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PERFORMANCE OF THE COBAS CT/NG TEST COMPARED TO THE APTIMA AC2 AND VIPER CTQ/GCQ ASSAYS FOR DETECTION OF *CHLAMYDIA TRACHOMATIS* AND *NEISSERIA GONORRHOEAE*

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ABSTRACT

The next generation amplification test for CT/NG (Roche cobas 4800), a fully automated system, was compared head-to-head using female samples with Gen-Probe Aptima Combo 2 and BD ProbeTec using Viper. Endocervical swabs, female urine and endocervical samples in liquid-based cytology medium were run on at least two of three platforms. A total of 4,316 samples were evaluated and 281 chlamydial and 69 gonococcal infections were identified. Estimates of sensitivity and specificity were obtained in comparison with the patient infection standard (PIS) and using latent class analysis (LCA). Chlamydia sensitivity estimates ranged from 86.9-95.6% using PIS and 97.6-98% using LCA. Specificity was $\geq$99.6% for all sample types. Sensitivity ranged from 95.6-100% using PIS and 96.9-100% using LCA for detection of gonococcal infections. Specificity for gonococcal infections was $\geq$99.8%. The cobas 4800 performance was equivalent to the comparator assays (all p-values>0.05) and the fully automated system provides high lab efficiency.

INTRODUCTION

Chlamydia trachomatis (CT) and *Neisseria gonorrhoeae* (NG) remain highly prevalent sexually transmitted infections (STI), control of which is a high priority for the Centers for Disease Control and Prevention (CDC)(5). The CDC STD Laboratory Diagnosis Guidelines recommend screening using highly sensitive and specific nucleic acid amplification assays (NAATs) (4) confirming that this class of testing is now considered the standard for diagnosis of these infections. NAATs have been commercially available for the last two decades and evidence of their performance characteristics is widely available (6, 7, 10-17). As chlamydia and gonorrhea screening programs continue to expand to meet the health care coverage benchmarks described by the US Preventive Task Force (USPTF), the CDC (5) and the Healthcare Effectiveness Data and Information Set (HEDIS), clinical diagnostic laboratories will experience increased volumes. In anticipation of this trend, diagnostic manufacturers have begun to develop next-generation fully automated platforms for CT/NG assays to increase specimen throughput and meet testing demands. Evaluation of these new assays provides critical information to laboratories considering moving into this field of diagnostics or considering an evidence-based platform change.

The **cobas**® CT/NG Test (Roche Molecular Systems, Pleasanton, CA) [c4800] is a new CT/NG assay that is designed for use in clinical laboratories. We compared the performance of this next-generation assay to two commercially available assays: Aptima Combo AC2 (Gen-Probe, San Diego, CA) [AC2] and BD Viper ProbeTec CT/GC Q^x Amplified DNA Assay (BD Diagnostics, Sparks, MD) [CTQ/GCQ] using the sample

types routinely collected from women in a variety of screening settings. Analyses were performed using multiple statistical methods in order to provide robust estimates of performance for each of the three assays.

METHODS

The female screening trial for CT/NG was a multicenter evaluation of the **cobas**® specimen collection kit and the c4800 performed on the **cobas**® 4800 system. Two FDA-cleared NAATs, AC2 and CTQ/GCQ, were used as comparator assays. The specimen collection sites were geographically diverse and included obstetrics-gynecology practices, family planning clinics, and STD clinics. Four sites performed all testing on the **cobas**® 4800 system. Results obtained in the female study population are described here; results obtained in the male population are described elsewhere (Taylor et al., in press).

Patient population

Inclusion criteria were 1) being at least14 years of age and 2) being eligible for routine CT/NG screening according to the routine practices at each enrollment site. Individuals were excluded from enrollment if any of the following criteria were met: i) previously enrolled in the study, ii) use of antimicrobial agents active against CT or NG during the preceding 21 days, iii) use of Replens, vaginal lubricant (Lil'Drug Store Products, Inc., Cedar Rapids, IA) within the previous 3 days, iv) history of hysterectomy or v) contraindication to Pap Test/cervical sampling. Participants were classified as symptomatic if they reported dysuria/pain during urination, coital

pain/difficulty/bleeding, discharge, or pelvic pain, abnormal vaginal discharge, or pelvic/uterine/ovarian pain. All other participants were classified as asymptomatic.

Specimen collection

From each female participant, specimens were collected in the following order: first-catch urine; a single vaginal swab (results described elsewhere; manuscript in preparation); 3 endocervical swabs using each manufacturer's sample collection device (in randomized order), and a sample suitable for liquid-based cytology (LBC) placed into PreservCyt medium (Cytyc Corp., Marlborough, MS). In cases where the LBC specimen was requested for Pap testing for routine patient care, the LBC specimen collection was performed prior to any other swab sampling. All urine specimens were divided into three aliquots and placed into each assay's transport tube. LBC samples were aliquoted into c4800 sample tubes and AC2 transport tubes prior to processing for Pap testing (prequot LBC). Following Pap processing in the laboratory, residual specimens were aliquoted into c4800 sample tubes (postquot LBC). These specimens were handles by cytology staff as routine specimens and no extra procedures were implemented to attempt to reduce cross-contamination. All specimens for comparator assays were stored and tested according to each manufacturer's package insert instructions.

cobas® CT/NG Test

The c4800 uses a dual target approach: CT primers CP102 and CP103 are used to target a sequence of approximately 206 nucleotides within the cryptic plasmid DNA of *C.*

105 *trachomatis*. In addition, CT primers CTMP101 and CTMP102 target a sequence of
106 approximately 182 nucleotides within the chromosomal DNA of *C. trachomatis*. NG
107 primers NG514 and NG519 target a sequence of approximately 190 nucleotides from a
108 highly conserved direct repeat region of *N. gonorrhoeae* called DR-9. In addition, another
109 set of NG primers, NG552 and NG579, target a second sequence of approximately 215
110 nucleotides identified as a conserved sequence variant from this region. DNA extracted from
111 samples using magnetic bead purification is added to the amplification mixture in a
112 multi-well plate, in which PCR amplification occurs fully automated. The CT/NG
113 Internal Control (IC) is a combination of two non-infectious recombinant plasmid DNAs,
114 each with primer binding regions identical to those of either the *C. trachomatis* or the *N.*
115 *gonorrhoeae* genomic target sequences. Both recombinant plasmid DNAs have an identical
116 randomized internal target sequence, and a unique probe binding region that differentiates the
117 IC from target amplicon to ensure independent amplification of both the IC and the *C.*
118 *trachomatis* and *N. gonorrhoeae* target DNAs. The IC is included in the c4800 and is
119 introduced into each sample on the **cobas**® 4800 system during sample processing prior to
120 lysis incubation. Selective amplification of target nucleic acid from the specimen is achieved in
121 the c4800 by the use of AmpErase (uracil-N-glycosylase) enzyme and deoxyuridine
122 triphosphate (dUTP). The c4800 utilizes real-time PCR technology. The use of dual
123 TaqMan probes labeled with FAM and BHQ for the detection of CT and HEX and BHQ
124 for the detection of NG provides for real-time detection of PCR product accumulation by
125 monitoring the emission intensity of fluorescent dyes released during the amplification
126 process. The amplification of CT targets, NG targets and the IC are measured
127 independently and at different wavelengths. This process is repeated for a designated

number of cycles, each cycle increasing the emission intensity of the individual reporter dyes.

Patient infected status for C. trachomatis and N. gonorrhoeae

For analyses patient infection status was defined as follows: participants were designated as being infected with CT or NG if at least 2 NAATs with different target regions gave positive results in the endocervical swab and/or the urine specimen. LBC results were not included in the calculation of the PIS since only one comparator test result was available. LBC results were compared to the PIS that was calculated based on urine and endocervical swab results from all three assays. For example, when LBC performance on the c4800 was assessed, results were compared to the other two NAAT results from urine and endocervical swabs. Additionally, localized infection could occur (e.g. a woman may have had multiple positive urine results and all negative endocervical swab results). In these cases, for analyses of urine performance the participant would have been classified as infected while in analyses of endocervical sample performance, including LBC, the participant would have been classified as uninfected. The reverse (all endocervical samples positive and all urine samples negative) was possible as well. Thus, each NAAT was evaluated using a PIS constructed based on results obtained in the two other NAATs used in the study (rotating PIS) It is worth noting that this differs from a strictly head-to-head comparison since a single positive sample by any comparator assay would be sufficient to categorize a participant as infected at that body site assuming there were other body sites positive by the other comparator. As an example, if a woman had a positive endocervical sample only by AC2 and only a positive urine sample by CTQ,

151 according to the PIS this participant would have been categorized as infected in both the
152 endocervical and urine analyses. Thus, the PIS maximized the number of infections
153 identified while taking into account the possibility of site-specific infections.
154

155 ***Statistical analysis***

156 The statistical analyses were chosen based on recommendations in the statistical guidance
157 from FDA (9) and in accordance with the guidelines published in Clinical and Laboratory
158 Standards Institute EP12-A2 (8) for evaluating qualitative test performance. Sensitivity
159 and specificity of c4800 were calculated separately for detection of CT and NG by using
160 the rotating PIS as the reference standard. The corresponding 2-sided 95% score (Wilson)
161 confidence intervals (CIs) were also estimated. Venn diagrams were plotted, separately
162 by sample type, to compare the list of matched clinical specimens (i.e., same sample type
163 for a given subject) tested positive from at least 1 NAAT. Fisher exact test was used to
164 assess the statistical significant difference in performance estimates between
165 symptomatic and asymptomatic groups. Latent class analysis (LCA) was also used to
166 estimate the sensitivity and specificity of each NAAT (1). LCA identifies underlying
167 characteristics, in this case infected and uninfected status as classes, and may be less
168 biased than the PIS which is strongly influenced by the test with the poorest performance.
169 To characterize the predictive values of positive results obtained with the c4800 by
170 varying the prevalence of CT and NG, the hypothetical estimates of positive predictive
171 value (PPV) was calculated. All analyses were performed using SAS/STAT® software
172 (SAS, Cary, NC) with α set to 0.05.
173

RESULTS

4479 women enrolled in the study. Of these, 17 were excluded because they did not meet the inclusion criteria or did not provide appropriate consent; 146 were considered non-evaluable because of errors in specimen collection, transport, and storage (Suppl. Figure 1). 4,316 (96.4%) female subjects were evaluable for CT and/or NG analyses. Patient characteristics are shown in Table 1. Among evaluable subjects, 355 and 351specimens were classified as non-evaluable respectively for the CT and NG primary analyses for a particular specimen type either because a specimen was not available for testing or because a specimen was repeatedly inhibitory (IC failure). Invalid results due to IC failure were observed in 1.12% and 0.02% of female urine and postquot LBC samples, respectively. There were no IC failures for the remaining sample types. These rates of inhibition are quite low and in the range of, or below the rates reported for some assays. The AC2 system does not utilize an assay control and therefore there are no data available on the frequency of occurrence of inhibition. AC2 failure rates ranged between 0.28 and 0.87% across all specimen types. Failure rates for the CTQ/GCQ assay ranged between 0.12 and 0.65% across all specimen types.

Start-up and daily maintenance time for the cobas 4800 was less than 30 minutes and total hands-on time for testing from start to finish was less than 40 minutes for 96 samples. The time to results was slightly less than 4 hours. Three runs of 96 samples could be performed in a single 9-hour shift with the results for the final set of 96 specimens available the next day.

197 ***C. trachomatis***

198 A total of 281 (6.5%) females were infected with CT based on the PIS. Symptoms were
199 reported in 59.4% (167/281) of infected women. Sensitivity and specificity of the c4800,
200 the AC2, and CTQ/GCQ assays for CT in females using the rotating PIS are presented by
201 sample type and symptom status in Table 2. Sensitivity of c4800 ranged from 86.9 -
202 95.6% and was similar by symptom status (all p-values >0.05). Regardless of symptom
203 status, specificity for CT was high, ranging from 99.6%-100.0% across all sample types.
204 Specificity did not differ significantly between the three molecular assays (all p-
205 values>0.05) with the exception of a significantly higher specificity of the c4800 and
206 QCT compared to AC2 in endocervical swabs in asymptomatic and symptomatic
207 individuals (overall p=0.0005).
208
209 Venn diagrams comparing all positive results, regardless of the final definition of
210 infection status, for CT across the three assays in endocervical swabs, liquid based
211 cytology, and urines obtained from female subjects are shown in Figure 1.
212 LCA estimated the sensitivity of c4800, AC2 and CTQ/GCQ to be 95.9, 98.3 and 98.8%,
213 respectively for detection of CT in endocervical swabs. For urine samples the LCA
214 estimates of sensitivity were 97.6, 98.4 and 98.0%. LCA estimated the specificity of all
215 three assays to be $\geq$ 99.3% regardless of sample type.
216

217 ***N. gonorrhoeae***

218 A total of 69 (1.6%) women were infected with NG and symptoms were reported in
219 66.7% (46/69) of these women. Sensitivity and specificity of c4800, AC2, and

CTQ/GCQ assays for NG in females using the rotating PIS are presented by sample type and symptom status in Table 3. Sensitivity in this analysis ranged from 95.6%-100.0%. Overall specificity was high, ranging from 99.8%-100.0% across all sample types. Performance characteristics did not differ significantly between the three molecular assays based on symptom status, order of sampling, clinic type, and PAP smear collection device (all p-values>0.05) with the exception of a significantly higher specificity of the c4800 and AC2 compared to CTQ/GCQ in endocervical swabs (overall p=0.021).

Venn diagrams comparing positive results for NG across the three assays in endocervical swabs, liquid based cytology, and urines are shown in Figure 2. LCA estimates of sensitivity were 98.5, 100 and 97.0% for the c4800, AC2 and CTQ/GCQ assays, respectively in endocervical samples. Specificity was estimated to be $\geq$ 99.7% for all assays. For urine samples the LCA-estimate sensitivity for NG was 100, 96.9 and 100% for c4800, AC2 and CTQ/GCQ, respectively with all specificity estimates >99.9%.

Positive predictive values

Hypothetical PPV of the cobas 4800 using genital samples for the detection of CT and NG were calculated at prevalence rates ranging from 1-50%, and are presented in Figure 3. For CT, the hypothetical PPV ranged from 77.3%-99.7% at prevalence rates ranging from 1%-50%. The corresponding ranges for NG were from 93.8%-99.9%.

DISCUSSION

The c4800 performance was equivalent to other currently available FDA-cleared assays for detection of chlamydia and gonorrhea infections in women. While the estimates of sensitivity for CT were consistently, but not significantly, lower than the estimates obtained for AC2 when comparing to the PIS, the LCA analysis showed high similarity in the performance. This is consistent with the broad confidence intervals for all estimates. Additionally, the specificity of the assay was consistently high with the c4800 assay. For CT, the specificity of the c4800 and QCT assays with endocervical swabs was higher than that of AC2, while for GC the QGC assay showed lower specificity. These statistically significant differences are likely to be the result of very high sample size of negative test results and not clinically meaningful.

The performance was not affected by presence or absence of symptoms making this a useful assay for both screening and diagnosis which is consistent with the CDC recommendations. Further, the system is suitable for use in a routine clinical laboratory because of the limited hands on-requirements, relatively rapid time to results, and throughput of approximately 388 samples per 9-hour shift. Additionally, the **cobas**® 4800 system can also perform high-risk human papilloma virus testing (3) thus, expanding the menu of the platform and increasing utility in the laboratory.

Use of dual targets for *C. trachomatis* ensures that the assay can detect variant chlamydial strains such as those describe in Sweden that contain a mutation in the cryptic plasmid (2). The c4800 assay has been evaluated against a large number of clinical

264 isolates including new variant strains and performs well. Including dual targets for *N.*
265 *gonorrhoeae* has also become the standard in next generation NAATs. The NG
266 specificity for this assay was $\geq$ 99.8%.
267
268 Since few laboratories have access to multiple platforms, head-to-head comparison data
269 may be difficult to obtain. Thus, reports of these findings are particularly relevant to
270 today's laboratory management needs. Head-to-head analysis of the positive results,
271 shown in Figures 1 and 2 provides an unbiased observation of the performance of all
272 three assays without assigning true- and false-positive status. Furthermore, we applied
273 LCA as a statistical technique for determining two features of a data set: first, the number
274 of classes which account for the values in the data, and secondly, the probability that a
275 result maps to one of those classes. Although a PIS attempts to classify individuals as
276 infected or uninfected, if a single assay has substantially higher sensitivity, this could be
277 misrepresented as lower specificity since no other assay will have matching positive
278 results for a small number of cases. LCA does not arbitrarily assign a true or false
279 infection status to results based on arbitrary predefined infection standard in the manner
280 that a PIS is developed and is therefore considered to provide a less biased estimate of
281 performance of new diagnostic assays. The LCA is not a rolling patient standard in that
282 it is performed only for those samples for which head-to-head results are available. It
283 moves beyond a head-to-head analysis such as a chi-square since LCA determines the
284 number of classes described by the data. For the data generated by this study, LCA
285 determined that the optimal number of classes was two (infected and uninfected) and
286 estimated high sensitivity and specificity for all three assays for both pathogens. Had

there been three classes identified by analysis, this would have been an indication of poor discriminatory ability, but such was not the case with these data. LCA is not designed for hypothesis testing and therefore cannot estimate differences in performance among the three methods, if any exist. Thus, the use of the PIS for comparison purposes is warranted. Use of multiple analytic approaches is a strength of the data presented here.

A limitation of the study was the relatively high prevalence of chlamydial infection in all study populations compared to the general population. We provided the estimated PPV of the c4800 tests in hypothetical populations of varying prevalence in order to provide a robust approximation of the performance in lower prevalence settings. The prevalence of gonococcal infection was lower and therefore was not affected by this limitation.

The prequot and postquot LBC performance was equivalent thus allowing this sample type to be used in any setting that handles LBC whether the samples require preparation for Pap testing before other testing or not. Appropriate caution in handling LBC samples prior to preparation for cytologic exam must be exercised in order to maintain the integrity of the specimens.

In conclusion, in both symptomatic and asymptomatic patients, the **cobas** 4800 CT/NG test offers high sensitivity, specificity, and positive predictive values for the direct, qualitative detection of CT and NG using endocervical swabs, urine specimens, and liquid-based cytology specimens collected in PreservCyt solution.

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PREVIOUS PRESENTATIONS

These data were presented in part at the following professional meetings: European Congress for Clinical Microbiology and Infectious Diseases Annual Meeting, Milan 2011, American Society for Microbiology Annual Meeting, New Orleans 2011, International Society for STD Research, Biennial Meeting, Quebec City 2011.

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412 **Figure legends:**

413

414 **Figure 1**. Venn diagrams comparing CT positive results across three assays in
415 endocervical swabs (A), liquid based cytology (B), and urines (C) obtained from female
416 subjects. 4223 (endocervical swabs), 4186 (liquid based cytology), and 4254 (urines)
417 subjects had all 3 valid results from c4800, AC2, and CT/GCQ assays
418

419 **Figure 2**. Venn diagrams comparing NG positive results across three assays in
420 endocervical swabs (A), liquid based cytology (B), and urines (C) obtained from female
421 subjects. 4221 (endocervical swabs), 4187 (liquid based cytology, and 4255 (urines)
422 subjects had all 3 valid results from c4800, AC2, and CT/GCQ assays
423

424 **Figure 3.** Hypothetical PPV based on Varying Population Prevalence for A) CT and B)
425 NG Results
426
427
428

429

Figure 1. Positive chlamydia results obtained by three platforms

A **B** **C**

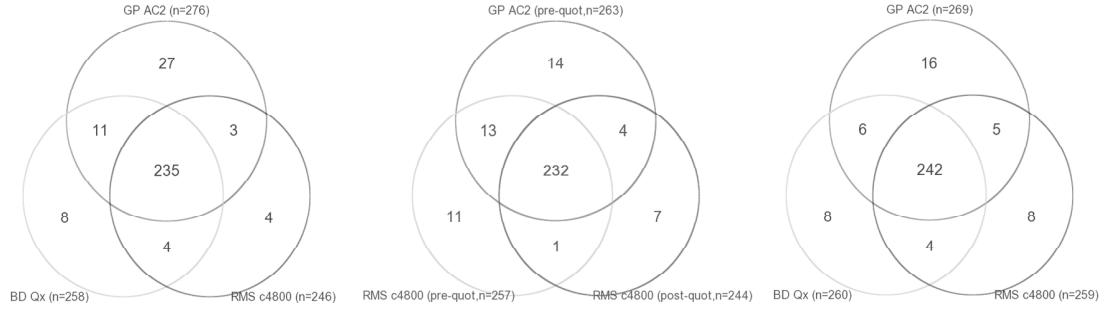

Figure 1. Venn diagrams comparing CT positive results across three assays in endocervical swabs (A), liquid based cytology (B), and urines (C) obtained from female subjects. 4223 (endocervical swabs), 4186 (liquid based cytology), and 4254 (urines) subjects had all 3 valid results from c4800, AC2, and CT/GCQ assays.

Figure 2. Positive gonorrhea results obtained by three platforms

A **B** **C**

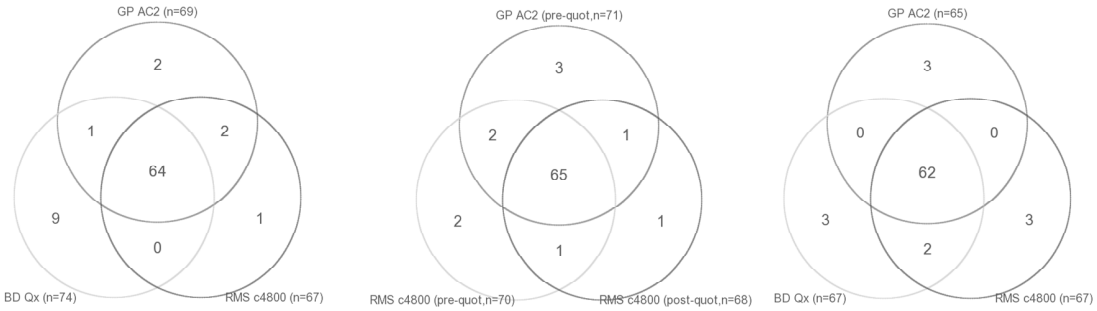

Figure 2. Venn diagrams comparing NG positive results across three assays in endocervical swabs (A), liquid based cytology (B), and urines (C) obtained from female subjects. 4221 (endocervical swabs), 4187 (liquid based cytology, and 4255 (urines) subjects had all 3 valid results from c4800, AC2, and CT/GCQ assays.

Figure 3A

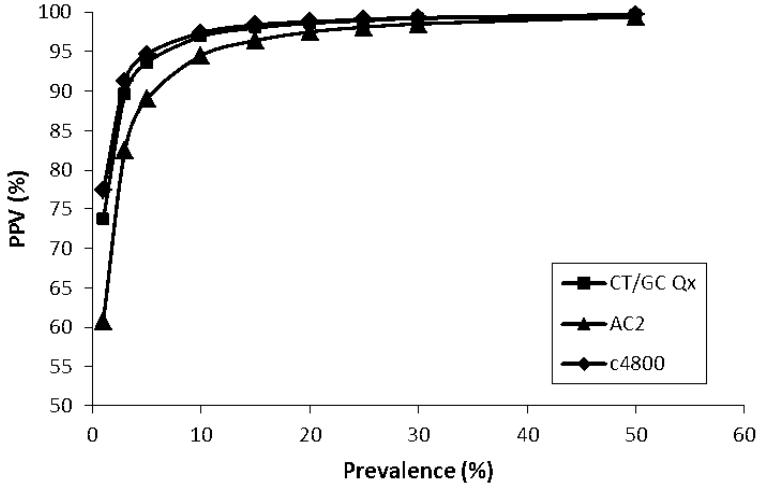

Figure 3A

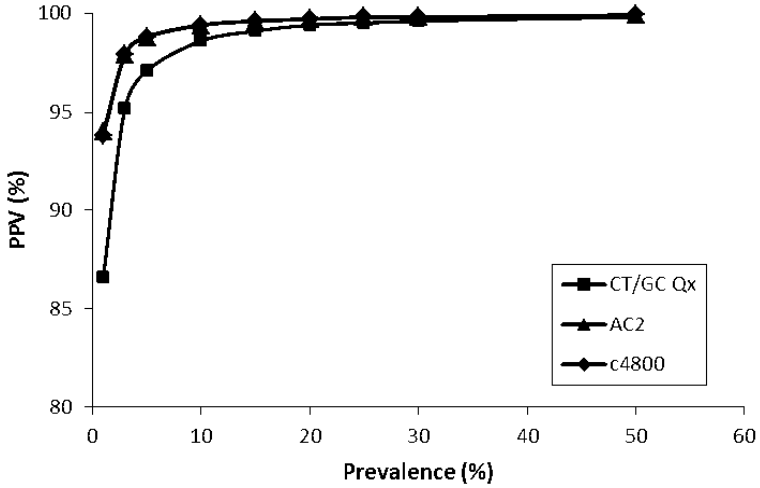

Table 1. Patient Characteristics and Disease Prevalence

Characteristic	Number of participants	CT Prevalence [CI][a]	NG Prevalence [CI][a]
Age (median)	25	--	--
Race[b]			
Black	1860 (43.1%)	9.4% [8.1%, 10.8%]	2.9% [2.2%, 3.7%]
White	2089 (48.4%)	3.4% [2.7%, 4.2%]	0.7% [0.4%, 1.1%]
Asian/Pacific Islander	122 (2.8%)	9.8% [5.7%, 16.4%]	0.8% [0.1%, 4.5%]
Other	245 (5.7%)	6.5% [4.1%, 10.3%]	0.4% [0.1%, 2.3%]
Hispanic Ethnicity	955 (22.1%)	4.7% [3.5%, 6.3%]	0.4% [0.2%, 1.1%]
Symptomatic[b]			
Yes	2024 (46.9%)	8.0% [6.9%, 9.3%]	2.3% [1.7%, 3.0%]
No	2292 (53.1%)	4.8% [4.0%, 5.8%]	1.0% [0.7%, 1.5%]
Clinic Type[b]			
Family Planning	1762 (40.8%)	7.5% [6.4%, 8.8%]	1.6% [1.1%, 2.3%]
OB/GYN Clinic	1079 (25.0%)	3.0% [2.1%, 4.2%]	0.1% [0.0%, 0.5%]
STD Clinic	1475 (34.2%)	7.3% [6.1%, 8.8%]	2.7% [2.0%, 3.7%]

[a]CI, (score) confidence interval
[b]p-value <0.01, Fisher exact significance test

Table 2. Clinical Performance for CT Detection by Sample Type and Symptom Status Compared to Patient Infected Status[a]

Sample Type	Symptom Status	Sensitivity (95% CI)[b]			Specificity (95% CI)		
		c4800	AC2	CTQ	c4800	AC2	CTQ
Endocervical Swab	Symptomatic	93.0% [146/157] (87.9%, 96.0%)	96.2% [153/159] (92.0%, 98.3%)	94.4% [153/162] (89.8%, 97.0%)	99.7% [1821/1827]* (99.3%, 99.8%)	98.9% [1843/1863] (98.3%, 99.3%)	99.6% [1849/1856]* (99.2%, 99.8%)
	Asymptomatic	89.5% [94/105] (82.2%, 94.0%)	97.1% [101/104] (91.9%, 99.0%)	96.2% [102/106] (90.7%, 98.5%)	100.0% [2163/2164]* (99.7%, 100.0%)	99.5% [2173/2185] (99.0%, 99.7%)	99.7% [2155/2162] (99.3%, 99.8%)
	Overall	91.6% [240/262] (87.6%, 94.4%)	96.6% [254/263] (93.6%, 98.2%)	95.1% [255/268] (91.9%, 97.1%)	99.8% [3984/3991]* (99.6%, 99.9%)	99.2% [4016/4048] (98.9%, 99.4%)	99.7% [4004/4018]* (99.4%, 99.8%)
Urine	Symptomatic	94.4% [153/162] (89.8%, 97.0%)	98.1% [152/155] (94.5%, 99.3%)	93.8% [152/162] (89.0%, 96.6%)	99.7% [1832/1838] (99.3%, 99.9%)	99.2% [1848/1862] (98.7%, 99.6%)	99.8% [1854/1857] (99.5%, 99.9%)
	Asymptomatic	89.1% [98/110] (81.9%, 93.6%)	92.5% [98/106] (85.8%, 96.1%)	96.2% [101/105] (90.6%, 98.5%)	99.8% [2165/2169] (99.5%, 99.9%)	99.8% [2181/2186] (99.5%, 99.9%)	99.7% [2161/2167] (99.4%, 99.9%)
	Overall	92.3% [251/272] (88.5%, 94.9%)	95.8% [250/261] (92.6%, 97.6%)	94.8% [253/267] (91.4%, 96.9%)	99.8% [3997/4007] (99.5%, 99.9%)	99.5% [4029/4048] (99.3%, 99.7%)	99.8% [4015/4024] (99.6%, 99.9%)
PreservCyt (Pre-Aliquot)	Symptomatic	95.6% [151/158] (91.1%, 97.8%)	96.8% [152/157] (92.8%, 98.6%)	–	99.7% [1826/1832] (99.3%, 99.8%)	99.6% [1838/1846] (99.1%, 99.8%)	–
	Asymptomatic	88.8% [95/107] (81.4%, 93.5%)	96.0% [97/101] (90.3%, 98.4%)		99.6% [2132/2141] (99.2%, 99.8%)	99.5% [2148/2159] (99.1%, 99.7%)	
	Overall	92.8% [246/265] (89.1%, 95.4%)	96.5% [249/258] (93.5%, 98.2%)		99.6% [3958/3973] (99.4%, 99.8%)	99.5% [3986/4005] (99.3%, 99.7%)	
PreservCyt (Post-Aliquot)	Symptomatic	91.6% [142/155] (86.2%, 95.0%)	–	–	99.7% [1806/1811] (99.4%, 99.9%)	–	–
	Asymptomatic	86.9% [93/107] (79.2%, 92.0%)			99.8% [2124/2129] (99.5%, 99.9%)		
	Overall	89.7% [235/262] (85.4%, 92.8%)			99.7% [3930/3940] (99.5%, 99.9%)		

[a] The patient infected status was constructed based on results obtained in the two other NAATs (rotating PIS using all endocervical swab and urine results, LBC results were not used to calculate the PIS, see Materials and Methods).

[b] CI, (score) confidence interval

*Pairwise comparisons that were statistically significant ($p<.05$); in all cases specificity was higher than estimates for AC2

Table 3. Clinical Performance for NG Detection by Sample Type and Symptom Status Compared to Patient Infected Status[a]

Sample Type	Symptom Status	Sensitivity (95% CI)[b]			Specificity (95% CI)		
		c4800	**AC2**	**GCQ**	**c4800**	**AC2**	**GCQ**
Endocervical Swab	Symptomatic	95.6% [43/45] (85.2%, 98.8%)	100.0% [46/46] (92.3%, 100.0%)	95.7% [45/47] (85.8%, 98.8%)	99.9% [1936/1938] (99.6%, 100.0%)	99.9% [1973/1974] (99.7%, 100.0%)	99.7% [1966/1971] (99.4%, 99.9%)
	Asymptomatic	95.7% [22/23] (79.0%, 99.2%)	100.0% [23/23] (85.7%, 100.0%)	91.3% [21/23] (73.2%, 97.6%)	100.0% [2246/2246] (99.8%, 100.0%)	100.0% [2266/2266] (99.8%, 100.0%)	99.8% [2241/2245] (99.5%, 99.9%)
	Overall	95.6% [65/68] (87.8%, 98.5%)	100.0% [69/69] (94.7%, 100.0%)	94.3% [66/70] (86.2%, 97.8%)	100.0% [4182/4184]* (99.8%, 100.0%)	100.0% [4239/4240]* (99.9%, 100.0%)	99.8% [4207/4216] (99.6%, 99.9%)
Urine	Symptomatic	97.6% [41/42] (87.7%, 99.6%)	97.6% [40/41] (87.4%, 99.6%)	95.3% [41/43] (84.5%, 98.7%)	99.9% [1955/1957] (99.6%, 100.0%)	99.9% [1977/1979] (99.6%, 100.0%)	100.0% [1977/1977] (99.8%, 100.0%)
	Asymptomatic	100.0% [23/23] (85.7%, 100.0%)	95.7% [22/23] (79.0%, 99.2%)	100.0% [23/23] (85.7%, 100.0%)	100.0% [2255/2256] (99.7%, 100.0%)	100.0% [2268/2269] (99.8%, 100.0%)	99.9% [2246/2249] (99.6%, 100.0%)
	Overall	98.5% [64/65] (91.8%, 99.7%)	96.9% [62/64] (89.3%, 99.1%)	97.0% [64/66] (89.6%, 99.2%)	99.9% [4210/4213] (99.8%, 100.0%)	99.9% [4245/4248] (99.8%, 100.0%)	99.9% [4223/4226] (99.8%, 100.0%)
PreservCyt (Pre-Aliquot)	Symptomatic	97.8% [45/46] (88.7%, 99.6%)	95.7% [44/46] (85.5%, 98.8%)	–	99.8% [1942/1945] (99.5%, 99.9%)	99.9% [1955/1957] (99.6%, 100.0%)	–

Sample Type	Symptom Status	Sensitivity (95% CI)[b]			Specificity (95% CI)		
		c4800	AC2	GCQ	c4800	AC2	GCQ
PreservCyt (Post-Aliquot)	Asymptomatic	95.7% [22/23] (79.0%, 99.2%)	100.0% [23/23] (85.7%, 100.0%)		100.0% [2225/2225] (99.8%, 100.0%)	99.9% [2236/2238] (99.7%, 100.0%)	
	Overall	97.1% [67/69] (90.0%, 99.2%)	97.1% [67/69] (90.0%, 99.2%)		99.9% [4167/4170] (99.8%, 100.0%)	99.9% [4191/4195] (99.8%, 100.0%)	
	Symptomatic	95.7% [44/46] (85.5%, 98.8%)			99.9% [1920/1921] (99.7%, 100.0%)		
	Asymptomatic	95.7% [22/23] (79.0%, 99.2%)	–	–	100.0% [2212/2213] (99.7%, 100.0%)	–	–
	Overall	95.7% [66/69] (88.0%, 98.5%)			100.0% [4132/4134] (99.8%, 100.0%)		

[a] The patient infected status was constructed based on results obtained in the two other NAATs (rotating PIS using all endocervical swab and urine results, LBC results were not used to calculate the PIS, see Materials and Methods).
[b] CI, (score) confidence interval
*Pairwise comparisons that were statistically significant (p<.05); in both cases, estimates were higher than those for GCQ.