CLIA Waiver by Application Approval Determination
Decision Summary

I. Document Number

CW240022

II. Parent Document Number

K242526

III. CLIA Waiver Type

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

IV. Applicant

Visby Medical, Inc.

V. Proprietary and Established Names

Visby Medical Respiratory Health Test

VI. Measurand (analyte)

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

VII. Sample Type(s)

Nasopharyngeal swabs
Anterior nasal swabs

VIII. Type of Test

Qualitative RT-PCR

IX. Test System Description

A Overview

The Visby Medical Respiratory Health Test (referred to as "Visby Test" hereafter) is a single-use (disposable), fully integrated, compact device containing a reverse transcription polymerase chain reaction (RT-PCR) based assay for qualitative detection and differentiation of RNA from

influenza A, influenza B, and SARS-CoV-2 viruses from nasopharyngeal swab (NPS) and anterior nasal swab (ANS) specimens.

The test system includes the Visby Medical Respiratory Health Test, the reusable Visby Medical power adaptor, the Visby Medical Respiratory Health Buffer Tube, and fixed-volume transfer pipette. Operators are instructed to collect the sample and elute it into the Visby Respiratory Health Buffer.

A test operator transfers the collection media (containing patient specimen) into the sample port of the device using the provided fixed-volume pipette and then slides a purple switch on the front of the device to both close the sample port and initiate the fully automated testing process. The device processes NPS and ANS samples by automatically performing all steps required to complete lysis, reverse transcription, polymerase chain reaction, and amplicon detection.

There are LED lights on the lower left corner of the device that are used to communicate the test status. Stable white light in the shape of a power symbol will appear indicating the test is plugged in. In addition, three progress lights to the right of the power light will each blink for ~10 minutes in sequence and then provide a stable white light to indicate the progression of the test. At the conclusion of the test, a green LED under “DONE” next to the white lights signals that the test run completed successfully.

When the run has completed, the operator visually interprets the status lights on the front of the device and the colorimetric output in the detection window. A valid test will have both an illuminated green LED under “DONE” and a purple spot adjacent to “RESULTS VALID” in the detection window. For valid tests, a purple spot next to the name of a pathogen indicates a positive result for that target.

In the case of a lack of a purple “RESULTS VALID” spot, the test is invalid, and the operator is instructed to repeat the test. If the subsequent test is also invalid, the operator is instructed to collect a new sample and repeat the test with a new device.

The device has built-in procedural controls. These include an internal process control and built-in electronic control. The internal control is designed to ensure that all steps in the testing process including reverse transcription, amplification of target sequences, and amplicon detection are working properly. The electronic control monitors if device is being operated outside of its temperature range or if there are any other device errors. The result of the internal process control is displayed in the detection window while the results of the electronic controls are displayed using the status lights.

Third-party external positive and negative controls are recommended for use with this test. These controls are not provided with the device and must be purchased separately by the customer directly from Microbiologics. The labeling states that the external controls “must be tested once with each new shipment received and once for each untrained operator.”

B Test System Components

The Visby Medical Respiratory Health Test kit includes sufficient reagents and consumables to test 10 samples or controls:

- Visby Medical Respiratory Health devices (10 each)
    - Containing lyophilized reagents
    - Each device is individually wrapped in an outer protective wrapper
- 1 box of Visby Medical Respiratory Health buffer tubes (10 each)
    - Each tube contains ~5.85 mL of buffer for collection and storage of swab specimens
- 1 box of individually wrapped fixed-volume disposable transfer pipette (12 each) for the addition of sample to the sample port
- Quick Reference Guide (QRG) (1 each)
- Sample collection instructions:
    - Nasopharyngeal swab collection instructions (1 each)
    - Anterior nasal swab collection instructions (1 each)

X. Specific Contents for CLIA Waiver

A Demonstrating "Simple":

Test System Characteristics:

- The test is unitized and automated: all reagents are encased in a plastic case where all analytical steps are executed automatically.
- Uses a direct specimen: nasopharyngeal swab or anterior nasal swab specimen that the operator places in a supplied tube containing a pre-measured amount of collection buffer. No additional sample processing is required.
- Needs only basic, non-technique-dependent specimen manipulation: the sample tube is inverted to mix the contents and an aliquot is transferred onto the test device using the provided fixed volume pipette.
- The device is packaged with a fixed volume pipette which ensures that an appropriate sample volume is loaded onto the device. Sample volume measurement is not needed for the operator.
- No reagent handling is required; all reagents are contained within the single use device.
- There is no operator intervention required during the analysis as all steps are automated and performed within the device.
- The device is for single use and there are no serviceable parts, thus no technical or electronic maintenance is required.
- The test requires no calibration or calculation.
- The test status is indicated by LED lights on the front of the device to indicate if the test is in progress, is completed, or error occurred.
- Contains a procedural control which, when positive, confirms that the test was properly executed.
- The results are interpreted visually.
- Contains a Quick Reference Guidance (QRG) sheet that is written in simple language at a 7th grade reading level and includes 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 assessment of the Visby Medical Respiratory Health Test was conducted in accordance with ISO 14971 to identify potential hazards, hazardous situations and the associated harms. The detailed analysis was included in the submission. Severity and probability were evaluated to generate an overall risk value. Elements considered included human factors, sample and device handling, storage, and environmental factors.

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:

a. *Mechanisms in Place to Prevent Erroneous Results:*

Internal Procedural Control:

The built-in Internal Control is an RT-PCR assay contained in the device that targets human beta-2 microglobulin (B2M) RNA. B2M is a component of the class I major histocompatibility complex (MHC) and is present in all nucleated human cells. The template for the assay comes from the human cells that are contained in the patient sample. The process control is designed to ensure that all steps in the testing process including reverse transcription, amplification of target sequences, and amplicon detection are working properly. A purple spot in the "RESULTS VALID" window indicates a successful internal process control. If a purple spot is not present in the "RESULTS VALID" window, then the test result is invalid.

Electronic Sensors:

The device firmware has built-in electronic controls that monitor the device during the run. If the run is successful, a green circle LED next to "DONE" illuminates when the testing process is complete and ready to read and turns off when the results read window timeframe has expired.

If an error is detected, the firmware will prevent the automated test process from being initiated or stop it from proceeding (depending on when the error is encountered). Error states are communicated to the user via the status lights located on the bottom left side of the top of the device, and are generated in the following conditions:

- The device is operated outside of the specified operating temperature (13-31°C).
- The power is interrupted during the test run or device is not receiving power.
- The purple slider was not completely closed or did not activate the test.
- The firmware detects a firmware error.
- The device has been plugged in for 2 hours, but the test has not been initiated.

Only tests with both a green circle LED next to "DONE" and a purple spot in the "RESULTS VALID" window are valid. In the case of an invalid test result, the user is instructed to retest the sample from the Visby Respiratory Health buffer tube with a new device, if the sample is within the stated specimen stability. If the leftover sample has exceeded the storage recommendations, the user is instructed to collect a new sample and retest on a new device.

Lockout Features:

The device is designed such that it is not possible to reuse it once a sample is loaded. This feature also prevents amplicon contamination of the work area by containing the testing materials in the device housing. The device can be plugged in either before or after the switch is closed. The action of closing the switch prevents reuse of the device, as follows.

- Once the sample port slider switch is closed, it cannot be pried open.
- Once the sample port slider switch is slid over and the sample port is closed, it is not possible to access the sample port.
- Once the device is plugged in and disconnected from power, it cannot be used again.

Fixed-Volume Transfer Pipette:

The device is packaged with a fixed volume pipette which ensures that an appropriate sample volume is loaded onto the device.

External Controls:

Ready-to-use external controls are available from Microbiologics and are recommended to be used with every new shipment and each new operator.

b. *Validating Fail-Safe and Failure Alert Mechanisms, Including External Control Procedures:*

Verification and validation of the software fail-safe and failure alert mechanisms was performed. Detailed software verification and validation documentation was included with the submission. The functionality of the safety features was evaluated in flex studies, as described below.

3. Flex Studies:

Flex Studies were performed to evaluate the robustness of the Visby Medical Respiratory Health Test and to variations in workflow and operating environment that may reasonably be expected to occur with untrained operators in the intended use CLIA Waived setting. Test conditions were designed based on a risk analysis of the complete test system and included conditions intended to verify the effectiveness of in-built controls, lock-out features and failure alerts.

The test samples used in flex studies were contrived in pooled swab matrix, confirmed to be negative for the target organisms prior to spiking. Each condition was evaluated testing a low positive sample (containing 2x Limit of Detection for each target organism as shown in **Table 1**) and a negative sample, each in five replicates (unless otherwise specified). The

testing was conducted according to the written test procedure for the Visby Medical Respiratory Health Test:

a. Swabs were placed in Visby Respiratory Health Buffer, and the tube was inverted 5 times to mix.
b. ~650 µL of Visby Respiratory Health Buffer with eluted sample was transferred into the Visby device using a fixed volume pipette.
c. The sample port was closed by sliding the switch upwards, and the device was plugged in. (Note: The Instructions for Use ask the operator to plug in the device prior to adding the sample. The internal testing process was modified to allow the sample to be loaded in a biosafety cabinet.)
d. After approximately 30 minutes, completion of the test was indicated by the green indicator light.
e. Per the Instructions for Use, an invalid result on the initial test was retested unless otherwise specified in the test case. Both results (initial invalid and retest result) were recorded.

Result interpretation is shown in **Table 2** below.

Table 1. Target Organisms Used in Studies

Virus	1x LoD (Copies/swab)	2x LoD (Copies/swab)
Influenza A 2009 H1N1, Brisbane/02/18 (live virus)	106	212
Influenza B Victoria, Washington/02/19 (live virus)	728	1456
SARS-CoV-2 (USA-WA1/2020) (inactivated virus)	100	200

Table 2. Visby Medical Respiratory Health Test Result Interpretation

Result	Indicator Light	"Results Valid" Spot	"FLU A" Spot	"FLU B" Spot	"COVID-19" Spot
Invalid	No Green Light [1]	N/A	N/A	N/A	N/A
Invalid	Green Light	Absent	N/A	N/A	N/A
Negative	Green Light	Present	Absent	Absent	Absent
Influenza A, Influenza B, SARS-CoV-2 Detected	Green Light	Present	Present	Present	Present

[1] For cases where the green indicator light is not illuminated after more than 30 minutes have passed, the test is invalid.

A brief description of each of the Flex Studies and the associated results is provided in **Table 3.** In most cases, the expected positive or negative results were observed under each of the test conditions, or the in-built fail-safe mechanisms or failure alerts were shown to function as intended to prevent reporting of erroneous results. However, six conditions across four flex studies were identified that were associated with multiple false-negative results for some

or all analytes:

Delayed Use of Test:
The assay was shown to be robust to delayed use of test if delayed by 30 minutes, one hour, or two hours when stored at ~75% relative humidity and 30°C. However, delayed use of test by six hours after device unpackaging when stored at ~75% relative humidity and 30°C, led to false negative results for all targets in one positive replicate and a false negative result for influenza B in another positive replicate. Additionally, delayed use of test by six hours after device unpackaging when stored at ~ 50% relative humidity and ~ 21-23°C, led to a false negative result for influenza A in one positive replicate. To reduce the likelihood that operators will delay using the test following device unpackaging, the Instructions for Use (IFU) and the Quick Reference Guide (QRG) include a warning to use the device immediately after unwrapping to ensure accurate results. The IFU also includes a warning statement that storage of the unwrapped device for an extended time prior to use may result in invalid or false negative results.

Specimen Stability:
The stability of the Visby Medical Respiratory Health Test analytes in nasopharyngeal and nasal swab matrix in Visby Respiratory Health Buffer was established through analytical studies. The claimed stability of such specimens for use with the Visby Medical Respiratory Health Test is two hours at 15-30 °C and two days at 2-8 °C. To support use of the Visby Medical Respiratory Health Test in a CLIA Waived setting, additional testing was performed to demonstrate the robustness of the assay system to storage of specimens under conditions outside those recommended in the device labeling, as described below.

To examine specimen stability duration, pooled negative nasal swab matrix was spiked with influenza A, influenza B, and SARS-CoV-2 for a final concentration of 2x LoD for each analyte. Contrived swab samples eluted in Visby Respiratory Health Buffer were stored for 0, 3, 4, 5, 8, or 24 hours. Five positive replicates and five negative replicates were prepared for each time point. All samples produced the final expected results for each of the target analytes. Another set of contrived swab samples were not stored eluted in Visby Respiratory Health Buffer but rather were stored “dry” for either 0, 1.5, 2, 4, 6, 8, or 24 hours. Five positive replicates and five negative replicates were prepared for each time point. All samples produced the final expected results for each of the target analytes.

To examine specimen stability temperatures, pooled negative nasal swab matrix was spiked with influenza A, influenza B, and SARS-CoV-2 for a final concentration of 2x LoD for each analyte. Contrived swab samples eluted in Visby Respiratory Health Buffer were stored at 40°C for 45 minutes. Five positive replicates and five negative replicates were prepared for this condition. All samples produced the final expected results for each of the target analytes. Another set of contrived swab samples were not stored eluted in Visby Respiratory Health Buffer but rather were stored “dry” at either -20°C or 40°C for one hour. All positive and negative replicates produced the final expected results when stored at -20°C for 1 hour. All negative replicates produced the final expected results when stored at 40°C for 1 hour. When stored at 40°C for one hour, one positive replicate produced a false negative for influenza B, while the

remaining four replicates produced the final expected results. Since dry swab storage is not a claimed storage condition for the test and because the device is intended for single use, it is unlikely that a swab sample would be stored for an extended period of time at elevated temperatures. To reduce the likelihood of occurrence, the IFU and the QRG in their respective sections on retests, state that if a sample has been stored under conditions that exceed the storage recommendations, to collect a new sample and repeat the test with a new device. The QRG also a warning that testing samples beyond these conditions can lead to inaccurate results.

The Visby Medical Respiratory Health Test appears robust to the conditions to which specimens are likely to be exposed prior to testing in the intended use environment.

Swab Inversion in Buffer:
The assay was shown to be robust to three inversions, five inversions, and vigorous shaking of the swab in Visby Respiratory Health Buffer prior to loading the sample for testing. However, no inversion of the swab in Visby Respiratory Health Buffer prior to loading the sample for testing resulted in a false negative for influenza B in one positive replicate and a false negative for SARS-CoV-2 in another positive replicate. Additionally, dipping the swab in Visby Respiratory Health Buffer and taking it out without swirling or inversion prior to loading the sample for testing resulted in a false positive for SARS-CoV-2. An investigation into this false positive determined that SARS-CoV-2 amplicon was present in the device. To reduce the likelihood of occurrence, the IFU and the QRG warns operators that failure to invert may lead to inaccurate results. The IFU also includes a warning statement that inverting the Visby Buffer Tube with a patient specimen less than five times can result in inaccurate results.

Device Positioning:
The assay should be operated on a flat surface with the device in its upright position. If the device is operated while positioned at a slight tilt of ten degrees, flex study testing showed the device still functioned as expected. However, if the device is operated while positioned at a 90 degree tilt on its horizontal edge with the lights up, erroneous results may occur. Other 90 degree tilt configurations examined as well as when the device is positioned upside down while operating resulted in invalid results. To reduce the likelihood of occurrence, the IFU and the QRG warns operators to place device on a level surface.

Table 3. Summary of Flex Studies and Fail Safe/Failure Alert Verification Testing

Specification (Category)	Test Condition	Fail Safe/ Failure Alert	Final Agreement		Final Invalid Results	Erroneous Results
			Negative	Positive		
Slider should be fully closed (Human Factors)	0% slider actuation (Slider left open)	Yes	0/5	0/5	10/10 Electronic Control Error	0/10
	50% slider actuation (Slider left halfway open)	Yes	0/5	0/5	10/10 Electronic Control Error	0/10
	90% slider actuation (Slider almost all the way closed)	Yes	2/5	0/5	8/10 Electronic Control Error	0/10

Specification (Category)	Test Condition	Fail Safe/ Failure Alert	Final Agreement		Final Invalid Results	Erroneous Results
			Negative	Positive		
	30-minute delay of slider actuation after loading sample	No	5/5	5/5	0/10	0/10
Device should be used immediately after unpackaging (Human Factors)	Device unwrapped and use delayed by 30 minutes (~75% RH, 30°C)	No	5/5	5/5	0/10	0/10
	Device unwrapped and use delayed by 1 hour (~75% RH, 30°C)	No	5/5	5/5	0/10	0/10
	Device unpackaged and use delayed by 2 hours (~75% RH, 30°C)	No	5/5	5/5	0/10	0/10
	Device unpackaged and use delayed by 6 hours (~75% RH, 30°C)	No	4/5	**2/5**	2/10	**2/10** [a]
	Device unpackaged and use delayed by 6 hours (~ 50% RH, ~ 21-23°C)	No	5/5	**4/5**	0/10	**1/10** [a]
Test Results should be interpreted within 2 hours of test run completion (Human Factors)	Correct interpretation 0 hours after test run completion	No	3/3	21/21 [b]	0/24	0/24
	Correct interpretation 2 hours after test run completion	Yes	3/3	21/21 [b]	0/24	0/24
	Correct interpretation 6 hours after test run completion	Yes	3/3	21/21 [b]	0/24	0/24
	Correct interpretation 24 hours after test run completion	Yes	3/3	21/21 [b]	0/24	0/24
650 µL sample volume should be added to device (Specimen Factors)	No sample added and slider completely closed	Yes	0/5		5/5	0/10
	200 µL sample input volume	Yes	0/5	0/5	10/10	0/10
	300 µL sample input volume	Yes	5/5	5/5	0/10	0/10
	450 µL sample input volume	Yes	5/5	5/5	0/10	0/10
	550 µL sample input volume	Yes	6/6	6/6	0/12	0/10
	750 µL sample input volume	No	6/6	6/6	0/12	0/10
	850 µL sample input volume	No	5/5	5/5	0/10	0/10
	1300 µL sample input volume	No	5/5	5/5	0/10	0/10
Nasopharyngeal or anterior nasal swab specimens should be tested (Specimen Factors)	Sputum	No	5/5	5/5	0/10	0/10
	Tongue Swab	No	5/5	5/5	0/10	0/10
	Throat Swab	No	5/5	5/5	0/10	0/10
	Saliva	No	0/5	0/5	10/10	0/10
Swabs should be eluted in Visby Respiratory Health Buffer (Specimen Factors)	UTM	No	0/5	0/5	10/10	0/10
	Water	No	5/5	5/5	0/10	0/10
	Saline	No	5/5	5/5	0/10	0/10
Swabs should be eluted into ~5.85 mL Visby Respiratory Health Buffer (Specimen Factors)	1 mL buffer input volume	No	5/5	5/5	0/10	0/10
	1.5 mL buffer input volume	No	5/5	5/5	0/10	0/10
	3 mL buffer input volume	No	5/5	5/5	0/10	0/10
	5.85 mL buffer input volume	No	5/5	5/5	0/10	0/10
	Control (invert 5 times)	No	5/5	5/5	0/10	0/10

Specification (Category)	Test Condition	Fail Safe/ Failure Alert	Final Agreement		Final Invalid Results	Erroneous Results
			Negative	Positive		
Swabs in buffer should be inverted 5x prior to loading sample (Specimen Factors)	Swirl 5 seconds, discard swab, and invert 5 times	No	5/5	5/5	0/10	0/10
	Swirl 10 seconds, discard swab, and invert 5 times	No	5/5	5/5	0/10	0/10
	No inversion	No	5/5	**3/5**	0/10	**2/10** [a]
	3 inversions	No	5/5	5/5	0/10	0/10
	Vigorous shaking	No	5/5	5/5	0/10	0/10
	Dip swab in and take out without swirling or inversion	No	**4/5**	5/5	0/10	**1/10** [a]
Operators should keep the device plugged in during entire run (Device Factors)	Removal and re-insertion of power cord ~5 minutes after test initiation	Yes	0/6	0/6	12/12 Electronic Control Failure	0/12
The assay is intended to operate between 13 to 31ºC and 5 to 80% RH (Environmental Factors)	9ºC (n=3 each at 5% RH and 95% RH)	Yes	0/6	0/6	12/12 Electronic Control Error	0/12
	11ºC (n=3 each at 5% RH and 95% RH)	No	6/6	6/6	0/12	0/12
	13ºC (n=3 each at 5% RH and 95% RH)	No	6/6	6/6	0/12	0/12
	15ºC (all replicates at ambient RH)	No	6/6	6/6	0/12	0/12
	31ºC (all replicates at ambient RH)	No	6/6	6/6	0/12	0/12
	33ºC (n=3 each at 5% RH and 95% RH)	No	6/6	6/6	0/12	0/12
	35ºC (n=3 each at 5% RH and 95% RH)	Yes	0/6	0/6	12/12 Electronic Control Error	0/12
	37ºC (n=3 each at 5% RH and 95% RH)	Yes	0/6	0/6	12/12 Electronic Control Error	0/12
The assay is intended to operate an elevation ≤ 10,000 feet above sea level (Environmental Factors)	-110 m (-361 feet)	No	6/6	6/6	0/12	0/12
	-30 m (-98 feet)	No	6/6	6/6	0/12	0/12
	1885 m (6184 feet, at 25.5°C)	No	6/6	6/6	0/12	0/12
	2970 m (9744 feet, at 20.3°C)	No	6/6	6/6	0/12	0/12
Operators should read results under nominal general office lighting conditions (i.e., 30-95 "foot candle" units) (Environmental Factors)	< 15 foot candles	No	3/3	21/21 [b]	0/24	0/24
	20-30 foot candles	No	3/3	21/21 [b]	0/24	0/24
	95-100 foot candles	No	3/3	21/21 [b]	0/24	0/24
The assay should be operated upright on a flat surface	10º tilt with lights down	No	5/5	5/5	0/10	0/10
	10º tilt with lights up	No	5/5	5/5	0/10	0/10
	90º horizontal edge with lights down	No	0/5	0/5	10/10	0/10
	90º horizontal edge with lights up	No	0/5	**0/5**	9/10	**1/10** [a]
	90º vertical edge with plug up	No	0/5	0/5	10/10	0/10

Specification (Category)	Test Condition	Fail Safe/ Failure Alert	Final Agreement		Final Invalid Results	Erroneous Results
			Negative	Positive		
(Environmental Factors)	Upside down	No	0/5	0/5	10/10	0/10
The assay is robust to vibration (Environmental Factors)	Inversion during reverse transcription: 2 minutes after test started	No	5/5	5/5	0/10	0/10
	Inversion during PCR: 10 minutes after test started	No	5/5	5/5	0/10	0/10
	Inversion during detection: 22 minutes after test started	No	5/5	5/5	0/10	0/10
	Vibration during run	No	5/5	5/5	0/10	0/10
	Dropped from 1 meter before run	No	5/5	5/5	0/10	0/10
	Dropped from 1 meter during run	No	5/5	5/5	0/10	0/10
Operators should store the test between 2ºC and 30ºC. If stored cold, devices should come to its minimum operating temperature of 55ºF (13ºC) prior to use (Environmental Factors)	Devices stored at < -15°C for 24 hours and then allowed to come to room temperature for 24 hours	No	5/5	5/5	0/10	0/10

[a] The workflow for the Visby Medical Respiratory Health Test was validated in a prospective Clinical Study with naïve operators and shown to be robust to user error. Therefore, in practice, the likelihood of obtaining false results due to this failure mode is considered low

[b] All possible combinations of targets were tested in positive contrived samples (i.e., each individual target, all pairwise combinations of targets, and all 3 targets in one) with 3 replicates each.

Overall, the Flex Studies demonstrated that the Visby Medical Respiratory Health Test is robust to foreseeable user-dependent variations in the assay workflow and that in-built assay controls and fail-safe and/or failure alert mechanisms are effective in preventing the generation of erroneous results due to operator error and/or use of the Visby Medical Respiratory Health Test outside the specified operating environmental conditions.

A Demonstrating "Insignificant Risk of an Erroneous Result" - Accuracy

1. Comparison Study:

a. *Study Design:*

i. Study Sites and Duration:

The performance of the Visby Medical Respiratory Health Test in the hands of untrained users was evaluated in a prospective clinical study that was performed at five sites. All five sites were considered representative of CLIA-waived intended use sites. The sites were geographically distributed across the United States and included a family urgent care, a

primary care pediatric clinic, two family practice clinics, and an outpatient research site. The study was conducted from May 2022 to February 2024.

ii. Subjects (Patients):

A total of 1,592 subjects were initially enrolled for the prospective clinical study, of which 1,501 subjects were included in the performance evaluation. The average age among study participants was 34 years, with a range between one month to 89 years of age. Specimens were collected according to the inclusion and exclusion criteria below.

Inclusion Criteria:

1. Suspected of respiratory viral infections and/or had signs and symptoms of respiratory infections.
2. Willing and able to provide written informed consent and/or assent as required by the reviewing IRB or IEC. Experimental Bill of Rights were documented for all study subjects enrolled in applicable states. Minors were defined as less than 18 years of age, unless otherwise required by state regulations.
3. Willing and able to provide all study specimens.

Exclusion Criteria:

1. Had a contraindication to the sample collection.
2. Had undergone a nasal wash or aspirate as part of standard of care on the day of the study visit.
3. Had undergone any form of antiviral treatment or therapy within two weeks of enrollment into this study.
4. Had been previously enrolled in this study.

iii. Operators:

A total of 18 untrained operators, representative of CLIA-waived users, participated in the clinical study with two to six operators per site (**Tables 4-6**). The education level of the operators included high school diploma, GED or equivalent, university education, and a graduate degree and included medical assistants, nurses and administrative staff performing various tasks relating to patient care or patient enrollment. The operators were representative of operators conducting testing at a CLIA Waived setting and did not receive any training on the use of the Visby Medical Respiratory Health Test.

Table 4. Influenza A Clinical Performance for the Visby Test vs. Comparator, by Operator

Operator	N	TP	FP	TN	FN	PPA	NPA
Site 1							
1	251	11	0	240	0	100.00%	100.00%
2	251	21	2	227	1	95.45%	99.13%
3	44	0	0	44	0	N/A	100.00%
4	136	3	0	133	0	100.00%	100.00%
5	60	12	0	48	0	100.00%	100.00%
6	49	8	0	40	1	88.89%	100.00%
Site 2							
1	26	0	0	26	0	N/A	100.00%
2	21	2	0	19	0	100.00%	100.00%
3	15	0	1	13	1	0.00%	92.86%

Operator	N	TP	FP	TN	FN	PPA	NPA
4	20	0	0	20	0	N/A	100.00%
Site 3							
1	77	5	2	70	0	100.00%	97.22%
2	81	7	2	72	0	100.00%	97.30%
3	88	8	1	79	0	100.00%	98.75%
Site 4							
1	221	4	1	216	0	100.00%	99.54%
2	7	0	0	7	0	N/A	100.00%
3	127	10	0	117	0	100.00%	100.00%
Site 5							
1	9	2	0	7	0	100.00%	100.00%
2	18	1	0	17	0	100.00%	100.00%

PPA=Positive Percent Agreement with comparator; NPA=Negative Percent Agreement with comparator; TP=true positive; FP=false positive; TN=true negative; FN=false negative

Table 5. Influenza B Clinical Performance for the Visby Test vs. Comparator, by Operator

Operator	N	TP	FP	TN	FN	PPA	NPA
Site 1							
1	251	0	0	251	0	N/A	100.00%
2	251	1	0	250	0	100.00%	100.00%
3	44	0	0	44	0	N/A	100.00%
4	136	0	0	136	0	N/A	100.00%
5	60	7	0	53	0	100.00%	100.00%
6	49	6	0	43	0	100.00%	100.00%
Site 2							
1	26	0	0	26	0	N/A	100.00%
2	21	0	0	21	0	N/A	100.00%
3	15	2	0	13	0	100.00%	100.00%
4	20	1	0	19	0	100.00%	100.00%
Site 3							
1	77	0	0	77	0	N/A	100.00%
2	81	0	0	81	0	N/A	100.00%
3	88	1	0	87	0	100.00%	100.00%
Site 4							
1	221	0	0	221	0	N/A	100.00%
2	7	0	0	7	0	N/A	100.00%
3	127	11	2	114	0	100.00%	98.28%
Site 5							
1	9	0	0	9	0	N/A	100.00%
2	18	1	1	16	0	100.00%	94.12%

PPA=Positive Percent Agreement with comparator; NPA=Negative Percent Agreement with comparator; TP=true positive; FP=false positive; TN=true negative; FN=false negative

Table 6. SARS-CoV-2 Clinical Performance for the Visby Test vs. Comparator, by Operator

Operator	N	TP	FP	TN	FN	PPA	NPA
Site 1							
1	251	49	4	198	0	100.00%	98.02%
2	251	53	3	194	1	98.15%	98.48%
3	44	6	0	38	0	100.00%	100.00%
4	136	22	2	111	1	95.65%	98.23%
5	60	14	0	45	1	93.33%	100.00%
6	49	14	0	35	0	100.00%	100.00%
Site 2							

1	26	3	1	22	0	100.00%	95.65%
2	21	1	0	19	1	50.00%	100.00%
3	15	1	0	14	0	100.00%	100.00%
4	20	1	0	19	0	100.00%	100.00%
Site 3							
1	77	8	0	69	0	100.00%	100.00%
2	81	7	0	74	0	100.00%	100.00%
3	88	2	0	86	0	100.00%	100.00%
Site 4							
1	221	42	2	174	3	93.33%	98.86%
2	7	1	0	6	0	100.00%	100.00%
3	127	17	0	110	0	100.00%	100.00%
Site 5							
1	9	1	0	8	0	100.00%	100.00%
2	18	4	0	14	0	100.00%	100.00%

PPA=Positive Percent Agreement with comparator; NPA=Negative Percent Agreement with comparator; TP=true positive; FP=false positive; TN=true negative; FN=false negative

iv. Samples:

One NP swab specimen or dual nostril AN swab specimen was collected from each eligible subject. The AN specimens could be self-collected under health care provider (HCP) observation (for subjects ≥ 14 years of age), or health care provider (HCP) collected, and all NP swabs were HCP collected. The swab specimen was placed directly in the Visby Respiratory Health Buffer and tested on-site by study operators using the Visby Medical Respiratory Health Test. The participating operators conducted the test by following the instructions in the Quick Reference Guide (QRG). The specimen was also tested at the reference laboratory for comparator testing.

v. Comparator Method (CM):

Comparator testing was performed using an FDA-cleared nucleic acid amplification test (NAAT) for influenza A and influenza B targets and a highly sensitive FDA-authorized laboratory-based RT-PCR EUA assay for the SARS-CoV-2 target.

b. *Results:*

A total of 1,592 subjects were initially enrolled. Study samples were excluded from the data analysis due to lack of a valid comparator result (n=53), lack of a valid Visby Medical Respiratory Health Test result (n=21), the subject was asymptomatic (n=10), or for protocol deviations (i.e., failure to complete a required procedure (n=4) or incorrectly filled out informed consent form (n=2)). Additionally, one subject withdrew from the study. This left 1,501 subjects for performance analysis.

Of the 1575 tests performed for these subjects, there were a total of 79 (5.0%, 95% CI = 4.0-6.2%) invalid results obtained on initial testing with the Visby Medical Respiratory Health Test. Of these, 21 were invalid upon retesting, for a final invalid rate of 1.3% (21/1575) with 95% CI (0.8-2.0%).

The clinical performance of the Visby Medical Respiratory Health Test with ANS and NPS specimens, when used by untrained operators in CLIA-waived settings, is shown in the three

tables below, expressed as percent agreement with the comparator method for positive and negative subjects.

Table 7. SARS-CoV-2 Clinical Performance for the Visby Test vs. Comparator, by Specimen Type

Specimen Type	N	TP	FP	TN	FN	PPA (95% CI)	NPA (95% CI)
NPS	829	130	7[a]	687	5[c]	96.3% (91.6-98.4%)	99.0% (97.9-99.5%)
ANS	672	116	5[b]	549	2[d]	98.3% (94.0-99.5%)	99.1% (97.9-99.6%)
Total	1501	246	12	1236	7	97.2% (94.4-98.7%)	99.0% (98.3-99.5%)

PPA=Positive Percent Agreement with comparator; NPA=Negative Percent Agreement with comparator; TP=true positive; FP=false positive; TN=true negative; FN=false negative

[a] 5 of 7 false positive NPS specimens tested positive and 2 tested negative with an alternate EUA molecular assay.

[b] 4 of 5 false positive AN specimens tested positive and 1 tested negative with an alternate EUA molecular assay.

[c] 3 of 5 false negative NPS specimens tested negative and 2 tested positive with an alternate EUA molecular assay.

[d] 1 of 2 false negative AN specimens tested negative and 1 tested positive with an alternate EUA molecular assay.

Table 8. Influenza A Clinical Performance for the Visby Test vs. Comparator, by Specimen Type

Specimen Type	N	TP	FP	TN	FN	PPA (95% CI)	NPA (95% CI)
NPS	829	33	4[a]	791	1[c]	97.1% (85.1-99.5%)	99.5% (98.7-99.8%)
ANS	672	61	5[b]	604	2[d]	96.8% (89.1-99.1%)	99.2% (98.1-99.7%)
Total	1501	94	9	1395	3	96.9% (91.3-98.9%)	99.4% (98.8-99.7%)

PPA=Positive Percent Agreement with comparator; NPA=Negative Percent Agreement with comparator; TP=true positive; FP=false positive; TN=true negative; FN=false negative

[a] 3 of 4 false positive NPS specimens tested positive and 1 tested negative with an alternate EUA molecular assay.

[b] 5 of 5 false positive AN specimens tested positive with an alternate EUA molecular assay.

[c] 1 false negative NPS specimen tested positive with an alternate EUA molecular assay.

[d] 2 false negative AN specimens tested positive with an alternate EUA molecular assay.

Table 9. Influenza B Clinical Performance for the Visby Test vs. Comparator, by Specimen Type

Specimen Type	N	TP	FP	TN	FN	PPA (95% CI)	NPA (95% CI)
NPS	829	15	2[a]	812	0	100% (79.6-100%)	99.8% (99.1-99.9%)
ANS	672	15	1[b]	656	0	100% (79.6-100%)	99.9% (99.1-100%)
Total	1501	30	3	1468	0	100% (88.7-100%)	99.8% (99.4-99.9%)

PPA=Positive Percent Agreement with comparator; NPA=Negative Percent Agreement with comparator; TP=true positive; FP=false positive; TN=true negative; FN=false negative

[a] 2 of 2 false positive NPS specimens tested positive with an alternate EUA molecular assay.

[b] 1 false positive AN specimen tested positive with an alternate EUA molecular assay.

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

The performance of the Visby Medical Respiratory Health 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. The low positive test samples were prepared individually for each target organism in pooled clinical swab matrix (previously determined to be negative for target analytes), by spiking with quantified organism stocks to target concentrations of 1x LoD, for each organism (**Table 1** above). The negative samples consisted of un-spiked negative matrix. The study was conducted at three CLIA-waived clinical sites with six operators (two operators at each site) testing the randomized and blinded panel in triplicate each day across six non-consecutive days. Three reagent lots were used in this study. Results were generated from a total of 108 replicates of each panel member (3 sites x 6 days x 2 operators/site x 3 replicates/operator/day). The results from the study are shown in **Table 10** below.

The study demonstrated that untrained users can perform the test accurately when testing samples with organism concentrations near the assay LoD.

Table 10. Summary of Results Testing Samples Near Assay LoD

Panel Member	% Agreement with Expected Results (Count)			Overall Agreement	
	Site 1	Site 2	Site 3	% Agreement (count)	95% CI
Influenza A 1x LoD	94.4 % (34/36)	100 % (36/36) [a]	88.9% (32/36)	94.4% (102/108) [a]	88.4%-97.4%
Influenza B 1x LoD	97.2 % (35/36)	100 % (36/36)	91.7% (33/36)	96.3% (104/108)	90.9%-98.6%
SARS-CoV-2 1x LoD	97.2% (35/36)	100 % (36/36)	88.9% (32/36)	95.4% (103/108)	89.6%-98.0%

Panel Member	% Agreement with Expected Results (Count)			Overall Agreement	
	Site 1	Site 2	Site 3	% Agreement (count)	95% CI
Negative	100 % (36/36)	100 % (36/36)	100 % (36/36)	100.0% (108/108)	96.6%-100.0%

[a] One influenza A sample was an unexpected positive for SARS-CoV-2.

3. External Control Lot-to-Lot Testing:

The reproducibility of three lots of the Microbiologics External Control Positive and Negative Swabs was evaluated, testing five replicates of the Negative Control and five replicates of the Positive Control for each of the three lots.

Table 11. External Control Lot-to-Lot Reproducibility Results

Control	Lot Name	Detection Rate
Positive	Lot 1	5/5
	Lot 2	5/5
	Lot 3	5/5
Negative	Lot 1	5/5
	Lot 2	5/5
	Lot 3	5/5

All valid tests returned expected results; there were two invalid results generated and the test was repeated in each case, returning a valid result.

4. Operator Questionnaire:

An operator questionnaire (total of 25 questions) was developed and filled out by the participating operators after the completion of the clinical study to assess the ease of use of the device as well as the user experience with the device during testing. One operator left before the end of the study and did not complete the questionnaire. The questionnaire was divided into two categories: 1) system set-up and operation and 2) results interpretation. The first set of questions asked operators to indicate agreement on a 1-5 scale (1=strongly disagree to 5=strongly agree), focusing on the ease of use. Based on the study operator responses, the Visby device was easy to set up and use by following the instructions provided in the QRG.

In the second part of the questionnaire, six questions asked operators to read and interpret various possible test results and control results when provided images. All 18 operators interpreted the results correctly for all interpretation questions with 100% accuracy. According to the operator responses, the potential results for the Visby Medical Respiratory Health Test are easy to understand and interpret.

B Labeling for Waived Devices

The labeling consists of:

1. Instructions for Use/Package Insert (IFU)

2. Quick Reference Guide (QRG)
3. AN Collection Instructions
4. NP Collection Instructions
5. Kit and individual device outer packaging labels

The following elements are appropriately present:

- The QRG is written in simple language and contains graphics which visually aid the user in processing samples.
- The IFU identifies the system as CLIA-waived.
- A statement informing the user that the test procedure must be followed as written to maintain the CLIA-waived status is present in the IFU.
- The IFU includes the statement that a Certificate of Waiver is required to perform the test in a waived setting.
- The IFU and QRG include instructions for performing Quality Control testing.
- Technical support telephone number is prominently displayed in both the IFU and QRG.
- All appropriate cautions regarding sample handling and processing are present.
- The labeling is sufficient and satisfies the requirements of 21 CFR Part 809.10.

XI. Benefit-Risk Considerations

Not applicable.

XII. Conclusion

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