Clinical Evaluation of the BD ProbeTec™ *Chlamydia trachomatis* Q^x Amplified DNA Assay on the BD Viper™ System With XTR™ Technology

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Background: This study evaluated the performance of the BD ProbeTec *Chlamydia trachomatis* Q^x (CTQ) Amplified DNA Assay on the BD Viper System with XTR Technology in a multicenter study.

Methods: Specimens were collected at 7 geographically diverse clinical sites from 1538 women and men attending sexually transmitted disease, family planning, and obstetrics and gynecology clinics. There were 1465 evaluable participants, 993 women and 472 men. CTQ assay results from female endocervical, self-collected vaginal, male urethral swab specimens, and male and female neat (unpreserved) urine specimens as well as those obtained using the Urine Preservative Transport (UPT) tube for the CTQ assay were compared with patient-infected status (PIS). PIS was determined based on the combined results from Aptima Combo 2 and BD ProbeTec ET CT Amplified DNA Assay.

Results: The sensitivity versus PIS for endocervical, vaginal, and both female urine samples was 91.3%, 96.5%, and 93.0%, respectively. The specificity for the same specimen types was 98.3%, 99.2%, and 99.4% (urine neat) and 99.2% (UPT), respectively. The sensitivity versus PIS for male urethral swabs and both male neat and UPT urine were 92.1% and 98%, respectively, with specificities of 98.4%, 99.2%, and 98.1%, respectively.

Conclusions: The CTQ assay demonstrated performance characteristics comparable with other commercially available nucleic acid-based tests such as Aptima Combo 2 and BD ProbeTec ET CT-Amplified DNA assay. Vaginal swabs and male urine specimens, the sample types recommended by the Centers for Disease Control for chlamydia screening, both performed at least as well as other sample types evaluated.

Many studies have demonstrated the excellent sensitivity and specificity of commercially available nucleic acid amplification tests (NAATs) for the identification of *Chlamydia trachomatis* in urine, endocervical, urethral, and vaginal swab specimens.[1–6] This multicenter study evaluated the clinical performance of the next generation assay BD ProbeTec *C. trachomatis* Q^x (CTQ) Amplified DNA Assay on the BD Viper System with XTR Technology in extracted mode (Viper) in comparison with the APTIMA Combo 2 (AC2) and the BD ProbeTec ET CT assay (PT) for endocervical, vaginal, urethral, and urine specimens collected, either neat (unpreserved urine) or in the BD Urine Preservative Transport (UPT) tube from symptomatic and asymptomatic men and women.

METHODS

Study Population

Participants were enrolled from 7 geographically diverse sites that included both traditionally high- and low-risk populations. Of these sites, 4 collected from sexually transmitted disease clinics, 2 collected from family planning (FP) and obstetrics and gynecology (OB/GYN) clinics, and 1 collected from both sexually transmitted disease and FP clinics. Seven sites collected from female subjects and 4 of the 7 sites collected from male subjects. Men and women between the ages of 17 to 64 years, who presented with urogenital symptoms or were being screened for Chlamydia (CT) and gonorrhea and who provided informed consent, were enrolled between November 26, 2007 and March 21, 2008. After exclusions for nonadherence to protocol and unavailable specimens, there were 993 women and 472 men who were included in the analysis. Participants were excluded if they had urinated within an hour of specimen collection or other specimen collection issues (n = 55), had taken antibiotics in the past 21 days (n =

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5), did not provide informed consent (n = 10), or were below the age requirement approved by each site's Institutional Review Board (n = 2). Three sites performed CTQ testing for specimens collected at their own site and/or specimens that were referred from the other collection sites. Reference method testing was performed at 5 of the 7 clinical centers and 2 sites served as specimen collection sites and performed no testing. One additional site served as a specimen-testing site only and performed no collection.

Specimen Collection, Transport, and Storage

For female participants, a first-catch urine sample was collected, followed by a self-collected vaginal swab, and then 3 endocervical swabs. For each male participant, 2 or 3 urethral swabs (collection center dependent) were obtained followed by a urine collection. Swab collection followed each manufacturer's collection kit instructions and used kit-specific collection and transport materials. Swab collection order was randomized throughout the study to minimize sample order variation. For female participants, 1 endocervical swab was tested on the reference AC2 assay, 1 swab was tested on the reference PT assay with an amplification control, and the third swab was tested using the CTQ assay. The self-collected vaginal swab was tested only with the CTQ assay. For the male participants from whom only 2 swabs were collected, the reference method was randomized at the collection stage to either the PT or the AC2 assay and the other swab specimen was always evaluated using the CTQ assay. The endocervical, vaginal, and urethral swabs for the PT and AC2 assays were stored and tested according to their respective manufacturer's package inserts.[2,5] For the CTQ assay, the urethral and endocervical swabs were stored at 2°C to 30°C for up to 14 days before testing. Vaginal swabs were stored at 2°C to 30°C for up to 7 days before testing.

Urine volume ranged from 20 to 69 mL, with the lower limit set by study design and manufacturer's package inserts while the upper limit was the maximum volume of the collection cup. Urine samples were divided into aliquots, and the samples from the urine specimen transport tube were tested with the AC2 assay (UTT), the PT assay (UPT urine) and the CTQ assay with neat and UPT urine. Urine processing, storage, and testing were performed in accordance with the respective manufacturer's package inserts.[2,5]

CTQ Assay

Immediately before testing, urine and swab specimens underwent a prewarm step at 114°C for 15 minutes to dissolve mucous and homogenize the specimen matrix. After cooling for 15 minutes, the specimens were loaded onto the Viper which then performed all steps necessary for extraction and amplification of target DNA without further user intervention. Each specimen was robotically transferred to an extraction tube that contained ferric oxide particles in a dissolvable film, together with a fluorescent-labeled extraction control oligonucleotide that underwent the same process as the specimens to confirm the validity of the extraction process for each individual specimen.

In the extraction tube, a high pH lysis buffer was used to disrupt bacterial cells and release their DNA into solution. An acidic buffer was then added to lower the pH and induce a positive charge on the ferric oxide particles, which in turn captured the negatively charged bacterial and extraction control DNA. The particles with bound DNA were washed and eluted using magnetic capture and a high pH buffer. Finally, a neutralization buffer was used to lower the pH of the extracted DNA solution to the optimum level for amplification of the target.

The neutralized ferric oxide eluates were then transferred from the extraction tube to priming microwells. When present, *C. trachomatis* DNA is then detected by strand displacement amplification of a specific target sequence in the presence of a fluorescent-labeled detector probe. The PT and the CTQ assays target different open reading frames (ORFs) within the cryptic plasmid of *C. trachomatis*. The PT targets a region within ORF 5, whereas the CTQ targets a region within ORF 3.[7] The priming wells contained the specific primers and probes for the target DNA sequences, as well as nucleotides and other reaction components necessary for strand displacement amplification. After rehydration with the ferric oxide eluate, the priming microwells were heated to 70°C. In parallel, amplification microwells that contained dried enzymes and stabilizing agents were prewarmed to the amplification temperature of 52.5°C. After 10 minutes, an aliquot of liquid from the priming microwells was transferred to the amplification microwells to initiate the amplification and detection reaction. Once transfer was complete, the robot automatically sealed the amplification microwells which were further moved into the fluorescent reader(s). Measurements of fluorescent signal were taken approximately once every minute over the course of an hour and positive/negative results were determined by comparing the maximum relative fluorescent signal obtained from a given specimen with a predetermined threshold.

Determination of PIS

The definition of the patient-infected status (PIS) for evaluation of CTQ performance was based on the reference swabs and urine specimen results obtained using the PT assay (DNA target) and AC2 assay (16S rRNA target) which allowed for 4 reference results, 2 from each system, except in the case of male subjects where CTQ performance were based on 3 reference results using 2 urine results, PT and AC2 and 1 reference swab result (PT or AC2). The definition required by the Food and Drug Administration (FDA) for submission considered a participant infected with *C. trachomatis* if a minimum of 1 positive result was reported by each of the reference NAAT assays (i.e., a positive from both the PT assay and AC2 assay).[7] Sensitivity analyses were performed as part of this study to assess the effect of alternate methods for defining PIS. For assay performance comparison, the performance characteristics of each reference method were estimated by using a PIS defined by a positive result from each of the other 2 assays (i.e., AC2 estimates were derived by comparison with a PIS that required a positive result from both the PT and CTQ assays, PT estimates were derived by comparison with a PIS that required a positive result from both the CTQ and AC2 assays). This method of a rotating PIS standard has been described as the most useful means of evaluating true assay performance.[8]

Data Analysis

For analysis, specimens were categorized by gender, presence or absence of genitourinary symptoms, and specimen type. The performance characteristics of the CTQ assay were calculated for each category by comparing assay results with PIS. Confidence intervals (95% confidence level) were calculated using the exact binomial distribution method. Comparison of performance, sensitivity, and specificity estimates were completed using logistic regression with Hochberg adjustment for multiple comparisons. Alpha-0.05 was used for all analyses.

RESULTS

A total of 1538 participants were enrolled in the study from 7 sites. After excluding noncompliant participants and specimens, data for 1465 participants (5388 specimens) were available for analysis. There were 993 women (68%) and 472 men (32%). Symptoms of CT/gonorrhea (burning/pain upon urination, abnormal discharge, coital pain/difficulty/bleeding, testicular or scrotum pain/swelling) were reported by the subject and were present in 543 women (55%) and 257 men (54%). Of all, 27 female participants were known to be pregnant. Of the 472 male participants, 62 had only 2 urethral swabs collected while the remainder provided 3 urethral swabs.

Chlamydial infection was identified in 115 (11.6%) women, with 50.4% (58/115) of these infections occurring in symptomatic women (Table 1). The overall prevalence in men was 21.4% (101/472), with the majority of infections (65.3% 66/101) identified from men with symptoms. Despite enrolling from FP and OB/GYN clinics, the prevalence of chlamydial infection in the study populations was high, ranging from 11.6% in women and 21.4% in men (Supplemental Digital Content, Table S1, online only, available at: http://links.lww.com/OLQ/A10). The effect of collection site was not significant for any specimen type on the CTQ assay (P values range from 0.1742 to 0.9312). Therefore, remaining analyses are for results pooled across all sites. For the CTQ assay, analysis was performed to look at the effects of specimen type, symptomatic status, and their interaction. These effects were not significant for either sensitivity or specificity of the CTQ assay (P value range, 0.0598–1.00).

Comparison of All NAATs

The results obtained with the rotating PIS for the PT and AC2 assays are shown in comparison with the estimates for CTQ, stratified by specimen types and symptomatic status in Table 1. Additional analyses were performed to compare sensitivity and specificity of the CTQ, PT, and AC2 assays. For specimen types where results were generated for CTQ, PT, and AC2 (i.e., female endocervical swabs and urine, male urethral swabs and urine), the overall performance of all assays was not significantly different (P values ranging from 0.3196 to 0.9577). However, when considering sensitivity and specificity separately for each of these 4 specimen types, differences were noted for sensitivity of endocervical swabs ($P = 0.0333$) (Fig. 1). The PT assay had significantly lower sensitivity than both AC2 and CTQ assays ($P = 0.0358$ and 0.0321, respectively) for endocervical swabs. There were no significant differences in sensitivity for female urine, male urethral, and male urine specimens or in specificity for any sample types (Figs. 1, 2).

Female Specimens

Sensitivity of the CTQ assay versus the PIS for endocervical swabs was 91.3% (105/115). Self-collected vaginal swabs identified 111/115 (96.5%) of all infections defined by the PIS, whereas urine samples detected 107/111 (93.0%) of infected women. The specificity of the CTQ assay was at least 98.0% for each sample type, with an overall specificity of 99.0% across combined specimen types (Table 1).

For women, 8 results were generated from each subject (AC2 swab and urine, PT swab and urine, CTQ endocervical, vaginal, UPT, and neat urine). For the 115 women identified as infected using the FDA-required PIS to which CTQ was compared, 87 (75.7%) were positive by all tests for every specimen type, whereas the remaining 27 (24.3%) had between 1 and 5 negative results despite being defined as infected. The overall sensitivity for combined female sample types is 93.5% (430/460) and overall specificity is 99.0% (3478/3512). Of the 10 CTQ endocervical swab-negative and PIS-positive results (Supplemental Digital Content, Table S2, online only, available at: http://links.lww.com/OLQ/A10), 9 occurred in participants in whom the AC2 and PT comparator assays also were positive only in the urine specimens, suggesting that CT infection in these women may have been localized to the urethra.

Looking at the women classified as negative using the FDA-required PIS, 9 may have been infected with CT (7/9 had 3 or more positive results), but may have been misclassified by use of this definition. Given that the sensitivity of AC2 has been described as higher than that of the PT assay, requiring a positive result from each of the assays is likely to underestimate true infections. Of the 9 women with possible infections, 4 had positive results from both the endocervical and urine samples, suggesting that these women were truly infected. When we recalculated the sensitivity of the CTQ assay using a PIS that classified these 4 women as infected, the overall sensitivity dropped slightly (from 93.5% to 92.9%), whereas the specificity increased somewhat (from 99.0% to 99.4%), but these changes were not statistically significant ($P = 0.300$ and 0.064, respectively). Similar results were obtained when we excluded these 9 women from evaluation (data not shown). This sensitivity analysis suggests that the estimates of performance characteristics are robust regardless of the definition of PIS.

Male Specimens

The CTQ assay sensitivity versus PIS with male urethral swabs was 92.1% (93/101) (Table 1). Specificity of the male urethral swab was 98.4% (365/371). Of the 6 CTQ positive swabs (365/371) with negative PIS, 4 were also positive by one of the reference assays or another specimen type (Supplemental Digital Content, Table S3, online only, available at: http://links.lww.com/OLQ/A10). Male neat and UPT urine specimens both had sensitivity of 98.0% (99/101). The CTQ assay specificity versus PIS for male UPT and unpreserved urine specimens were 98.1% (364/371) and 99.2% (368/371), respectively. Three of the 7 CTQ-positive UPT and all 3 CTQ-positive neat specimens with negative PIS were positive by another NAAT or another specimen type.

Urine Volume

The recommended urine volume was between 20 and 25 mL, but allowed up to 69 mL, which was the limit of the urine-collection cup. On the basis of the previous reports of the effect of urine volume of test sensitivity, an analysis was conducted to verify that the performance of the urine specimens was not affected by urine volume (Supplemental Digital Content, Table S4, online only, available at: http://links.lww.com/OLQ/A10). Urine volume was not associated with false-negative results for either the neat or UPT urine specimen types (P values of 0.6056 and 0.9827 for neat and UPT urine, respectively).

Inhibitory Samples

There were 19 initial indeterminate urine results from the PT, of which 7 resolved as negative and 12 remained as indeterminate (shown as "I", Supplemental Digital Content, Tables S2 and S3, online only, available at: http://links.lww.com/OLQ/A10). All 19 specimens were negative by CTQ and AC2.

DISCUSSION

In this multicenter trial, a second generation assay, CTQ on the Viper, was evaluated. CTQ assay results collected from

TABLE 1. Assay Performance Compared to PIS

			Sensitivity			Specificity		
Gender	Specimen Type	Symptom	CTQ	PT	AC2	CTQ	PT	AC2
Female specimens	Endocervical	Symptomatic	89.7 (52/58) (78.8, 96.1)	79.0 (49/62) (66.8, 88.3)	88.1 (52/59) (77.1, 95.1)	98.6 (478/485) (97.0, 99.4)	99.4 (477/480) (98.2, 99.9)	98.5 (475/482) (97.0, 99.4)
	Endocervical	Asymptomatic	93.0 (53/57) (83.0, 98.1)	86.4 (51/59) (75.0, 94.0)	92.9 (52/56) (82.7, 98.0)	98.0 (385/393) (96.0, 99.1)	100.0 (379/379) (99.0, 100.0)	99.0 (389/393) (97.4, 99.7)
	Endocervical	Total	91.3 (105/115) (84.6, 95.8)	82.6 (100/121) (74.7, 88.9)	90.4 (104/115) (83.5, 95.1)	98.3 (863/878) (97.2, 99.0)	99.7 (856/859) (99.0, 99.9)	98.7 (864/875) (97.8, 99.4)
	Vaginal*	Symptomatic	94.8 (55/58) (85.6, 98.9)	NA	NA	99.0 (481/486) (97.6, 99.7)	NA	NA
	Vaginal	Asymptomatic	98.2 (56/57) (90.6, 100.0)	NA	NA	99.5 (390/392) (98.2, 99.9)	NA	NA
	Vaginal	Total	96.5 (111/115) (91.3, 99.0)	NA	NA	99.2 (871/878) (98.4, 99.7)	NA	NA
	Female urine	Symptomatic	91.4 (53/58) (81.0, 97.1)	83.6 (51/61) (71.9, 91.8)	94.9 (56/59) (85.9, 98.9)	99.0 (480/485) (97.6, 99.7)	99.6 (478/480) (98.5, 99.9)	98.3 (476/484) (96.8, 99.3)
	Female urine	Asymptomatic	94.7 (54/57) (85.4, 98.9)	89.8 (53/59) (79.2, 96.2)	98.2 (55/56) (90.4, 100.0)	99.5 (391/393) (98.2, 99.9)	99.7 (384/385) (98.6, 100.0)	99.5 (392/394) (98.2, 99.9)
	Female urine	Total	93.0 (107/115) (86.8, 96.9)	86.7 (104/120) (79.3, 92.2)	96.5 (111/115) (91.3, 99.0)	99.2 (871/878) (98.4, 99.7)	99.7 (862/865) (99.0, 99.9)	98.9 (868/878) (97.9, 99.5)
	Female neat urine†	Symptomatic	93.1 (54/58) (83.3, 98.1)	NA	NA	99.0 (480/485) (97.6, 99.7)	NA	NA
	Female neat urine	Asymptomatic	93.0 (53/57) (83.0, 98.1)	NA	NA	100.0 (393/393) (99.1, 100.0)	NA	NA
	Female neat urine	Total	93.0 (107/115) (86.8, 96.9)	NA	NA	99.4 (873/878) (98.7, 99.8)	NA	NA
	All types	Symptomatic	92.2 (214/232) (88.0, 95.3)	81.3 (100/123) (73.3, 87.8)	91.5 (108/118) (85.0, 95.9)	98.9 (1919/1941) (98.3, 99.3)	99.5 (955/960) (98.8, 99.8)	98.4 (951/966) (97.5, 99.1)
	All types	Asymptomatic	94.7 (216/228) (91.0, 97.3)	88.1 (104/118) (80.9, 93.4)	95.5 (107/112) (89.9, 98.5)	99.2 (1,559/1,571) (98.7, 99.6)	99.9 (763/764) (99.3, 100.0)	99.2 (781/787) (98.3, 99.7)
	All types	Total	93.5 (430/460) (90.8, 95.6)	84.6 (204/241) (79.5, 89.0)	93.5 (215/230) (89.5, 96.3)	99.0 (3,478/3,512) (98.6, 99.3)	99.7 (1,718/1,724) (99.2, 99.9)	98.8 (1,732/1,753) (98.2, 99.3)
Male specimens	Urethral	Symptomatic	93.9 (62/66) (85.2, 98.3)	86.9 (53/61) (75.8, 94.2)	95.0 (57/60) (86.1, 99.0)	97.9 (187/191) (94.7, 99.4)	100.0 (177/177) (97.9, 100.0)	97.7 (168/172) (94.2, 99.4)
	Urethral	Asymptomatic	88.6 (31/35) (73.3, 96.8)	86.1 (31/36) (70.5, 95.3)	90.9 (30/33) (75.7, 98.1)	98.9 (178/180) (96.0, 99.9)	98.9 (173/175) (95.9, 99.9)	98.8 (166/168) (95.8, 99.9)
	Urethral	Total	92.1 (93/101) (85.0, 96.5)	86.6 (84/97) (78.2, 92.7)	93.5 (87/93) (86.5, 97.6)	98.4 (365/371) (96.5, 99.4)	99.4 (350/352) (98.0, 99.9)	98.2 (334/340) (96.2, 99.3)
	Male urine	Symptomatic	97.0 (64/66) (89.5, 99.6)	94.0 (63/67) (85.4, 98.3)	98.5 (64/65) (91.7, 100.0)	97.4 (186/191) (94.0, 99.1)	99.5 (188/189) (97.1, 100.0)	97.9 (188/192) (94.8, 99.4)
	Male urine	Asymptomatic	100.0 (35/35) (90.0, 100.0)	97.2 (35/36) (85.5, 99.9)	97.2 (35/36) (85.5, 99.9)	98.9 (178/180) (96.0, 99.9)	99.4 (173/174) (96.8, 100.0)	100.0 (179/179) (98.0, 100.0)
	Male urine	Total	98.0 (99/101) (93.0, 99.8)	95.1 (98/103) (89.0, 98.4)	98.0 (99/101) (93.0, 99.8)	98.1 (364/371) (96.2, 99.2)	99.4 (361/363) (98.0, 99.9)	98.9 (367/371) (97.3, 99.7)
	Male neat urine†	Symptomatic	97.0 (64/66) (89.5, 99.6)	NA	NA	99.5 (190/191) (97.1, 100.0)	NA	NA
	Male neat urine	Asymptomatic	100.0 (35/35) (90.0, 100.0)	NA	NA	98.9 (178/180) (96.0, 99.9)	NA	NA
	Male neat urine	Total	98.0 (99/101) (93.0, 99.8)	NA	NA	99.2 (368/371) (97.7, 99.8)	NA	NA
	All types	Symptomatic	96.0 (190/198) (92.2, 98.2)	90.6 (116/128) (84.2, 95.1)	96.8 (121/125) (92.0, 99.1)	98.3 (563/573) (96.8, 99.2)	99.7 (365/366) (98.5, 100.0)	97.8 (356/364) (95.7, 99.0)
	All types	Asymptomatic	96.2 (101/105) (90.5, 99.0)	91.7 (66/72) (82.7, 96.9)	94.2 (65/69) (85.8, 98.4)	98.9 (534/540) (97.6, 99.6)	99.1 (346/349) (97.5, 99.8)	99.4 (345/347) (97.9, 99.9)
	All types	Total	96.0 (291/303) (93.2, 97.9)	91.0 (182/200) (86.1, 94.6)	95.9 (186/194) (92.0, 98.2)	98.6 (1,097/1,113) (97.7, 99.2)	99.4 (711/715) (98.6, 99.8)	98.6 (701/711) (97.4, 99.3)

In this table, each assay was evaluated compared with a patient infection standard (PIS) based on the other two assays. The CTQ assay on the Viper was compared with positives identified by the BD ProbeTec (PT) assay and the Gen-Probe AC2 (AC2) assay, PT was compared with a combination of CTQ and AC2 and AC2 was compared with CTQ and PT. For cells with NA, the sample type listed was not tested in the assay under evaluation.

*No comparator test results were available for vaginal specimens.

†All comparator urine samples were placed in manufacturer's urine transport tube: this was the only urine sample tested without a urine preservative.

CTQ indicates BD ProbeTec *Chlamydia trachomatis* Q^x.

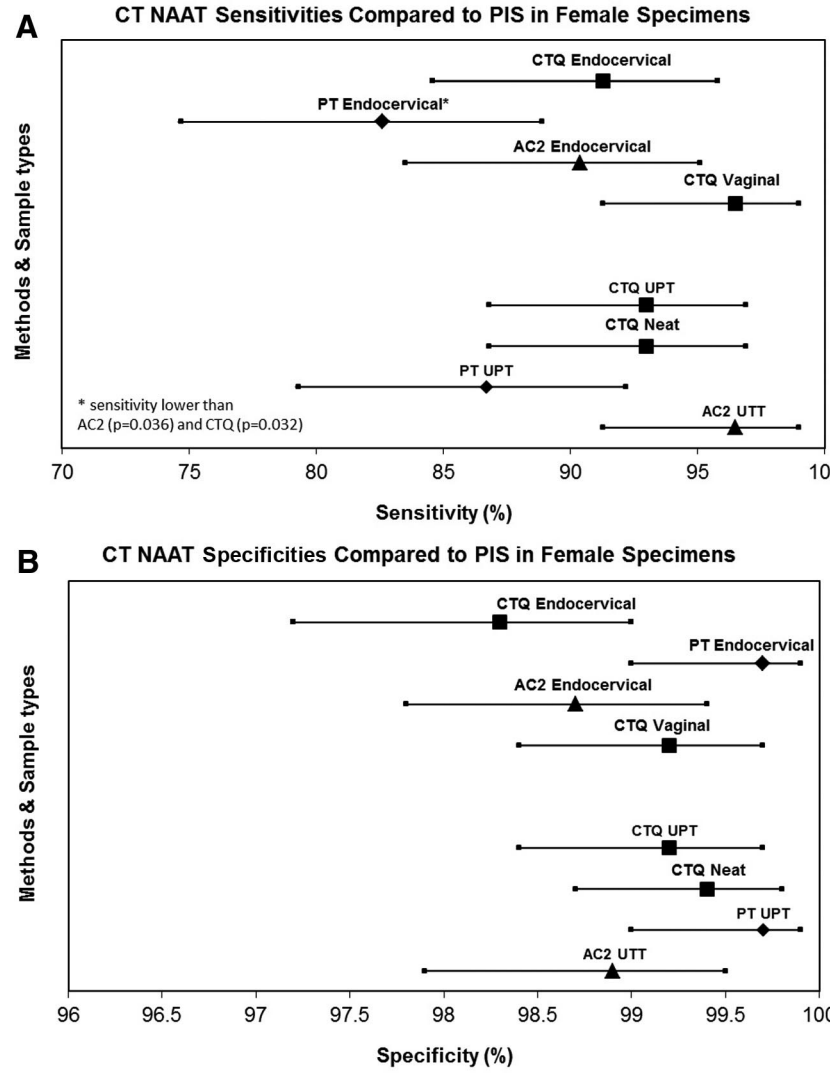

Figure 1. Comparative performance of all assays using female specimens. AC2 UTT is the urine sample in the AC2 transport medium. A, Sensitivity estimate based on rotating standard with 95% CI and (B) specificity estimates with 95% CI.

clinician-collected female endocervical, self-collected vaginal and male urethral swab specimens, and male and female UPT and neat urine specimens were compared with a PIS determined by testing specimens with the AC2 and PT reference assays. In this evaluation, the performance of the CTQ assay was excellent, with sensitivities ranging from 91.3% to 98.0% across all specimen types for both men and women and specificities ranging from 98.3% to 99.4%. The performance characteristics were similar to, or better than, those for the comparator assays (Figs. 1, 2). Performance of the CTQ assay was not affected by the presence or absence of symptoms in the study subjects, suggesting that the test is useful for routine screening populations.

It is worth noting that despite inclusion of FP and OB/GYN clinics, the prevalence of CT was quite high across all study sites. It is unclear if this is a function of self-selection bias in clients who were willing to participate in a study that collected multiple samples, or if the prevalences are truly representative of the clinics' population. In either case, because all of the assays performed equivalently, and the PT and AC2 assays, have been evaluated in lower prevalence populations, we expect that the CTQ assay performs equally well in those settings.

A strength of this study was the use of 2 reference nucleic acid assays, each with a different amplification target from the CTQ assay to classify study subjects as infected or not. Requiring that each comparator assay be positive from at least 1 specimen provides a potentially conservative infected patient standard.[1,2,5] For example, this approach results in modestly higher sensitivity estimates and lower specificity estimates than does an infected patient standard that requires only that any 2 of 4 tests be positive.[8] Our observations concurred with these expectations. Although we did not see a significant decrease in sensitivity with alternate PIS definitions, we did note a significant increase in specificity. This, in conjunction with the similarity of performance of the reference assays, suggests that our performance estimates are robust.

Findings from this study also revealed that neat urine performed as well as UPT-treated urine using the CTQ assay, and there was no effect of urine volume on performance. These are important findings as they simplify urine specimen collection testing on the BD Viper platform, as simple urine samples

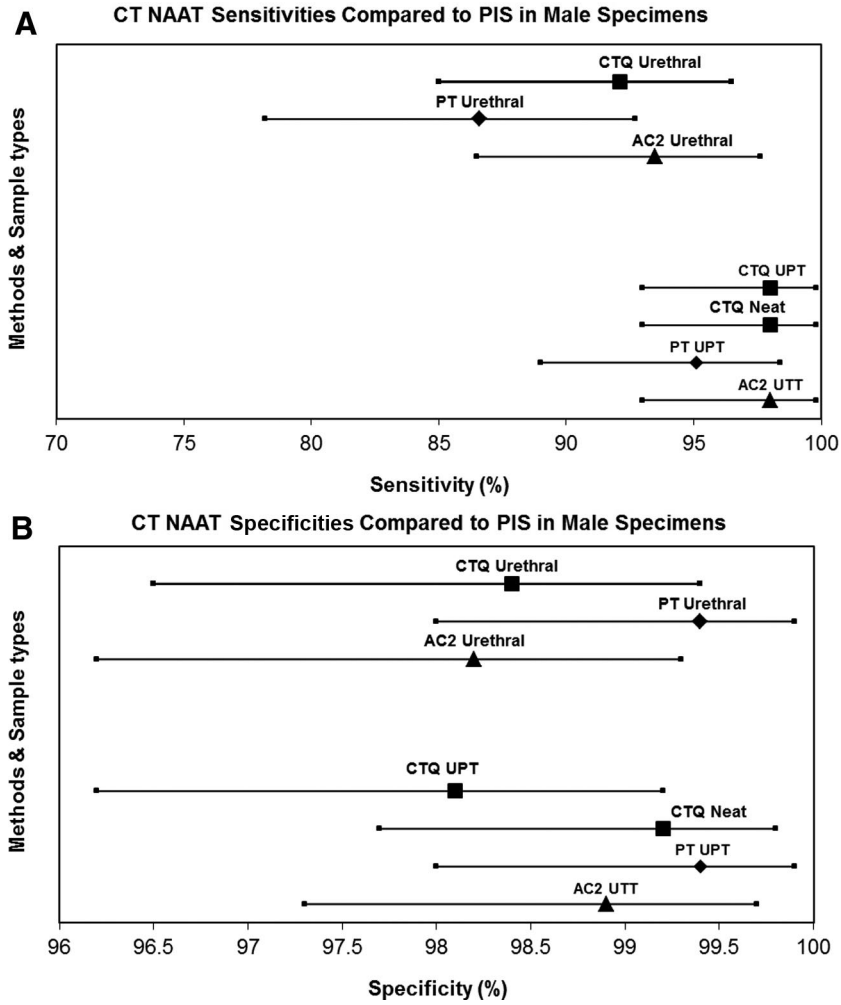

Figure 2. Comparative performance of all assays using male specimens. AC2 UTT is the urine sample in the AC2 transport medium. A, Sensitivity estimate based on rotating standard with 95% CI and (B) specificity estimates with 95% CI.

can be used without the necessity for a preservative and a urine-processing tube and there are no limitations on the urine volume collected. These factors continue to make urine an extremely convenient sample for the screening and detection of CT. Although there have been reports of lower sensitivity in samples with higher urine volume,[9] this was likely not observed in this study because the effect of urine volume should have affected all assays equally. The urine volumes measured in this study reflected those typically obtained in clinical settings.

For female participants, apparent localization of infection to the endocervix or the urethra has been reported in the literature and was also noted in this study.[10–13] The main effect of this is to modestly lower sensitivity estimates for the test endocervical swab when the infected patient is positive only in the urine and vice versa, when the infected patient is positive only in the endocervix. The percent agreement when restricting analysis to a single specimen type suggests that the assay performance is high for both endocervical and urine samples. The notion that negative result from 1 site denotes no infection cannot always be true.

Although the goal of any diagnostic assay is to detect all infected patients, our ability to do so is affected by site-specific infections. Vaginal swabs routinely perform at least as well as endocervical or urine samples, and often provide slightly better sensitivity as noted in this evaluation (Table 1). These data support previous studies of other NAATs using vaginal swabs that have demonstrated excellent performance.[14–18] For this protocol, the vaginal swabs were all self-obtained by the participants, thus demonstrating not only the excellent performance and acceptability of the sample type itself, but also the reliable quality of self-obtained samples. These findings confirm earlier reports suggesting that this is an excellent sample type.[17–20] This is an important finding from a public health perspective. This is consistent with recommendations that vaginal swabs should be the specimen type of choice for routine chlamydial screening.[21]

NAATs continue to be the most sensitive method of detecting CT and have become the standard of care in most screening and clinical settings. Performance assessments of the AC2 and PT assays have been published.[2,5] The overall sensitivity of the CTQ assay in this study was 94.5% (95% CI, 92.6%–96.0%) and the specificity was 98.9% (95% CI, 98.6%–99.2%). Thus, this next generation assay performs comparably with other currently available assays and can be recommended for use with vaginal, endocervical, and urethral swabs, and UPT and neat urine specimens from men and women.

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