AMERICAN SOCIETY FOR MICROBIOLOGY | Journal of Clinical Microbiology®

BACTERIOLOGY

Use of Nucleic Acid Amplification Testing for Diagnosis of Extragenital Sexually Transmitted Infections

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ABSTRACT Nucleic acid amplification testing (NAAT) is the preferred method to detect *Chlamydia trachomatis* and *Neisseria gonorrhoeae*, but no commercial tests are cleared by the U.S. Food and Drug Administration for use with extragenital swab samples. This study evaluated the performance of the Gen-Probe Aptima Combo2 assay (Aptima) and the Cepheid Xpert CT/NG assay (Xpert) to detect *C. trachomatis* and *N. gonorrhoeae* in rectal and pharyngeal samples from 224 men and 175 women reporting a history of anal receptive sexual intercourse. Discordant results between the NAATs were repeated using the assays APTIMA CT or APTIMA GC, which target alternate primers, as the confirmatory tests. *C. trachomatis* was detected from 59 rectal swabs and 8 pharyngeal samples, with 97.7% and 99.5% agreement between the two test systems, respectively. For *C. trachomatis*, Xpert was 95% sensitive (95% CI, 86 to 99%) and Aptima was 92% sensitive (95% CI, 81 to 97%) from rectal swabs, while both systems were 100% sensitive from pharyngeal samples. *N. gonorrhoeae* was detected from 30 rectal and 40 pharyngeal samples, with 99.5% and 97.5% agreement between the two test systems. The sensitivity of Xpert for *N. gonorrhoeae* from rectal swabs was 100% (95% CI, 88 to 100%) versus 93% (95% CI, 78 to 99%) for Aptima. From pharyngeal swab samples, Xpert was 98% sensitive (95% CI, 87 to 99.9%) versus 93% (95% CI, 80 to 98%) for Aptima. For *C. trachomatis*, neither system was >95% sensitive from the rectum, though both were >99.5% specific. For *N. gonorrhoeae*, Xpert had higher sensitivity than Aptima, but with more false positives from pharyngeal samples.

KEYWORDS *Chlamydia trachomatis*, NAAT, *Neisseria gonorrhoeae*, pharyngeal, rectal

Received 17 April 2017 **Returned for modification** 11 May 2017 **Accepted** 27 June 2017

Accepted manuscript posted online 5 July 2017

Citation Cosentino LA, Danby CS, Rabe LK, Macio I, Meyn LA, Wiesenfeld HC, Hillier SL. 2017. Use of nucleic acid amplification testing for diagnosis of extragenital sexually transmitted infections. J Clin Microbiol 55:2801–2807. https://doi.org/10.1128/JCM.00616-17.

Editor Erik Munson, Marquette University

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The Centers for Disease Control and Prevention (CDC) recommends annual screening of all sexually active women aged <25 years and at-risk men who have sex with men (MSM) (1). Culture detection of *Neisseria gonorrhoeae* has long been available for the detection of rectal or oropharyngeal gonococcal infection (1), but implementation of nucleic acid amplification testing (NAAT) for this pathogen has been reported to double its detection from rectal samples and increase its detection from pharyngeal samples 5-fold (2). Several studies using various platforms, including the Becton Dickinson Probetec ET, the Gen-Probe Aptima Combo2, and the Roche Cobas Amplicor PCR, have shown that NAAT for detection of *Chlamydia trachomatis* and *N. gonorrhoeae* in rectal and pharyngeal samples is more sensitive than culture (2, 3, 4, 5, 6, 7). NAAT is less labor-intensive than culture and provides quick turnaround of results, leading to quicker treatment.

While NAAT has become the preferred method for detection of *C. trachomatis* and *N. gonorrhoeae* due to its high sensitivity and specificity, no commercial test systems have been cleared by the U.S. Food and Drug Administration (FDA) for use with extragenital specimens. To date, no company has performed the clinical studies to

provide evidence of high-quality test performance with extragenital samples, a requirement for FDA clearance. A clinical trial funded by the NIH (clinicaltrials.gov NCT02870101) is currently evaluating the performance of commercially available NAATs for detection of pharyngeal and rectal *C. trachomatis* and *N. gonorrhoeae* aimed at providing sufficient evidence for FDA clearance for each platform to be used for extragenital testing.

The burden of extragenital gonorrhea and chlamydial infections is high. A study evaluating rectal swab samples sent to a commercial testing laboratory from 52,063 U.S. men aged 15 to 60 years found that 8.8% had rectal gonorrhea and 11% had rectal chlamydia (8). Studies have shown that urogenital screening alone misses infections in men and women who are positive in extragenital sites only (9, 10, 11, 12, 13, 14).

The Gen-Probe Aptima Combo2 assay was FDA cleared in 2005 for use in genital and urine samples and relies on transcription-mediated amplification. The Cepheid Xpert CT/NG assay, a real-time PCR assay, was FDA cleared in 2012 for use in female endocervical swabs and vaginal swabs and female and male urine specimens from symptomatic and asymptomatic patients (15). There are limited data on the comparability of these test systems for detection of extragenital infections. One study used residual Aptima samples from 409 rectal swabs and found a good correlation between the two test systems, despite the need to dilute the sample to overcome the incompatibility of the Aptima buffer with the Xpert system (16). One small study including 144 men reported that Xpert was less sensitive than Aptima, but the number of samples positive for either *N. gonorrhoeae* or *C. trachomatis* was quite limited (17).

The primary objective of the present study was to compare the performance of Xpert to that of the Aptima Combo2 Assay for detection of *C. trachomatis* and *N. gonorrhoeae* from rectal samples obtained from both men and women reporting a history of receptive anal intercourse. A secondary objective was to assess the performance of these tests in pharyngeal samples. Urogenital samples were also collected to evaluate the proportion of total infections which occurred only at extragenital sites.

RESULTS

A total of 399 participants were enrolled in the study between March 2014 and April 2015. The study population consisted of 224 men and 175 women. The median age of the men was 26 years (range, 18 to 62 years), and 70.5% of the men were Caucasian. The median age of the women was 27 years (range, 18 to 49 years) and 52% identified themselves as Caucasian (Table 1). Among the men enrolled, 22.8% had *N. gonorrhoeae* at any site, and the prevalence of this pathogen at extragenital sites was 17.4%. *N. gonorrhoeae* infection was less common among women (4.8% at any site) and rare in extragenital sites only (Table 1). For *C. trachomatis*, 21.9% of men had this pathogen at any site and 17.4% were positive only at extragenital sites. While the prevalence of *C. trachomatis* detection was similar overall in women (17.6%) and men, women were substantially less likely to have this pathogen at only extragenital sites (2.3%).

As shown in Table 2, there was 97.7% agreement for rectal swab detection of *C. trachomatis* using the two NAAT systems (kappa = 0.91). Of the 59 confirmed positive rectal swab samples for *C. trachomatis*, 51 samples were positive by both systems (Table 2). Eight of the 9 discordant samples were confirmed to be positive for *C. trachomatis* using the alternate primers (Aptima CT test). The one false-positive *C. trachomatis* test was with the Xpert system. Based on the resolution of the discordant results, for rectal swabs, the estimated sensitivities and specificities for *C. trachomatis* were 94.9% (95% confidence interval [CI], 85.9 to 98.9%) and 99.7% (95% CI, 98.4 to 100%) for Xpert and 91.5% (95% CI, 81.3 to 97.2%) and 100% (95% CI, 98.9 to 100%) for Aptima.

Of the 399 participants, 5 pharyngeal specimens were excluded due to an error or invalid result in the Xpert system, leaving 394 evaluable samples. Overall, there was a 99.5% agreement between the two NAAT systems for pharyngeal samples, although the study power was limited by the low number of chlamydial infections. Of the 8 *C. trachomatis* confirmed-positive pharyngeal samples, all were detected by both NAAT systems, but there were also 2 false-positive samples by the Xpert system (Table 2).

TABLE 1 Characteristics of 399 study participants stratified by sex

Characteristic	No. of men (%) (n = 224)	No. of women (%) (n = 175)	P value
Age (median, range) (yrs)	26 (18, 62)	27 (18, 49)	0.24
Predominant race			0.001
Caucasian	158 (70.5)	91 (52.0)	
Black	49 (21.9)	65 (37.1)	
Other	17 (7.6)	19 (10.9)	
Enrollment clinic type			<0.001
STD[a] clinic	216 (96.4)	137 (78.3)	
Hospital based	8 (3.6)	38 (21.7)	
Lifetime history of sexual activity			0.30
Men only	171 (76.3)	125 (71.4)	
Both men and women	53 (23.7)	50 (28.6)	
Confirmed positive infections			
Positive for *N. gonorrhoeae*			
Any site	51 (22.8)	6 (4.8)	<0.001
Rectal	26 (11.6)	4 (2.3)	<0.001
Pharyngeal	37 (16.7)	4 (2.3)	<0.001
Urogenital	12 (5.4)	5 (2.9)	0.32
Detected at extragenital sites only	39 (17.4)	1 (0.6)	<0.001
Positive for *C. trachomatis*			
Any site	49 (21.9)	22 (17.6)	0.02
Rectal	39 (17.4)	20 (11.4)	0.12
Pharyngeal	6 (2.7)	4 (2.3)	>0.99
Urogenital	10 (4.5)	17 (9.7)	0.03
Detected at extragenital sites only	39 (17.4)	4 (2.3)	<0.001

[a]STD, sexually transmitted disease.

After the resolution of the discordant results, for pharyngeal swabs the estimated sensitivities and specificities for *C. trachomatis* were 100% (95% CI, 63.1 to 100%) and 99.5% (95% CI, 98.1 to 99.9%) for Xpert and 100% (95% CI, 63.1 to 100%) and 100% (95% CI, 99.0 to 100%) for Aptima.

The results for the comparison of the two NAAT systems for detection of *N. gonorrhoeae* are shown in Table 3. There was 99.5% agreement (kappa = 0.96) between the two test systems for the 30 gonococcal infections detected from the rectum, with 2 rectal gonococcal infections not detected by the Aptima system. The estimated

TABLE 2 Performance of Xpert and Aptima in the detection of *Chlamydia trachomatis* from rectal, pharyngeal, and urogenital samples before discordant resolution

Sample site (n)	Xpert result	Aptima result(s)		Kappa	% agreement
		No. positive	No. negative		
Rectal (399)				0.91	97.7
	Positive	51	6		
	Negative	3	339		
Pharyngeal (394)[a]				0.89	99.5
	Positive	8	2		
	Negative	0	384		
Urine (224)				0.95	99.6
	Positive	9	0		
	Negative	1	214		
Vaginal (170)[a]				0.69	95.3
	Positive	10	1		
	Negative	7	152		

[a]Five pharyngeal samples and five vaginal samples were excluded because of invalid test results obtained using Cepheid.

TABLE 3 Performance of Xpert and Aptima in the detection of *Neisseria gonorrhoeae* from rectal, pharyngeal, and urogenital samples before discordant resolution

Sample site (*n*)	Xpert result	Aptima result(s)		Kappa	% agreement
		No. positive	No. negative		
Rectal (399)				0.96	99.5
	Positive	28	2		
	Negative	0	369		
Pharyngeal (394)[a]				0.86	97.5
	Positive	36	9		
	Negative	1	348		
Urine (224)				1.00	100
	Positive	12	0		
	Negative	0	212		
Vaginal (170)[a]				0.89	99.4
	Positive	4	0		
	Negative	1	165		

[a]Five pharyngeal samples and five vaginal samples were excluded because of invalid test results obtained using Cepheid.

sensitivities and specificities were 100% (95% CI, 88.4 to 100%) and 100% (95% CI, 99.0 to 100%) for Xpert and 93.3% (95% CI, 77.9 to 99.2%) and 100% (95% CI, 99.0 to 100%) for Aptima. Of the gonococcal infections detected from pharyngeal samples, 36 were detected by both NAAT systems (97.5% agreement). Of the 10 discordant results, there were 6 false-positive and one false-negative *N. gonorrhoeae* test results for Xpert, and 3 false-negative results for Aptima. After resolution of these discordant results, for pharyngeal swabs the estimated sensitivities and specificities for *N. gonorrhoeae* were 97.5% (95% CI, 86.8 to 99.9%) and 98.3% (95% CI, 96.3 to 99.4%) for Xpert and 92.5% (95% CI, 79.6 to 98.4%) and 100% (95% CI, 99.0 to 100%) for Aptima.

Urine and vaginal swab samples were collected concurrently with extragenital samples and comparisons of the two test systems are shown in Tables 2 and 3. For urine samples from men, there were 10 chlamydial and 12 gonococcal infections, with 99.6% agreement between the two NAAT systems for *C. trachomatis* and 100% agreement for *N. gonorrhoeae*. However, the sensitivity of Xpert was substantially lower for detection of *C. trachomatis* from vaginal swabs. Of 17 true-positive *C. trachomatis* vaginal swab samples, only 10 vaginal swabs were positive by both methods, a 95.3% agreement (kappa = 0.69). During the study, the Xpert system had an invalid rate of 18.8% in the first lot of cartridges used across all sample types. Combined, the other two new lots had an invalid rate of only 2.6%. However, the low sensitivity of Xpert vaginal samples for *C. trachomatis* was observed in all three lots of test cartridges included in this study. The volume of residual vaginal sample was too limited to allow for direct sequencing of pathogens from the samples. After resolution of discordant results, for vaginal swabs the estimated sensitivities and specificities for *C. trachomatis* were 58.8% (95% CI, 32.9 to 81.6%) and 99.3% (95% CI, 96.4 to 100%) for Xpert and 100% (95% CI, 80.5 to 100%) and 100% (95% CI, 97.6 to 100%) for Aptima.

Rectal chlamydial and gonococcal infections were common in this study population, with 17% of men and 11% of the women having rectal *C. trachomatis* infection and 12% of men and 2% of women having rectal *N. gonorrhoeae* infection (Table 1). In contrast, pharyngeal infections due to *C. trachomatis* were infrequent, occurring in only 2% of men and women. Pharyngeal *N. gonorrhoeae* infection was more common in men, with 16.7% versus only 2.3% in women. Urogenital-only testing would have missed 80% of the *C. trachomatis*-positive and 77% of the *N. gonorrhoeae*-positive samples in males. In contrast, 82% of the total chlamydial infections and 83% of the total gonococcal infections in women were detected using the vaginal sample alone (14).

DISCUSSION

The primary objective of this study was to compare the performance of Aptima and Xpert for detection of sexually transmitted infections from rectal samples. Our study, which included 59 rectal samples positive for *C. trachomatis* and 30 samples positive for *N. gonorrhoeae*, demonstrated that the Xpert and Aptima systems had 97.7% agreement for detection of *C. trachomatis* and 99.5% agreement for *N. gonorrhoeae* from rectal swabs. The Xpert and Aptima systems had similar sensitivities and specificities and both systems performed well for detection of rectal infections.

Our secondary objective was to compare the performance of the two test systems for detection of these pathogens from pharyngeal swabs. Although pharyngeal chlamydial infections were infrequent, there was a 99.5% agreement between the two test systems for *C. trachomatis*. However, for *N. gonorrhoeae*, Xpert had higher sensitivity than Aptima, but with more false positives from pharyngeal samples. One possible cause for the higher number of discrepancies in the pharyngeal results for *N. gonorrhoeae* may be due to cross-reaction with commensal *Neisseria* species, as reported by Tabrizi et al. (18) in an evaluation of six commercial NAAT tests. The Xpert system uses two targets to reduce false-positive reactions due to commensal *Neisseria* species. In an *in vitro* study, Tabrizi et al. (19) demonstrated excellent specificity of assay using Xpert for *N. gonorrhoeae* isolates. However, it is possible that oropharyngeal samples may include adequate numbers of cross-reacting organisms which bind to both targets, resulting in a false-positive result for *N. gonorrhoeae* with Xpert. In a previously published study comparing Xpert and Aptima in 144 men reporting sex with men, no false-positive gonococcal infection results from the pharynx were reported, although they reported that Aptima was superior to Xpert for detection of extragenital gonococcal and chlamydial infections (17).

In our study, the frequency of pharyngeal infection due to *C. trachomatis* was low in both men and women, but 16.7% of the men tested positive for *N. gonorrhoeae* in the pharynx. Park et al. (20) also showed a substantial burden of pharyngeal infections, with a rate of 1.7% *C. trachomatis* positivity and 5.8% *N. gonorrhoeae* positivity among men who have sex with men. Most pharyngeal *N. gonorrhoeae* infections will remain undetected unless NAAT is used. Further, recent studies have reported that undetected pharyngeal gonorrhea can increase the risk of anal infection when saliva is used as a lubricant for anal sex (21).

The study showed good agreement between the two NAAT systems for urine samples from men, but the Xpert had a high number of false-negative vaginal swab results for *C. trachomatis*. Nevertheless, 5 of the 8 women who had false-negative vaginal Xpert results for *C. trachomatis* had concordant rectal chlamydial infection by both NAAT systems, suggesting that these women likely had chlamydial infections. No explanation for the low sensitivity of Xpert for detection of *C. trachomatis* for vaginal swabs was identified, and the poor performance spanned across all three lots of test cartridges used. Other studies (22, 23) reported sensitivity of 97 to 98% for detection of *C. trachomatis* from vaginal swabs using Xpert.

Rectal *C. trachomatis* and *N. gonorrhoeae* infections were common among both men and women reporting a history of receptive anal intercourse (RAI) in our study. As previously reported from this study population, nearly 80% of chlamydial and gonococcal infections in men and 16 to 18% of these infections in women were detected only in the pharynx or rectum (14). Extragenital testing for STIs should be considered, especially for men who have sex with men, in whom most infections occur at extragenital sites. This study demonstrated that both Xpert and Aptima can be used with appropriate validation for detection of *C. trachomatis* and *N. gonorrhoeae* from rectal samples.

MATERIALS AND METHODS

This is a secondary analysis of a study evaluating the patterns of extragenital chlamydia and gonorrhea in men and women reporting a lifetime history of ever having receptive anal sex (14). Samples were collected from participants 18 to 62 years of age who attended the Sexually Transmitted Diseases Clinic at the Allegheny County Health Department (ACHD) and Magee-Womens Hospital of the University

of Pittsburgh Medical Center (UPMC) and who reported a lifetime history of receptive anal intercourse. Written informed consent approved by the University of Pittsburgh Institutional Review Board, Pittsburgh, PA, was obtained from all participants prior to initiation of study procedures. Participants were excluded if they reported use of oral antibiotics over the previous 7 days, use of rectal douche or other rectal products in the past 24 h, and, if female, use of vaginal douche or vaginal product in the previous 24 h. A questionnaire was administered asking a series of questions about age, ethnicity and sexual activity history and symptoms.

For rectal testing, clinicians collected samples using the Aptima Unisex collection swab (Aptima, Aptima Combo 2; Hologic Inc., Bedford, MA) and a second swab was collected using the Xpert CT/NG vaginal/endocervical specimen collection kit (Xpert, Cepheid Innovation, Sunnyvale, CA). The order of collection of the two NAAT swabs was predetermined by computer randomization. The swabs were inserted by clinicians approximately 2.5 cm above the anal margin and placed into the appropriate transport media. Two pharyngeal swabs were collected from each lateral posterior wall, including tonsillar crypts, and the pharyngeal arc. For males, urine samples were obtained as a first-pass collection at least 1 h after the last void. For women, vaginal swabs were obtained by clinicians without placement of a speculum. Samples were transported to the laboratory within 24 h. Once at the laboratory, specimens were processed as recommended in the package insert for each test kit, Gen-probe (Aptima Combo 2 package insert IN0037-04 Rev A; Gen-Probe Inc., San Diego, CA) and Xpert (GeneXpert package insert CXCT/NG-CE-10; Cepheid Innovation, Sunnyvale, CA). A total of 5 lots of the Aptima Combo 2 kits and 3 lots of the Xpert test cartridges were used to complete all the testing. Any Aptima- or Xpert-positive *C. trachomatis* or *N. gonorrhoeae* result, including a discrepant test result, was verified using the appropriate Aptima CT or Aptima GC assay, which targets different nucleic acid sequences.

The study was designed to ensure a sample size providing at least 20 samples positive for *C. trachomatis* and *N. gonorrhoeae* from rectal swabs. The performance of the test systems from pharyngeal swabs was a secondary study objective. Data analyses were conducted with SPSS statistical software, release 23.0 (IBM Corp., Armonk, NY). Descriptive statistics, including median, range, and frequency distributions were performed for all demographic and risk behavior characteristics. *P* values were calculated using Fisher's exact or Mann-Whitney U tests. Sensitivity and specificity of the two test systems are presented along with 95% confidence intervals calculated using binomial exact methods. The kappa statistic was used to quantify agreement between the two assays for detecting *C. trachomatis* and *N. gonorrhoeae* in samples from each anatomical site.

ACKNOWLEDGMENTS

Cepheid and Hologic donated the reagents for *C. trachomatis* and *N. gonorrhoeae* testing but they were not involved in the design of the study, analysis, and interpretation of the data and preparation or approval of the manuscript.

The Microbicide Trials Network is funded by the National Institute of Allergy and Infectious Diseases (UM1AI068633, UM1AI106707), with cofunding from the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development and the National Institute of Mental Health, all components of the U.S. National Institutes of Health.

S.L.H. has received consultation fees from Merck, Symbiomix, and Cepheid and receives grant funding from Becton Dickinson. No other authors have conflicts of interest.

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