CLIA Waiver by Application Approval Determination

Decision Summary

A. Document Number

CW220001

B. Parent Document Number

K220407

C. CLIA Waiver Type:

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

D. Applicant

Visby Medical

E. Proprietary and Established Names

Visby Medical Sexual Health Test

F. Measurand (analyte)

Chlamydia trachomatis (CT) DNA,
Neisseria gonorrhoeae (NG) DNA, *and*
Trichomonas vaginalis (TV) DNA

G. Sample Type(s)

Female Vaginal Swabs (self-collected in healthcare settings)

H. Type of Test

Qualitative, Polymerase Chain Reaction (PCR)

I. Test System Description

1. Overview

Similar to the previously FDA cleared and CLIA waived Visby Medical Sexual Health Click Test ("Click Test", K200748/CW200003), the Visby Medical Sexual Health Test ("Visby Test") is a single-use (disposable), fully integrated and compact device containing a PCR-based assay for direct qualitative detection and differentiation of DNA from CT, NG, and TV from female vaginal swab samples. The Visby Test is a modified version of the Click Test with the purpose to further simplify the user interface and to

enable the automated manufacture process of the Click Test. The Visby Test has no changes to the PCR primer and probe sequences, reagent formulations, detection method, or result interpretation as compared to the Click Test. Validations presented in the following sections demonstrate that the Visby Test continues to perform as previously demonstrated and the CLIA waived status remains unchanged.

The Visby Test consists of a main test unit which houses the core sample processing modules where all analytical steps take place: lysis, mixing, polymerase-chain reaction (PCR), and detection. The device is run by firmware. The device further contains the fluidic and thermal elements required to perform these functions (syringe pump, rotary valve, controller printed circuit board (PCB) and firmware). A lyophilized control organism pellet containing *Neisseria subflava* (NS) is located underneath the sample port, inaccessible to the device user. Two additional lyophilized pellets, one containing the polymerase and the other containing the primers required for PCR, are co-located within the mixing chamber. The liquid detection reagents (horseradish peroxidase (HRP), substrate, and wash buffer) are contained within the reagent canisters. None of the internal components are accessible by the operator.

The disposable, fixed volume pipette is used to transfer approximately 650 µL of the sample to the lysis module through an input port, simultaneously hydrating the control organism pellet. The lysis module is designed to lyse the organism contained in both the sample and the control pellet.

As the liquid moves through the fluidic pathway, the mixture is thermally cycled to amplify the select pathogenic targets. After PCR, the biotinylated amplified product (if present) is hybridized to a probe which is bound at specific locations along the flow channel. A colorimetric reaction follows as a result of enzyme-linked reaction between streptavidin-bound HRP and biotinylated amplified product to form a visible purple precipitate. The operator thus observes color change at the specific locations corresponding to targets present in the sample, with each colored spot indicating a positive test result.

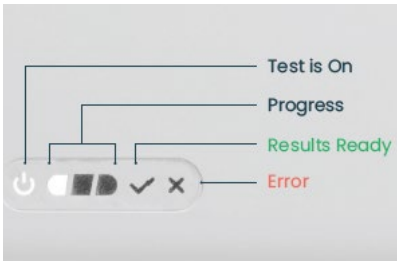
2. Test Components

The device contains all of the hardware and reagents required to perform the test. Each kit contains 10 test devices, 12 single use disposable fixed-volume transfer pipettes and 12 biohazard bags. The unit is outlet powered with a reusable power adaptor that is packaged separately.

Samples are collected by the patient using the Visby Vaginal Specimen Collection Kit, packaged separately. As shown below, each individual vaginal specimen collection kit contains a tube of collection media and a single use, sterile collection swab.

3. Workflow

Plug the Visby Power Adaptor into the device power port. Upon receipt of the sample, the test operator uses the transfer pipette to load a fixed volume of the sample into the sample port (below on the left) before sliding the switch upward in a firm and swift motion to completely close the sample port and start the test (below in the middle). Subsequently, the first progress indicator light blinks, indicating that the test starts (below on the right). The three progress lights will each blink for ~ 10 minutes in sequence and then provide a stable white light to indicate the progression of the test. In approximately 30 minutes, a green check mark lights up indicating that the test run has completed successfully. A 'Red X' during or at the end of the run indicates a run failure.



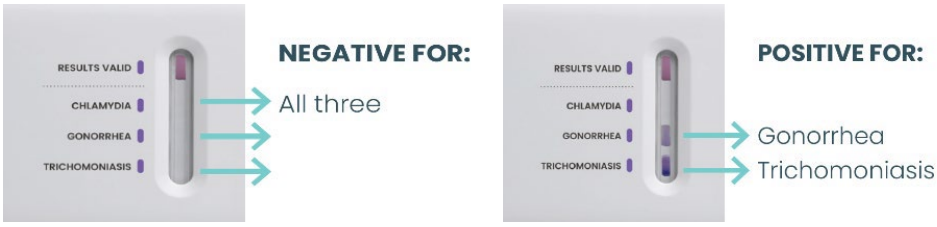
4. Result Interpretation

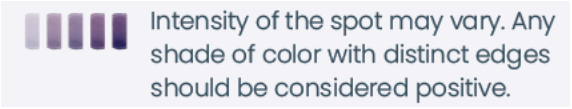
When the run has completed, the operator visually interprets the status lights on the front of the device and the colorimetric output in the detection window. As shown in the figure

below, a valid test will have both an illuminated green check mark and a purple spot adjacent to “RESULTS VALID” in the detection window as shown below.

In the case of a “Red X” error and/or no spot adjacent to “RESULTS VALID” in the detection window (see figure below), 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.

For valid tests, a purple spot next to the name of a pathogen indicates a positive result for that target. The following figures illustrate examples of valid test results, including a negative result for all pathogens (upper left figure) and a positive result for NG and TV (upper right figure). In addition, any shade of purple with distinct edges next to the target is a positive result (figure on the bottom).





5. External Controls

Third-party, single-use, liquid 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 the manufacturer. The controls are manufactured by ZeptoMetrix Corporation (Buffalo, NY). The positive controls are prepared from purified microorganisms (CT, NG, and TV) that are grown in microbial culture. Once purified, the microorganism is chemically treated to alter its surface proteins, resulting in an intact microorganism that is unable to bind, penetrate, or infect a host cell. Controls are run in the same manner as clinical samples. The labeling states that the external controls "must be tested once with each new shipment received and once for each untrained operator."

J. 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: self-collected vaginal swab which the patient 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.
- The reagents inside of the device are stable and can be stored at a wide range of temperatures (2-30°C)
- 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.
- 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.

K. Demonstrating "Insignificant Risk of an Erroneous Result"- Failure Alerts and Fail-safe Mechanisms

1. Risk Analysis

A comprehensive risk assessment of the Visby 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 to eliminate the error. If the error could not be eliminated, then fail-safe and failure alert mechanisms were implemented. Finally, labeling was used to alert user to potential errors or to caution users to potential harms.

When appropriate, the effectiveness of risk mitigations was verified and/or validated. The majority of the verification and validation testing occurred as part of the firmware validation for electronic controls or by flex studies that stressed the functional limits of the test system.

2. Fail-Safe and Failure Alert Mechanisms

1) The Visby Test was designed with the following fail-safe and failure alert mechanisms to prevent erroneous results, which are similar to the features of the Visby Click Test.

Internal Procedural Control

The built-in Internal Control monitors for failure in lysis, PCR or the detection phase. It is a lyophilized pellet of *Neisseria subflava* which is located between the sample port and the lysis chamber. When the sample is added to the sample port, it allows re-hydration of the Internal Control pellet as it moves along the microfluidic pathway to the lysis chamber. If this process does not proceed correctly, the purple "RESULTS VALID" spot in the detection window will be absent and the test 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 check mark LED turns on when the test is successfully completed and ready to read and turns off when the results read window timeframe has expired. This is unchanged from the Click Test.

If an error is detected, the firmware will stop the test and illuminate a red LED light that displays a Red X to notify the user of the error. Red X errors are generated in the following conditions:

- The device is operated outside of the specified operating temperature (13-33°C).
- The power is interrupted during the test run.
- The firmware detects that the heaters or motors are not operating within specifications.
- The firmware detects a firmware error.

- The device has been plugged in for 2 hours, but the test has not been initiated.

Note: This is a new failure alert mechanism in the Visby Test.

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. All the same lockout features are present in the Visby Test as were in the Click Test. In the Visby Test, the user interface has been modified from pushing three buttons (Click Test) and then plugging in the device to start the run, to closing one switch (Visby Test). For the Visby Test, the device can be plugged in either before or after the switch is closed. The lockout features are listed below:

- 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.

External Controls

Ready-to-use external controls are available from Zeptometrix and are recommended to be used with every new shipment and each new operator. The external controls are unchanged from the Click Test.

2) 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

The flex studies were based on the hazard analysis and evaluated conditions that presented a low but potential risk of obtaining an incorrect result due to operator errors performing the procedure (human factors) or to environmental conditions outside of the intended use specifications.

The test samples used in flex studies were contrived in pooled vaginal swab matrix, confirmed to be negative for the target organisms prior to spiking. Each flex study condition was evaluated testing a low positive sample (3x LoD for each target organism) and a negative sample, each in five replicates (unless otherwise specified). The testing was conducted according to the written test procedure for the Visby Test.

Unless specified in the flex test descriptions, the prepared samples were tested in accordance with the Instructions for Use of the Visby Sexual Health Test. The steps of the testing process are as follows:

- ~650 µL of sample was transferred into the Visby device using a fixed volume pipette.
- The slider was pushed upwards in a firm, swift motion to close the sample port.
- The Visby Power Adapter was plugged into the device power port to start the test (the power adapter can be plugged into the device either before or after sample addition and sample port closure).
- After approximately 27 minutes, completion of the test was indicated by the indicator light (Green ✓ or Red X).

Examples of result interpretation are shown below.

Result	Indicator Light	Results Valid Spot	CT Spot	NG Spot	TV Spot
Invalid – Red X	Red X	N/A	N/A	N/A	N/A
Invalid – Control Failure	Green ✓	Absent	N/A	N/A	N/A
CT, NG, and TV Detected	Green ✓	Present	Present	Present	Present
Negative (CT, NG, and TV Not Detected)	Green ✓	Present	Absent	Absent	Absent

3.1 Human Factors

3.1.1 Incomplete Slider Actuation and Delayed Operations

Per the Visby Test Instructions for Use (IFU) and QRG, users are instructed to slide the switch upwards in a firm, swift motion to close the sample port and start the test after sample is added. The slider both closes the sample port (so that patient sample cannot leak out of the device) and initiates the test.

This flex test evaluated the impact of incomplete closure of the slider which is expected to result in Red X errors because the firmware generates a Red X if the test run is not initiated (by the closed slider) within two hours of plugging into power.

The flex test also evaluated the impact of not closing the slider immediately after loading the sample. This was expected to have no impact on the test results as the sample is stable under storage at room temperature for four hours and the sample has only rehydrated the lyophilized internal control organism (but not the lyophilized PCR reagents) prior to test initiation.

The following variations to the nominal condition of operation were tested:

- 0% slider actuation (slider left open)
- 50% slider actuation (slider left halfway open)

- 95% slider actuation (slider almost all the way closed)
- Sample loaded and slider actuation delayed for 30 minutes
- Sample loaded and slider actuation delayed for 1 hour 45 minutes

All conditions were tested with five negative replicates and five low positive (3x LoD of CT, NG, and TV) replicates.

The results are summarized in the table below.

Condition	# Correct Results / # Tested		# Erroneous Results / # Tested	# Invalid Results / # Tested
	Negative Sample	Low Positive Sample (3x LoD CT, NG, and TV)		
0% slider actuation (Slider left open)	0/5	0/5	0/10	10/10 Red X
50% slider actuation (Slider left halfway open)	0/5 [1]	0/5 [1]	0/10	10/10 Red X
95% slider actuation (Slider almost all the way closed)	5/5	5/5	0/5	0/10
30-minute delay of slider actuation after loading sample	5/5	5/5	0/5	0/10
1 hour 45-minute delay of slider actuation after loading sample	5/5	5/5	0/5	0/10

[1] One device was found to inadvertently have 100% slider actuation. The sample was retested at the correct test condition of 50% slider actuation and returned the expected Red X error result.

In this study, devices with inadequate slider actuation had expected Red X errors. When the slider was closed almost all the way (~95%), all devices returned expected results.
The device is robust to errors related to inadequate slider closure or delay in test initiation after loading sample. The device either provided the expected results or the existing engineering controls avoided generation of erroneous test results.

A separate flex test also evaluated the impact of removing the device from the foil wrapper prior to starting the test. The study was performed with five negative and five low positive replicates. The devices were removed from the foil wrapper and tests were started eight hours later. The results are shown below and suggest that this condition did not impact the device performance.

Condition	# Correct Results / # Tested		# Erroneous Results / # Tested	# Invalid Results / # Tested
	Negative Sample	Low Positive Sample (3x LoD CT, NG, and TV)		

Test started eight hours after device's removal from foil wrapper	5/5	5/5	0/5	0/10

3.1.2 Delayed Visual Interpretation of Results

Per the Visby Test IFU and QRG, users are instructed to interpret the test results within two hours post the completion of the test. The green check mark status light on the device is maintained for these two hours and afterward it turns off indicating that the time limit for reading the results has passed. Despite this built-in control, extended timelines to interpret the result were evaluated at six and 24 hours post completion of the test.

Eight different samples representing possible results (a triple negative, three single positives, three double positives, and a triple positive) were prepared and tested. The results were visually interpreted by three operators at different timepoints post completion of the test in a randomized order.

The results are summarized in the table below.

Input sample	**Device Result Visual Output**	**# Correct User Interpretations / # Tests Read**				**# of Incorrect User Interpretations / # Tests Read**
		Time after completion of the run				
		0 hours	**2 hours**	**6 hours**	**24 hours**	
Negative (no spiked organisms)	Negative for CT, NG, TV	3/3	3/3	3/3	3/3	0/96
Low Positive (3X LoD) CT	CT detected	3/3	3/3	3/3	3/3	
Low Positive (3X LoD) CT and NG	CT and NG detected	3/3	3/3	3/3	3/3	
Low Positive (3X LoD) CT and TV	CT and TV detected	3/3	3/3	3/3	3/3	
Low Positive (3X LoD) NG	NG detected	3/3	3/3	3/3	3/3	
Low Positive (3X LoD) NG and TV	NG and TV detected	3/3	3/3	3/3	3/3	
Low Positive (3X LoD) TV	TV detected	3/3	3/3	3/3	3/3	
Low Positive (3X LoD) CT, NG, and TV	CT, NG, and TV detected	3/3	3/3	3/3	3/3	

Based on data presented above, the results of the device remain visible and accurate for up to 24 hours. This study indicates that the device is robust to errors related to delayed reading of the test results.

3.1.3 Sample Loading Volume

Per the Visby Test IFU and QRG, users are instructed to use the fixed volume pipette (650 µL) to load the test sample onto the device. Failure to add the correct sample volume can impact the test result as the patient sample should effectively rehydrate the lyophilized reagents. For this flex study, specimen volumes were tested at a range from 300 µL to 1300 µL. The condition where no sample was added was also tested.

All conditions were tested with five negative and five low positive (3X LoD of CT, NG, and TV) replicates, with the specified volume loaded by a calibrated pipette. Note that conditions that were also used for internal design verification testing were tested with six replicates instead of five.

The results are summarized in the table below.

Condition	**# Correct Results / # Tested**		**# Erroneous Results / # Tested**	**# Invalid Results / # Tested**
	Negative Sample	**Low Positive Sample (3x LoD CT, NG, and TV)**		
No sample added and slider completely closed	0/5		0/5	5/5
300 µL sample input volume	3/5	3/5	0/10	4/10
400 µL sample input volume	5/5	5/5	0/10	0/10
500 µL sample input volume	5/5	5/5	0/10	0/10
600 µL sample input volume	5/6 [1]	5/6 [1]	0/12	2/12 Control Failure
700 µL sample input volume	6/6	6/6	0/12	0/12
1300 µL sample input volume	5/5	5/5	0/10	0/10
[1] One device returned an initial invalid result (Control Failure). Result was valid upon retesting the sample.				

This evaluation demonstrates that devices tested with input volumes of 400 µL to 1300 µL produced expected results. Devices tested with a volume of 300 µL produced expected results or invalid results. Failure to add sample to device resulted in control failure for all cases.

This study indicates that the device is robust to errors related to variation in sample loading volume and the existing controls manage to avoid generation of erroneous test results.

3.1.4. Extended Sample Storage (Delayed Testing)

Per the Visby Test IFU and QRG, samples can be stored for up to 4 hours at room temperature (15°C - 30°C), up to 4 hours under refrigeration (2°C - 8°C), or for up to 90 days when frozen (< -15°C). This flex study tested samples that were stored at room temperature (30°C) or refrigeration (4°C) for longer than the recommended time frame to assess the possibility of erroneous results under extended storage conditions. For each time point, five negative and five low positive (2x LoD of CT, NG, and TV) replicates were tested. Frozen samples were evaluated by testing five aliquots of both a negative and a low positive (3x LoD of CT, NG, and TV) sample prepared previously and stored frozen (< -15°C) for eight months prior to this test.

The results are summarized in the following table.

Storage Conditions	**Time Point**	**# Correct Results / # Tested**		**# Erroneous Results / # Tested**	**# Invalid Results / # Tested**
		Negative Sample	**Low Positive Sample (2x LoD of CT, NG, and TV, for frozen samples, 3xLoD)**		
< -15°C	8 months	5/5	5/5	0/10	0/10
N/A	Fresh (T_0)	5/5	5/5	0/10	0/10
4°C	24 – 30 hours	5/5	5/5	0/10	0/10
	72 – 78 hours	5/5	4/5 [1]	0/10	1/10 Control Failure
	7 – 10 days	5/5	5/5	0/10	0/10
30°C	24 – 30 hours	4/5 [2]	5/5	0/10	1/10 Red X
	72 – 78 hours	5/5	5/5	0/10	0/10
	7 – 10 days	5/5	4/5 [3]	1/10	0/10

[1] One device returned an initial invalid result (Control Failure). Correct result was returned upon retesting the sample.
[2] One device returned an initial invalid result (Red X Error). Correct result was returned upon retesting the sample.
[3] One out of the five devices loaded with a low positive sample had a false negative result for TV; the device had the expected positive results for CT and NG.

Devices tested with vaginal samples stored beyond the claimed specimen stability produced the expected results up to three days when kept at room temperature, up to seven days of refrigerated

storage, and up to eight months when stored frozen. Therefore, the data showed that the risk of erroneous results is low, even when the samples are stored slightly outside the recommended conditions. However, storing samples at room temperature significantly longer than four hours (i.e., more than three days) may lead to erroneous results. This is mitigated by stating in the QRG and IFU that samples should be stored at room temperature only up to four hours.

3.1.5 Incorrect Specimens

The Visby Test is intended for self-collected vaginal swab in clinical setting. This flex study evaluated the impact of incorrect specimen type on the test result. For this test, negative samples consisted of aliquoted water or undiluted pooled male urine. Positive samples were prepared by spiking water or the undiluted pooled male urine with 3x LoD of CT, NG, and TV. Each sample was tested in five replicates.

The results are summarized in the following table.

Sample	**# Correct Results / # Tested**		**# Erroneous Results / # Tested**	**# Invalid Results / # Tested**
	Negative Sample	**Positive Sample (3x LoD CT, NG, and TV)**		
Water	5/5	5/5	0/10	0/10
Male Urine	0/5	0/5	0/10	10/10 Control Failure

Undiluted male urine caused control failure invalids possibly due to inhibition of the PCR assay. The inhibition of the internal process control by the incorrect specimen type demonstrated adequate control as no erroneous results were observed.

3.1.6 Vaginal Swab Elution

The Visby Medical Vaginal Self Collection Kit Instructions direct the patient to place the head of the swab into the collection tube and tap the swab against the bottom of the tube for 15 seconds, then discard the swab. The specimen presumed to contain the eluted target(s) is then provided to the test operator. This study examined the effect of inadequate swab elution that may be caused by not following the sample handling instructions.

The study was performed by applying a mixture of CT, NG, and TV organisms onto a swab at the concentration that would create the 3x LoD sample in units/mL, assuming 100% elution of the organisms off the swab into the collection media. Five swabs were tested. The following elution methods were simulated:

- Control (tap for 15 seconds, discard swab)
- Tap 5 seconds, discard swab
- Tap 10 seconds, discard swab
- Place swab in media for 5 seconds (no tapping), discard swab
- Place swab in media for 10 seconds (no tapping), discard swab

All conditions were tested with 5 low positive (3x LoD of CT, NG, and TV) replicates. Negative samples were not tested.

The results are summarized in the following table.

Elution Condition	# Correct Results / # Tested	# Erroneous Results / # Tested	# Invalid Results / # Tested
	Low Positive Sample (3x LoD CT, NG, and TV)		
Control (tap for 15 seconds, discard swab)	5/5	0/5	0/5
Tap 5 seconds, discard swab	5/5	0/5	0/5
Tap 10 seconds, discard swab	5/5	0/5	0/5
Place swab in media for 5 seconds (no tapping), discard swab	5/5	0/5	0/5
Place swab in media for 10 seconds (no tapping), discard swab	5/5	0/5	0/5

This study demonstrates that the Visby Test returned the expected results with variations in elution techniques, indicating that the device is robust to errors related to swab elution into the collection media.

3.2 Environmental factors

3.2.1 Operational Temperature and Humidity

Per the Visby Test IFU and QRG, users are instructed to run the test between 13 to 33ºC and at 5-80% relative humidity. Performance of the Visby Test when operated at combination of extremes of temperature and humidity was evaluated.

For each condition, three negative and three positive replicates (CT, NG, and TV triple positive) were evaluated. Since the environmental testing vendors do not allow the use of potentially infectious materials, it was not possible to use samples that contained human matrix or live pathogen. Therefore, negative samples were composed of Visby Medical's sample collection media without the addition of clinical matrix. The positive samples were composed of inactivated target organisms at concentrations near 3xLoD.

The results are summarized in the following table.

Various Temperatures Combined with Low Relative Humidity (RH)				
Condition	**# Correct Results / # Tested**		**# Erroneous Results / # Tested**	**# Invalid Results / # Tested**
	Negative Sample	**Low Positive Sample (approximately 3x LoD CT, NG, and TV)**		
9°C, 5% RH	0/3	0/3	0/6	6/6 Red X
11°C, 5% RH	3/3	2/3 [1]	0/6	1/6 Control Failure
13°C, < 17% RH [2]	3/3	3/3	0/6	0/6
33°C, 5% RH	3/3	3/3	0/6	0/6
35°C, 5% RH	0/3	0/3	0/6	6/6 Red X
37°C, 5% RH	0/3	0/3	0/6	6/6 Red X

[1] One device returned an initial invalid result (Control Failure). Retesting the sample returned a correct result.
[2] The environmental chamber could not reach 5% RH at 13°C at the beginning. The device run started at 17% RH and ended at 5.9% RH approximately 30 minutes later.

Note: The accuracy of the environmental chamber used in this study is +/- 2°C.

Various Temperatures Combined with High Relative Humidity (RH)				
Condition	**# Correct Results / # Tested**		**# Erroneous Results / # Tested**	**# Invalid Results / # Tested**
	Negative Sample	**Low Positive Sample (approximately 3x LoD CT, NG, and TV)**		
9°C, 95% RH	0/3	0/3	0/6	6/6 Red X
11°C, 95% RH	3/3	3/3	0/6	0/6
13°C, 95% RH	3/3	3/3	0/6	0/6
33°C, 95% RH	3/3	3/3	0/6	0/6
35°C, 95% RH	0/3	0/3	0/6	6/6 Red X
37°C, 95% RH	0/3	0/3	0/6	6/6 Red X

Note: The accuracy of the environmental chamber used in this study is +/- 2°C.

The results of this flex testing indicate that the Visby Test will generate invalid results when the temperature is < 11°C (for instance, when the device is refrigerated or undergoes cold temperature) or > 33°C at both low (5% RH) and high (95% RH) humidity conditions. Within the operating condition boundaries of 13°C and 33°C, as well as at the low end of the range (11°C), the device will generate the expected results in both low (5% RH) and high (95% RH) humidity conditions.

The above studies demonstrate that the built-in temperature and humidity sensors prevent the device from operating outside of the temperature specifications and that the device will not generate erroneous results when exposed to extreme temperatures and humidity conditions.

3.2.2 Geographic Altitude

Per the Visby Test IFU and QRG, users are instructed to run the test between -300 to 9500 ft altitude. This flex study tested the performance of the device when operated outside of this range.

For this study, in order to use non-infectious material, negative samples were composed of Visby Medical's sample collection media without the addition of clinical matrix. The positive samples were composed of inactivated target organisms at concentrations near 3xLoD.
Five negative and five positive replicates were tested.

Altitude Condition **(Temperature Controlled at 23°C unless specified)**	**# Correct Results / # Tested**		**# Erroneous Results / # Tested**	**# Invalid Results / # Tested**
	Negative Sample	**Low Positive Sample (approximately 3x LoD CT, NG, and TV)**		
-102 m (-335 feet)	5/5	5/5	0/10	0/10
-34 m (-112 feet)	5/5	5/5	0/10	0/10
1905 m (6250 feet)	5/5	5/5	0/10	0/10
2984 m (9790 feet)	5/5	5/5	0/10	0/10

Results of this testing indicate that the risk of erroneous results is low when the device is operated at the altitude levels outside of the device specification.

3.2.3 Visibility of Indicator Signals (Lighting Conditions)

Per the Visby Test IFU and QRG, users are instructed to read results under nominal general office lighting conditions of 30-95 "foot candle" (fc) units. This study evaluated the impact of suboptimal lighting on the ability of the user to correctly interpret the test results. Lighting conditions were evaluated at low lighting conditions (< 15 and 20-30 fc) as well as high lighting conditions (95-125 fc):

- < 15 fc was achieved by moving the devices away from any windows and turning down all lights in the laboratory.
- 20-30 fc was achieved by moving the devices away from any windows and adjusting the light to approximately halfway by using the dimmer switch.
- 95-125 fc was achieved by holding a LED focus spotlight 15.5 inches directly above the bench where the devices were placed.

Eight different samples representing possible results (a triple negative, three single positives, three double positives, and a triple positive) were prepared and tested. Three operators interpreted the test results in a randomized order under each lighting condition.

Additionally, the impact of suboptimal lighting on the ability of the user to correctly interpret the LED status light signals was evaluated:

- 20-30 fc was achieved by moving the devices away from any windows and adjusting the light to approximately halfway by using the dimmer switch.
- 95-125 fc was achieved by holding a LED focus spotlight 15.5 inches directly above the bench where devices were placed.
- ≥ 130 fc was achieved by holding a LED focus spotlight 12.5 inches directly above the bench where devices were placed. This high lighting condition was tested to ensure that an operator correctly interprets the LED signals in extreme high lighting conditions without erroneously interpreting the LEDs.

Three different operators interpreted the LED signals (white, green and red) from four devices under each of the above lighting conditions.

The results are summarized in the following table.

Lighting Conditions – Test Results					
Input sample	**Device Result Visual Output**	**# Correct User Interpretations / # Tests Read**			**# of Incorrect User Interpretations / # Tests Read**
		Lighting Condition			
		< 15 fc	**20-30 fc**	**95-125 fc**	
Negative (no spiked organisms)	Negative for CT, NG, TV	3/3	3/3	3/3	0/72
Low Positive (3X LoD) CT	CT detected	3/3	3/3	3/3	
Low Positive (3X LoD) CT and NG	CT and NG detected	3/3	3/3	3/3	
Low Positive (3X LoD) CT and TV	CT and TV detected	3/3	3/3	3/3	
Low Positive (3X LoD) NG	NG detected	3/3	3/3	3/3	
Low Positive (3X LoD) NG and TV	NG and TV detected	3/3	3/3	3/3	
Low Positive (3X LoD) TV	TV detected	3/3	3/3	3/3	
Low Positive (3X LoD) CT, NG, and TV	CT, NG, and TV detected	3/3	3/3	3/3	

Lighting Conditions – LED Signals				
LED Signal	**# Correct User Interpretations / # Tests Read**			**# of Incorrect User Interpretations / # Tests Read**
	Lighting Condition			
	20-30 fc	**95-125 fc**	**≥ 130 fc**	
Power Icon	12/12	12/12	12/12	

Progress 1	12/12	12/12	12/12	0/216
Progress 2	12/12	12/12	12/12	
Progress 3	12/12	12/12	12/12	
Green Check	12/12	12/12	12/12	
Red X	12/12	12/12	12/12	

The results demonstrate that the test results and LED signals can be interpreted correctly under variable lighting conditions.

3.2.4 Device Positioning

Per the Visby Test IFU and QRG, users are instructed to run the device on a flat surface. The syringe module inside the device drives the movement of the sample fluid through the sample processing, amplification, and detection modules by pushing and pulling the fluid at specified rates that were determined based on a device positioned on a flat surface. If the test unit is positioned at an angle that is not flat, the intended flow rates may not be achieved and therefore this may impact the performance of the test. This flex study evaluated the performance of device under the following non-level positioning.

a. 10° tilt with the lights down

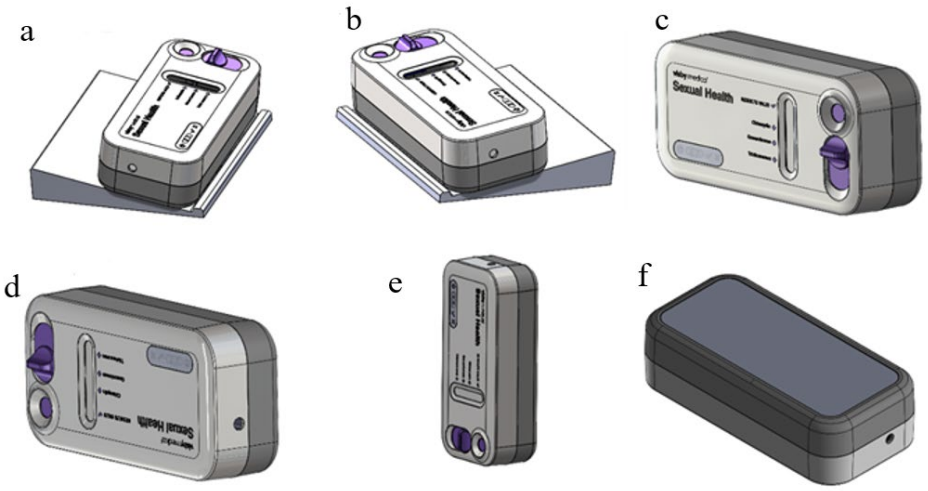
b. 10° tilt with the lights up

c. 90° rotation on the horizontal edge with lights down

d. 90° rotation on the horizontal edge with light up

e. 90° rotation on the vertical edge with plug up

f. Upside down



All conditions were tested with five negative and five low positive (3x LoD of CT, NG, and TV) replicates. Each sample was loaded, the slider was closed, and the device was positioned according to the following prior to being plugged in to initiate the test.

The results are summarized in the following table.

Condition	# Correct Results / # Tested		# Erroneous Results / # Tested	# Invalid Results / # Tested
	Negative Sample	Low Positive Sample (3x LoD CT, NG, and TV)		
a. 10º tilt with lights down	5/5	5/5	0/10	0/10
b. 10º tilt with lights up	5/5	5/5	0/10	0/10
c. 90º horizontal edge with lights down	0/5	0/5	0/10	10/10 Control Failure
d. 90º horizontal edge with lights up	4/5 [1]	1/5 [2,3]	4/10 [2,3]	1/10 [1] Control Failure
e. 90º vertical edge with plug up	0/5	0/5	0/10	10/10 Control Failure
f. Upside down	0/5	0/5	0/10	10/10 Control Failure

[1] One device returned an initial invalid result (Control Failure). Correct result was returned upon retesting the sample.

[2] One out of the five devices loaded with a low positive sample showed an erroneous result that was false negative for NG; the device had the expected positive results for CT and TV.

[3] Three out the five devices loaded with low positive sample showed erroneous results that were negative for all three targets.

This evaluation demonstrates that the Visby Test is robust to being operated when tilted to 10° in either direction (conditions a and b). When placed at 90° with the lights down (such that the writing on the device is readable, condition c), vertically at 90° (condition e), or upside down (condition f) devices returned an invalid result through a control failure where no spot developed next to the “RESULTS VALID” location on the detection window. These positions disrupted the fluid movement during the test such that the test could not be performed correctly; the internal control functioned properly to guard against erroneous results.

When placed at 90° with the lights up (such that the writing on the device is upside down, condition d), devices returned the expected results for six out of ten samples. Four low positive samples tested had false negative results. The tests were investigated and determined that the PCR amplification in the device performed as expected. The false negative results were due to the device positioning impacting the fluid flow of the detection reagents such that the enzyme-linked colorimetric assay did not perform as expected at the target locations. While this error

of placing a device 90° on its edge with the lights up is not an intuitive misuse case, the product labeling has been updated to include a warning that placing the device in this position can result in erroneous test results.

3.2.5 Device Agitation and Movement During Analysis

Per the Visby Test IFU and QRG, users are instructed to not disturb the device once testing begins and to not use devices that have been dropped. This study evaluated the performance of the device when disturbed during each phase of testing, including experiencing vibration throughout the test run, as well as when it experienced a drop before or during the test. All conditions were tested with five negative and five low positive (3x LoD of CT, NG, and TV) replicates.

The first part of this study evaluated if moving the device at designated time points during the automated process had an impact on device performance. For these test conditions, the device was inverted and held in that position for five to seven seconds at the designated time points, and then the device was returned to its proper orientation on the flat benchtop surface.

- Two minutes after test initiation: inversion during lysis
- Ten minutes after test initiation: inversion during PCR
- 22 minutes after test initiation: inversion during detection

The second part of this study evaluated the impact of vibration during the test. To imitate vibration of large machinery adjacent to the device, vortex mixers were turned on in a biosafety cabinet (BSC) while devices were running within an approximate 12-inch radius of the vortex mixers inside the BSC. The metal surface of a BSC translates vibrational forces from the vortex mixers into the devices more rigorously than a normal hard table surface would.

The last part of the study evaluated the impact of dropping the device as the following:

- Dropped from a height of one meter before loading the sample and initiating a run
- Dropped from a height of one meter during the run

The results are summarized in the following table.

Condition	**# Correct Results / # Tested**		**# Erroneous Results / # Tested**	**# Invalid Results / # Tested**
	Negative Sample	**Low Positive Sample (3x LoD CT, NG, and TV)**		
Inversion during lysis: two minutes after test started	4/5 [1]	5/5	0/10	1/10 Red X
Inversion during PCR: ten minutes after test started	5/5	5/5	0/10	0/10
Inversion during detection: 22 minutes after test started	4/5 [1]	5/5	0/10	1/10 Red X

Vibration during run	3/5 [2]	5/5	0/10	2/10 Red X
Dropped from one meter before run	5/5	5/5	0/10	0/10
Dropped from one meter during run	4/5 [3]	1/5[4]	0/10	5/10 (three Red X and one Control Failure)

[1]One device returned an invalid result (Red X Error) due to power interruption because the device became unplugged during the inversion.
[2] Two devices returned an invalid result (Red X Error) due to power interruption because the device became unplugged due to vibration).
[3] One device returned an invalid result (Red X Error) due to power interruption because the device became unplugged when it was dropped.
[4] Four devices returned an invalid result (three Red X Errors, one Control Failure).

This flex test demonstrates that the device enduring the above tested misuses will not result in erroneous test results. As part of the fail-safe and failure alert mechanisms, invalid result (Red X) will occur if the devices become unplugged due to these disruptions.

3.2.6 Functionality After Freezing

Per the Visby Test IFU and QRG, users are instructed to store the test kits between 36°F and 86°F (2°C and 30°C). In case of refrigeration or other exposure to cold temperatures, users are asked to ensure that the device is allowed to fully come back to room temperature prior to use. Users are also instructed to not freeze the device. This flex test evaluated the impact of freezing on the performance of the device.

The devices were stored at < -15°C for 24 hours and then allowed to come to room temperature for 24 hours. Following this pretreatment, five devices were tested with negative samples and another five devices were tested with low positive (3x LoD of CT, NG, and TV) samples.

The results are summarized in the following table.

Sample	**Correct Results / # Tested**	**# Erroneous Results / # Tested**	**# Invalid Results / # Tested**
Negative	5/5	0/5	0/5
Positive Sample (3x LoD CT, NG, and TV)	5/5	0/5	0/5

All tests returned expected results, demonstrating that one freeze-thaw cycle does not adversely affect the device performance.

3.2.7 Power Fluctuation

Per the Visby Test IFU and QRG, the device should be plugged in, loaded with sample, and the slider pushed upwards to completely close the sample port. Users are instructed to keep the system plugged in during the entire run. Because power interruptions will disrupt the automated testing process, the firmware has a built-in failure alert mechanism to produce a Red X error when a power interruption is detected. In this flex test, the power fluctuation was simulated by removal and re-insertion of the power cord approximately 5 minutes after the test was initiated.

This condition was only tested with five negative replicates as the expected result was Red X error. The results are summarized in the following table:

Sample	# Correct Results / # Tested	# Erroneous Results / # Tested	# Invalid Results / # Tested
Negative Sample	0/5	0/5	5/5 Red X

All five tests returned the Red X error after the power cord was re-inserted, indicating that this failure alert mechanism was properly implemented in the device.

L. Demonstrating "Insignificant Risk of an Erroneous Result" –Accuracy

1. Method Comparison between the Visby Test and the Click Test:

   To demonstrate that the performance of the Visby Test is comparable to that of the Click Test, the following three studies were conducted:

   - Testing performed at external CLIA waived sites. A total of 102 remnant vaginal swab specimens, a subset from the previous clinical study for Click Test, were tested with the Visby Test at external CLIA waived sites by untrained operators.
   - In-house testing with trained operators. A second clinical comparison study was performed to supplement the number of positive specimens tested, with a focus on testing a sample set representing a natural distribution of the pathogen loads in the intended use population, including those with low target levels. Therefore, for this study, all remnant specimens, with sufficient volume and patient consent, from the previous clinical study of the Click Test were included in this study. The above specimens were further supplemented with archived and banked frozen self-collected samples from two other previous studies conducted by Visby Medical. These specimens were tested in house by trained operators.
   - In-house study with contrived samples at near LoD target concentrations. Each individual sample was prepared in an individual negative clinical matrix, with the intention to mimic the patient specimen as much as possible.

1) Testing at external CLIA waived sites

Archived frozen vaginal swab patient samples that had been previously characterized in the clinical study for the Click Test were tested. The frozen samples were de-identified, randomized, and blinded to the study staff and test operators. The panel included 30 CT positive, 20 NG positive, 30 TV positive and 33 specimens that were negative for all three pathogens. The testing was performed with Visby Tests at three external study sites in the United States representing CLIA waived settings by a total of six untrained operators (two operators at each site). Operators thawed the samples and tested them on-site by following the instructions in the QRG. The performance of the Visby Test was evaluated by comparing the Visby Test results to the results from Click Test recorded during the previous clinical study. Of the 102 specimens (seven of these samples are double positive and two are triple positive) included in the study, two (2.4%) had an initial invalid test result. One sample was excluded from the performance evaluation because it was invalid upon retesting. There were no additional exclusions. Please refer to the following table for performance evaluation.

Visby Test vs. Click Test Performance Comparison (External CLIA Waived Sites, Testing Archived Specimens)

Target	N	TP	FP	TN	FN	PPA (95% CI)	NPA (95% CI)
CT	101	30	0	71	0	100.0% (88.6%-100.0%)	100% (94.9%-100.0%)
NG	101	20	0	81	0	100.0% (83.9%-100.0%)	100.0% (95.5%-100.0%)
TV	101	30	3	68	0	100.0% (88.6%-100.0%)	95.8% (88.3%-98.6%)

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

2) In-house testing with trained operators

All specimens meeting the inclusion criteria (see below) from the previous clinical study of Click Test were included in this study. To obtain a sufficient number of positive specimens (by the comparator assays) for each analyte, these specimens were further supplemented with archived and banked frozen self-collected samples meeting the same inclusion criteria from two other previous studies conducted by Visby Medical. Target negative patient specimens were also included in this study, and all positive and negative specimens were de-identified, randomized and blinded to the operators.

The inclusion criteria for this study:

- Self-collected vaginal swab in Visby Medical Collection Media from a female subject ≥14 years of age at the time of specimen collection.
- Age and symptomatic status of the subject at the time of specimen collection is available.
- Swab specimen was obtained from a collection wherein the subject consented/assented to future unspecified research use.

- Minimum sample volume of 1.0 mL.

A total of 359 de-identified archived frozen self-collected vaginal swab specimens were provided to the operators for testing. One specimen had less than 1 mL of volume and thus could not be tested. Of the remaining 358 specimens, seven were excluded from performance evaluation due to the following listed reasons.

- One specimen had invalid result for both the initial and retest on the Visby Test and thus was excluded.
- Five specimens yielded invalid results on the Visby Test and there was insufficient volume to run a retest, thus these specimens were excluded.
- One specimen had invalid result for both the initial and retest on the Click Test and thus was excluded.

Therefore, a total of 351 specimens were included in the performance evaluation.

Testing with Visby devices was conducted by seven trained operators internally at Visby Medical over seven days under variable lighting conditions throughout the day. When sufficient sample volume was present, specimens were tested on both the Click Test and the Visby Test to allow for performance comparison. Specimens without sufficient volume (27/351) to run on both devices were tested only on the Visby Test, and the original Click Test results from the previous Click Test clinical studies were used for the comparison. Please refer to the following table for performance estimates calculations.

Visby Test vs. Click Test Performance Comparison (Internal Testing of Archived Specimens)

Visby Test vs. Click Test							
Target	**N**	**TP**	**FP**	**TN**	**FN**	**PPA**	**NPA**
CT	351	116	3	232	0	100.0% (95% CI: 96.8% - 100.0%)	98.7% (95% CI: 96.3% - 99.6%)
NG	351	34	0	317	0	100.0% (95% CI: 89.9% - 100.0%)	100.0% (95% CI: 98.8% - 100.0%)
TV	351	100	4	240	7[a]	93.5% (95% CI: 87.1% - 96.8%)	98.4% (95% CI: 95.9% - 99.4%)

a. TV PIS (based on the original clinical study for Click Test) for all seven specimens were negative.

Note that 4 CT, 3 NG, and 6 TV positive specimens tested at the external sites in the first study had sufficient volume and were also tested in the internal study. Therefore, a total of 142 CT, 51 NG and 124 TV positive patient specimens (by the comparator assays) were tested in the above two Clinical Comparison studies.

The initial invalid rate for Visby Test is 3.1% (11/358) and the final invalid rate is 0.3% (1/353, five out the 11 initial invalids did not have sufficient volume for retest).

3) In-house testing with contrived samples

20 contrived samples with target concentrations at 1.5x LoD (N=6), 2x LoD (N=10) and 3x LoD (N=4) for each analyte were prepared in individual negative clinical matrices. An additional 20 negatives, prepared with pooled negative clinical matrix collected from consented subjects under IRB-approved protocols, were included in the study. All samples were randomized, blinded and tested on both the Click Test as well as the Visby Test in-house. All 80 contrived samples yielded valid results (no initial invalids) and were included in the final data analysis. The results of this study are presented in the table below.

Visby Test vs. Click Test Performance Comparison (Internal Testing of Contrived Samples)

Target	Concentration	Correct Results/Total Tested	
		Click Test	Visby Test
CT Serovar H (VR-879)	1.5x LoD	6/6	5/6
	2x LoD	10/10	9/10
	3x LoD	4/4	4/4
NG (ATCC 49226)	1.5x LoD	5/6	5/6
	2x LoD	9/10	10/10
	3x	4/4	4/4
TV (ATCC 30001)	1.5x	6/6	6/6
	2x	10/10	10/10
	3x	4/4	4/4
Negative	N/A	20/20	19/20[a]

a. One device was unexpectedly positive for TV.

2. Device Performance with Analyte Concentrations Near the Assay LoD

Device performance with analyte concentrations near the assay LoD was evaluated as part of the reproducibility study of the Visby Test. The study was conducted at three CLIA waived study sites by a total of six untrained operators (two operators at each site). Each operator tested the panel (see table below) three times each testing day, over six non-consecutive days. The panel

consisted of seven members prepared by spiking cultured organisms into negative pooled clinical vaginal swab matrix. Three reagent lots were used in this study.

Panel Members Tested for Reproducibility Study

Panel Member	Target Concentration
CT Moderate Positive	4xLoD
CT Low Positive	**1xLoD**
NG Moderate Positive	4xLoD
NG Low Positive	**1xLoD**
TV Moderate Positive	4xLoD
TV Low Positive	**1xLoD**
Negative	No target

A summary of percent agreement with expected results for each panel member by site and overall is presented in the following table. The results of this study are comparable to the results observed for the Click Test (refer to K200748). This study demonstrated that untrained users could perform the Visby Test accurately, including testing samples with organism concentrations near the assay LoD.

Panel Member	Site 1	Site 2	Site 3	Overall Agreement	
	% Agreement with Expected Results (No. Correct/ Total Tested)			% Agreement	95% CI
CT Moderate Positive	100.0% (36/36)	100.0% (36/36)	100.0% (36/36)	100.0% (108/108)	96.6%-100.0%
CT Low Positive	**97.2% (35/36)**	**100.0% (36/36) [a]**	**100.0% (36/36)**	**99.1% (107/108)**	**94.9%-99.8%**
NG Moderate Positive	100.0% (36/36)	100.0% (36/36)	97.2% (35/36)	99.1% (107/108)	94.9%-99.8%
NG Low Positive	**100.0% (36/36)**	**100.0% (36/36)**	**100.0% (36/36)**	**100.0% (108/108)**	**96.6%-100.0%**
TV Moderate Positive	100.0% (36/36)	100.0% (36/36)	100.0% (36/36)	100.0% (108/108)	96.6%-100.0%
TV Low Positive	**97.2% (35/36)**	**97.2% (35/36) [b]**	**94.4% (34/36)**	**96.3% (104/108)**	**90.9%-98.6%**
Negative	97.2% (35/36) [c]	100.0% (36/36)	97.2% (35/36) [c]	98.1% (106/108)	93.5%-99.5%

[a] One CT Low Positive sample was unexpectedly also positive for TV
[b] One TV Low Positive sample was unexpectedly also positive for CT
[c] One sample was unexpectedly positive for TV

3. Operator Questionnaire

A questionnaire was developed and administered to the participating operators at the end of the previously described clinical comparison study (first study listed above) between the Visby Test and the Click Test, conducted at external CLIA waived sites by untrained users, to assess the ease of use of the device as well as the user experience with the device during testing. The questionnaire was divided into two categories: (1) system set-up and operation,

and (2) results interpretation. Overall, based on the operators' responses, the system was easy to use, and the instructions were easy to follow. One operator commented that "a few tests had very faint positives, but overall this was a small number of devices".

For the "result interpretation" part, each of the six operators interpreted the results correctly for all seven result situations with 100% accuracy. According to the operator responses, the results for the Visby Test are easy to understand and interpret.

M. Labeling for Waived Devices

The labeling consists of:

1. Instructions for Use (IFU or Package Insert)
2. Quick Reference Guide (QRG)
3. Vaginal Specimen Collection Kit Package Insert
4. Vaginal Specimen Self-Collection Instructions

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 labeling 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.
- The QRG includes instructions for performing Quality Control testing.
- Technical support telephone number is prominently displayed.
- All appropriate cautions regarding sample handling and processing are present.
- The labeling includes the statement that a Certificate of Waiver is required to perform the test in a waived setting.
- The labeling is sufficient and satisfies the requirements of 21 CFR Part 809.10.

N. Conclusion:

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