Performance of the APTIMA Combo 2 Assay for Detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in Female Urine and Endocervical Swab Specimens

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The greater sensitivity of nucleic acid amplification tests (NAATs) for *Chlamydia trachomatis* and *Neisseria gonorrhoeae* permits the use of urine and other noninvasive specimens, which can increase the reach and decrease the costs of public health screening programs aimed at controlling these infections. This study evaluated the performance of the APTIMA Combo 2 assay, a multiplex assay based on the transcription-mediated amplification reaction, for the simultaneous detection of both pathogens in endocervical swab and urine specimens from females. Combo 2 assay results were compared with patient infected status, which were available by using other commercial NAATs. Sensitivity and specificity for *C. trachomatis* were 94.2 and 97.6%, respectively, in swabs and 94.7 and 98.9%, respectively, in first-catch urine (FCU). Sensitivity and specificity for *N. gonorrhoeae* were 99.2 and 98.7%, respectively, in swabs and 91.3 and 99.3%, respectively, in FCU. The assay reliably detected both infections in coinfected patients. The Combo 2 assay can be recommended for use with endocervical swab and urine specimens from females, especially for screening tests for asymptomatic women in sexually transmitted disease surveillance programs. This Food and Drug Administration-cleared assay can be a useful tool in efforts to reduce the prevalence and incidence of *C. trachomatis* and *N. gonorrhoeae* infections in sexually active women and to prevent their costly and serious sequelae.

Chlamydia trachomatis has been shown by many studies to be highly prevalent in all socioeconomic groups in the developed world. Prevalence in the United States is among the highest in the developed world (11). *Neisseria gonorrhoeae* infections continue to be prevalent in certain geographic areas and among particular risk groups, but local prevalence can vary widely (11). Approximately 3 million new infections with chlamydia and 1 million with gonococci occur each year in the United States. Coinfection with both pathogens is not uncommon, requiring the evaluation of local epidemiology to influence decisions to screen for only or both organisms (5, 7). Young women are most at risk for serious sequelae, such as pelvic inflammatory disease (PID), ectopic pregnancy, and infertility (5, 7). Chlamydial PID is the most important preventable cause of infertility and adverse pregnancy outcome. *N. gonorrhoeae* infections are also a major cause of PID, infertility, and ectopic pregnancy, and both facilitate the transmission of human immunodeficiency virus (6, 13).

Because the majority of chlamydial infections may be asymptomatic, it is recommended by the Centers for Disease Control and Prevention that sexually active adolescents and women who are ≤ 24 years of age be screened for chlamydia at least once yearly (5, 7). Because of the high incidence in certain areas, some recommendations have been made to screen asymptomatic women twice yearly (2–4).

The sensitivity of nucleic acid amplification tests (NAATs) has been demonstrated to be superior to that of culture and direct specimen tests for the detection of these pathogens (1, 12, 17, 29–31). Specificity has also been excellent. Their greater sensitivity permits the use of specimens other than endocervical swabs for women and urethral swabs for men. Urine and vaginal swabs, which can be self administered, are both more acceptable from the patient's point of view and easier and less costly to collect. Numerous studies have reported excellent performance of amplification assays with urine for *C. trachomatis* (8, 12, 14, 16, 17, 25, 30–32, 36), for *N. gonorrhoeae* (29, 34), and for both simultaneously (multiplex assays) (1, 9, 27, 37). A few studies have reported good results for *C. trachomatis* with vaginal or vulvar swabs (18, 19, 33, 35). These specimens are far better for screening asymptomatic populations. Their use with NAATs for detection of these organisms can facilitate early treatment of the infected patient, prevent transmission to partners and infants, reduce the health care costs associated with the sequelae of infection, and identify individuals at risk for other bacterial and viral sexually transmitted diseases (STDs), including human immunodeficiency virus (11).

Since the advent of NAATs, community-based screening programs for *C. trachomatis* and *N. gonorrhoeae* have proven

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effective in detecting infection and reducing prevalence (14, 27). With the more sensitive tests, the return on the public health investment in screening programs is higher. A study of the cost-effectiveness of screening for *C. trachomatis* using culture, direct tests, and amplification tests suggested that the amplification tests provided the greatest cost benefit by permitting noninvasive specimen collection and preventing the greatest number of PID cases (20, 23, 24).

One commercially available and Food and Drug Administration (FDA)-cleared assay, APTIMA Combo 2, utilizes the amplification technology of transcription-mediated amplification (TMA). The Gen-Probe TMA system targets rRNA, which is present in high copy number in all infected cells and in the bacteria, in order to increase the sensitivity of the assay. The original assay has been modified to improve specificity, minimize inhibition, and allow the simultaneous detection of *C. trachomatis* and *N. gonorrhoeae*. We report on the clinical performance of this new multiplex APTIMA Combo 2 assay with endocervical swabs and urine specimens from female patients at seven clinical sites in the United States.

MATERIALS AND METHODS

Study design. This study was undertaken to evaluate the performance of the APTIMA Combo 2 assay, a multiplex NAAT recently cleared by the FDA for the simultaneous detection of *C. trachomatis* and *N. gonorrhoeae* in clinical specimens. Specimens from females were tested for *C. trachomatis* and *N. gonorrhoeae* by Combo 2 and by other assays. Performance characteristics were calculated by comparing assay results with the patient's infected status.

Trial sites and populations. Endocervical swab and first-catch urine (FCU) specimens were collected from 18- to 35-year-old females (mean age of 28 years and median age of 25 years) at seven geographically diverse clinical sites in the United States with both medium and high prevalence of *C. trachomatis* and *N. gonorrhoeae* infections, including STD clinics, family planning clinics, and obstetrics and gynecology clinics. The trial sites included Baltimore, Md., the Florida State Department of Health, Houston, Tex., Birmingham, Ala., New Orleans, La., Stockton, Calif., and San Francisco, Calif. The trial was conducted between March 2000 and August 2000. The patient's symptomatic status and whether she was pregnant were recorded. Informed consent was documented where required by the site's institutional review board. Patient confidentiality was maintained as required by law.

Specimen collection procedures. Patients provided at least 25 ml of FCU and four endocervical swab specimens (one each for testing by *N. gonorrhoeae* culture, Combo 2, and two comparator NAATs). The FCU specimen was collected before the swab specimens. The *N. gonorrhoeae* culture swab was collected before the other swabs. The collection order of the subsequent swabs was determined by a randomization protocol.

Collection, storage, and transport of the *N. gonorrhoeae* culture swab followed the standard operating procedure of the local clinic site. All presumptively positive gonorrhea isolates were confirmed as *N. gonorrhoeae* by biochemical or immunological tests according to local protocols. All other specimens were collected, stored, and transported to the laboratory according to each assay manufacturer's instructions. Specimens were not frozen before testing.

Specimens were excluded if the patient had urinated within 1 h before providing the specimen or had taken antibiotics within the previous 21 days or if collection, storage, or transport requirements were not met.

Assays and testing algorithm. The APTIMA Combo 2 assay (Gen-Probe, Inc., San Diego, Calif.) employs the TMA technology, in which rRNA target molecules from *C. trachomatis* and from *N. gonorrhoeae* are isolated and specific regions are amplified by using a separate capture oligomer and a unique set of primers for each target. There is no cross-reactivity with other species of chlamydia or neisseria. The *C. trachomatis* target is the 23S rRNA, and the GenBank accession numbers are U68443 and M59178. These numbers are for both the 16S and 23S rRNA sequences. The *C. trachomatis* confirmatory assay targets the 16S rRNA. The *N. gonorrhoeae* target for Combo 2 is the 16S rRNA, and the GenBank accession number is X07714. The *N. gonorrhoeae* confirmatory assay targets a different region of the *N. gonorrhoeae* 16S rRNA molecule and does not detect the *N. gonorrhoeae* primary Combo 2 amplicon.

TABLE 1. Testing algorithm

Organism	Swab test format	FCU test format
C. trachomatis	Combo 2	Combo 2
	LCx	LCx
	Amplicor	Amplicor
N. gonorrhoeae	Combo 2	Combo 2
	LCx	LCx
	Culture	

Target sequences are isolated and separated from the specimen matrix, which may contain amplification inhibitors, by specific capture onto the surface of magnetic particles ("target capture"). Simultaneous detection of the rRNA amplicons is achieved using a dual kinetic assay that employs single-stranded DNA probes labeled with two different types of acridinium ester molecules. Differences in the light-off kinetics of these acridinium ester molecules allows the detection and differentiation of two analytes in a single reaction.

The LCx *C. trachomatis* assay and the LCx *N. gonorrhoeae* assay (Abbott Laboratories, Abbott Park, Ill.) use the ligase chain reaction to amplify a region of the *C. trachomatis* plasmid DNA and the *opa* gene from *N. gonorrhoeae*. The ligase chain reaction product is detected by the Abbott LCx analyzer.

The Amplicor and Cobas Amplicor *C. trachomatis* tests (Roche Diagnostic Systems, Inc., Branchburg, N.J.) are PCR-based assays.

All specimens were tested for *C. trachomatis* and *N. gonorrhoeae* by both the Combo 2 and LCx tests and for *C. trachomatis* by the Amplicor test. In addition, swab specimens were tested for *N. gonorrhoeae* by culture. Refer to Table 1 for the testing algorithm. All assays were performed according to their respective manufacturer's instructions by laboratory staff with demonstrated proficiency in that method. Each laboratory followed its standard procedures for *N. gonorrhoeae* culture.

Confirmatory testing. Combo 2-positive specimens from patients classified as not infected (see paragraph below) were investigated further in masked testing together with a subset of negative specimens at Gen-Probe by confirmatory TMA assays. These confirmatory TMA assays use primers and probes that target different rRNA sequences to differentiate between amplicon contamination and the true presence of the pathogen in the specimen. The accuracy of the confirmatory TMA assays was demonstrated by testing specimens with concordant results, i.e., positive or negative by the Combo 2 and the comparator assays (results not shown).

Determination of patient infected status. Patients were classified as infected or not infected based on the results of the comparator assays. For *C. trachomatis*, a patient was classified as infected when at least two positive results were obtained by the comparator assays in any combination of specimen type (e.g., both swab and FCU by either assay, swabs by both assays, FCU by both assays, or swab by one assay and FCU by the other). A patient was classified as not infected when all comparator assay results were negative. A patient was classified as having inconclusive infected status (two patients) when results were positive by one comparator assay on one specimen type; these patients were excluded from the data analysis. A patient was considered to have evaluable *C. trachomatis* data when she could be classified as either infected or not infected and when a Combo 2 result was available for at least one specimen type.

For *N. gonorrhoeae*, a patient was classified as infected when the culture result was positive or when both the LCx swab and FCU results were positive. A patient was classified as not infected when culture and LCx on both specimen types were negative. A patient was classified as having inconclusive infected status (five patients) when LCx results were positive on only one specimen type; these patients were excluded from the data analysis. A patient was considered to have evaluable *N. gonorrhoeae* data when she could be classified as either infected or not infected and when a Combo 2 result was available for at least one specimen type.

Data analysis. Specimens were categorized by type and the presence or absence of patient symptoms. The performance characteristics of the Combo 2 assay (sensitivity, specificity, positive predictive value [PPV], and negative predictive value [NPV]) were calculated for each category by comparing assay results with the patient infected status. Confidence intervals (95% confidence level) were calculated using the exact binomial distribution method.

TABLE 2. Number of patients with evaluable results by specimen type and patient symptomatic status

Specimen type and symptomatic status	No. of patients with:	
	C. trachomatis	N. gonorrhoeae
Swab		
Symptomatic	819[a]	881[b]
Asymptomatic	569	596[c]
Unknown	1	2
Total for swab	1,389	1,479
FCU		
Symptomatic	821	883
Asymptomatic	569	599
Unknown	1	2
Total for FCU	1,391	1,484

[a] Does not include two patients for whom only FCU results were available.
[b] Does not include two patients for whom only FCU results were available.
[c] Does not include three patients for whom only FCU results were available.

RESULTS

A total of 1,391 patients had evaluable *C. trachomatis* results. Of these, there were two patients for whom only FCU results were available; sufficient data were available for determination of their infected status (Table 2).

A total of 1,484 patients had evaluable *N. gonorrhoeae* results. Of these, there were five patients for whom only FCU results were available; sufficient data were available for determination of their infected status (Table 2).

The numbers of patients classified as infected or not infected with each pathogen are shown in Table 3. Overall, *C. trachomatis* prevalence was 15.0% and ranged from 7.5 to 31% among the seven sites. Overall, *N. gonorrhoeae* prevalence was 8.6% and ranged from 2.1 to 16.2%.

The results of testing by the Combo 2 assay compared with patient infected status, stratified by specimen type and patient symptomatic status, are shown in Table 4 for *C. trachomatis* and in Table 5 for *N. gonorrhoeae*. Overall, the Combo 2 assay showed a sensitivity and specificity of 94.2 and 97.6%, respectively, in *C. trachomatis* swab specimens and of 94.7 and 98.9%, respectively, in *C. trachomatis* FCU specimens. The PPV and NPV of the Combo 2 assay for *C. trachomatis* were 87.4 and 99.0%, respectively, in swab specimens and 93.8 and 99.1%, respectively, in FCU specimens.

Of 28 apparently false-positive *C. trachomatis* swab specimens, 22 were positive by the confirmatory TMA assay. Of 13 apparently false-positive *C. trachomatis* FCU specimens, 3 were positive by the confirmatory TMA assay.

Overall, the Combo 2 assay showed a sensitivity and specificity of 99.2 and 98.7%, respectively, in *N. gonorrhoeae* swab specimens and of 91.3 and 99.3%, respectively, in *N. gonorrhoeae* FCU specimens. Of 17 apparently false-positive *N. gonorrhoeae* swab specimens, 15 were positive by the confirmatory TMA assay. The PPV and NPV of the Combo 2 assay for *N. gonorrhoeae* were 88.1 and 99.9%, respectively, in swab specimens and 92.1 and 99.2%, respectively, in FCU specimens.

Of 10 apparently false-positive *N. gonorrhoeae* FCU specimens, 6 were positive by the confirmatory TMA assay. One *N.*

gonorrhoeae FCU specimen from an asymptomatic patient was not available for confirmatory testing.

A total of 32 patients with evaluable *C. trachomatis* results and 34 patients with evaluable *N. gonorrhoeae* results were pregnant. Table 6 shows the results of testing specimens from pregnant patients by the Combo 2 assay compared with patient infected status for both pathogens and both specimen types. For *C. trachomatis*, the Combo 2 assay showed a sensitivity and specificity of 100 and 95.8%, respectively, on swab specimens and 100 and 100%, respectively, on FCU specimens from pregnant patients. Of all specimens from all pregnant patients, only one specimen, a *C. trachomatis* swab, gave an apparent false result (apparent false positive); this specimen was positive by the TMA confirmatory assay. For *N. gonorrhoeae*, Combo 2 results were concordant with patient infected status in all specimens from pregnant patients (100% sensitivity and 100% specificity for both specimen types).

A total of 56 patients were found to be coinfected with *C. trachomatis* and *N. gonorrhoeae* (4.0% of patients with evaluable *C. trachomatis* results and 3.8% of patients with evaluable *N. gonorrhoeae* results). Of patients infected with *C. trachomatis*, 26.9% were coinfected with *N. gonorrhoeae*. Of patients infected with *N. gonorrhoeae*, 44.1% were coinfected with *C. trachomatis*. For coinfected patients, the Combo 2 swab gave *C. trachomatis*-negative and *N. gonorrhoeae*-positive results for two patients; the Combo 2 FCU specimen gave *C. trachomatis*-positive and *N. gonorrhoeae*-negative results for four patients and *C. trachomatis*-negative and *N. gonorrhoeae*-positive results for two patients (Table 7).

DISCUSSION

In this multicenter trial, the APTIMA Combo 2 assay performed with extreme accuracy for the detection of both *C. trachomatis* and *N. gonorrhoeae* in swab and urine specimens from symptomatic and asymptomatic females. The Combo 2 assay targets the same region of the *C. trachomatis* rRNA molecule that was developed with the earlier Gen-Probe AMPLIFIED *C. trachomatis* assay, previously documented in clinical studies (8, 12, 17, 30). The ability to screen for both *C. trachomatis* and *N. gonorrhoeae* simultaneously with no cross-interference is highly desirable.

The performance of the Combo 2 assay for *C. trachomatis* was comparable to or higher than that reported for other NAATs in both swab and FCU specimens (15, 17, 31, 32, 36, 38). Performance was more or less identical in both specimen types. Performance was higher in asymptomatic than in symptomatic women.

TABLE 3. Number of patients infected and not infected

Bacterium	No. of patients	
	Infected	Not infected
C. trachomatis	208[a]	1,183[b]
N. gonorrhoeae	127	1,357[c]

[a] Includes one patient for whom only FCU results were available.
[b] Includes one patient for whom only FCU results were available.
[c] Includes five patients for whom only FCU results were available.

TABLE 4. Results of *C. trachomatis* testing by the Combo 2 assay versus infection status in all patients ($n = 1,391$)

Specimen type and symptomatic status	n	No. of patients who were:				Sensitivity[g]	Specificity[g]	PPV (%)	NPV (%)
		Infected		Not infected					
		Combo 2 positive	Combo 2 negative	Combo 2 positive	Combo 2 negative				
Swab									
Symptomatic	819[a]	133	11	22[b]	653	92.4 (86.7–96.1)	96.7 (95.1–97.9)	85.8	98.3
Asymptomatic	569	61	1	6[c]	501	98.4 (91.3–100)	98.8 (97.4–99.6)	91.0	99.8
Unknown	1	1	0	0	0	NC[d]	NC	NC	NC
Total	1,389	195	12	28	1,154	94.2 (90.1–97.0)	97.6 (96.6–98.4)	87.4	99.0
FCU									
Symptomatic	821	136	9	8[e]	668	93.8 (88.5–97.1)	98.8 (97.7–99.5)	94.4	98.7
Asymptomatic	569	60	2	5[f]	502	96.8 (88.8–99.6)	99.0 (97.7–99.7)	92.3	99.6
Unknown	1	1	0	0	0	NC	NC	NC	NC
Total	1,391	197	11	13	1,170	94.7 (90.7–97.3)	98.9 (98.1–99.4)	93.8	99.1

[a] Does not include two patients for whom only FCU results were available.
[b] All 22 specimens were available for confirmatory testing; 18 were positive.
[c] All six specimens were available for confirmatory testing, four were positive.
[d] Not calculated.
[e] All eight specimens were available for confirmatory testing; two were positive.
[f] All five specimens were available for confirmatory testing; one was positive.
[g] Values are percentages. Values in parentheses are 95% confidence intervals.

The performance of the Combo 2 assay for *N. gonorrhoeae* was comparable to or higher than that reported for other NAATs in both swab and FCU specimens (29, 34, 36, 38). The sensitivity of the assay for *N. gonorrhoeae* was somewhat lower in FCU specimens than in swabs (91.3 versus 99.2%); however, it compares well with the results of other NAATs with urine (9). The sensitivity appeared to be lower for asymptomatic women than for symptomatic women in this study.

Among pregnant patients, the virtually perfect performance of the assay is encouraging. Others have reported decreased sensitivity of NAATs in specimens from pregnant women (26, 28), although this has been not reported by others (15). Although amplification inhibition has been reported with beta-

human chorionic gonadotropin in spiking experiments, it does not appear to interfere with the APTIMA Combo 2 assay (28).

Previous studies have demonstrated that screening programs for STDs are cost-effective in direct proportion to the number of infections that are detected and treated before infection is transmitted or sequelae develop (10, 21, 22, 24). The higher the predictive value of the test, both positive and negative, the greater the value of testing. In this trial, many of the specimens that were apparently false positive by the Combo 2 had positive results by the confirmatory assay: for *C. trachomatis*, 22 of 28 (78.6%) swabs and 3 of 13 (23%) FCU samples; for *N. gonorrhoeae*, 15 of 17 (88.2%) swabs and 4 of 10 (40%) FCU sam-

TABLE 5. Results of *N. gonorrhoeae* testing by the Combo 2 assay versus infection status in all patients ($n = 1,484$)

Specimen type and symptomatic status	n	No. of patients who were:				Sensitivity[a]	Specificity[a]	PPV (%)	NPV (%)
		Infected		Not infected					
		Combo 2 positive	Combo 2 negative	Combo 2 positive	Combo 2 negative				
Swab									
Symptomatic	881[b]	94	0	15[c]	772	100 (96.2–100)	98.1 (96.9–98.9)	86.2	100
Asymptomatic	596[d]	31	1	2[e]	562	96.9 (83.8–99.9)	99.6 (98.7–100)	93.9	99.8
Unknown	2	1	0	0	1	NC[f]	NC		NC
Total	1,479	126	1	17	1,335	99.2 (95.7–100)	98.7 (98.0–99.3)	88.1	99.9
FCU									
Symptomatic	883	87	7	7[g]	782	92.6 (85.3–97.0)	99.1 (98.2–99.6)	92.6	99.1
Asymptomatic	599	28	4	3[h]	564	87.5 (71.0–96.5)	99.5 (98.5–99.1)	90.3	99.3
Unknown	2	1	0	0	1	NC	NC		NC
Total	1,484	116	11	10	1,347	91.3 (85.0–95.6)	99.3 (98.6–99.6)	92.1	99.2

[a] Values are percentages. Values in parentheses are 95% confidence intervals.
[b] Does not include two patients for whom only FCU results were available.
[c] All 15 specimens were available for confirmatory testing; 13 were positive.
[d] Does not include three patients for whom only FCU results were available.
[e] Both specimens were available for confirmatory testing; both were positive.
[f] Not calculated.
[g] All seven specimens were available for confirmatory testing; four were positive.
[h] Of these three specimens, two were available for confirmatory testing; both were negative.

TABLE 6. Results of testing by the Combo 2 assay versus infection status in pregnant patients

Bacterium and specimen type	n	No. of patients who were:				Sensitivity (%)	Specificity (%)
		Infected		Not infected			
		Positive	Negative	Positive	Negative		
C. trachomatis							
Swab	32	8	0	1[a]	23	100	95.8
FCU	32	8	0	0	24	100	100
N. gonorrhoeae							
Swab	34	8	0	0	26	100	100
FCU	34	8	0	0	26	100	100

[a] This specimen was positive by the confirmatory TMA assay.

ples. These specimens may have been true positives. Thus, the specificities reported in this study may be artificially low if the apparently false-positive specimens that were positive in the Combo 2 confirmatory assay were true positives. Accepting a Combo 2 confirmatory positive result as indicating a true positive would significantly increase the apparent specificities for both specimen types and both organisms.

Apparent false-positive results are to be expected when the new test is more sensitive than the comparator test. The confirmatory TMA assay in this trial used primers and probes that target different rRNA sequences. Extensive testing has demonstrated that the confirmatory assay distinguishes between pathogen and amplicon contamination in the sample and its ability to detect the target molecule in clinical specimens. The confirmatory *C. trachomatis* assay gave positive results for 97 and 98% of concordant positive specimens; the confirmatory *N. gonorrhoeae* assay gave positive results for 100% of concordant positive specimens (data not shown). We therefore regarded the results of the confirmatory TMA assays as reliable. Further evaluation of the PPV of this test is warranted, especially in low-prevalence populations.

The ability to detect *C. trachomatis* and *N. gonorrhoeae* with great accuracy in urine and other noninvasively collected specimens can dramatically increase the number of women who can be screened while decreasing the cost of screening. This approach to large-scale screening programs has the potential to significantly impact the epidemic of STDs in the United States and other, less developed countries.

The Combo 2 assay can be recommended for use with urine specimens from females, especially in screening tests for asymptomatic women who are participating in STD surveillance programs. This FDA-cleared assay will provide clinicians and laboratorians with the necessary tools to significantly re-

duce the prevalence and incidence of *C. trachomatis* and *N. gonorrhoeae* infections in sexually active women, as well as prevent their costly and serious sequelae.

TABLE 7. Results of testing by the Combo 2 assay in coinfected patients (n = 56)

Specimen type	n	No. of patients who were *C. trachomatis* positive and *N. gonorrhoeae* positive (%)
Swab	56[a]	54 (96.5)
FCU	56[b]	50 (89.5)

[a] Two specimens were *C. trachomatis* negative and *N. gonorrhoeae* positive.
[b] Four specimens were *C. trachomatis* positive and *N. gonorrhoeae* negative; two specimens were *C. trachomatis* negative and *N. gonorrhoeae* positive.

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