



Published in final edited form as:

Sex Transm Dis. 2017 July ; 44(7): 398–400. doi:10.1097/OLQ.0000000000000627.

Comparative evaluation of two nucleic acid amplification tests for the detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* at extragenital sites

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Keywords

Neisseria gonorrhoeae; *Chlamydia trachomatis*; extragenital; nucleic acid amplification testing

Chlamydia trachomatis (CT) and *Neisseria gonorrhoeae* (NG) are among the most common sexually transmitted infections (STIs), with over 200 million new infections worldwide in 2012.^{1,2} In the United States (U.S.) in 2015, there were over 1.5 million reported CT cases and nearly 400,000 NG cases.³

CT and NG infections are curable with antibiotics. Routine screening, timely treatment and partner treatment are mainstays of disease control. However, CT and NG infections are frequently asymptomatic and therefore go undetected and untreated if screening tests are not performed.^{4–6} Extragenital sites may be important reservoirs for CT and NG in the population, which can serve to perpetuate the spread of these infections. Among MSM, greater than 70% of extragenital NG infections and over 85% of extragenital CT infections are detected in the absence of urethral infection, warranting routine screening at extragenital sites in addition to urethral screening.⁷

Studies have shown that nucleic acid amplification tests (NAATs) perform better than other testing strategies available for CT and NG detection.^{8–12} The CDC currently recommends NAATs for the detection of urethral, vaginal/cervical, rectal, and pharyngeal CT and NG infection.⁴ However, despite widespread practice, no NAAT platforms are currently FDA-approved for use with extragenital specimens. Clinical Laboratory Improvement Amendments (CLIA)-defined performance specifications have been established by many laboratories to allow for the use of NAATs on extragenital specimens for clinical management. In the literature, laboratory-based NAATs for the detection of CT and NG

infections, such as Aptima Combo 2[®] (Hologic, Inc., San Diego, CA) used as the reference in this study, have been used for extragenital infection identification.^{13,14} Those NAATs require expensive instrumentation, central laboratory processing, and often require days for turn-around of results.

Because point-of-care testing may allow for earlier treatment, newer assays like the Xpert[®] CT/NG assay (Cepheid, Sunnyvale, CA) should be evaluated and compared with other commonly used NAATs for the detection of extragenital infection.¹⁵⁻¹⁷ We evaluated the performance of the Xpert[®] CT/NG assay for use with extragenital specimens at a sexual health clinic in Hollywood, CA.

Study population

A convenience sample of men seeking routine sexual health testing were recruited by trained staff at the AIDS Healthcare Foundation (AHF) Wellness Clinic in Hollywood, California, between September 2015 and November 2016. Potential participants were informed about the risks, benefits and alternatives of study participation.

Specimen collection

Following the clinic standard of care, participants self-collected rectal swabs and trained staff collected pharyngeal swabs using the manufacturers' specimen collection kits (Xpert CT/NG Swab Collection Kits [Cepheid, Sunnyvale, CA] and Aptima Unisex Swab Specimen Collection Kits [Hologic, Inc., San Diego, CA]). For rectal specimen collection, participants were instructed to insert the specimen collection swab into the anal canal and rotate for 15–30 seconds. For pharyngeal specimen collection, the specimen collection swab was inserted into the mouth without touching the lips, teeth, tongue, or cheeks, and the tonsillar area was swabbed from side to side gently and quickly. Extragenital specimens were stored until tested according to manufacturer directions at 2–30 degrees Celsius.

One specimen from each anatomic site was tested at the AHF laboratory using Xpert CT/NG[®] assay on the GeneXpert[®] instrument (Cepheid, Sunnyvale, CA). The first swabs from each anatomic site collected were transported for testing using the Aptima Combo 2[®] assay (Hologic, Inc., San Diego, CA) on the Panther platform at the Los Angeles County Public Health Laboratory. The Aptima Combo 2[®] assay is used as the clinical standard of care test and was conducted within 5 – 10 business days of specimen collection. The Xpert CT/NG[®] assay was run with 1–2 business days of specimen collection, because the specimens were run in batches.

Test methods

The reference test was the Aptima Combo 2[®] assay (from here referred to as Aptima), which utilizes transcription-mediated amplification (TMA) to amplify the target rRNA and uses a Dual Kinetic Assay to detect the amplicon. The Aptima assay detects a specific region of the 23S rRNA from CT and a specific region of the 16S rRNA from NG. Results from the Aptima test are routinely used by AHF for patient management and were communicated to the clinicians and participants. The standard of care at AHF currently does not include

pharyngeal CT testing and therefore pharyngeal CT results were not available for analysis. Laboratory staff that performed the Aptima test were not aware of symptom status or results from the comparator test.

The Xpert® CT/NG assay (from here referred to as Xpert) is run on the GeneXpert® System and can be implemented in clinical settings without the need for central laboratory processing. That assay is run in approximately 90 minutes and results are displayed in tabular and graphic formats on a computer system. The GeneXpert® System has three main internal quality control mechanisms to ensure ideal test functioning and conditions.¹⁸ One of those internal quality control mechanisms is the sample adequacy control (SAC), which detects the presence of the gene encoding hydroxymethylbilane synthase (HMBS), a single-copy human cellular housekeeping gene, to monitor whether the sample contains human DNA. A negative SAC indicates that inadequate numbers of human cells were present in the sample, which can be due to sample degradation, insufficient mixing, or because of an inadequately collected specimen. The primers and probes in the Xpert CT/NG Assay detect chromosomal sequences in the CT and NG bacteria, with one target for CT and two different targets for NG. Both of the NG targets need to be positive for the Xpert CT/NG Assay to return a positive NG result. The testing methods occurred in accordance with the manufacturer directions. Xpert results were neither used for clinical management nor were communicated to the participants. Indeterminate Xpert test results were rerun once and those specimens that failed to show presence of human DNA or were incomplete pairs were excluded from analysis.

Data analysis

We used descriptive statistics to summarize the results and concordance between the two assays using Aptima as the reference. Concordance was measured using the percent agreement between the two assays. Positive percent agreement was determined by the percent of Xpert positive of those Aptima positive. Negative percent agreement was determined by the percent of Xpert negative of those Aptima negative. We calculated 95% confidence intervals (CIs) using the exact binomial method. We stratified analyses by symptom status in which participants were considered symptomatic if they were treated with at least ceftriaxone at their screening visit, prior to receipt of diagnostic test result. All analyses were conducted using SAS v9.4 (Cary, NC).

Ethics

Ethical approval for this study was granted by the Institutional Review Board at the University of California Los Angeles IRB#15-000740. Verbal informed consent was obtained from all participants.

Results from APTIMA testing were available for 396 rectal CT, 394 rectal NG, and 451 pharyngeal NG tests. Of those, 393 rectal CT, 391 rectal NG, and 448 NG pharyngeal were valid on the Xpert test.

The number of specimens positive using the Xpert assay were: 44 CT rectal, 40 NG rectal, and 35 NG pharyngeal while the number of specimens positive by the reference test, Aptima, were: 49 CT rectal, 43 NG rectal, and 34 NG pharyngeal [Table 1].

The concordance between the two assays was 97.7% (95% CI: 95.7%, 99.0%) for CT rectal tests, 98.2% (95% CI: 96.4%, 99.3%) for NG rectal tests and 98.4% (95% CI: 96.8%, 99.4%) for NG pharyngeal tests [Table 2]. The positive percent agreement and negative percent agreement values are displayed in Table 2. In addition, the results are stratified by symptom status in Table 2.

We conducted a concordance study between two extragenital NAATs. We found that the performance of the Xpert test, a point-of-care CT/NG NAAT that can have results available within 2 hours, in extragenital specimens was highly similar to a laboratory-based NAAT. We stratified by symptom status, however there was no statistically significant difference observed between groups. We also calculated positive percent agreement and negative percent agreement, however these values should be interpreted with caution as there was no tiebreaker test performed to resolve discordant results and therefore the true disease status for each participant is unknown. We found that our results show similar concordance between the Aptima and Xpert tests to a previous study¹⁹, however both studies were similarly limited in the lack of a third assay as a tiebreaker result and limited in the number of positive specimens assessed. The sensitivity and specificity of the Xpert CT/NG with FDA approved specimen types, vaginal swab, endocervical swab and male and female urine are very high.¹⁶ That high performance for detection of urogenital infections support our findings of good performance in extragenital specimens

The implementation of the GeneXpert instrument provides a NAAT assay that can be used with smaller instrumentation and at the point-of-care.¹⁶ Xpert assays have improved turnaround time to allow for earlier treatment for infectious diseases.²⁰

Our study is subject to several limitations. We were unable to calculate sensitivity and specificity because we did not use a third assay for the tiebreaker and therefore cannot determine the true infected status of participants. The sample size of positive test results was small, which led to wide confidence intervals for positive percent agreement. Further research is needed to better understand the true performance of both assays for the detection of extragenital CT and NG infections.

In conclusion, we found that the Xpert CT/NG assay can be used for rectal detection of CT and NG infections and pharyngeal NG infections. The Xpert assay can improve turnaround time for results and may allow for same-day testing and treatment for those common and curable STIs, which may help reduce continued transmission of those infections.

Acknowledgments

Funding Statement: CCB acknowledges funding from Fogarty International Center 5R25TW009343 FIC. Cepheid provided all testing materials.

We would like to thank Cepheid for the donation of test equipment and supplies and the study participants for their contributions toward this study. In addition, we thank Rosalba Castro the laboratory technician at AIDS Healthcare

Foundation and Kathryn Salow a research associate at AIDS Healthcare Foundation for their valuable contributions toward the success of this study.

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Comparison of Xpert and APTIMA assays for detection of extragenital *Neisseria gonorrhoeae* and *Chlamydia trachomatis*.

Table 1

	Rectal <i>Chlamydia trachomatis</i>				Rectal <i>Neisseria gonorrhoeae</i>				Pharyngeal <i>Neisseria gonorrhoeae</i>			
	APTIMA positive		APTIMA negative		APTIMA positive		APTIMA negative		APTIMA positive		APTIMA negative	
	Xpert positive	Xpert negative	Xpert positive	Xpert negative	Xpert positive	Xpert negative	Xpert positive	Xpert negative	Xpert positive	Xpert negative	Xpert positive	Xpert negative
Asymptomatic	31	5	2	290	24	3	2	297	25	0	3	350
Symptomatic	11	2	0	52	14	2	0	49	6	3	1	60
TOTAL	42	7	2	342	38	5	2	346	31	3	4	410

Table 2

Concordance between Xpert CT/NG Assay and the reference tests Aptima Combo 2 with 95% confidence intervals calculated using the exact binomial method.

	Positive Percent Agreement [*]	Negative Percent Agreement [†]	Concordance [‡]
Asymptomatic			
Rectal <i>Chlamydia trachomatis</i>	86.1% (70.5–95.3)	99.3% (97.6–99.9)	97.9% (95.7–99.1)
Rectal <i>Neisseria gonorrhoeae</i>	88.9% (70.8–97.7)	99.3% (97.6–99.9)	98.5% (96.5–99.5)
Pharyngeal <i>Neisseria gonorrhoeae</i>	100% (86.3–100)	99.2% (97.5–99.8)	99.2% (97.7–99.8)
Symptomatic			
Rectal <i>Chlamydia trachomatis</i>	84.6% (54.6–98.1)	100% (93.2–100)	96.9% (89.3–99.6)
Rectal <i>Neisseria gonorrhoeae</i>	87.5% (61.7–98.5)	100% (92.8–100)	96.9% (89.3–99.6)
Pharyngeal <i>Neisseria gonorrhoeae</i>	66.7% (29.9–92.5)	98.4% (91.2–100)	94.3% (86.0–98.4)
TOTAL			
Rectal <i>Chlamydia trachomatis</i>	85.7% (72.8–94.1)	99.4% (97.9–99.9)	97.7% (95.7–99.0)
Rectal <i>Neisseria gonorrhoeae</i>	88.4% (74.9–96.1)	99.4% (97.9–99.9)	98.2% (96.4–99.3)
Pharyngeal <i>Neisseria gonorrhoeae</i>	91.2% (76.3–98.1)	99.0% (97.5–99.7)	98.4% (96.8–99.4)

* Positive percent agreement was determined by the percent of Xpert CT/NG positive of those Aptima Combo 2 positive

† Negative percent agreement was determined by the percent of Xpert CT/NG negative of those Aptima Combo 2 negative

‡ Concordance was determined by the percent agreement between the Xpert CT/NG and Aptima Combo 2 results.