CLIA Waiver by Application Approval Determination

Decision Summary

A. Document Number

CW200003

B. Parent Document Number

K200748

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 Click Test

F. Measurand (analyte)

Chlamydia trachomatis DNA,
Neisseria gonorrhoeae DNA, *and*
Trichomonas vaginalis 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

The Visby Medical Sexual Health Click Test is a single-use (disposable), compact device containing a PCR-based assay for qualitative detection and differentiation of DNA from *Chlamydia trachomatis* (CT), *Neisseria gonorrhoeae* (NG) and *Trichomonas vaginalis* (TV). The test system 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 and has no external

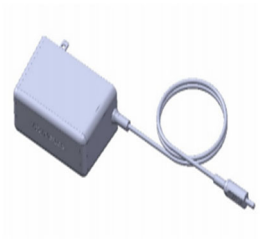
interfaces. The device further contains the fluidic and thermal elements required to perform these functions (syringe pump, rotary valve, controller printed circuit board (PCB) and firmware). A lyophilized control organism pellet containing *Neisseria subflava* (NS) is located underneath the sample port, 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 test device contains all of the hardware and reagents required to perform the test. Each kit contains 10 test devices, and 10 single use disposable fixed-volume transfer pipettes. 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

Upon receipt of the sample, the test operator uses the transfer pipette to load a fixed volume of the sample into the unit before sliding the sample port closed and depressing the three buttons in sequence (see below). The test is then connected to the external power adapter and a stable white light will appear, indicating the test is running.

A. Close Button 1 by sliding the cap to the right.

B. Push Button 1 all the way down

C. Push Button 2 all the way down to unlock button 3.

D. Push Button 3 all the way down to pierce the reagent seals. Use two thumbs, push firmly.
Note: All buttons are flat at this point, as shown.

E. Plug in the test. A stable white light will appear indicating the test is running. The test must remain plugged in through Step 3.

There are three LED lights (white circle, green check mark, and red X) on the top of the test device that are used to communicate the test status.

After approximately 27 minutes, a green LED check mark will appear to indicate the test had completed running.

4. Result Interpretation

The unit is designed to clearly indicate the colorimetric spot location for each of the pathogenic targets and the positive control, for a total of four possible spots (reaction zones). The following image shows a valid test (purple rectangle visible next to the Control), positive for NG and TV and negative for CT.

Although the hue and intensity of the color of the spot may vary, any colored spot should be considered positive, as long as the shape is filled with color and the spot has distinct edges.

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) in the same buffer as used in the Visby Medical Vaginal Specimen Collection kits. The positive controls are prepared from purified microorganisms (CT, NG, and TV) that are grown in microbial culture. Once purified, the microorganism is chemically and/or enzymatically 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, starting from the post-collection eluted swab. The labeling states that the 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 cartridge 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 reagents 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 cartridge.
- 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 three 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 instruction 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 Medical Sexual Health Click test was conducted in accordance with ISO 14971 to identify potential hazards and 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

The Visby Medical Sexual Health Click Test was designed with several fail-safe and failure alert mechanisms to prevent erroneous results.

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, Control failure invalidates the results.

Electronic Sensors

The device has multiple sensors which will stop the test from proceeding if a failure is detected after the power plug is connected, e.g.:

- Temperature sensor controls for out-of-range internal temperature,
- Electronic sensors control, among other, for:
  a. hardware failure, e.g., heaters or motors failure,
  b. lack or interruption of electrical power,
  c. software failure
- Liquid sensor in the mixing chamber must detect liquid when the device is plugged in

When an error occurs, the RED X error light appears on the top of the device.

Lockout Features

The device is designed such that it is not possible to reuse it once a sample is loaded.

1. Once the Buttons are depressed, they cannot be pried open
2. Once Button 1 is slid over and the sample port is closed, it is not possible to access the sample port.
3. 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 Zeptometrix and are recommended to be used with every new shipment and each new operator.

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 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:

a. Sample was transferred to the sample port with the transfer pipette (unless specified otherwise).
b. The sample port was closed.
c. The three buttons on the Visby device were depressed, in sequence, including a two-finger push for Button 3.
d. The device was plugged in to the power adapter.
e. The results were visually interpreted after the green check mark LED light appeared (after ~27 minutes).

Result interpretation is shown below.

Result	Indicator Light	Control 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
Test in Progress	White	N/A	N/A	N/A	N/A

Human Factors

Incorrect Order of Operations

The test procedure for the Visby test instructs the operator to use the device immediately after unpacking. Users are then instructed to load the sample and to push Buttons 1-3 in sequence without a delay, including a two-finger push for Button 3. After sample loading and pushing the buttons, the device is then plugged in to the power adapter.

After the sample was loaded, the following conditions were evaluated, using a low positive and negative sample, each tested in five replicates for each condition.

1. Plug in and perform the test as per instructions

a. All tests returned expected results; no erroneous or invalid results were returned.

2. 10 second delay in pushing Button 3
    a. All tests returned expected results; no erroneous or invalid results were returned.
3. One-minute delay in pushing Button 3
    a. All tests returned expected results; no erroneous or invalid results were returned.
4. Single finger push to the top of Button 3
    a. Invalid results were returned for all tests (Control failure) due to failure to puncture the reagent canisters under Button 3.
5. Single finger push to the middle of Button 3
    a. All tests returned expected results; no erroneous or invalid results were returned.
6. Single finger push to the bottom of Button 3
    a. Invalid results were returned for all tests (Control failure) due to failure to puncture the reagent canisters under Button 3.
7. Device unpacked and use delayed by 8 hours
    a. All tests returned expected results; no erroneous or invalid results were returned.
8. Incomplete push of Button 1
    a. Invalid results were returned for four out of 10 tests (three RED X errors and one Control failures). In 60% of cases, expected results were returned, while invalid results occurred in some cases when insufficient sample volume was introduced.
9. Not pushing Button 1 (pressing Buttons 2 and 3 only)
    a. The testing was limited to 3 devices as a RED X error was expected. Invalid results were returned for all tests (RED X error). The device includes a lock out mechanism to prevent pushing of Buttons 2 or 3 prior to Button 1, therefore, this situation is not expected to be a source of error in actual practice.
10. No sample loaded, Buttons 1-3 pressed and device plugged in
    a. The testing was limited to 3 devices as a RED X error was expected. Invalid results were returned for all tests (RED X error).
11. No detection reagent present in the device (test was modified, and reagents were removed from under Button 3). Nominal instructions followed.
    a. The testing was limited to 3 devices as Control failure was expected.
    b. Invalid results were returned for all tests (Control failure).

The results from the study are summarized below.

	Order of Operations			
Condition	**No. Correct Results/No. Tested**		**No. Erroneous Results/No. Tested**	**No. Invalid Results/No. Tested**
	Negative Sample	**Low Positive Sample (CT, NG, and TV)**		
Nominal (per QRI)	5/5	5/5	0/10	0/10
Plug in before loading and pressing buttons	5/5	5/5	0/10	0/10
10 second delay in Button 3 push	5/5	5/5	0/10	0/10
1 minute delay in Button 3 push	5/5	5/5	0/10	0/10
Single finger push of Button 3, top	0/5	0/5	0/10	10/10 (Control Failure)
Single finger push of Button 3, middle	5/5	5/5	0/10	0/10
Single finger push of Button 3, bottom	0/5	0/5	0/10	10/10 (Control Failure)
Use delayed 8 hours after unpacking	5/5	5/5	0/10	0/10
Inadequate Button 1 push	2/5	4/5	0/10	4/10 (Red X or Control Failure)
Not pushing Button 1	0/3	N/A	0/3	3/3 (Red X)
No sample loaded	0/3	N/A	0/3	3/3 (Red X)
No detection reagent	0/3	N/A	0/3	5/5 (Control Failure)

Additional testing was conducted to evaluate the risk of erroneous results when the device is plugged in before the sample is added, Button 1 is pressed, and pressing of Buttons 2 and 3 is delayed.

1. Plug in before loading and pressing the buttons in sequence without delay (Control condition).
    a. All tests returned expected results; no erroneous or invalid results were returned.
2. Plug in before loading, pressing Button 1 and waiting 10 minutes, then pressing Buttons 2 and 3 in sequence.
    a. All tests returned expected results; no erroneous or invalid results were returned.
3. Plug in before loading, pressing Button 1 and waiting 20 minutes, then pressing Buttons 2 and 3 in sequence.
    a. Four tests returned expected results and one test was invalid; no erroneous results were returned.

4. Plug in before loading, pressing Button 1 and waiting 25 minutes, then pressing Buttons 2 and 3 in sequence.
    a. One test returned expected results and four tests were invalid; no erroneous results were returned.
5. Plug in before loading, pressing Button 1 and not pressing Buttons 2 and 3 at all.
    a. This condition was evaluated with three replicates.
    b. All tests returned invalid results; no erroneous results were returned.

When the plug is inserted, all electronic sensors are activated. The test process begins when Button 1 is pressed, which initiates the release and flow of the reagents. The detection reagents are released after Buttons 2 and 3 are pressed. If the device is plugged in before there is liquid in the lysis chamber (which occurs after pushing Button 1), the electronic sensors will signal a failure and invalid results will be returned. Failure to push Buttons 2 and 3 will result in failure to release the detection reagents from the internal canisters and will therefore cause an invalid test as the detection reagents are required to form the purple color at the control spot. Plugging in the device after all the buttons are pressed mitigates the potential for invalid results in a situation where the user might get distracted after pressing Button 1 and does not press Buttons 2 and 3 in a timely manner.

The studies demonstrated that even if the user does not follow the instructions of plugging in the device after all buttons are pressed, there is no risk of erroneous results.

The summary of results from this study is shown below.

Condition	No. Expected Results/No. Tested	No. Invalid Results/No. Tested	No. Erroneous Results/No. Tested
No Delay (Control Condition)	5/5	0/5	0/5
Delay by 10 minutes	5/5	0/5	0/5
Delay by 20 minutes	4/5	1/5	0/5
Delay by 25 minutes	1/5	4/5	0/5
Buttons 2 and 3 Not Pressed	0/3	3/3	0/3

The results from the studies indicate that the device is robust to errors related to inadequate pushing of the buttons, delays in test initiation and plugging in the test prior to pushing the buttons; the existing controls effectively guard against erroneous test results.

a. Time of Reading Results

The test procedure for the Visby test instructs the operator to interpret the test results within 2 hours of the completion of the test, i.e., after the green light appears. To assist with instruction, the green check mark status light on the top of the device is maintained for 2 hours after the completion of the run. After the 2 hours, the green check mark turns off, indicating that the time limit for reading the results has passed. Despite this built-in control, the ability to interpret results correctly beyond that time was evaluated at 6 and 24 hours after completion of the test by having 3 different operators interpret the test results in a randomized order.

This study was conducted with 8 different unique samples (spiked with whole organisms into Visby Collection Media at ~3x LoD), each tested in one replicate. The samples were contrived to represent a permutation of the possible results: one negative, 3 unique single positive samples, 3 unique double positive samples, and one triple positive sample.

The results from this study are summarized below.

Time of Reading Results						
Input sample	**Device Result Visual Output**	**Correct User Interpretations/Total Reads**				**No. Incorrect User Interpretation/Total Readss**
		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 CT	CT detected	3/3	3/3	3/3	3/3	
Low Positive CT and NG	CT and NG detected	3/3	3/3	3/3	3/3	
Low Positive CT and TV	CT and TV detected	3/3	3/3	3/3	3/3	
Low Positive NG	NG detected	3/3	3/3	3/3	3/3	
Low Positive NG and TV	NG and TV detected	3/3	3/3	3/3	3/3	
Low Positive TV	TV detected	3/3	3/3	3/3	3/3	
Low Positive CT, NG, and TV	CT, NG, and TV detected	3/3	3/3	3/3	3/3	

All tests returned expected results. The study demonstrated that the results of the Visby Medical Sexual Health Click Test remain visible and accurate for up to 24 hours.

b. Specimen Volume

The test procedure instructs the operator to use the provided fixed volume pipette (650 µL) to load the test sample into the device. This study evaluated the potential for erroneous results when the operator does not follow the directions and either pours the sample into the port or uses another method of transfer. For this study, a range of sample volumes was evaluated by adding measured volumes of low positive and negative samples, each tested in at least five replicates.

Devices tested with volumes of 500 µL to 1300 µL produced expected results for all samples. One invalid result (control failure) was returned for the 400 µL sample. Invalid results are not unexpected with low sample volume due to failure to properly rehydrate the lyophilized reagents. No erroneous results were observed. The summary of the results is shown below.

Sample	Sample Volume Variability						
	400 µL	500 µL	600 µL	700 µL	1300 µL	No. of Erroneous Results/No. Tested	No. of Invalid Results/No. Tested
	No. Correct Results/No. Tested						
Negative	5/5	5/5	6/6	6/6	5/5	0/27	0/27
Low Positive (CT, NG, TV)	4/5	5/5	6/6	6/6	5/5	0/27	1/27 (Control failure)

This study indicates that the device is robust to errors related to variation in sample volume added to the Visby test; the existing controls effectively guard against erroneous test results.

c. Specimen Handling (delayed testing)

The Visby test is intended to be used with samples collected shortly before testing, so as to render the results while the patient is waiting. However, in some situations the testing could be delayed due to workload factors. Although the sample stability was demonstrated up to 4 hours at room temperature or in refrigerator, and up to 90 days when frozen, this study evaluated the risk of erroneous results when the samples were stored beyond the claimed stability.

Frozen storage (< -15ºC) was evaluated with 20 low positive samples (i.e., 2x LoD) and 5 moderately positive samples (i.e., 10x LoD). The samples were prepared in pooled clinical vaginal sample matrix.

Storage at 4ºC, and 30ºC was evaluated with a low positive sample and a negative sample, each tested in 5 replicates at several timepoints beyond the claimed stability intervals. The table below summarizes the results of the study.

Delayed Testing/ Specimen Stability			
Storage Conditions	**Time-point**	**Positive Sample Tests No. Correct/No. Tested**	**Negative Sample Tests No. Correct/No. Tested**
< -15°C	180 – 186 days	20/20	N/A
4°C	24 – 30 hours	5/5	5/5
	72 – 78 hours	5/5	5/5
	7 – 10 days	5/5	5/5
30°C	24 – 30 hours	5/5	5/5
	72 – 78 hours	5/5	5/5
	7 – 10 days	4/5	5/5

Devices tested with vaginal samples stored beyond the claimed specimen stability produced the expected results up to 3 days when kept at room temperature, up to 7 days of refrigerated storage, and up to 6 months (180 days) when stored frozen. There were no erroneous results returned indicating that the Visby device can accurately detect the target organisms in samples stored beyond the recommended length of time.

d. Incorrect Specimen

For this study, the low positive samples were contrived in (a) water, and (b) in male urine. Also, un-spiked water and male urine samples were tested as Negative controls. All samples were tested in 5 replicates; all tests returned correct results. The summary of the results is shown below.

	Testing Incorrect Sample Types			
Sample	**Correct Results**		**No. of Erroneous Results/No. Tested**	**No. of Invalid Results/No. Tested**
	Negative Sample	**Positive Sample**		
Water	5/5	5/5	0/10	0/10
Urine (male)	5/5	5/5	0/10	0/10

This study demonstrates that the Visby device can tolerate alternate samples. However, the Instructions for Use clearly indicate that the test should be used with female vaginal swabs only.

e. Swab Elution (this is performed by the patient)

The swab elution is performed by the patient at the time of specimen collection. The collection 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 provided to the test operator is the liquid in the collection tube, presumed to contain the eluted targets. This study examined the effect of inadequate swab elution that may be caused by not following the collection instructions, i.e., not "tapping" of the swab for 15 seconds as directed. 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:

1. Control (tap for 15 seconds, discard swab)
2. Place swab in media for 15 seconds with no tapping, then discard the swab
3. Place swab in media and tap for 5 seconds, then discard the swab
4. Place swab in media and tap for 10 seconds, then discard the swab
5. Place swab in media for 5 seconds with no tapping, then discard the swab
6. Place swab in media for 10 seconds with no tapping, then discard the swab

All conditions under evaluation returned expected results; no erroneous results were observed. The summary of results is shown in the table below.

Condition	Swab Elution		
	No. Correct/No. Tested	No. Erroneous Results/No. Tested	No. Invalid Results/No. Tested
Control (tap for 15 seconds)	10/10	0/10	0/10
Tap for 5 seconds	5/5	0/5	0/5
Immerse for 5 seconds, no tapping	5/5	0/5	0/5
Immerse and Tap for 10 seconds	5/5	0/5	0/5
Immerse for 10 seconds, no tapping	5/5	0/5	0/5
Immerse for 15 seconds, no tapping	5/5	0/5	0/5

The results showed that the elution of the target takes place even if the tapping procedure is not performed as directed, as long as the swab is placed in the collection medium. Based on the results of the clinical study, the elution of the targets did not appear to be a problem, as the assay performed with high sensitivity.

f. Movement during analysis

The test procedure instructs the operator to run the device on a flat surface and not to disturb it once testing begins. This study evaluated the impact of disturbance of the unit during the different phases of the analytical process as the sample is subjected to lysis, target amplification and detection. To that end, the device was inverted and then returned to its normal position after 1 minute of test initiation (lysis), after 10 minutes of test initiation (amplification), after 21 minutes of test initiation (detection) and inverting the device after the test initiation and leaving it upside down for the duration of the test. The study included a low positive sample and a negative sample, each run in five replicates for each condition. There were 11 invalid results, and no erroneous results were returned. The summary of results is shown below.

Sample	Movement during Analysis					
	Inversion after 1 min (during lysis)	Inversion after 10 min (during PCR)	Inversion after 21 min (during detection)	Upside down entire run	No. Erroneous Results/No. Tested	No. Invalid Results/No. Tested
	No. Correct Results/No. Tested					
Negative Sample	5/5	5/5	5/5	**0/5**	0/20	**5/20**
Low Positive Sample (CT, NG,TV)	5/5	4/5	5/5*	**0/5**	0/20	6/20

The study demonstrated that the device is not vulnerable to movement during analysis and that the device design prevents erroneous results should the device were disturbed after the test has been initiated. Additionally, the study showed that placing the device upside down will generate invalid results, but no erroneous results, indicating that the built-in mitigations effectively guard against erroneous results.

g. Dropping of the Device

The test procedure instructs the operator not to use devices that have been dropped. The impact of a drop on the test unit prior to use was evaluated to understand its performance when it experienced a drop from a one-meter height onto a hard surface (a) prior to loading the sample and initiating the run, and (b) during the run. The study was conducted with a low positive sample and a negative sample, each tested in five replicates for each condition. Of the 20 tests performed, one invalid result was returned; no erroneous results were generated. All other tests returned expected results.

	Dropping of the Device while Handling			
Sample	**Dropped Before Run Initiation**	**Dropped After Run Initiation**	**No. Erroneous Results/No. Tested**	**No. Invalid Results/No. Tested**
	No. Correct Results/No. Tested			
Negative Sample	5/5	4/5	0/10	1/10
Low Positive Sample (CT, NG,TV)	5/5	5/5	0/10	0/10

This study demonstrates that the device is not sensitive to being dropped and the existing controls will prevent generation of erroneous test results if the user drops the device either before or during the run.

h. Device Storage

The package insert for the Visby device instructs the user to store the kits between 18°C and 30°C and not to freeze the devices. This study evaluated the impact of freezing on the performance of the device after an inadvertent storage in a freezer. The devices were frozen at <-15°C for 24 hours and then allowed to come to room temperature prior to running samples. Five negative and five low positive samples (3x LoD of CT, NG, and TV) were tested (a total of 10 devices). All tests returned expected results, demonstrating that one freeze-thaw cycle does not adversely affect the device performance.

	Freezing/thawing the Device Prior to Use		
Sample	**No. Correct Results/ No. Tested**	**No. Erroneous / No. Tested**	**No. Invalid/ No. Tested**
Negative	5/5	0/5	0/5
Low Positive Sample (CT, NG, and TV)	5/5	0/5	0/5

Environmental Conditions

i. Operational Temperature and Humidity

The Visby test has operational specifications clearly stated in the labeling, namely that the testing should be performed at temperatures ranging between 18ºC and 30ºC with relative humidity (RH) between 30% and 80%. This study evaluated the device performance when operated outside of the specified boundaries. Because the device is engineered with internal sensors, this study demonstrated that the device would generate invalid results and the RED X error light will appear when exposed to temperatures outside of the specifications. A range of temperatures, including 10°C and 35°C (representative of extremes of temperature), was evaluated both at 20% RH and 95% RH. The study was conducted by running a negative sample in 3 replicates at each condition under evaluation (no positive samples were tested because the device was expected to generate an error regardless of the nature of the sample). All tests returned expected invalid results with the RED X light appearing. The study results are summarized below.

Temp	**Extremes of Relative Humidity**			
	Relative Humidity		**No. Erroneous Results**	**No. Invalid Results**
	95%	**20%**		
10°C	Red X (3/3)	Red X (3/3)	0/6	6/6
13°C	Red X (3/3)	Red X (3/3)	0/6	6/6
15°C	Red X (3/3)	Red X (3/3)	0/6	6/6
30°C	Red X (3/3)	Red X (3/3)	0/6	6/6
32°C	Red X (3/3)	Red X (3/3)	0/6	6/6
35°C	Red X (3/3)	Red X (3/3)	0/6	6/6

Additional testing was conducted to demonstrate that the built-in temperature sensor will prevent the device from proceeding when the temperature is just beyond the boundaries of specifications with RH within specifications. This study was conducted with low positive and negative samples, each run in at least five replicates. The results demonstrated that invalid results will be generated, and the RED X error light will appear, when the operational temperature is outside of the specifications. The study results are summarized below.

Sample	Temp	Extremes of Operational Temperature and Humidity			
		Relative Humidity		No. Erroneous Results No. Tested	No. Invalid Results No. Tested
		30%	80%		
Negative	16°C	Red X (5/5)	Red X (5/5)	0/10	10/10
	17°C	Negative (6/6)	Negative (6/6)	0/12	0/12
	29°C	Negative (6/6)	Negative (6/6)	0/12	0/12
	30°C	Red X (5/5)	Red X (5/5)	0/10	10/10
Positive Control Sample	16°C	Red X (5/5)	Red X (5/5)	0/10	10/10
	17°C	Positive (6/6)	Positive (6/6)	0/12	0/12
	29°C	Positive (6/6)	Positive (6/6)	0/12	0/12
	30°C	Red X (5/5)	Red X (5/5)	0/10	10/10

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

The above studies demonstrated 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 a range of humidity conditions.

j. Geographic Altitude (atmospheric pressure)

The labeling for the Visby device states that the testing should be performed at altitudes between 98 feet below and 5400 feet above the sea level. This study evaluated the Visby device performance when operated at high and low altitudes under normal (room) ambient temperature and also under environmental temperature at the upper boundary of the specification. The study was conducted with negative and positive samples, each tested in at least five replicates. No erroneous results were returned at any of the conditions tested. A total of 3 invalid results were obtained.

Sample	Temp		High Altitude			
		Elevation			No. Erroneous Results No. Tested	No. Invalid Results No. Tested
		2000 meters (> 6000*) ft	1800 meters (5906 ft)	-80 m (-292 ft)		
Negative	23°C	Negative (3/5)	Negative (5/5)	ND	0/10	2/10 (Control failure)
	29°C	Negative (5/6)	Negative (6/6)	Negative (6/6)	0/12	1/12 (Control failure)
Positive Control Sample	23°C	Positive (5/5)	Positive (5/5)	ND	0/10	0/10
	29°C	Positive (6/6)	Positive (6/6)	Positive (6/6)	0/12	0/12

ND= Not Done
*Testing at 23°C was done at 6562 ft while testing at 29°C was done at 6070 ft.

The study demonstrates that the device design prevents generation of erroneous results even when operating at an altitude outside of the specifications.

k. Ambient Lighting

Because the test results are visually read by the operator, good lighting is important. This study evaluated the impact of suboptimal lighting on the ability of the user to correctly interpret the test results. The varied lighting conditions were defined in "foot candles" (fc) units and achieved by adjusting all lights in a brightly lit room (>130 fc). A low (dim) lighting condition was created by turning down all lights below 25 fc. The table below approximates the ambient light conditions relative to the foot candle units.

Conditions	Level of Illumination	Foot Candles
Dim (Storage Room)	All lights in the room were turned down	<25 fc
General (Office)	The lights were adjusted to approximately halfway on.	25-30 fc
Bright (Laboratory)	All blinds were opened up to allow natural sunlight in the middle of the day.	95-100 fc
Outdoor (Overcast)	Additional light was used to illuminate the top surface of the device	≥130 fc

This study was conducted with eight different unique samples (spiked with whole organisms into Visby Collection Media at ~ 3x LoD), to create different permutations of possible test results. Each sample was tested in one replicate (the same samples were used in the Time of Reading the Results study described above). The test device were then given to three operators, in a blinded and randomized manner, for interpretation under three lighting conditions corresponding to (a) dim, (b) general (office-like), and (c) bright (laboratory-like) light environments. All tests were interpreted correctly by each of the three operators under the range of lighting conditions.

The results from this study are summarized below.

Lighting Conditions – Interpretation of Test Results					
Sample	Device Result Visual Output	No. Correct/No. Total Lighting Condition			No. of Erroneous User Interpretations/ No. Total
		< 25 fc	25-30 fc	95-100 fc	
Negative (no spiked organisms)	Negative for CT, NG, TV	3/3	3/3	3/3	0/72
Low Positive CT	CT detected	3/3	3/3	3/3	

Low Positive CT and NG	CT and NG detected	3/3	3/3	3/3	
Low Positive CT and TV	CT and TV detected	3/3	3/3	3/3	
Low Positive NG	NG detected	3/3	3/3	3/3	
Low Positive NG and TV	NG and TV detected	3/3	3/3	3/3	
Low Positive TV	TV detected	3/3	3/3	3/3	
Low Positive CT, NG, and TV	CT, NG, and TV detected	3/3	3/3	3/3	

The results demonstrate that the signal generated by the device is discernable under a range of lighting conditions and allows for accurate interpretation by the operator.

Additional testing was conducted to assess the ability of the operator to visualize and interpret the LED status light signals beyond the nominal lighting conditions of 30-95 fc. In this study, four different operators interpreted the LED signals (white, green and red) from three units (for a total of 12 reads per signal) under each of the following lighting conditions: (a) general (office-like), (b) bright (laboratory-like), and (c) outdoor (overcast). All users were able to correctly discern the LED lights.

The summary of results from this study is shown below.

Lighting Conditions – LED Signals				
LED Signal	**No. Correct/No. Total**			**No. of Erroneous User Interpretations/No. Total**
	Lighting Condition			
	25-30 fc	**95-100 fc**	**≥ 130 fc**	
White	12/12	12/12	12/12	0/108
Green	12/12	12/12	12/12	
Red	12/12	12/12	12/12	

The results demonstrated that the LED signals are clearly visible to different individuals even under variable lighting conditions.

l. Device Positioning

The test procedure instructs the user to place the Visby device on a flat surface. This study evaluated the impact of placing the device at an angle and performing the test. As the device is small enough to be held in a hand, positioning of the device at a 90° angle while the test is running was included in the evaluation. The following angles were tested:

- 10º tilt (face down)
- 10º tilt (face tilting up)
- 90º rotation on the horizontal edge with the buttons down
- 90º rotation on the horizontal edge with buttons up
- 90º rotation on the vertical edge with plug up

The testing was conducted with low positive and negative samples, each tested in five replicates for each condition. No erroneous results were returned at any of the conditions tested. A total of 20 invalid results (Control failure) were obtained.

Sample	Non-level Positioning						
	10° tilt, Face Down	10° tilt, Face Up	90° tilt Buttons Down	90° tilt Buttons Up	90° tilt (Vertical, Plug Up)	No. Erroneous Results/No. Tested	No. Invalid Results/No. Tested
	No. Correct Results/No. Tested						
Negative	5/5	5/5	0/5	5/5	0/5	0/25	10/25 (Control Failure)
Low Positive (CT, NG, TV)	5/5	5/5	0/5	5/5	0/5	0/25	10/25 (Control Failure)

The study results indicate that the device design prevents generation of erroneous results even when the device is operated in a tilted position.

m. Vibrations

The effect of vibrations on the integrity of the device was evaluated by subjecting the devices to a series of vibrations (low, medium, and high-level vibrations) prior to testing. The devices were positioned in three different orientations and the vibrations were maintained as shown below.

Positioning of Device	Low Level Vibrations 3.92 m/s^2 rms	Medium Level Vibrations 5.30 m/s^2 rms	High Level Vibrations 6.87 m/s^2 rms
	Duration of Exposure		
Time with base down orientation (mm:ss)	13:20	5:00	1:40
Time with side down orientation (mm:ss)	13:20	5:00	1:40
Time with end down orientation (mm:ss)	13:20	5:00	1:40

Following the exposure to vibrations, the devices were used to test six negative samples, and six low positive samples (two samples per analyte), for a total of 12 tests. All devices returned valid and expected results, suggesting that the devices maintain integrity when exposed to environmental vibrations.

n. Power Fluctuations

The test procedure instructs the user to transfer the sample into the sample port, press the

three buttons in sequence and then the device should be plugged in. The directions further state to keep the device plugged in during the entire time the test is running. In this flex study, the impact of power fluctuation on the Visby device was simulated by removal and re-insertion of the power cord during the analysis. The testing was conducted with 12 negative specimens by transferring the collection media into the sample port for processing.

All 12 tests returned the expected invalid results where the Red X error light came on after the power cord was re-inserted. No erroneous results were returned.

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

1. Comparison Study

a. Study Design

a. Study Sites and Duration

The study was conducted at 14 clinical sites representative of CLIA waived testing facilities over a period of 10 months, from February 2019 to December 2019. The sites were geographically distributed across the United States and included an OB/GYN physician's office, Sexual Health clinics, Primary Care clinics, a Public Health Clinic, a university Student Health clinic, an HIV/AIDS clinic, and STD clinics. The observed prevalence of each pathogen at each clinical site and overall is shown below.

	Prevalence (determined by the Comparator Results)		
Site	**CT**	**NG**	**TV**
1	1.6% (3/185)	0.5% (1/184)	0.0% (0/184)
2	3.9% (9/233)	1.3% (3/236)	18.9% (42/222)
3	1.5% (1/66)	1.5% (1/67)	4.6% (3/65)
4	27.2% (72/265)	9.3% (25/269)	9.8% (26/266)
5	6.6% (4/61)	4.9% (3/61)	13.3% (8/60)
6	8.6% (23/268)	0.7% (2/269)	0.7% (2/269)
7	3.7% (12/326)	0.9% (3/330)	11.2% (37/330)
8	0.0% (0/15)	6.7% (1/15)	13.3% (2/15)
9	6.8% (4/59)	1.7% (1/59)	6.8% (4/59)
10	0.0% (0/51)	0.0% (0/51)	5.9% (3/51)
11	6.4% (5/78)	2.6% (2/78)	3.9% (3/77)
12	10.5% (6/57)	1.8% (1/57)	1.8% (1/57)
13	11.1% (7/63)	1.6% (1/63)	4.8% (3/63)
14	14.9% (7/47)	2.1% (1/47)	6.4% (3/47)
Total	8.6% (153/1774)	2.5% (45/1786)	7.8% (137/1765)

b. Subjects (Patients)

The study subjects were prospectively enrolled females, 14 years of age and older, who self-collected vaginal swab specimens using the Visby Vaginal Collection Kit. The average age among study participants was 34 years, with a range between 14 to 80 years of age.

A total of 1899 subjects were initially enrolled, of which 1881 met the study inclusion criteria. Of those, 1789 females (929 symptomatic and 860 asymptomatic) were included in the performance evaluation.

c. Operators

A total of 32 untrained operators, representative of CLIA waived users, participated in the study. The education level of the operators included high school diploma, university education, GED or equivalent, and a graduate degree and included medical assistants, nurses and administrative staff performing various tasks relating to patient care or patient enrollment. The operators had no prior training or experience with CLIA high or moderate complexity laboratory testing and did not receive any training on the use of the Visby test.

d. Samples

Each female self-collected one vaginal swab using the Visby Sexual Health Vaginal Specimen Collection Kit. Samples were then handed over to the participating study operators who tested them on-site using the Visby Medical Sexual Health Test. The testing was performed by following the instructions in the Quick Reference Guide (QRG); no other materials or instructions were provided to the operators.

Three additional vaginal swabs were collected from each female by a licensed clinician and were sent to one central laboratory for comparator testing with three FDA cleared nucleic acid amplification tests (NAATs) detecting CT, NG and TV.

e. Comparator Method (CM)

The results of the Visby Test were compared to a composite comparator result comprised of results of three FDA-cleared nucleic acid amplification tests (NAATs), testing clinician collected vaginal swabs.

A positive composite comparator result (CCR) for CT and NG was defined as at least two positive results from the three comparator assays. The performance estimates for CT and NG are shown as percent agreement with the comparator result.

For TV, vaginal swabs are the optimal specimens in determination of infection by this pathogen, therefore, testing vaginal swabs with three FDA-cleared assays for

TV defines patient infected status (PIS). For this reason, the performance estimates for TV are shown as sensitivity and specificity.

b. Results

Of the 1789 subjects tested, 28 samples were excluded from the data analysis due to lack of a valid Visby test result. Additional, 64 subjects' results were excluded due to protocol deviations, such as improper execution of the Visby test or mishandling of the specimens (e.g., delayed testing). Samples were also excluded from the data analysis due to lack of a valid comparator test result (CT=15, NG=3 and TV=24). There were a total of 119 invalid results obtained on initial testing with the Visby test, for an overall invalid rate of 6.55% (119/1817), with 95% CI (5.5%-7.8%).

The clinical performance of the Visby Test with vaginal 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.

Clinical Performance of the Visby Test for CT vs. CCR, by Symptom Status

Symptom Status	N	TP	FP	TN	FN	PPA (95% CI)	NPA (95% CI)
Symptomatic	918	95	26	795	2	97.9% (92.8%-99.4%)	96.8% (95.4%-97.8%)
Asymptomatic	856	54	10	790	2	96.4% (87.9%-99.0%)	98.8% (97.7%-99.3%)
Overall	1774	149	36	1585	4	97.4% (93.5%-99.0%)	97.8% (96.9%-98.4%)

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

Clinical Performance of the Visby Test for NG vs. CCR, by Symptom Status

Symptom Status	N	TP	FP	TN	FN	PPA (95% CI)	NPA (95% CI)
Symptomatic	929	25	8	896	0	100% (86.7%-100%)	99.1% (98.3%-99.6%)
Asymptomatic	857	19	8	829	1	95.0% (76.4%-99.1%)	99.0% (98.1%-99.5%)
Overall	1786	44	16	1725	1	97.8% (88.4%-99.6%)	99.1% (98.5%-99.4%)

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

Clinical Performance of the Visby Test for TV vs. PIS, by Symptom Status

Symptom Status	N	TP	FP	TN	FN	Sensitivity (95% CI)	Specificity (95% CI)
Symptomatic	916	83	35	797	1	98.8% (93.6%-99.8%)	95.8% (94.2%-97.0%)
Asymptomatic	849	53	18	778	0	100% (93.2%-100%)	97.7% (96.5%-98.6%)
Overall	1765	136	53	1575	1	99.3% (96.0%-99.9%)	96.7% (95.8%-97.5%)

TP=true positive; FP=false positive; TN=true negative; FN=false negative

2. Device Performance with Analyte Concentrations Near the Assay LoD

The performance of the Visby 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 vaginal swab matrix (previously determined to be negative for CT, NG, TV), by spiking with quantified organism stocks to target concentrations of 1x LoD, for each organism. 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 of four samples twice a day over five days.

The results from the study are shown in the table below.

Summary of Results Testing Samples Near Assay LoD

	Site 1	Site 2	Site 3	Overall Agreement	
Panel Member	% Agreement with Expected Results (No. Correct/ No. Tested)			% Agreement	95% CI
CT Low Positive (16.0 EB/mL)	100% (20/20)	100% (20/20)	100% (20/20)	100% (60/60)	94.0%-100%
NG Low Positive (6.2 CFU/mL)	95.0% (19/20)	95.0% (19/20)	100% (20/20)	96.7% (58/60)	88.6%-99.1%
TV Low Positive (1.2 Troph/mL)	100% (20/20)	95.0% (19/20)	95.0% (19/20)	96.7% (58/60)	88.6%-99.1%
Negative	100% (18/18)*	100% (20/20)	100% (20/20)	100% (58/58)	93.8%-100%

*Two samples had invalid results and were omitted from the analysis.

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

3. External Control Lot-to-lot Testing

The reproducibility of three lots of the Zeptometrix External Controls was evaluated, testing 5 replicates of the Negative Control and five replicates of the Positive Control of each of the three lots.

Control	Lot Name	No. Expected Results/ No. Tested
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 three invalid results generated and the test was repeated in each case, returning a valid result.

4. Operator Questionnaire

A questionnaire was developed and administered to the participating operators at the end of the study 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. Three operators commented that Button 3 was difficult to depress, leading to adding emphasis to the step in the procedure which requires two thumbs (both hands) to push the button down effectively all the way down. In the second part, one operator misinterpreted four out of seven images, one operator misinterpreted an invalid result, failing to observe lack of the Control line; one operator misinterpreted a valid result, citing failure of the internal Control, and two operators correctly recognized an invalid result but cited the wrong reason for the failure, i.e., mistaking the "device error" (Red X) for the "Control failure", (or vice versa). The observation led to adding emphasis to the Result section of the test procedure that, when confirming the validity of the test, the user should observe both the green check mark and the presence of the purple rectangular spot next to the Control.

M. Labeling for Waived Devices

The labeling consists of:

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

2. The following elements are appropriately present:

- The QRI 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 QRI 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. Benefit-Risk Considerations

There has been a significant increase in sexually transmitted infections in recent years, raising a global public health concern. As many patients remain asymptomatic and the infections, if left untreated, can lead to severe reproductive health complications, such as infertility, ectopic pregnancy and congenital infection, as well as an increased risk of acquiring and transmitting HIV, prompt and appropriate treatment is essential in combatting the spread of these STIs. Currently, there is only one CLIA waived test for *C. trachomatis* and *N. gonorrhoeae* resulting in limited access to testing for sexually transmitted infections in POC settings. The majority of testing for these diseases is still predominantly performed in high or moderate complexity laboratories, which necessitates sending off specimens and thus delaying the results for at least several days, leading to many patients not returning ("lost to follow up") to obtain treatment. The Visby Sexual Health Test, designed to detect the most common of these pathogens in women, i.e., *C. trachomatis, N. gonorrhoeae* and *T. vaginalis,* has been shown to be easy to use by non-laboratory personnel and to have comparable clinical performance to the laboratory-based nucleic acid amplification tests. The test provides an actionable result in less than 30 minutes, allowing the clinician to start treatment with appropriate antibiotics immediately, during a single patient encounter.

FDA believes that bringing POC devices for the detection of the common sexually transmitted pathogens into the CLIA waived healthcare settings presents a significant step towards slowing the infection rates for these pathogens. The broadened access to testing afforded by the Visby test to non-traditional healthcare settings, such as doctor's offices, community-based clinics, planned-parenthood clinics, health department clinics and other free-standing counseling testing sites operating under a CLIA Certificate of Waiver, will increase the number of patients

screened and treated for these infections, lowering the numbers of untreated cases of STIs and reducing the burden of serious sequalae. Additionally, providing testing in CLIA waived settings will lower the rates of transmission of these infections and decrease the use of empiric antibiotics, which contribute to growing antimicrobial resistance among isolates of *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, and other bacteria.

For those reasons, FDA concluded that the benefits of the device in the CLIA waived healthcare settings outweigh the risks associated with the device.

O. Conclusion:

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