# Comparative Evaluation of 2 Nucleic Acid Amplification Tests for the Detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* at Extragenital Sites

*Claire C. Bristow, PhD, MPH, MSc,* Mark R. McGrath, MPH,† Adam C. Cohen, PhD, MPH,† Laura J. Anderson, MPH,‡ Kristie K. Gordon, BA,†§ and Jeffrey D. Klausner, MD, MPH‡§*

**Abstract:** *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (NG) infections are frequently asymptomatic, requiring highly accurate diagnostic tests and proper management to prevent further transmission. We compared two nucleic acid tests, Xpert® CT/NG (Cepheid, Sunnyvale, CA) point-of-care platform and at an offsite clinical laboratory with Aptima Combo 2® (Hologic, Inc., San Diego, CA) assay, for the detection of extragenital infection in patients at an STI clinic in Hollywood, CA.

We calculated concordance between the two assays and used the exact binomial method to calculate 95% confidence intervals (CIs) for each specimen type and pathogen.

The concordance between the two assays was 97.7% (95% CI: 95.7%,99.0%) for 393 paired CT rectal results, 98.2% (95% CI: 96.4%,99.3%) for 391 paired NG rectal results and 98.4% (95% CI: 96.8%,99.4%) for 448 paired NG pharyngeal results.

The performance of Xpert® CT/NG assay in point-of-care testing in extragenital specimens was highly similar to the laboratory-based platform.

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

*Chlamydia trachomatis* 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 men who have sex with men, more than 70% of extragenital NG infections and more than 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]

From the *Division of Global Public Health, Department of Medicine, University of California San Diego, La Jolla, CA; †AIDS Healthcare Foundation, Los Angeles, CA; ‡UCLA Fielding School of Public Health, Los Angeles, CA; and §UCLA David Geffen School of Medicine, Los Angeles, CA

The authors thank Cepheid for the donation of test equipment and supplies and the study participants for their contributions toward this study. In addition, the authors thank Rosalba Castro, the laboratory technician at AIDS Healthcare Foundation, and Katheryn Salow, a research associate at AIDS Healthcare Foundation, for their valuable contributions toward the success of this study.

Author C.C. Bristow acknowledges funding from Fogarty International Center 5R25TW009343 FIC. Cepheid provided all testing materials.

Conflict of interest: None declared.

Correspondence: Claire C. Bristow, PhD, MPH, MSc, University of California San Diego, 9500 Gilman Dr, 0507, La Jolla, CA 92093-0507. E-mail: cbristow@ucsd.edu.

Received for publication January 23, 2017, and accepted March 12, 2017.

DOI: 10.1097/OLQ.0000000000000627



Studies have shown that nucleic acid amplification tests (NAATs) perform better than other testing strategies available for CT and NG detection.[8–12] The Centers for Disease Control and Prevention currently recommends NAATs for the detection of urethral, vaginal/cervical, rectal, and pharyngeal CT and NG infections.[4] However, despite widespread practice, no NAAT platforms are currently Food and Drug Administration–approved for use with extragenital specimens. Clinical Laboratory Improvement Amendments–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 turnaround 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] and Aptima Unisex Swab Specimen Collection Kits [Hologic, Inc]). For rectal specimen collection, participants were instructed to insert the specimen collection swab into the anal canal and rotate for 15 to 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°C to 30°C.

One specimen from each anatomic site was tested at the AHF laboratory using Xpert CT/NG assay on the GeneXpert instrument (Cepheid). The first swabs from each anatomic site collected were transported for testing using the Aptima Combo 2 assay (Hologic, Inc) on the Panther platform at the Los Angeles County Public Health Laboratory. The Aptima Combo 2 assay is

**TABLE 1.** Comparison of Xpert and APTIMA Assays for Detection of Extragenital *Neisseria gonorrhoeae* and *Chlamydia trachomatis*

| | Rectal *Chlamydia trachomatis* | | | | Rectal *Neisseria gonorrhoeae* | | | | Pharyngeal *Neisseria gonorrhoeae* | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 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 |

used as the clinical standard of care test and was conducted within 5 to 10 business days of specimen collection. The Xpert CT/NG assay was run with 1 to 2 business days of specimen collection because the specimens were run in batches.

## TEST METHODS

The reference test was the Aptima Combo 2 assay (hereinafter referred to as Aptima), which uses transcription-mediated amplification to amplify the target rRNA and uses 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 who performed the Aptima test were not aware of symptom status or results from the comparator test.

The Xpert CT/NG assay (hereinafter 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 3 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, which detects the presence of the gene encoding hydroxymethylbilane synthase, a single-copy human cellular housekeeping gene, to monitor whether the sample contains human DNA. A negative sample adequacy control 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 2 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 2 assays using Aptima as the reference. Concordance was measured using the percent agreement between the 2 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, before receipt of diagnostic test result. All analyses were conducted using SAS version 9.4 (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

**TABLE 2.** Concordance Between Xpert CT/NG Assay and the Reference Tests Aptima Combo 2 with 95% CIs Calculated Using the Exact Binomial Method

| | Positive Percent Agreement* | Negative Percent Agreement† | Concordance‡ |
|---|---|---|---|
| Asymptomatic | | | |
| Rectal *C. trachomatis* | 86.1% (70.5–95.3) | 99.3% (97.6–99.9) | 97.9% (95.7–99.1) |
| Rectal *N. gonorrhoeae* | 88.9% (70.8–97.7) | 99.3% (97.6–99.9) | 98.5% (96.5–99.5) |
| Pharyngeal *N. gonorrhoeae* | 100% (86.3–100) | 99.2% (97.5–99.8) | 99.2% (97.7–99.8) |
| Symptomatic | | | |
| Rectal *C. trachomatis* | 84.6% (54.6–98.1) | 100% (93.2–100) | 96.9% (89.3–99.6) |
| Rectal *N. gonorrhoeae* | 87.5% (61.7–98.5) | 100% (92.8–100) | 96.9% (89.3–99.6) |
| Pharyngeal *N. gonorrhoeae* | 66.7% (29.9–92.5) | 98.4% (91.2–100) | 94.3% (86.0–98.4) |
| Total | | | |
| Rectal *C. trachomatis* | 85.7% (72.8–94.1) | 99.4% (97.9–99.9) | 97.7% (95.7–99.0) |
| Rectal *N. gonorrhoeae* | 88.4% (74.9–96.1) | 99.4% (97.9–99.9) | 98.2% (96.4–99.3) |
| Pharyngeal *N. gonorrhoeae* | 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.

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, whereas 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 2 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 2 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 the 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 study[19]; 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 Food and Drug Administration–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.

## REFERENCES


1. World Health Organization, Dept. of Reproductive Health and Research. Global incidence and prevalence of selected curable sexually transmitted infections—2008, 2012.
2. Newman L, Rowley J, Vander Hoorn S, et al. Global estimates of the prevalence and incidence of four curable sexually transmitted infections in 2012 based on systematic review and global reporting. PLoS One 2015; 10:e0143304.
3. Centers for Disease Control and Prevention. Sexually Transmitted Disease Surveillance 2015. Atlanta: US Department of Health and Human Services, 2016.
4. Centers for Disease Control and Prevention. Recommendations for the laboratory-based detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae*—2014. MMWR Recomm Rep 2014; 63:1–19.
5. Garrett NJ, McGrath N, Mindel A. Advancing STI care in low/middle-income countries: Has STI syndromic management reached its use-by date? Sex Transm Infect 2017; 93:4–5.
6. Goldenberg SD, Finn J, Sedudzi E, et al. Performance of the GeneXpert CT/NG assay compared to that of the Aptima AC2 assay for detection of rectal *Chlamydia trachomatis* and *Neisseria gonorrhoeae* by use of residual Aptima Samples. J Clin Microbiol 2012; 50:3867–3869.
7. Patton ME, Kidd S, Llata E, et al. Extragenital gonorrhea and chlamydia testing and infection among men who have sex with men—STD Surveillance Network, United States, 2010-2012. Clin Infect Dis 2014; 58:1564–1570.
8. Black CM, Marrazzo J, Johnson RE, et al. Head-to-head multicenter comparison of DNA probe and nucleic acid amplification tests for *Chlamydia trachomatis* infection in women performed with an improved reference standard. J Clin Microbiol 2002; 40:3757–3763.
9. Gaydos CA, Quinn TC, Willis D, et al. Performance of the APTIMA Combo 2 assay for detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in female urine and endocervical swab specimens. J Clin Microbiol 2003; 41:304–309.
10. Gaydos CA, Theodore M, Dalesio N, et al. Comparison of three nucleic acid amplification tests for detection of *Chlamydia trachomatis* in urine specimens. J Clin Microbiol 2004; 42:3041–3045.
11. Martin DH, Cammarata C, Van Der Pol B, et al. Multicenter evaluation of AMPLICOR and automated COBAS AMPLICOR CT/NG tests for *Neisseria gonorrhoeae*. J Clin Microbiol 2000; 38:3544–3549.
12. Van Der Pol B, Quinn TC, Gaydos CA, et al. Multicenter evaluation of the AMPLICOR and automated COBAS AMPLICOR CT/NG tests for detection of *Chlamydia trachomatis*. J Clin Microbiol 2000; 38: 1105–1112.
13. Gratrix J, Singh AE, Bergman J, et al. Evidence for increased *Chlamydia* case finding after the introduction of rectal screening among women attending 2 Canadian sexually transmitted infection clinics. Clin Infect Dis 2015; 60:398–404.
14. Danby CS, Cosentino LA, Rabe LK, et al. Patterns of extragenital *Chlamydia* and gonorrhea in women and men who have sex with men reporting a history of receptive anal intercourse. Sex Transm Dis 2016; 43:105–109.
15. Dize L, West S, Williams JA, et al. Comparison of the Abbott m2000 RealTime CT assay and the Cepheid GeneXpert CT/NG assay to the Roche Amplicor CT assay for detection of *Chlamydia trachomatis* in ocular samples from Tanzania. J Clin Microbiol 2013; 51:1611–1613.
16. Gaydos CA. Review of use of a new rapid real-time PCR, the Cepheid GeneXpert® (Xpert) CT/NG assay, for *Chlamydia trachomatis* and *Neisseria gonorrhoeae*: results for patients while in a clinical setting. Expert Rev Mol Diagn 2014; 14:135–137.
17. Gaydos CA, Van Der Pol B, Jett-Goheen M, et al. Performance of the Cepheid CT/NG Xpert Rapid PCR test for detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae*. J Clin Microbiol 2013; 51: 1666–1672.
18. Bristow CC, Adachi K, Nielsen-Saines K, et al. Characteristics of the sample adequacy control (SAC) in the Cepheid Xpert® CT/NG Assay in female urine specimens. Journal of Microbiology & Experimentation 2014; 1:26.
19. Geiger R, Smith DM, Little SJ, et al. Validation of the GeneXpert® CT/NG Assay for use with male pharyngeal and rectal swabs. Austin J HIV AIDS Res 2016; 3. pii:1021.
20. Wingrove I, McOwan A, Nwokolo N, et al. Diagnostics within the clinic to test for gonorrhoea and chlamydia reduces the time to treatment: A service evaluation. Sex Transm Infect 2014; 90:474.