, influenza A, and/or influenza B infection if used in conjunction with other clinical and epidemiological information, and laboratory findings. SARS-CoV-2, influenza A, and influenza B viral nucleic acid are generally detectable in NPS and ANS specimens during the acute phase of infection. Positive results do not rule out co-infection with other organisms. The agent(s) detected by the cobas SARS-CoV-2 & Influenza A/B may not be the definitive cause of disease. Negative results do not preclude SARS-CoV-2, influenza A, and/or influenza B infection. The results of this test should not be used as the sole basis for diagnosis, treatment, or other patient management decisions.
Test Technology	Same	Multiplexed Real-time Reverse Transcription Polymerase Chain Reaction (RT-PCR)
Test Processes	Same	Automated
External Controls	Yes – commercially available	Yes – sold separately as the cobas SARS-CoV-2 & Influenza A/B Quality Control Kit
Reagents / Kit Components	Included in VELO Respiratory Test Cartridge, with no user involvement required	Included in Liat assay tube, with no user involvement required
Detection	Fluorescence detected automatically by the instrument (VELO Instrument), not reliant on user judgement	Fluorescence detected automatically by the instrument (cobas Liat System), not reliant on user judgement

Test Results	Same	Qualitative
Test Format	Same	Single use
General Device Characteristic Differences		
Specimen Type(s)	Anterior nasal swabs	Nasopharyngeal swabs and anterior nasal swabs
Time to Result	In 10 minutes or less	In approximately 20 minutes
Sample Processing	Specimen swab loaded directly into VELO Respiratory Test Cartridge where direct elution followed by thermal lysis nucleic acid release before RT-PCR	Specimen swab eluted into transfer media (VTM/UTM) before being loaded into the Liat assay tube, followed by chemical based nucleic acid extraction and purification before RT-PCR
Internal Control	Endogenous Sample Process Control	Exogenous Sample Process Control
Instrument Systems	VELO Instrument	cobas Liat System

VI Standards/Guidance Documents Referenced:

1. IEC 61010-1:2016
2. IEC 61010-1 Edition 3.1 2017-01 CONSOLIDATED VERSION 19-34
3. IEC 61010-2-010:2019
4. IEC 61010-2-101:2018
5. IEC 61326-2-6:2021
6. IEC 61000
7. ISO 15223-1 Fourth edition 2021-07
8. ISO 20417 First edition 2021-04 Corrected version 2021-12
9. ISO 14971 Third Edition 2019-12
10. ISO 23640:2015
11. ISO/IEC 14443A
12. IEC 62304 Edition 1.1 2015-06 CONSOLIDATED VERSION
13. ANSI AAMI IEC 62366-1:2015+AMD1:2020 (Consolidated Text)
14. CLSI EP17-A2
15. CLSI EP25 2nd Edition
16. ISTA 3A 2018
17. CLSI EP12 3rd Edition
18. 21 CFR 866.3981 - Special Controls
19. FDA Guidance: "Establishing the Performance Characteristics of In Vitro Diagnostic Devices for the Detection or Detection and Differentiation of Influenza Viruses" (July 15, 2011)
20. FDA Guidance: "Respiratory Viral Panel Multiplex Nucleic Acid Assay - Class II Special Controls Guidance for Industry and FDA Staff" (October 9, 2009)
21. FDA Guidance: "Instrumentation for Clinical Multiplex Test Systems - Class II Special Controls Guidance for Industry and FDA Staff" (March 10, 2005)

22. FDA Guidance: "Design Considerations for Pivotal Clinical Investigations for Medical Devices" (November 7, 2013) - Section 8: Diagnostic Clinical Performance Studies
23. FDA Guidance: "Guidance on Informed Consent for In Vitro Diagnostic Device Studies Using Leftover Human Specimens that are Not Individually Identifiable" (April 25, 2006)
24. FDA Guidance: "Significant Risk and Nonsignificant Risk Medical Device Studies" (January 2006)
25. FDA Guidance: "Financial Disclosure by Clinical Investigators" (February 2013)
26. FDA guidance "Reprocessing Medical Devices in Health Care Settings: Validation Methods and Labeling" (March 17, 2015)

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/Reproducibility:

a. *Precision:*

Precision of the VELO Respiratory Test was evaluated over 12 total days, conducted by two operators at a single site. A 3-member panel of contrived nasal swabs was evaluated, consisting of a true negative (no analyte) swab, a low positive (2x LoD of all three targets) swab, and a moderate positive (4x LoD of all three targets) swab. 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 one lot of VELO Respiratory Test cartridges, tested by 2 operators each performing 2 replicates/run and 2 runs per day, for a total of 12 days, resulting in a total of 96 replicates per panel member. Four VELO Instruments were utilized during this study. Results are summarized in **Tables 1-3**. The results of the study demonstrate acceptable test variability.

Table 1. Summary of % Agreement with expected results (95% CI) by Operator in the Precision Study

Analyte	Panel member	Operator 1 n/N[1]	Operator 2 n/N[1]	Overall % Agreement with Expected Results (n/N[1])	95% CI
SARS-CoV-2	Negative	48/48	48/48	100% (96/96)	96.2-100%
	Low positive 2x LoD	47/48	47/48	97.9% (94/96)	92.7-99.4%
	Moderate positive 4x LoD	47/48	48/48	99.0% (95/96)	94.3-99.8%
Influenza A	Negative	48/48	48/48	100% (96/96)	96.2-100%
	Low positive 2x LoD	48/48	47/48	99.0% (95/96)	94.3-99.8%
	Moderate positive 4x LoD	48/48	48/48	100% (96/96)	96.2-100%

Influenza B	Negative	48/48	48/48	100% (96/96)	96.2-100%
	Low positive 2x LoD	48/48	47/48	99.0% (95/96)	94.3-99.8%
	Moderate positive 4x LoD	48/48	48/48	100% (96/96)	96.2-100%

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

Table 2. Summary of % Agreement with expected results (95% CI) by VELO Instrument in the Precision Study

Analyte	Panel member	VELO Instrument n/N[1]				Overall % Agreement with Expected Results (n/N[1])	95% CI
		1	2	3	4		
SARS-CoV-2	Negative	25/25	22/22	24/24	25/25	100% (96/96)	96.2-100%
	Low positive 2x LoD	23/24	23/24	25/25	23/23	97.9% (94/96)	92.7-99.4%
	Moderate positive 4x LoD	23/23	23/23	25/26	24/24	99.0% (95/96)	94.3-99.8%
Influenza A	Negative	25/25	22/22	24/24	25/25	100% (96/96)	96.2-100%
	Low positive 2x LoD	23/24	24/24	25/25	23/23	99.0% (95/96)	94.3-99.8%
	Moderate positive 4x LoD	23/23	23/23	26/26	24/24	100% (96/96)	96.2-100%
Influenza B	Negative	25/25	22/22	24/24	25/25	100% (96/96)	96.2-100%
	Low positive 2x LoD	24/24	24/24	24/25	23/23	99.0% (95/96)	94.3-99.8%
	Moderate positive 4x LoD	23/23	23/23	26/26	24/24	100% (96/96)	96.2-100%

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

Table 3. Summary of % Agreement with expected results (95% CI) by Day in the Precision Study

Analyte	Panel member	Day n/N[1]												Overall % Agreement with Expected Results (n/N[1])	95% CI
		1	2	3	4	5	6	7	8	9	10	11	12		
SARS-CoV-2	Negative	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	100% (96/96)	96.2-100%
	Low positive 2x LoD	8/8	**7/8**	8/8	8/8	8/8	8/8	8/8	8/8	**7/8**	8/8	8/8	8/8	97.9% (94/96)	92.7-99.4%
	Moderate positive 4x LoD	8/8	8/8	8/8	**7/8**	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	99.0% (95/96)	94.3-99.8%
Influenza A	Negative	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	100% (96/96)	96.2-100%
	Low positive 2x LoD	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	**7/8**	8/8	8/8	8/8	99.0% (95/96)	94.3-99.8%
	Moderate positive 4x LoD	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	100% (96/96)	96.2-100%
Influenza B	Negative	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	100% (96/96)	96.2-100%

	Low positive 2x LoD	8/8	8/8	8/8	8/8	8/8	**7/8**	8/8	8/8	8/8	8/8	8/8	8/8	99.0% (95/96)	94.3-99.8%
	Moderate positive 4x LoD	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	100% (96/96)	96.2-100%

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

b. *Reproducibility:*

The reproducibility of the VELO Respiratory Test was evaluated 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) swab, a low positive (2x LoD of all three targets) swab, and a moderate positive (4x LoD of all three targets) swab. 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. Results are summarized in **Table 4**. No significant differences between sites, instruments, lots or operators were observed. The results of the study demonstrate acceptable test reproducibility.

Table 4. Summary of % Agreement with expected results (95% CI) by Site in the Reproducibility Study

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)	95.9-100%
	Low positive 2x LoD	30/30	29/30	29/30	97.8% (88/90)	92.3-99.4%
	Moderate positive 4x LoD	30/30	30/30	30/30	100% (90/90)	95.9-100%
Influenza A	Negative	30/30	30/30	30/30	100% (90/90)	95.9-100%
	Low positive 2x LoD	30/30	30/30	30/30	100% (90/90)	95.9-100%
	Moderate positive 4x LoD	30/30	30/30	30/30	100% (90/90)	95.9-100%
Influenza B	Negative	30/30	30/30	30/30	100% (90/90)	95.9-100%
	Low positive 2x LoD	30/30	30/30	30/30	100% (90/90)	95.9-100%
	Moderate positive 4x LoD	30/30	30/30	30/30	100% (90/90)	95.9-100%

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

The mean and variability analysis between sites, operators, lots, days, runs, within-runs, and overall (total) for Ct values is shown in **Table 5**. Overall %CV was ≤ 4.73%. Overall variability was low, and the study demonstrates assay variability within an acceptable range.

Table 5: Summary of Reproducibility Results (Cq Variability Analysis)

Analyte	Panel Member Conc.	n/N[1]	Mean Cq[2]	Total		Between Lot		Between Site		Between Operator		Between Day		Between Run	
				SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
SARS-CoV-2	2x LoD	88/90	29.4	0.85	2.87	0.32	1.10	0.09	0.31	0.18	0.61	0.16	0.56	0.25	0.86
	4x LoD	90/90	28.7	0.78	2.72	0.04	0.14	0.33	1.16	0.52	1.81	0.18	0.64	0.25	0.87
Flu A	2x LoD	90/90	28.8	0.91	3.16	0.37	1.29	0.06	0.21	0.14	0.47	0.08	0.26	0.25	0.88
	4x LoD	90/90	27.2	0.89	3.21	0.34	1.22	0.16	0.59	0.33	1.20	0.14	0.51	0.24	0.87
Flu B	2x LoD	90/90	30.3	1.44	4.73	0.32	1.06	0.19	0.64	0.52	1.71	0.47	1.56	0.52	1.71
	4x LoD	90/90	29.2	0.98	3.35	0.10	0.34	0.31	1.07	0.47	1.60	0.28	0.95	0.31	1.08

[1]n is number of tests with expected results. N is the total number of valid tests
[2] According to the VELO Instrument detection algorithm, not all targets reported as 'Detected' have a Cq value assigned and recorded in logs. Analysis here is completed for all available Cq values.
Cq = quantification cycle, SD = standard deviation, %CV = percent coefficient of variation

2. Linearity:

Not applicable. This is a qualitative assay.

3. Analytical Specificity/Interference:

a. *Analytical Reactivity (Inclusivity):*

Wet Testing: The inclusivity of the VELO Respiratory Test was established through wet testing by evaluating ten strains of SARS-CoV-2, ten strains of Influenza A H1N1 (including 5 pandemic 2009 strains), ten strains of Influenza H3N2 and eight strains of Influenza B, as shown in **Table 6**. These strains are in addition to the SARS-CoV-2, Influenza A H1N1, Influenza H3N2 and Influenza B strains used in the analytical sensitivity study (**Table 10**). Inactivated strains were used for SARS-CoV-2 and cultured virus were used for influenza strains. Each virus was spiked into pooled negative nasal matrix at analyte concentrations of 3x LoD. This spiked material was then transferred onto a swab and tested on three devices.

Of the 38 viruses tested, 33 were successfully detected in 3/3 devices for the expected strain (influenza A, influenza B or SARS-CoV-2) on the VELO Respiratory Test at 3x LoD. Influenza A H1N1 was detected in 3/3 devices at 4x LoD for strains A/NY/01/09 and A/Denver/1/1957, 5x LoD for strain A/Swine/Iowa/15/, and 6x LoD for strains A/California/08/2009 and A/New Jersey/8/1976. The results from this study demonstrate that the VELO Respiratory Test run on the VELO instrument is capable of detecting multiple strains of influenza A, influenza B and SARS-CoV2 at the concentrations tested.

Table 6. Analytical Reactivity (Inclusivity) Wet-Testing Study Results

Virus	Strain	Concentration per swab	Number detected/Number Tested (% detected)
Influenza A H1N1 (including pandemic 2009 strains)	A/California/07/09	7.5 $TCID_{50}$	3/3 (100%)
	A/NY/01/09	7.5 $TCID_{50}$	5/6 (83%)
		10 $TCID_{50}$	3/3 (100%)
	A/Victoria/4897/2022	9,000 cp	3/3 (100%)
	A/Wisconsin/67/2022	9,000 cp	3/3 (100%)
	A/California/08/2009	84 $CEID_{50}$	4/6 (67%)
		140 $CEID_{50}$	5/6 (83%)
		168 $CEID_{50}$	3/3 (100%)
	A/Denver/1/1957	84 $CEID_{50}$	5/6 (83%)
		112 $CEID_{50}$	3/3 (100%)
	A/New Jersey/8/1976	84 $CEID_{50}$	0/3 (0%)
		140 $CEID_{50}$	5/6 (83%)
		168 $CEID_{50}$	3/3 (100%)
	A/NWS/33	84 $CEID_{50}$	3/3 (100%)
	A/Solomon Island/3/2006	84 $CEID_{50}$	3/3 (100%)
	A/Swine/Iowa/15/30	84 $CEID_{50}$	3/6 (50%)
		140 $CEID_{50}$	3/3 (100%)
Influenza A H3N2	A/Aichi/2/68	236.4 $CEID_{50}$	3/3 (100%)
	A/Brisbane/10/07	236.4 $CEID_{50}$	3/3 (100%)
	A/California/122/2022	9,000 cp	3/3 (100%)
	A/Switzerland/9715293/13	7.5 $TCID_{50}$	3/3 (100%)
	A/Sydney/5/1997	236.4 $CEID_{50}$	3/3 (100%)
	A/Texas/50/12	7.5 $TCID_{50}$	3/3 (100%)
	A/Thailand/8/2022	9,000 cp	3/3 (100%)
	A/Uruguay/716/2007	236.4 $CEID_{50}$	3/3 (100%)
	A/Victoria/361/2011	236.4 $CEID_{50}$	3/3 (100%)
	A/Wisconsin/67/05	7.5 $TCID_{50}$	3/3 (100%)
Influenza B	B/Lee/1940	26.4 $CEID_{50}$	3/3 (100%)
Influenza B Victoria Lineage	B/Michigan/1/2021	9,000 cp	3/3 (100%)
	B/Brisbane/60/2008	26.4 $CEID_{50}$	3/3 (100%)
	B/Nevada/03/2011	26.4 $CEID_{50}$	3/3 (100%)
	B/Hong Kong/330/2001	26.4 $CEID_{50}$	3/3 (100%)
Influenza B Yamagata Lineage	B/Guangdong/120/00	3 $TCID_{50}$	3/3 (100%)
	B/Massachusetts/2/12	3 $TCID_{50}$	3/3 (100%)
	B/Texas/6/11	3 $TCID_{50}$	3/3 (100%)
SARS-CoV-2	2019-nCoV/USA-WA1/2020	9,000 cp	3/3 (100%)
	Alpha (B.1.1.7) VOC202012/01	9,000 cp	3/3 (100%)
	Beta (B.1.351) VOC202012/02	9,000 cp	3/3 (100%)
	Gamma (P.1)	9,000 cp	3/3 (100%)

Virus	Strain	Concentration per swab	Number detected/Number Tested (% detected)
	Delta (B.1.617.2) VOC21APR-02	9,000 cp	3/3 (100%)
	BA.2.12.1; USA/NY-Wadsworth-22014351- 01/2022	1.5 $TCID_{50}$	3/3 (100%)
	BA.4.6; USA/MDHP35538/2022	1.5 $TCID_{50}$	3/3 (100%)
	BF.7; USA/NY-Wadsworth-22042128-01/2022	1.5 $TCID_{50}$	3/3 (100%)
	XBB; USA/CA-Stanford-109_S21/2022	1.5 $TCID_{50}$	3/3 (100%)
	XBB.1.5; USA/NY-Wadsworth-22061020-01/2022	1.5 $TCID_{50}$	3/3 (100%)

In Silico Analysis: The inclusivity of the VELO Respiratory Test was evaluated in May 2025 using *in silico* analysis of the forward primers, reverse primers, and probes for the SARS-CoV-2 Orf1ab and M targets using sequences available in the NCBI and GISAID gene databases. The *in silico* analysis of 137,277 SARS-CoV-2 sequences, including variants of concern (VOC), variants under investigation (VUI) and variants under monitoring (VUM), indicate that 100% of SARS-CoV-2 sequences analyzed had no changes within the primer and/or probe binding regions that would be predicted to affect the performance of both assay targets. Therefore, all known SARS-CoV-2 variants are predicted to be detected.

b. *Cross-reactivity:*

The analytical specificity (cross-reactivity) of the VELO Respiratory Test was evaluated by testing a cohort of fifty-two (52) non-targeted microorganisms (including viruses, bacteria and fungi) that have a similar genome to influenza A virus, influenza B virus and SARS-CoV-2 or are reasonably likely to be present in the clinical sample (**Table 7**). Panels were composed of up to three different non-target microorganisms spiked into pooled negative nasal matrix at $\geq 1x10^5$ units/mL (for viruses) and $\geq 1x10^6$ units/mL (for bacteria and fungi). Each panel was tested in triplicate. Exclusivity was determined if 0/3 replicates returned a detected result for each of the three (3) viral targets. None of the evaluated organisms demonstrated cross-reactivity with the VELO Respiratory Test at the tested concentrations listed in **Table 7**, with each returning 0/3 replicates detected for each of the three viral targets.

Table 7. Non-targeted Microorganisms Evaluated in the Cross-reactivity and Microbial Interference Wet-Testing Studies

Microorganism	Concentration (per mL)
Adenovirus Type 1[a]	$3.45x10^6$ $TCID_{50}$
Adenovirus Type 7[a]	$3.37x10^6$ $TCID_{50}$
Adenovirus Type 10[a]	$9.6x10^5$ $TCID_{50}$
Adenovirus Type 21	$2x10^5$ $TCID_{50}$
Human Coronavirus OC43	$2x10^5$ $TCID_{50}$

Microorganism	Concentration (per mL)
Human Coronavirus 229E	$2x10^5$ copies
Human Coronavirus NL63	$2.13x10^5$ $TCID_{50}$
Human Coronavirus HKU1[b]	$2x10^5$ copies
MERS-CoV[c]	N/A[e]
Cytomegalovirus	$2.02x10^5$ $TCID_{50}$
Enterovirus Coxsackievirus CV-A16	$2x10^5$ $TCID_{50}$
Enterovirus D68	$2x10^5$ $TCID_{50}$
Enterovirus Type 71	$2x10^5$ $TCID_{50}$
Epstein Barr Virus	$2x10^5$ $TCID_{50}$
Human parainfluenza Type 1	$1.6x10^5$ $TCID_{50}$
Human parainfluenza Type 2	$2x10^5$ $TCID_{50}$
Human parainfluenza Type 3	$2x10^5$ $TCID_{50}$
Human parainfluenza Type 4	$2x10^5$ $TCID_{50}$
Measles	$1.9x10^5$ $TCID_{50}$
Human Metapneumovirus Type 1A	$2x10^5$ $TCID_{50}$
Mumps virus	$1.1x10^5$ $TCID_{50}$
Respiratory syncytial virus A1998/3-2	$2x10^5$ $TCID_{50}$
Respiratory syncytial virus A Long	$2x10^5$ $TCID_{50}$
Respiratory syncytial virus B	$2x10^5$ $TCID_{50}$
Rhinovirus A50, A2	$1.33x10^5$ $TCID_{50}$
Rhinovirus 20, 15-CV19	$2x10^5$ $TCID_{50}$
Aspergillus fumigatus	$4.65x10^5$ CFU
Aspergillus niger	$2x10^6$ CFU
Bordatella parapertussis	$2x10^6$ CFU
Bordetella pertussis	$2x10^6$ CFU
Candida albicans	$2x10^6$ CFU
Chlamydia pneumoniae	$2x10^6$ IFU
Corynebacterium xerosis	$2x10^6$ CFU
Escherichia coli	$2x10^6$ CFU
Fusobacterium necrophorum	$2x10^6$ CFU
Hemophilus influenzae	$2x10^6$ CFU
Klebsiella pneumoniae	$2x10^6$ CFU
Lactobacillus acidophilus	$5.79x10^6$ CFU
Legionella pneumophila	$2x10^6$ CFU
Moraxella catarrhalis	$2x10^6$ CFU

Microorganism	Concentration (per mL)
Mycoplasma genitalium[c]	$2x10^6$ CFU
Mycobacterium tuberculosis[d]	$2x10^6$ copies
Mycoplasma pneumoniae	$2x10^6$ CCU
Neisseria meningitidis	$2x10^6$ CFU
Neisseria mucosa	$2x10^6$ CFU
Pneumocystis jirovecii (PJP)[b]	$2x10^6$ copies
Pseudomonas aeruginosa	$2x10^6$ CFU
Staphylococcus aureus	$2x10^6$ CFU
Staphylococcus epidermis	$2x10^6$ CFU
Streptococcus pneumoniae	$2x10^6$ CFU
Streptococcus pyogenes	$2x10^6$ CFU
Streptococcus salivaris	$2x10^6$ CFU

[a] When tested as part of the cross-reactivity study this panel of Adenoviruses (each virus spiked at $2x10^5$ $TCID_{50}$/mL) resulted in 1/3 replicates detected for one of the VELO Respiratory Test targets. When tested individually at the higher concentrations listed, 0/3 were detected for each of the three viral targets.
[b] Synthetic nucleic acid.
[c] Inactivated whole organism.
[d] Genomic nucleic acid.
[e]Swabs contrived with 8 µl of NATrol MERS-CoV Stock (Ct 25.7).

c. *Microbial Interference:*

Microbial interference was evaluated for the VELO Respiratory Test by testing a cohort of fifty-two (52) non-targeted microorganisms (including viruses, bacteria and fungi) that have a similar genome to influenza A virus, influenza B virus and SARS-CoV-2 or are reasonably likely to be present in the clinical sample (**Table 7**). All samples were prepared in pooled negative nasal matrix and tested with panels of up to three different non-target microorganisms in the presence of the test target analytes, SARS-CoV-2 (BetaCoV/Australia/VIC01/2020), influenza A (A/Hong Kong/8/68) and influenza B (B/Wisconsin/1/2010), co-spiked at 3x LoD. Non-target microorganisms were spiked at $\geq 1x10^5$ units/mL (for viruses) and $\geq 1x10^6$ units/mL (for bacteria and fungi. Each panel was tested in triplicate. Absence of microbial interference was determined if 3/3 replicates returned a detected result for each of the three (3) target analytes. None of the evaluated microorganisms demonstrated interference with the VELO Respiratory Test at the tested concentrations listed in **Table 7**, with each returning 3/3 replicates detected for each of the three viral targets.

d. *Competitive Interference:*

The impact of competitive interference, caused by co-infections with on-panel analytes, was evaluated for the VELO Respiratory Test by testing contrived samples containing high concentration of SARS-CoV-2, influenza A or influenza B strains in the presence of either one or both of the other target viruses at 3x LoD prepared in pooled negative nasal matrix.

For this study, competitive interference was assessed using one strain each of SARS-CoV-2 (USA-WA1/2020) and influenza B (B/Wisconsin/1/2010) and two strains of influenza A (A/PR/8/34 - H1N1 strain and A/Hong Kong/8/68 - H3N2 strain). Testing was performed in triplicate. Absence of competitive interference was determined if all replicates for the low concentration (3x LoD) target(s) yielded positive results.

The study showed that influenza B at $1x10^6$ copies/swab inhibited detection of influenza A (H1N1) at 3x LoD, in the presence of SARS-CoV-2 at 3x LoD (**Table 8**). Subsequent testing using samples consisting of influenza A (H1N1) at 3x LoD, in the presence of influenza B at concentrations ranging from $1x10^5$ – $1.5x10^6$ copies/swab demonstrated no competitive interference (**Table 8**). No competitive interference was observed for the other potential co-infections evaluated at the concentrations tested (**Table 8**).

Table 8. Competitive Interference Results Summary

Viral Targets in Sample			Detection Rate		
Influenza A	**Influenza B**	**SARS-CoV-2**	**Influenza A**	**Influenza B**	**SARS-CoV-2**
H3N2 $1x10^6$ cp/swab	3xLoD	3x LoD	3/3	3/3	3/3
H1N1 $1x10^6$ cp/swab	3xLoD	3x LoD	3/3	3/3	3/3
H3N2 3x LoD	N/A	$1x10^6$ cp/swab	3/3	0/3	3/3
H1N1 3x LoD	3x LoD	$1x10^6$ cp/swab	3/3	3/3	3/3
H3N2 3x LoD	$1x10^6$ cp/swab	N/A	3/3	3/3	0/3
H1N1 3x LoD	$1x10^6$ cp/swab	3x LoD	2/3	3/3	3/3
H1N1 3x LoD	$1x10^6$ cp/swab	N/A	3/3	3/3	0/3
	$1x10^5$ cp/swab	N/A	3/3	3/3	0/3
	$9x10^5$ cp/swab	N/A	3/3	3/3	0/3
	$1.5x10^6$ cp/swab	N/A	3/3	3/3	0/3

*cp/swab = copies/swab

e. *Interfering Substances:*

The performance of the VELO Respiratory Test was evaluated in the presence of endogenous and exogenous substances that may be commonly found in ANS specimens. A total of 22 potentially interfering endogenous and exogenous substances (**Table 9**) were tested at or above clinically relevant levels in pooled negative nasal matrix in the presence and absence of test target analytes. Positive samples were prepared by co-spiking the test target analytes, SARS-CoV-2 (BetaCoV/Australia/VIC01/2020), influenza A (A/Hong Kong/8/68) and influenza B (B/Wisconsin/1/2010) at 3x LoD in pooled negative nasal matrix containing an individual exogenous or endogenous substance. Negative samples consisted of pooled negative nasal matrix containing an individual exogenous or endogenous substance. Each

sample was tested in triplicate.

Three of the substances tested in the study, blood (15% v/v), Triamcinolone (11 µg/swab) and Otravine Extra Dual Relief Nasal Spray (15% v/v; active ingredients listed in **Table 9**) gave false negative results with the influenza A target at the initial concentration tested. Mucin (5% w/v) gave a false negative result with the influenza B target and the anti-viral drug Zanamivir (7.5 mg/mL) gave 2 false positive results, one for the SARS-CoV-2 target and one for the influenza B target, and a false negative result for the influenza A target. The FluMist nasal vaccine was not tested as cross-reactivity with the influenza test targets is expected. Additional testing at lower test substance concentrations were performed to determine the concentration where interference is no longer observed. No interference was observed for any of the substances tested at the concentrations noted in **Table 9**.

Table 9. Exogenous/Endogenous Interfering Substances Study Results

Potential Interferant	Product	Active Ingredient(s)	Concentration
Blood (human)	N/A	None Specified	10% v/v [a]
Leukocytes	N/A	None Specified	1 x 10^6 cells/swab
Mucin: bovine submaxillary gland, type I-S	N/A	Purified mucin protein	2% w/v [b]
Nasal spray or drops	Zicam Intense Sinus Relief	Oxymetazoline HCl (0.05% w/v) / Menthol	15% v/v
	Phenylephrine	Phenylephrine	0.03 µg/mL
	Calpol saline nasal spray	Sodium chloride (0.9%) with preservatives	15% v/v
Nasal corticosteroids	Pirinase Hayfever once daily spray	Fluticasone propionate (50 µg/spray)	15% v/v
	Boots Adult Hay fever relief	Beclomethasone (50 µg/spray)	15% v/v
	Dexamethasone	Dexamethasone	12 µg/mL
	Flunisolide	Flunisolide	16 µg/swab
	Triamcinolone	Triamcinolone	10 µg/swab [c]
	Benacort Hayfever Relief Nasal Spray	Budesonide (64 µg/spray)	15% v/v
	Clarinaze Allergy Control 0.05% Nasal Spray	Mometasone furoate (50 µg/spray)	15% v/v
Nasal gel	Zicam, Powerful Allergy Relief	Sulfur / Luffa opperculata / Galphimia glauca / Histaminum hydrochloricum	15% v/v
Sore throat and cough lozenges	Ultra Chloraseptic Spray	Benzocaine (0.71%)	15% v/v
Anti-viral drugs	N/A	Zanamivir	6.0 mg/mL[d]
	N/A	Oseltamivir phosphate	0.4 µg/mL
Antibiotics	N/A	Mupirocin	1.5 µg/mL
	N/A	Tobramycin	33 µg/mL
Zinc (common ingredient in nasal sprays)	Zinc chloride	Zinc	0.1 mg/mL
Nicotine or Tobacco	Nicorette (Nicotine 0.5	Nicotine	15% v/v

Potential Interferant	Product	Active Ingredient(s)	Concentration
	mg/spray)		
Decongestant	Otravine Extra Dual Relief Nasal Spray	Xylometazoline hydrochloride / Ipratropium bromide	13.5% v/v [a]

a Potential interference observed with 15% v/v.
b Potential interference observed with 5 and 2.5% w/v.
c Potential interference observed 11 µg/swab.
d Potential interference observed with 7.5 and 6.75 mg/mL.
Note: FluMist Quadrivalent was not evaluated as cross-reactivity with targets is expected.

4. Assay Reportable Range:

Not applicable.

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

a. *Controls – External Control Evaluation:*

The assay contains an endogenous sample and process control (SPC) that serves as an Internal Control (IC) and commercially available external positive and negative controls. For more information, see Section **IV.C.5. Quality Control**, above.

b. *In-use Test Cartridge Hold Time - Specimen Stability:*

In accordance with the VELO Respiratory Test labeling, patient specimens should be tested immediately after collection for optimal test performance, however, sample storage and handling were evaluated for VELO Respiratory Test to support the following:

- Specimen loaded Test Cartridges are stable up to 30 minutes at room temperature (15-30°C/59-86°F).

c. *Kit Shelf-life Stability:*

Real-time kit stability data for the VELO Respiratory Test supports the recommended shelf-life stability claim of ambient room temperature storage claim (15-30°C / 59-86°F) for up to 9 months.

d. *In-use Test Cartridge Stability – Open Pouch:*

In accordance with the VELO Respiratory Test labeling, the sealed VELO Respiratory Test Cartridge foil pouch should be opened at the time of sample loading, however, VELO Respiratory Test Cartridge stability once removed from the foil pouch was evaluated for the VELO Respiratory Test to support the following:

- The VELO Respiratory Test Cartridge is stable for up to one hour after opening the foil pouch at room temperature (15-30°C/59-86°F).

6. Detection Limit:

The limit of detection (LoD) of the VELO Respiratory Test was evaluated for eight viral strains: two Influenza A H1N1 strains (A/PR/8/34 and A/New Cal/20/99), two Influenza A H3N2 strains (A/Hong Kong/8/68 and A/South Australia/55/14), Influenza B Yamagata Lineage (B/Wisconsin/1/2010), Influenza B Victoria Lineage (Malaysia/2506/04), SARS-CoV-2 (Beta CoV/Australia/VIC01/2020), and inactivated Omicron BA.5 (hCoV-19/USA/COR-22-06-3113/2022). Testing used pooled negative nasal matrix with multiple lots of VELO Respiratory Test Cartridges and VELO Instruments.

The preliminary LoD was established as the minimum concentration yielding 3/3 "Detected" results in a limited dilution series for each target viral strain per cartridge lot. The confirmatory LoD was determined by testing the preliminary LoD plus at least one higher and one lower concentration with multiple replicates across ≥3 days. The confirmatory LoD was the lowest concentration consistently detected at ≥95% across both cartridge lots. A final LoD validation was performed in which the confirmatory LoD for each strain was tested with a third cartridge lot using 20 replicates. The final confirmatory LoDs (**Table 10**) represent testing across three cartridge lots with 60 total replicates achieving ≥95% detection rates. Co-analyte spiked samples were also evaluated and showed equivalent LoDs to single analyte samples.

Table 10. Confirmed LoD for Influenza A, Influenza B and SARS-CoV-2

Virus	Strain	Source/ Product Type	LoD Copies (cp), IU or $TCID_{50}$ / swab
SARS-CoV-2	BetaCoV/Australia/VIC01/2020	NIBSC Inactivated	3,000 IU/swab
	hCoV-19/USA/COR-22-06-3113/2022	Zeptometrix Inactivated	0.5 $TCID_{50}$/swab
Influenza A H1N1	A/PR/8/34	ATCC Live	3,000 cp/swab [a]
	A/New Cal/20/99	Zeptometrix Live	2.5 $TCID_{50}$/swab
Influenza A H3N2	A/Hong Kong/8/68	ATCC Live	3,000 cp/swab [b]
	A/South Australia/55/14	Zeptometrix Live	2.5 $TCID_{50}$/swab
Influenza B (Yamagata)	B/Wisconsin/1/2010	ATCC Live	3,000 cp/swab [c]
Influenza B (Victoria)	Malaysia/2506/04	Zeptometrix Live	1.0 $TCID_{50}$/swab

[a]Equivalent to 28.0 $CEID_{50}$/swab
[b]Equivalent to 78.8 $CEID_{50}$/swab
[c]Equivalent to 8.8 $CEID_{50}$/swab

7. Assay Cut-Off:

For each reaction chamber, the respective target is detected using a detection algorithm which monitors the fluorescence signal generated at each PCR cycle and uses pre-determined

criteria to establish if amplification has occurred. While 'Detected' targets can be reported before completion of all 42 PCR cycles, if the algorithm criteria have been met (Cq value determined), 'Not Detected' targets can only be reported after all 42 PCR cycles are completed and analyzed.

8. Accuracy (Instrument):

   Not Applicable.

9. Carry-Over:

   An analytical study was performed to assess potential carryover or cross-contamination in the single-use, self-contained VELO Respiratory Test Cartridge by testing high positive and no template samples in an alternating fashion on the same VELO Instrument. The high positive samples consisted of a single viral target, either SARS-CoV-2 (USA-WA1/2020- ATCC inactivated), influenza A (H1N1 - A/PR/8/34 – live/ H3N2 - A/Hong Kong/8/68 - live) or influenza B (B/Wisconsin/1/2010 - live), prepared by spiking a swab at $1x10^6$ copies/swab in pooled negative nasal matrix. The no template samples were prepared by spiking a swab with nuclease free water only. A no template sample was followed by a positive sample alternating 8 times before running a final no template sample to give 17 runs per VELO instrument. The study was repeated on five VELO Instruments for a total of 40 positive and 45 no template samples. The high positive samples yielded 100% the expected detected result for the target analyte in the sample and not detected for the other two target analytes. The no template samples yielded either 93% the expected invalid result or not detected (7%) for all viral targets. These results demonstrated that there is an acceptable, low likelihood of cross-contamination between samples when the VELO Respiratory Test is performed on the VELO instrument according to the instructions for use.

B Comparison Studies:

1. Method Comparison with Predicate Device:

   Not Applicable.

2. Matrix Comparison:

   The objective of this study was to establish equivalent performance of the VELO Respiratory Test between the two matrices used in the analytical studies: pooled negative nasal matrix (NNM) and simulated nasal matrix. Simulated nasal matrix was formulated using vials of AmpliRun Negative Respiratory Swab Matrix negative control material (Vircell) rehydrated with a solution of bovine mucin. For this study, SARS-CoV-2 (BetaCoV/Australia/VIC01/ 2020), influenza A (A/Hong Kong/8/68) and influenza B (B/Wisconsin/1/2010) swabs were co-spiked for both matrices at 2x and 5x LoD. Negative swabs were also prepared for both matrices and included in the evaluation. For both matrices, 10 replicates of the negative samples, 30 replicates of the positive swabs prepared at 2x LoD and 10 replicates of the positive swabs prepared at 5x LoD were tested. The acceptance criteria to demonstrate equivalency was ≥95% detection for samples at 2x LoD, 100% detection for the samples at 5x LoD for each target, and 0% detection of the negative samples. The results obtained in

this study are summarized in **Table 11**. The data demonstrated equivalent performance of the test with both the pooled negative nasal matrix and simulated nasal matrix.

Table 11. Matrix Equivalency

Virus	Concentration	% Detected (Rate)	
		NNM	Simulated matrix
SARS-CoV-2	5x LoD	100% (10/10)	100% (10/10)
Flu A	5x LoD	100% (10/10)	100% (10/10)
Flu B	5x LoD	100% (10/10)	100% (10/10)
SARS-CoV-2	2x LoD	97% (29/30)	100% (30/30)
Flu A	2x LoD	100% (30/30)	97% (29/30)
Flu B	2x LoD	100% (30/30)	100% (30/30)
SARS-CoV-2	Negative	0% (0/10)	0% (0/10)
Flu A	Negative	0% (0/10)	0% (0/10)
Flu B	Negative	0% (0/10)	0% (0/10)

C Clinical Studies:

1. Prospective Study:

The clinical performance of the VELO Respiratory Test to detect influenza A, influenza B, and SARS-CoV-2 was evaluated in a prospective clinical study using paired anterior nasal swab (ANS) specimens collected from individuals with signs and symptoms of upper respiratory viral infection. Testing of clinical samples was performed with the VELO Respiratory Test in nine (9) CLIA waived healthcare facilities (e.g., physician offices, primary care / outpatient clinics, and urgent care centers) in various geographical locations with 15 untrained test operators. The results of all three viral targets were compared to results from an FDA-cleared, CLIA waived RT-PCR assay (comparative reference method).

Prospective clinical specimens were collected and tested between December 2024–March 2025. Initial enrollment in the prospective clinical study included 1,815 anterior nasal swab specimens. Of these, 97 specimens were excluded from the performance analysis for major protocol deviations. **Table 12** provides a summary of the demographic information for the remaining 1,718 subjects enrolled in the clinical study.

Table 12. Subject Demographics- Prospective Symptomatic Population

Characteristics	Symptomatic Subjects
Total, N	1718
Age (years)	
Mean	41.0
Standard Deviation	17.97
Median	41
Range (minimum – maximum)	0 – 92
Age Group (Years), n (%)	
≤ 12	78 (4.5%)
13 to ≤18	96 (5.6%)

19 to <55	1130 (65.8%)
≥55	414 (24.1%)
Sex, n (%)	
Male	677 (39.4%)
Female	1041 (60.6%)
Ethnicity, n (%)	
Hispanic / Latino	191 (11.1%)
Not Hispanic / Not Latino	1516 (88.2%)
Note Reported	10 (0.6%)
Unknown	1 (0.1%)
Race, n (%)	
American Indian / Alaskan Native	30 (1.7%)
Asian	20 (1.2%)
Black / African-American	389 (22.6%)
Native Hawaiian / Pacific Islander	3 (0.2%)
White	1225 (71.3%)
Other	12 (0.7%)
More than one race	32 (1.9%)
Not Reported	7 (0.4%)

In addition, of the 1,718 evaluable specimens collected in the prospective study a further 6 specimens were excluded either due to invalid comparator tests results for all three target analytes or the patient subsequently withdrew from the study. 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 of each analyte, 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 and the performance is summarized in **Table 13**. Three (3) influenza A / SARS-CoV-2 coinfections were detected by both the VELO Respiratory Test and the primary reference method.

Table 13. 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.

2. 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 test results for influenza B for the VELO Respiratory Test and a comparator RT-PCR assay and the performance is summarized in **Table 14**.

Table 14. 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	71.7 – 98.9	82/82	100	95.5 – 100

3. Other Clinical Supportive Data (When 1. and 2. Are Not Applicable):

Not Applicable.

D Clinical Cut-Off:

Not Applicable.

E Expected Values/Reference Range:

The positivity for SARS-CoV-2, Flu A and Flu B, as determined by the VELO Respiratory Test, are shown below (**Table 15**), stratified by the study site.

Table 15: Overall Positivity Rates Observed During the Clinical Study Stratified by Site

Clinical Site ID	Site Location	SARS-Cov-2			Influenza A			Influenza B		
		Total No.	No. Pos.	Exp. Value	Total No.	No. Pos.	Exp. Value	Total No.	No. Pos.	Exp. Value
Overall		**1,670**	**157**	**9.4%**	**1,677**	**354**	**21.1%**	**1,665**	**18**	**1.1%**
1	Riverside CA	138	1	0.7%	138	26	18.8%	138	6	4.3%
2	Orange City FL	73	3	4.1%	73	14	19.2%	73	1	1.4%
3	DeLand FL	20	2	10.0%	20	1	5.0%	20	0	0.0%
4	Brooklyn NY	5	0	0.0%	5	0	0.0%	5	0	0.0%
5	Gulfport MS	232	5	2.2%	232	13	5.6%	232	0	0.0%
6	Birmingham AL	379	49	12.9%	380	121	31.8%	377	5	1.3%
7	Birmingham AL	595	90	15.1%	600	157	26.2%	592	6	1.0%
8	Tulsa OK	215	6	2.8%	215	19	8.8%	215	0	0.0%
9	Salt Lake City, UT	13	1	7.7%	14	3	21.4%	13	0	0.0%

F Other Supportive Instrument Performance Characteristics Data:

Not Applicable.

VIII Proposed Labeling:

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

IX Conclusion:

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

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