Title

Performance of a single-use, rapid, point-of-care PCR device for the detection of Neisseria gonorrhoeae, Chlamydia trachomatis, and Trichomonas vaginalis: a cross-sectional study

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A Cross Sectional Study of Performance of a single use rapid point-of-care PCR device for the detection of *Neisseria gonorrhoeae*, *Chlamydia trachomatis* and *Trichomonas vaginalis*

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Abstract

Background: Timely detection and treatment are important for the control of *Neisseria gonorrhoeae* (NG), *Chlamydia trachomatis* (CT) and *Trichomonas vaginalis* (TV). The objective of this study was to measure performance of the Visby Medical Sexual Health single use point-of-care (POC) polymerase chain reaction (PCR) device.

Methods: Women aged 14 years and above were enrolled at 10 clinical sites for a cross-sectional, single-visit study to provide self-collected vaginal swab for testing with the investigational device. Results were compared to the patient infected status (PIS) derived from clinician-collected vaginal specimens based on concordance of Aptima Combo2 and TV with BD Probetec™ NG/CT and TV assays. If they did not match then BD MAX CT/GC/TV was used as a tiebreaker. The primary outcome was sensitivity and specificity for the detection of NG, CT and TV with 95% confidence intervals (95% CI). Subgroup analyses included outcomes by symptomatic status.

Findings: 1585 participants were enrolled yielding 1457 evaluable results for CT, 1468 for NG and 1449 for TV. The observed sensitivities were: CT 97.6% (95% CI 93.2–99.2); NG 97.4% (95% CI 86.5–99.5); and TV 99.2% (95% CI 95.5–99.9). Observed specificities were: CT 98.3% (95% CI 97.5–98.9); NG 99.4% (95% CI 98.9–99.7); and TV 96.9% (95% CI 95.8–97.7).

Interpretation: This study utilized an innovative rapid, easy to use, single use POC device to detect NG, CT and TV infections suitable for use in a clinical office. We observed excellent positive and specificity compared to the PIS reference supporting this device as an advance in the development of rapid diagnostics for STIs and other infectious diseases.

Funding: NIAID DMID

Summary:

A novel single use rapid point-of-care molecular test detected vaginal *Neisseria gonorrhoeae*, *Chlamydia trachomatis* and *Trichomonas vaginalis* infections with high accuracy.

Keywords

Amplified POC test; chlamydia; gonorrhea; trichomonas; self-collected vaginal specimens; Polymerase chain reaction; *Neisseria gonorrhoeae* ; *Chlamydia trachomatis* ; *Trichomonas vaginalis* ; sexually transmitted infection; diagnosis

Introduction

Nucleic acid amplification tests (NAATs) are the standard for quality clinical services to detect *Neisseria gonorrhoeae* (NG) and *Chlamydi*a *trachomatis* (CT) *and Trichomonas vaginalis* (TV) infections in the genital tract. [1] However, most NAATs require complex

laboratory instrumentation, which limits the ability to provide immediate diagnosis and treatment decisions. As a result, standard practices can err in treating some individuals who are not infected if antibiotics are given empirically for syndromic management, or by not treating infected individuals lost to follow up when the results are available. [2,3] If a diagnostic test could be performed at the point of care (POC) for curable sexually transmitted infections (STIs), this could alleviate inappropriate antibiotic use, treat everyone that presents with an infection, and reduce anxiety of waiting for test results for patients. In fact, accurate rapid POC tests have been identified as one of the innovations needed to regain traction against increasing rates of sexually transmitted diseases [4–6].

The challenge of developing POC STI tests for CT, NG and TV in women is that up until recently the most compact and rapid tests were immunoassay lateral flow devices, which rarely achieved clinical sensitivity over 90% with a few exceptions such as OSOM trichomonas rapid test and the aQcare Chlamydia TRF kit.[7] The most robust advancement in POC STI testing in women was compact NAAT technology that can be used at the POC such as the GeneXpert [8]. Those instruments can run one to many samples and give results in less than 90-minutes; however, that turn-around-time is not within a typical clinical visit, are fairly expensive and require stable electricity. Newer table top platforms, retaining high sensitivity and specificity for NG and CT (none currently have TV) have reduced that time to results in 30 minutes getting closer to the 20 minutes most patients find acceptable. [9,10]

There are currently no POC tests that are close to FDA-clearance that can detect all common STIs at the point of service with an easy-to-use self-contained system that can deliver results in under 30 minutes. [6] The Visby Medical Sexual Health Test is an innovative single use rapid nucleic acid based diagnostic test for the detection of NG, CT and TV infections that can be performed at the POC, without complex instrumentation and give a result in less than 30 minutes. We performed a large clinical study to determine the performance of the Visby Medical Sexual Health Test to detect NG, CT and TV from self-collected vaginal specimens.

Methods

Study design, setting, and population

We conducted a cross-sectional, single-visit study of women ≥14 years at 10 clinics across 7 US states (ClinicalTrials.gov Identifier: NCT03852316). Sites included sexual transmitted disease clinics, student health clinics, primary care clinics, academic clinical research centers and private clinical research organizations located in the states of California, Florida, Illinois, Maryland, Mississippi, Nevada and Pennsylvania from February 2019 until January 2020. We offered enrollment to consecutive women who were both symptomatic and asymptomatic. Participant inclusion criteria were: a) Willing and able to give voluntary written informed consent (parent/legal guardian consent for minors); b) Female at birth (including pregnant and breastfeeding women); c) Age ≥14 years at the time of enrollment; d) Able to read and understand the procedural information provided for the study; e) Able and willing to follow all study procedures, including performing self-collection of one vaginal swab and permitting a licensed health care provider to collect three additional vaginal swabs.

Exclusion criteria included: a) Having a medical condition, serious intercurrent illness, or other circumstance that, in the investigator's judgment, could jeopardize the subject's safety, or could interfere with study procedures; b) Prior enrollment in this study; c) Use of antiperspirants and deodorants or the following vaginal products: douches, washes, lubricants, vaginal wipes, vaginal moisturizers, or feminine hygiene spray in the genital area, within 48 hours prior to enrollment.

This study included collaboration between the device manufacturer, the National Institute of Allergy and Infectious Diseases of the National Institutes of Health, and the STAR STI Clinical Trial Group. The study was approved by instutional review board(s) that included a combination of a central IRB and local IRBs as required by the sites.

Description of investigational device

The Visby Medical Sexual Health Test device (see device image in Figure 1) is a single-use, disposable, fully integrated, rapid, compact instrument containing a polymerase chain reaction (PCR)-based assay for the qualitative detection and differentiation of deoxyribonucleic acid (DNA) from NG, CT, and TV. The device is intended to detect NG, CT, and TV DNA in a variety of environments without the need for complex off-board sample processing or instrumentation.

The device is meant to be used by any clinic personnel using the Quick Start Instructions: input sample, activate device, and visually read colorimetric results in less than 30 minutes. A patient vaginal swab self-collection kit with transport media is provided with the product. Once the patient collects the swab sample, it is placed into a vial containing collection medium. A healthcare professional then uses a transfer pipette to transfer 650 μL of the sample-containing media into the device input port. The transfer pipette automatically meters the sample volume needed for the device. Buttons 1, 2 and 3 are pressed in succession. After button 3 is pressed and the device is plugged in, a white LED light turns on indicating the reaction is in progress. At this point, the remaining device operation is automatic, including pathogen lysis/DNA release, PCR amplification and detection of amplified products. When the reaction is complete, the LED light turns green indicating to the user that the results are ready. The results should be read within 2 hours. If the sample is positive, a colorimetric change (from white to purple) is visible in the appropriate spot for the pathogen. If the test run is valid a results for all three organisms are readable.

The Visby Sexual Health test contains all the reagents and instrumentation required to perform a single PCR-based test. The device stores buffers and reagents on-board for release at the correct time. Printed circuit boards (PCBs) control temperature and time the movement of the motors and liquid flow. Sample processing occurs on the device using a combination of heat and chemical lysis to release pathogen DNA. PCR amplification is achieved by mixing the inactivated sample with lyophilized PCR reagents and then performing continuous flow PCR using a serpentine shaped plastic-molded fluidic circuit that allows rapid heating and cooling. Biotin-labeled PCR primers specifically amplify NG, CT and TV genes. A detection flow cell has oligonucleotide capture probes which hybridize to the amplified pathogenic target (supplementary table 1). A colorimetric signal is generated on the flow cell when horseradish peroxidase HRP-linked to streptavidin

binds biotin-labeled amplicon/capture probe pairs, and catalyzes the conversion of 3, 3', 5, 5'-tetramethylbenzidine, resulting in a purple precipitate. The presence of target pathogen in the sample thus leads to a white-to-purple color change on the detection flow cell.

A control non-pathogenic Neisseria species is lyophilized and present in the lysis chamber of the device and serves as the positive internal process control. Lyophilized PCR primers that amplify the Neisseria internal control is included with primers for NG,CT and TV. This monitors the test for effective sample prep, PCR amplification, and detection. If all elements in the Sexual Health Test function properly, the positive control spot will produce a purple color. The test is considered valid if there is a purple signal in the control window and a green check mark is visible on the test that indicates the test ran without an error.

Study procedures

Consecutive participants who met enrollment criteria were consented and assessed for clinical symptoms of infection and asked about recent medication and topical vaginal product use. Participants were then provided the manufacturer's self-collection instructions that they would use to self-collect a vaginal specimen using the manufacturer's urogenital specimen collection kit containing a swab and transport media. An untrained operator would receive the specimen and run the device using the provided Operators Guide without coaching or instruction. According to protocol, the test would need to run within 2 hours of collection. If the result was invalid (no signal in control window) or error (did not receive the green check that device operated correctly), a second test would be run—also within 2 hours from collection. Meanwhile, a licensed health care provider would collect three randomly ordered vaginal swabs (to account for the possible impact of collection order on the comparator assay performance). The study health care provider recorded any signs of infection. Swabs were stored and transported per manufacturer guidelines for vaginal collection and testing, and specimen collection was standardized for all study sites.

Central laboratory procedures

The study used three comparator systems: Hologic Aptima™ Combo2 [11] and Aptima *Trichomonas vaginalis* Assays [12]; BD Molecular Diagnostics BD ProbeTec™ CT/GC Qx Amplified DNA Assay [13] and BD ProbeTec™ *Trichomonas vaginalis* Qx Assay [14]; and BD Molecular Diagnostics BD MAX™ CT/GC/TV [15] assuring three distinct nucleic acid amplification targets for each organism. Hologic Aptima Trichomonas is not FDA cleared for self-collected vaginal specimens so all PIS comparators were clinician collected for consistency.

One reference laboratory (Molecular Testing Labs [MTL], Vancouver, Washington) processed and tested all of the comparator assays for the study. The laboratory conducted quality control and quality assurance procedures according to the manufacturers' recommendations and in compliance with the College of American Pathology. Trained laboratory staff processed swabs, tested the specimens, and interpreted the results according to each manufacturer's instructions on the respective FDA-cleared assays. Staff repeated initial equivocal, invalid, or otherwise indeterminate results once per manufacturer recommendations before classifying as positive, negative, equivocal, or invalid/unresolved.

Laboratory testing staff were blinded to clinical information and the investigational device results.

Determination of the Patient Infected Status (PIS)

All participants had their specimens resulted from the Aptima™ and Probetec™ systems. If there was discordance between those two results then the BD MAX™ CT/GC/TV result was used as a tiebreaker. To determine the PIS, there needed to be agreement between two of the three comparator assay results. If at least two comparator results did not match, the PIS was considered indeterminate. Possible outcomes of the PIS by organism included infected, not infected and indeterminate. We defined PIS definitions *a priori* in the study protocol.

Statistical methods

The analytic population were those who were confirmed to have met inclusion/exclusion criteria and had specimens collected and run according to protocol. Specimens needed to have a test initiated within two hours for the Sexual Health Test and for comparators within the stated manufacturer's stability window. If results were missing from the Sexual Health Test, the data were not included in the analysis. If the PIS were indeterminate or results were missing from the comparators that was required to determine a PIS for an organism (e.g. either the Aptima™ or Probetec™, but only for the BD MAX™ CT/GC/TV when a tiebreaker was needed), then the results for that participant were excluded from the analysis only for the organism without a valid positive or negative PIS result.

If participants reported "Yes" to any of the following symptoms on the Clinical Symptoms Assessment case report form, the subject was classified as 'Symptomatic': Unusual vaginal discharge, vaginal irritation (itching, burning, soreness), lower abdominal/pelvic pain, painful urination, increased urinary frequency, abnormal bleeding/spotting, pain or bleeding with sex/intercourse. Otherwise, the subject was classified as "Asymptomatic".

For each organism, sensitivity, specificity, accuracy (total agreement), positive predictive value and negative predictive value for the Visby Medical Sexual Health investigational device against the PIS were calculated, with 95% Wilson confidence intervals (Cls).[16] The study was designed with a target sample size to achieve 120 CT, 120 TV and 45 NG. The sample size was determined by the numbers required for a sensitivity of 95% with a lower confidence bound of 90% for CT and NG and 85% for TV. The sample size was also to meet a specificity fo at least 95% with a lower bound of 90%. In sub-analyses test characteristics were determined for those who were symptomatic and asymptomatic.

Results

There were 1585 women enrolled with broad demographic and geographic representation that includes ages 14–80 years (Table 1). Women were included who were pregnant and menstruating. An investigational device was run for 1,555 of the participants with 1,444 having a valid first test and an additional 88 valid results with a repeat test for a total of 1,532 (98.5% (95% CI 97.8–99.1)) for the Visby Medical Sexual Health Test with 154 (10.1% (95% CI 8.6–11.7)) positive for CT, 50 (3.3% (95% CI 2.5–4.3) positive for NG and 178 (11.6% (95% CI 10.1–13.3)) positive for TV (see Figure 2). In 1579 participants a PIS

could be determined for at least one organism with 136/1579 positive for CT (8.6% (95% CI 7.3–10.1)), 44 /1578 positive for NG (2.8% (95% CI 2.0–3.6)) and 137/1579 positive for TV (8.7% (95% CI 7.4–10.2)). In the final evaluable results, there were: 1457 (93.7%) available for CT, 1468 (94.4%) for NG and 1,449 (93.2%) for TV. Figure 1 lists the number by reason for exclusion from analysis that included i) protocol deviations for study procedures (consenting error, incorrect collection of specimens, controls not performed correctly prior to testing, testing outside the 2 hour window, no device available, mislabeled specimen, did not complete study visit), ii) violation of study inclusion/exclusion discovered after visit occurred in two participants (repeat participant, use of product in prior 48 hours in the exclusion list), iii) no result available from investigation device (device not run or all runs within testing window were invalid/error) and/or iv) no result for PIS (lacking two comparators in agreement for any reason).

Table 2 shows the diagnostic performance measures for the investigational device by organism when compared to the PIS. For *CT* the sensitivity was 97.6% (95% CI 93.2–99.2) and the specificity was 98.3% (95% CI 97.5–98.9). For NG the sensitivity was 97.4% (95% CI 86.5–99.5) and the specificity was 99.4% (95% CI 98.9–99.7). For TV the sensitivity was 99.2% (95% CI 95.5–99.9) and specificity was 96.9% (95% CI 95.8–97.7). A planned stratified analysis was performed for symptomatic/asymptomatic status found similar sensitivity and specificity (see table 2). For all evaluable results, the Visby Medical Sexual Health Test agreed with the PIS (accuracy) in 98.3% (95% CI 97.5–98.8) of results for CT, 99.4% (95% CI 98.8–99.7) for NG, and 97.1% (95% CI 96.1– 97.8) for TV. Table 3 shows the agreement with each comparator where positive agreement was highest with Hologic and BD Max for NG and CT and BD Probetec and BD Max for TV. Table 4 also presents PPV and NPV across a range of pre-specified hypothetical prevalences of interest adjusting the study specific values via Bayes Rule.

Device Invalid Rates and Usability

We conducted a second Sexual Health Test if the first was invalid and the specimen was still within the two-hour window. The first test was invalid in 111/1,555 (7.1%) and 23/1,555 (1.5%) were invalid on both the first test and a retest (Supplementary Table 2).

Of 27 operators, 4 left study before they completed a user survey. Of those that completed the survey on usability (n=23) where they were asked about ease of use (Supplementary Table 3), all operators responded that they would agree or strongly agree that “It was easy to set up the device”, “The instructions for the device were easy to follow” and “Overall, it was easy to run the device”. For the question “It was easy to see and understand the test results.” 21/23 responded that they agreed or strongly agreed and two were neutral

Discussion

This study demonstrated that a single use rapid device to detect NG, CT and TV from a self-collected vaginal specimen in women was highly accurate while performed by clinic staff without laboratory training. The device also delivered a valid test result for 98.5% of the samples provided. Results from this study were used to support a regulatory submission and Clinical Laboratory Improvement Act (CLIA) waiver submission to the FDA for the

first rapid, simple, molecular point-of-care testing device for STIs. This represents a major step forward for the detection of STIs that may result in more timely and accurate patient care, improved control of these infections and reduction in STI complications.[17] Widespread use of the rapid device would allow for treatment decisions in real time. Such use has been shown to improve appropriate treatment and reduce unnecessary antibiotic exposure.[18,19] Given the rising burden of STIs, a simple POC device with those characteristics is highly desirable.[20] This device is the first to apply the advances in molecular and microfluidic technology towards true POC highly accurate and rapid testing that can be applied to many prevalent infectious diseases globally.[21]

Compared to the Visby Medical Sexual Health Test, other POC test systems—such as the binx io and GeneXpert—require samples to be placed in a desktop PCR instrument that once loaded will run for 30–45 minutes or longer.[8,22,23] The Visby Medical device is compact, transportable, scalable and because it is single-use does not require maintenance. Samples can be run sequentially as patients are seen in real time for one or many patients. While other technologies are in development,[6,24] this device may be the first to be cleared for such use. The Visby Medical Sexual Health Test meets many of the desirable characteristics defined by the World Health Organization for a point-of-care device: Sensitive, Specific, User-friendly, Rapid and robust, Equipment-free and Deliverable to end-users.[17] Most importantly, we observed excellent sensitivity and specificity when compared to the PIS reference for the detection of NG, CT and TV. Our findings are similar to published estimates of the performance for NAATs for STIs.[12,14,15,25,26] The closest to a true POC PCR test include binx io and GenXpert that have performed studies on self collected vaginal specimens although none cover CT, NG and TV. The binx io device had sensitivity and specificity for CT (n=51) were 96.1% and 97.7%.[9] The GenXpert had sensitivity and specificity values for CT (n=79) of 98.7%, and 99.4%; and for NG (n=22) were 100% and 99.9%.[8]

Operators of the test were untrained and used an enclosed Quick Start Instructions to operate the Visby Medical Sexual Health Test. They found the test easy to use and successfully operated the tests. These results suggest that the test may meet CLIA-waiver requirements.

Strengths of this study include a diverse study population of participants from across geographic and clinical sites. To minimize bias, all PIS devices had different amplification targets and methodologies to avoid the results being systematically aligned. Weaknesses of the study include the moderate number of NG cases, which may have impacted the precision of the observed sensitivity. Finding a substantial number of NG cases in women is a challenge and the study did try to go to higher prevalence areas and recall women who had tested NG positive in clinic to come in for a study visit. Because the PIS composite reference method required at least two comparator assays to agree, limitations in the performance of the comparator assays had the potential to affect the observed device accuracy. We did observe greater specimen positivity using the test device than the PIS and there was differences in agreement between devices but not clearly supporting higher sensitivity. There were very few false negative results when compared to the PIS for NG and in the one case in the analysis of a NG false negative the stored device image revealed a probable misinterpretation by the operator when later reviewed by study staff. Also, this

study did not use self-collected specimens for the comparator assays but did for the test device, the positivity would also be biased in favor of the test device because self-collected specimens for women are more sensitive.[27]

To adopt use of this or any POC test for STIs, clinics will need to incorporate the training and timing into their workflows. It will be important for clinicians to understand that positive predictive values of STI tests vary by organism and that a positive value for an organism in a low prevalence setting (PPV is less than 90%) there should an interpretation of the result within context and possibly further confirmation by a second test. For usability, it will also be important that this technology to be validated with samples from all genders and anatomic sites.

Conclusion

In summary, this study evaluated a new “first-in-class” diagnostic point-of-care device for the detection of STIs in women. The Visby Medical Sexual Health Test had excellent agreement with other FDA-approved laboratory-based assays for the detection of NG, CT and TV and should be suitable for a CLIA-waived test environment. This device has major potential for the rapid detection of STIs in clinical settings.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Research in context

Evidence before this study

A PubMed search for articles up to April 2020 that were available on "point of care diagnostics for *Chlamydia trachomatis* (CT), *Neisseria gonorrhoeae* (NG), and *Trichomonas vaginalis* (TV)". The most recent review of POC testing in 2016 found 33 publications with 13 articles evaluating test performance. This review identified should sensitive and specific POC tests are available for CT, NG, and TV, but only one of these (GeneXpert) used nucleic acid amplification methods that provide the highest sensitivity. Since then one additional test (binx io) has also been published on. However both of these use desk top machines that have higher complexity of operation, require regular maintenance, do not detect TV, can do only one run at a time, and take longer than most patients are willing to wait.

Added value of this study

This study establishes a new class of diagnostic devices that are nucleic acid amplification based, single use, rapid (<30 minutes), and simple to use. This device is potentially the new gold standard of POC tests for infectious diseases such as STIs, influenza, and coronavirus where rapid turnaround are key. The findings show that sensitivity and specificity of a true POC test can be the same as a laboratory based test. This device runs a self-collected vaginal sample to provide an in-clinic accurate diagnostic test for NG, CT and TV that can lead to treatment in the same visit. For the public health control of STIs the implications for this device are an advancement to treat everyone that needs treatment (and avoiding unnecessary speculative treatment) and minimize time to treatment that will reduce transmission and complications.

Implications of all the available evidence

The development of rapid point of care test for STIs and other infectious diseases represents an important need in medicine and public health.

Figure 1.
Visby Medical Sexual Health Test

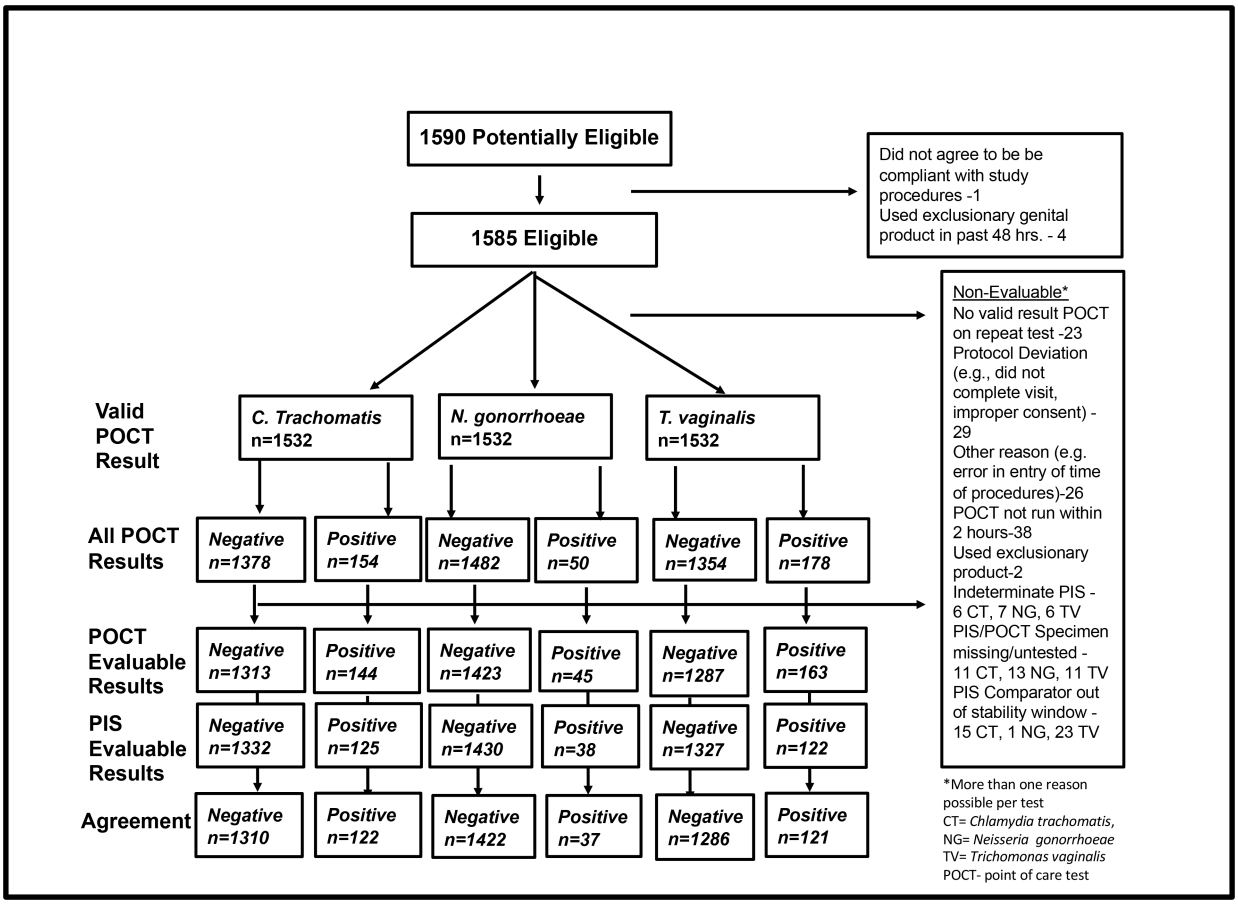

Figure 2.

Study flow diagram in an evaluation of the Visby Medical Sexual Health Test, a rapid point-of-care test (POCT) for the detection of *Chlamydia (C.) trachomatis*, *Neisseria (N.) gonorrhoeae* and *Trichomonas (T.) vaginalis.*

Table 1.

Characteristics of enrolled study participants in an evaluation of the Visby Medical Sexual Health Test, a rapid point-of-care molecular assay for the detection of *Chlamydia trachomatis, Neisseria gonorrhoeae* and *Trichomonas vaginalis*.

Variable	Characteristic	n	%
Sex	Female	1585	100
Ethnicity	Not Hispanic or Latino	1168	74
	Hispanic or Latino	310	20
	Unknown	107	7
Race	American Indian or Alaska Native	12	<1
	Asian	86	5
	Native Hawaiian or Other Pacific Islander	6	<1
	Black or African American	876	55
	White	414	26
	Multi-Racial	70	4
	Unknown	121	8
Age, years	14–17	9	<1
	18–25	564	36
	26–35	366	23
	36–45	225	14
	46–55	225	14
	>55	196	12
Study site	University of California San Diego Antiviral Research Center (CA)	186	12
	Stroger Hospital of Cook County – CORE (Chicago, IL)	243	15
	San Francisco Department of Public Health (CA)	71	5
	Philadelphia Department of Public Health (PA)	281	18
	University of Mississippi Medical Center (Jackson, MS)	62	4
	Florida International University (Miami, FL)	279	18
	AXIS Clinical Trials (Los Angeles, CA)	375	24
	Johns Hopkins University Rangos (Baltimore,MD)	16	1
	South Florida Clinical Trials (Hialeah, FL)	12	<1
	Impact Clinical Trial (Las Vegas, NV)	60	4

Table 2:

Test Performance of Visby Medical Sexual Health Test by Organism and Symptomatic status in an evaluation of a rapid pointof-care molecular test for the detection of *Chlamydia trachomatis, Neisseria gonorrhoeae* and *Trichomonas vaginalis*.

Symptomatic Status	Organism	Click Device Result	PIS Designation			Sensitivity (95% CI[a])	Specificity (95% CI[a])	Accuracy (95% CI[a])
			Positive	Negative	Total			
			n	n	n			
Symptomatic and Asymptomatic Combined	CT (N=1457)	Positive	122	22	144	97.6 (93.2, 99.2)	98.3 (97.5, 98.9)	98.3 (97.5, 98.8)
		Negative	3	1310	1313			
		Total	125	1332	1457			
	NG (N=1468)	Positive	37	8	45	97.4 (86.5, 99.5)	99.4 (98.8, 99.7)	99.2 (95.5, 99.9)
		Negative	1	1422	1423			
		Total	38	1430	1468			
	TV (N=1449)	Positive	121	41	162	99.4 (98.9, 99.7)	96.9 (95.8, 97.7)	97.1 (96.1, 97.8)
		Negative	1	1286	1287			
		Total	122	1327	1449			
Symptomatic	CT (N=735)	Positive	77	15	91	98.7 (93.0, 99.8)	97.7 (96.3, 98.6)	97.8 (96.5, 98.7)
		Negative	1	643	644			
		Total	77	658	735			
	NG (N=746)	Positive	20	4	24	100 (83.9, 100)	99.4 (98.6, 99.8)	99.5 (98.6, 99.8)
		Negative	0	722	722			
		Total	20	726	746			
	TV (N=734)	Positive	72	28	100	98.6 (92.6, 99.8)	95.8 (93.9, 97.1)	96.0 (94.4, 97.2)
		Negative	1	633	634			
		Total	73	661	734			
Asymptomatic	**CT (N=722)**	**Positive**	46	7	53	95.8 (86.0, 98.8)	99.0 (97.9, 99.5)	98.8 (97.6, 99.3)
		Negative	2	667	669			
		Total	48	674	722			
	NG (N=722)	**Positive**	17	4	21	94.4 (74.2, 99.0)	99.4 (98.5, 99.8)	99.3 (98.4, 99.7)
		Negative	1	700	701			
		Total	18	704	722			
	TV (N=715)	**Positive**	49	13	62	100 (92.7, 100)	98.0 (96.7, 98.9)	98.2 (96.9, 98.9)
		Negative	0	653	653			
		Total	49	666	715			

[a]As determined by patient-infected status designation.

The denominator for estimates is based on subjects in the Evaluable population for the specified organism (N). 95% CI = 95% Wilson confidence interval.

Table 3:

Test Performance of Visby Medical Sexual Health Test by Organism Against Each Comparator

Organism	Comparator	Click Device Result	Click Result			Positive agreement(95% CI[a])	Negative agreement(95% CI[a])	Accuracy (95% CI[a])
			Positive	Negative	Total			
			n	n	n			
Chlamydia	**Hologic**	Positive	120	3	123	97.6 (93.1, 99.2)	98.2 (97.3, 98.8)	98.1 (97.3, 98.7)
		Negative	24	1308	1332			
		Total	144	1332	1455			
	Probetec	Positive	120	10	130	92.3 (86.4, 95.8)	99.2 (97.3, 98.8)	97.7 (96.8, 98.3)
		Negative	24	1303	1327			
		Total	144	1313	1457			
	BD Max	Positive	55	1	56	98.2 (90.6, 99.7)	98.0 (96.3, 98.9)	98.0 (95.8, 97.7)
		Negative	9	442	451			
		Total	64	443	507			
Neisseria	**Hologic**	Positive	36	1	37	97.3 (86.2, 99.5)	99.4 (98.8, 99.7)	99.3 (98.7, 99.6)
		Negative	9	1419	1428			
		Total	45	1420	1466			
	Probetec	Positive	35	7	42	83.3 (69.4,91.4)	99.3 (98.7, 99.6)	98.8 (98.2, 99.3)
		Negative	10	1413	1423			
		Total	45	1420	1465			
	BD Max	Positive	19	0	19	100.0 (83.2, 100)	99.2 (98.0, 99.7)	99.2 (98.0, 99.7)
		Negative	4	497	501			
		Total	23	497	520			
Trichomonas	**Hologic**	**Positive**	123	27	150	82.0 (75.1, 87.3)	97.2 (96.1, 98.0)	95.6 (94.4, 96.6)
		Negative	37	1259	1296			
		Total	160	1286	1446			
	Probetec	**Positive**	117	5	122	95.9 (90.8, 98.2)	96.6 (95.5, 97.5)	96.6 (95.5, 97.4)
		Negative	45	1282	1327			
		Total	162	1287	1449			
	BD Max	**Positive**	39	0	39	100 (91.0, 100)	96.6 (94.5, 97.9)	96.8 (94.9, 98.0)
		Negative	16	448	464			
		Total	55	448	503			

The denominator for estimates is based on subjects in the Evaluable population for the specified organism, BD Max was not run for every sample and was only used for tiebreaker or in some cases run if sample was going to become past testing window and other comparators results were not completed.. 95% CI = 95% Wilson confidence interval.

Table 4.

Positive Predictive Value (PPV) and Negative Predictive Value (NPV) Estimates Across Range of Hypothetical Prevalences by Organism in an evaluation of a rapid point-of-care molecular test for the simultaneous detection of *Chlamydia (C.) trachomatis, Neisseria (N.) gonorrhoeae* and *Trichomonas (T.) vaginalis*.

Hypothetical Prevalence (%)	C.trachomatis		N. gonorrhoeae		T. vaginalis	
	PPV	NPV	PPV	NPV	PPV	NPV
1	37.4	>99.9	63.7	>99.9	24.5	>99.9
2	54.7	>99.9	78.0	>99.9	39.6	>99.9
5	75.7	99.9	90.2	99.9	62.8	>99.9
10	86.8	99.7	95.1	99.7	78.1	>99.9
20	93.7	99.4	97.8	99.3	88.9	99.8