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Detection of *Neisseria gonorrhoeae* and *Chlamydia trachomatis* in Genitourinary Specimens from Men and Women by a Coamplification PCR Assay

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A coamplification PCR test for the direct detection of *Neisseria gonorrhoeae* and *Chlamydia trachomatis* in urethral and endocervical swabs and urine samples from men and women was compared to standard culture techniques. Processed specimens were amplified in single reaction tubes containing primers for both organisms, and PCR products were detected by a colorimetric microwell plate hybridization assay specific for each pathogen. Of 344 specimens from men, 45 (13.1%) urine specimens were PCR positive for *C. trachomatis*, 51 (14.8%) urethral swab specimens were PCR positive, and 29 urethral swab specimens (8.4%) were culture positive. After analysis of discrepancies, the resolved sensitivity and specificity of PCR for *C. trachomatis* were 96.2 and 99.3%, respectively, in urethral swab specimens, compared to 88.2 and 98.6% for urine specimens. Of the 192 specimens from women, 28 (14.6%) urine specimens were PCR positive for *C. trachomatis*, 32 (16.7%) endocervical specimens were PCR positive, and 19 (9.9%) endocervical specimens were culture positive. After analysis of discrepancies, the resolved sensitivity and specificity of PCR for *C. trachomatis* for endocervical specimens were both 100% compared to 100 and 99.4%, respectively, for urine specimens from women. In men, 68 (19.8%) urine specimens were PCR positive for *N. gonorrhoeae*, 73 (21.2%) urethral swabs were PCR positive, and 59 (17.2%) urethral swabs were culture positive. After analysis of discrepancies, the resolved sensitivity and specificity of PCR for *N. gonorrhoeae* were 97.3 and 97.0%, respectively, for urethral specimens compared to 94.4 and 98.5% for urine specimens. In women, 18 (9.4%) urine specimens were PCR positive for *N. gonorrhoeae*, 23 (12.0%) were endocervical swab PCR positive, and 15 (7.8%) endocervical specimens were culture positive. After analysis of discrepancies, the resolved sensitivity and specificity of PCR for *N. gonorrhoeae* were 100 and 99.4%, respectively, for endocervical specimens compared to 90.0 and 95.9% for female urine specimens. These results indicate that a multiplex PCR is highly sensitive for detecting both *C. trachomatis* and *N. gonorrhoeae* from a single urine or genital swab, providing a more cost-effective way of screening multiple pathogens.

Neisseria gonorrhoeae and *Chlamydia trachomatis* are recognized as two of the most prevalent sexually transmitted bacterial infections (5). Worldwide, there is an estimated annual incidence of 25 million cases of gonorrhea and 50 million cases of chlamydia (16). In an effort to prevent the spread of these diseases, increased attention is being focused on early diagnosis and treatment of symptomatic or asymptomatic infected individuals. In men, *C. trachomatis* causes 40 to 50% of cases of nongonococcal urethritis, making it one of the most common sexually transmitted diseases (STD) in heterosexual males (20). In women, chlamydia is a major cause of pelvic inflammatory disease leading to infertility and ectopic pregnancy and can result in conjunctivitis and pneumonia in newborn infants exposed during passage through an infected birth canal (17). Symptoms and complications of gonorrhea are similar to those of chlamydia, and a substantial proportion of infected individuals, especially women, are asymptomatic (9). For both organisms, asymptomatic persons serve as a reservoir of infection, and since coinfection is common, symptoms may overlap, making clinical diagnosis difficult.

Conventional diagnosis of *N. gonorrhoeae* infection requires isolation on selection media or observation of gram-negative diplococci in Gram smears of genital discharge, or urethral or cervical swabs. Although culture is relatively inexpensive and highly sensitive, it is logistically complicated. A pelvic examination is required for women, and insertion of a urethral swab is required for men. Diagnosis of *C. trachomatis* infection is frequently based on isolation in tissue culture. This procedure requires careful specimen collection and stringent transport conditions and requires at least 48 to 72 h to perform. Similar to the routine diagnosis of gonorrhea, detection of chlamydia usually involves an invasive procedure. Since 30 to 70% of all chlamydial and gonococcal infections may be asymptomatic, routine, noninvasive screening of individuals at risk for chlamydial or gonococcal infection is highly desirable. Treatment is relatively simple and straightforward, with a 7-day course of doxycycline twice a day or a single dose of azithromycin for *C. trachomatis*, and a single dose of a quinolone or cephalosporin for *N. gonorrhoeae* (3, 14, 21). A rapid, easily performed, and accurate diagnostic test that can be performed on noninvasive samples would facilitate early recognition and treatment of these infections and thus ultimately reduce partner infections.

Recent studies have demonstrated that molecular amplification assays such as PCR and ligase chain reaction (LCR) have high sensitivity and specificity for detection of either gonorrhea or chlamydia in a variety of sample types including urethral and endocervical swabs and urine (2, 7, 11, 16, 17, 19, 21, 22). However, they require separate processing and ampli-

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fication techniques for each pathogen. In order to improve the efficiency of specimen processing and amplification, we evaluated a duplex PCR for *N. gonorrhoeae* and *C. trachomatis* that simultaneously detects both pathogens in a single amplified swab or urine specimen.

MATERIALS AND METHODS

Patient population. From February 1995 through April 1995, 344 male and 192 female patients attending two Baltimore City STD clinics were prospectively enrolled with informed consent. The study protocol was approved by both the Johns Hopkins University and the Baltimore City Health Department ethical review boards. For men, a urethral swab specimen for chlamydia was collected by inserting a narrow-shafted Dacron-tipped swab 2 to 3 cm into the urethra and then placed in chlamydia transport vials containing sucrose-phosphate buffer, 10% fetal bovine serum, and antibiotics. An additional urethral swab was obtained for *N. gonorrhoeae* culture and plated on Thayer-Martin medium. Fifteen milliliters of first-voided urine (FVU) was then collected in a sterile 50-ml screw-cap plastic cup. For women, one endocervical swab was obtained and placed in chlamydia transport medium. An additional endocervical swab was collected for *N. gonorrhoeae* culture. An FVU sample was collected from the women following the completion of their pelvic examination and the collection of all other specimens. The FVU specimens were transported at room temperature and later stored at 4°C for up to 96 h before urine processing. Chlamydia transport vials were stored at −70°C for 12 to 24 h until they were processed for culture. All PCR swab testing utilized the same chlamydia transport vial that was collected for chlamydia culture. In order to limit freezing and thawing the specimen several times, the PCR aliquot was taken at the same time the culture was inoculated (13).

Chlamydia culture. Tissue culture was performed in 96-well microtiter plates by using McCoy cell monolayers pretreated with DEAE-dextran as described previously (22). A 100-μl aliquot of transport medium was inoculated into each of two microtiter wells (total volume, 200 μl). After 48 h both wells were evaluated for chlamydia inclusions by using immunofluorescence staining. One well was stained with monoclonal antibody to *C. trachomatis* major outer membrane protein (MOMP) (Microtrak chlamydia culture reagent; Syva, San Jose, Calif.), and the other well was stained with antilipopolysaccharide monoclonal antibody (Sanofi Diagnostics Pasteur, Chaska, Minn.).

Gonorrhea culture. Modified Thayer-Martin plates were directly inoculated from the swab as described previously (15). Cultures were examined following overnight incubation at 34 to 36°C at 10% CO_2. Typical colonies containing gram-negative diplococci and giving a positive oxidase reaction were presumptively identified as *N. gonorrhoeae*. The identities of presumptive *N. gonorrhoeae* isolates were confirmed by using fluorescent-antibody staining (Baxter Scientific, McGaw Park, Ill.).

PCR. PCR was performed on urogenital and urine swab specimens by using a prototype, rapid, nonradioactive PCR-based assay (Roche Molecular Systems, Branchburg, N.J.) according to investigational test procedures. For urethral and endocervical swab specimens, a 100-μl sample of each chlamydia 2-sucrose-phosphate transport medium (CTM) was diluted with an equal volume of AMPLICOR CT/NG lysis buffer. The resulting lysed specimen was then mixed with 200 μl of AMPLICOR CT/NG specimen diluent.

Urine specimens from men and women were processed by pretreating 500 μl of urine with 500 μl of AMPLICOR CT/NG urine wash buffer for 15 min at 37°C to solubilize potential inhibitors. The sediment was collected by high-speed centrifugation and resuspended in 250 μl of AMPLICOR CT/NG lysis buffer. After a 10-min incubation at room temperature, 250 μl of AMPLICOR CT/NG specimen diluent was added and the specimens were centrifuged at 12,000 × *g* for 10 min. The supernatant was used for PCR analysis.

Fifty microliters of the processed patient sample or control was then added to each PCR tube containing 50 μl of the PCR master mix. The master mix is a buffered solution containing two pairs of biotin-labeled primers for simultaneous amplification of *C. trachomatis* (target sequence, 207-bp fragment in cryptic plasmid) and *N. gonorrhoeae* (target sequence, 201-bp fragment in putative cytosine methyltransferase gene), *Taq* polymerase, deoxynucleoside triphosphates (including dUTP substituted for TTP), and AmpErase (uracil-*N*-glycosylase) to prevent carryover contamination (12). PCR amplification was carried out for 35 cycles with the GeneAMP 9600 thermocycler (Perkin-Elmer Cetus, Norwalk, Conn.).

After amplification, the PCR products were denatured with a weak sodium hydroxide solution and then added to a microtiter plate containing either an immobilized *C. trachomatis*-specific DNA probe or an immobilized *N. gonorrhoeae*-specific DNA probe. After a 1-h incubation at 37°C, the plates were washed to remove unbound material and an avidin-horseradish peroxidase conjugate was added. Following a 15-min incubation at 37°C, the plates were washed. Peroxide substrate solution containing tetramethylbenzidine was then added, and the plates were incubated for 10 min at room temperature. Reactions were stopped by the addition of a weak acid solution, and the optical density of the reaction mix was measured by a spectrophotometer at 450 nm. Optical density values greater than or equal to 0.8 were considered positive; those less than 0.2 were considered negative. Specimens with optical density values between 0.2 and 0.8 were retested in duplicate. If two of the three test results (original and duplicate repeats) were found to be ≥0.25, the sample was considered positive for chlamydia or gonococcal DNA by PCR. If two of the three test results (original and duplicate repeats) were found to be <0.25, the sample was considered negative for chlamydia or gonococcal DNA by PCR. Negative controls were required to have an optical density value of <0.2, and the positive control had to have an optical density ≥2.0 for the test to be considered valid.

Analysis of discrepant results. For *C. trachomatis* specimens that were PCR positive and culture negative, two additional tests were utilized to resolve the discrepancies: direct fluorescent antibody (DFA) staining of CTM sediment and MOMP-based PCR performed on the CTM or urine specimen. This expanded reference standard has been utilized in the past to help resolve discrepancies, particularly among tests being evaluated that might be more sensitive than culture (1, 6, 7, 10, 11, 16). For *N. gonorrhoeae* specimens that were PCR positive and culture negative, PCR was repeated by performing a confirmatory 16S rRNA PCR assay on the CTM or urine specimen.

RESULTS

Chlamydia in men. A total of 344 matched urethral and urine specimens were available for evaluation from male participants. *C. trachomatis* was detected by urethral culture in 29 (8.4%) of 344 male specimens. As shown in Table 1, PCR performed on urine was positive for *C. trachomatis* in 23 of the 29 culture-positive men. Similarly, PCR performed on urethral swabs was positive for *C. trachomatis* in 27 of the 29 culture-positive men. In addition, PCR was positive for *C. trachomatis* in 26 urine and 26 urethral swab specimens obtained from culture-negative men (Table 1). Twenty-two of the 26 urine specimens and 24 of the 26 urethral swab specimens were confirmed positive by either DFA or MOMP PCR. Thus, PCR performed on urine detected 45 of the 51 men who were either positive by culture or confirmed positive by urine PCR, yielding an 88.2% sensitivity and a 98.6% specificity (Table 1). Similarly, PCR performed on urethral swabs detected 51 of the 53 men who were either positive by culture or confirmed positive by urethral PCR and yielded a 96.2% sensitivity and 99.3% specificity (Table 1).

Because two PCR tests were performed on each man, we were able to identify infections that would not have been detected had we tested a urine specimen only or a urethral swab specimen only. To identify all infected men, we compared urine and urethral swab PCR results from culture-negative men. Both the urine and the urethral swab specimens were PCR positive for 19 men; all of these were confirmed by DFA or MOMP PCR. In seven men, the urine specimen was PCR positive and the corresponding urethral swab specimen was PCR negative; three of these seven specimens were confirmed by DFA or MOMP PCR. In another seven men, the urethral swab specimen was PCR positive and the corresponding urine specimen was PCR negative; five of these seven specimens were confirmed by DFA or MOMP PCR. Thus, 27 infections were detected among culture-negative men, to give a total of 56 patient infections (Table 1). Compared to total resolved patient infections, PCR for *C. trachomatis* exhibited 80.4% sensitivity and 98.6% specificity for urine specimens from men and 91.1% sensitivity and 99.3% specificity for urethral swab specimens from men (Table 2). In contrast, culture had a sensitivity of 51.8%.

Chlamydia in women. A total of 192 matched endocervical and urine specimens were collected and available for evaluation from women. *C. trachomatis* was detected by endocervical culture in 19 (9.9%) of 192 women. As shown in Table 1, PCR performed on urine and endocervical swabs was positive for *C. trachomatis* in all 19 culture-positive women. Similarly, PCR was positive for *C. trachomatis* in an additional 10 urine and 13 endocervical swab specimens obtained from culture-negative women (Table 1). Nine of the 10 urine specimens and all 13 endocervical swab specimens were confirmed positive by either

TABLE 1. Comparison of PCR with culture for *C. trachomatis* for 344 specimens from men and 192 specimens from women before and after discrepant analysis[a]

Specimen type and PCR result	No. of specimens with the following result by culture:		No. of specimens resolved[b]:		No. of patients resolved[c]:	
	Positive	Negative	Positive	Negative	Positive	Negative
Men						
Urine						
Positive	23	26	45	4	45	4
Negative	6	289	6	289	11	284
Urethral						
Positive	27	26	51	2	51	2
Negative	2	289	2	289	5	286
Women						
Urine						
Positive	19	10	28	1	28	1
Negative	0	163	0	163	6	157
Cervical						
Positive	19	13	32	0	32	0
Negative	0	160	0	160	2	158

[a] Discrepant analysis was performed on PCR-positive, culture-negative samples.
[b] PCR-positive specimens were resolved positive if culture was positive, DFA was positive, or MOMP PCR was positive.
[c] PCR-negative, culture-negative specimens were resolved positive if the alternative specimen from the same patient was PCR positive and was resolved by either DFA or MOMP PCR testing.

DFA or MOMP PCR. Thus, PCR performed on urine detected all 28 women who were either positive by culture or confirmed positive by urine PCR and yielded 1 false-positive result (Table 1), for a sensitivity and specificity of 100 and 99.4%, respectively. PCR performed on endocervical swabs detected all 32 women who were either positive by culture or confirmed positive by urethral PCR and yielded no false-positive results (Table 1), for a sensitivity and specificity of 100% each.

To identify all infected women, we compared urine and endocervical swab PCR results from culture-negative women. Both the urine and endocervical swab specimens were PCR positive for seven women; all of these were confirmed by DFA or MOMP PCR. In three women, the urine specimen was PCR positive and the endocervical swab was PCR negative; two of these three specimens were confirmed by DFA or MOMP PCR. In another six women, the endocervical swab specimen was PCR positive and the corresponding urine specimen was PCR negative; all six specimens were confirmed by DFA or MOMP PCR. Thus, a total of 15 patient infections were observed in culture-negative women, to give a total of 34 patient infections (Table 1). Compared to total resolved patient infections, PCR for *C. trachomatis* exhibited 82.4% sensitivity and 99.4% specificity for urine specimens from women and 94.1% sensitivity and 100% specificity for endocervical swab specimens (Table 2). In contrast, the sensitivity of culture was 55.9% (Table 2).

Gonorrhea in men. *N. gonorrhoeae* was detected by urethral culture in 59 (17.2%) of 344 men. As shown in Table 3, PCR performed on urine was positive for *N. gonorrhoeae* in 55 of the 59 culture-positive men. Similarly, PCR performed on urethral swabs was positive for *N. gonorrhoeae* in 57 of the 59 culture-positive men. In addition, PCR was positive for *N. gonorrhoeae* in 17 urine and 24 urethral swab specimens obtained from culture-negative men (Table 3). Thirteen of the 17 urine specimens and 16 of the 24 urethral swab specimens were confirmed positive by 16S rRNA PCR. Thus, PCR performed on urine detected 68 of the 72 men who were either positive by culture or confirmed positive by urine PCR and yielded a 94.4% sensitivity (Table 3). Similarly, PCR performed on urethral swabs detected 73 of the 75 men who were either positive by culture or confirmed positive by urethral PCR, yielding a 97.3% sensitivity (Table 3).

To identify all infected men, we compared urine and urethral swab PCR results from culture-negative men. Both the urine and the urethral swab specimens were PCR positive for 13 men; 11 of these 13 specimens were confirmed by 16S rRNA PCR. In four men, the urine specimen was PCR positive and the corresponding urethral swab specimen was PCR negative; two of these four specimens were confirmed by 16S rRNA PCR. In another 11 men, the urethral swab specimen was PCR positive and the corresponding urine specimen was PCR negative; 5 of these 11 specimens were confirmed by 16S rRNA PCR. Thus, 18 patient infections were observed in culture-negative men to give a total of 77 patient infections (Table 3). Compared to total resolved patient infections, PCR for *N. gonorrhoeae* exhibited 88.3% sensitivity and 98.5% specificity for male urine specimens and 94.8% sensitivity and 97.0% specificity for male urethral swab specimens (Table 4). In contrast, the sensitivity of culture was 76.6% (Table 4).

Gonorrhea in women. *N. gonorrhoeae* was detected by endocervical culture in 15 (7.8%) of 192 women. As shown in Table 3, PCR performed on urine was positive for *N. gonorrhoeae* in 13 of the 15 culture-positive women. Similarly, PCR performed on endocervical swabs was positive for *N. gonorrhoeae* in all 15 culture-positive women. In addition, PCR was positive for *N. gonorrhoeae* in an additional 12 urine and 9 endocervical swab specimens obtained from culture-negative women (Table 3). Five of the 12 urine specimens and 8 of the 9 endocervical swab specimens were confirmed positive by 16S rRNA PCR. Thus, PCR performed on urine detected 18 of 20 women who were either positive by culture or confirmed positive by urine PCR and yielded a 90.0% sensitivity (Table 3). PCR performed on endocervical swabs detected all 23 women who were either positive by culture or confirmed positive by urethral PCR, yielding a 100% sensitivity (Table 3).

To identify all infected women, we compared urine and endocervical swab PCR results from culture-negative women. Both the urine and the endocervical swab specimens were PCR positive for five women; all of these were confirmed by 16S

TABLE 2. Resolved performance characteristics for detection of *C. trachomatis* by PCR for gender and specimen type

Test and sample	Per specimen		Per patient	
	Sensitivity (%)	Specificity (%)	Sensitivity (%)	Specificity (%)
Male (n = 344)				
PCR				
Urine	88.2	98.6	80.4	98.6
Urethral	96.2	99.3	91.1	99.3
Culture, urethral	51.8	100	51.8	100
Female (n = 192)				
PCR				
Urine	100	99.4	82.4	99.4
Endocervical	100	100	94.1	100
Culture, endocervical	55.9	100	55.9	100

TABLE 3. Comparison of PCR with culture for *N. gonorrhoeae* for 344 specimens from men and 192 specimens from women before and after discrepant analysis

Specimens type and PCR result	No. of specimens with the following result by culture:		No. of specimens resolved[a]:		No. of patients resolved[b]:	
	Positive	Negative	Positive	Negative	Positive	Negative
Men						
Urine						
Positive	55	17	68	4	68	4
Negative	4	268	4	268	9	263
Urethral						
Positive	57	24	73	8	73	8
Negative	2	261	2	261	4	259
Women						
Urine						
Positive	13	12	18	7	18	7
Negative	2	165	2	165	5	162
Cervical						
Positive	15	9	23	1	23	1
Negative	0	168	0	168	0	168

[a] PCR-positive specimens were resolved positive if culture was positive or 16S rRNA PCR was positive.

[b] PCR-negative, culture-negative specimens were resolved positive if the alternative specimen from the same patient was PCR positive and resolved by 16S rRNA PCR testing.

rRNA PCR. In seven women, the urine specimen was PCR positive and the corresponding endocervical swab specimen was PCR negative; none of these seven specimens was confirmed by 16S rRNA PCR. In another four women, the endocervical swab specimen was PCR positive and the corresponding urine specimen was PCR negative; three of these four specimens were confirmed by 16S rRNA PCR. Thus, a total of 8 patient infections were observed in culture-negative women, to give a total of 23 patient infections (Table 3). Compared to total resolved patient infections, PCR for *N. gonorrhoeae* exhibited 78.3% sensitivity and 95.9% specificity for female urine specimens and 100% sensitivity and 99.4% specificity for endocervical swab specimens (Table 4). In contrast, the sensitivity of culture was 65.2% (Table 4).

DISCUSSION

The prevalence of gonorrhea for men and women in inner-city STD clinics is approximately 9 to 15%. The prevalence of chlamydial infection ranges from 3 to 8% among asymptomatic men and women to 10 to 20% among adolescents attending STD clinics (18). Laboratory-based diagnosis, treatment, and contact tracing for these infections have been recommended by the Centers for Disease Control (4). Until recently the laboratory standard for diagnosis of *C. trachomatis* was in vitro cell culture isolation. However, following the development of molecular amplification assays such as PCR and LCR, the sensitivity of culture has been estimated to range from 50 to 85% (10). In our laboratory sensitivities ranged from 52 to 56% for chlamydia and from 65 to 77% for gonorrhea compared to PCR. The conventional laboratory diagnosis of *N. gonorrhoeae* is isolation of culture and demonstration of intracellular gram-negative diplococci in Gram-stained smears. As with *C. trachomatis*, adequacy of the sample is a limitation in diagnosis since only viable organisms can be detected. False-negative results have been attributed to poor specimen storage, transport problems, and inhibition of growth by the components of selective media (8).

Urine screening is important because it represents a noninvasive method for detection of pathogens in asymptomatic at-risk populations. Urine screening for chlamydia by EIA or DFA has proven to be of low sensitivity and specificity and is not routinely recommended for use with urine (6). In contrast, PCR and LCR assays utilizing urine, endocervical, or urethral samples have proven to be highly sensitive and specific and provide a method for screening both symptomatic and asymptomatic individuals for *C. trachomatis* and *N. gonorrhoeae* (7, 8, 16). Various studies have demonstrated PCR sensitivities for chlamydia infection in male urine following discrepant analysis to range from 93 to 97%, with specificities of 99% or greater (1, 10). In this study, the sensitivity and specificity of chlamydia detection by PCR in urine specimens from men were 88.2 and 98.6%, respectively. In the analysis of matched urine and urethral swab specimens from men, the combination of screening both samples by PCR increased the sensitivity to 100%. Similarly, the total number of infected patients increased when results for both specimen types were considered together. Because of sample-to-sample variation, only the swab or only the urine specimen was positive in some patients; such patients may not have been identified if only a single specimen from each patient had been analyzed. This effect is especially pronounced in women because swab and urine specimens are obtained from different anatomical sites. Because of this specimen-to-specimen variation, the apparent sensitivity of PCR performed on urine specimens from men was 80.4% when calculated on a per-patient basis instead of 88.2% when calculated on the traditional per-specimen basis. Likewise, the apparent sensitivity of PCR performed on urethral swab specimens from men was 91.1% when calculated on a per-patient basis, instead of 96.2%.

The sensitivity and specificity of chlamydia PCR performed on female urine after resolution on a per-specimen basis were 100 and 99.4%, respectively, compared to 55.9% sensitivity with culture. Because of sample-to-sample variation, the apparent sensitivity of chlamydia PCR performed on female urine was 82.4% when calculated on a per-patient basis. PCR sensitivity performed on endocervical swabs was 94.1% when calculated on a per-patient basis.

The resolved sensitivity of *N. gonorrhoeae* PCR was 94.4% for male urine specimens and 97.3% for male urethral swab specimens, much higher than the culture sensitivity for *N. gonorrhoeae* in men, which was 76.6%. As was observed for

TABLE 4. Resolved performance characteristics for detection of *N. gonorrhoeae* by PCR for gender and specimen type

Test and sample	Per specimen		Per patient	
	Sensitivity (%)	Specificity (%)	Sensitivity (%)	Specificity (%)
Male (n = 344)				
PCR				
Urine	94.4	98.5	88.3	98.5
Urethral	97.3	97.0	94.8	97.0
Culture, urethral	76.6	100	76.6	100
Female (n = 192)				
PCR				
Urine	90.0	95.9	78.3	95.9
Endocervical	100	99.4	100	99.4
Culture, endocervical	65.2	100	65.2	100

chlamydia, specimen-to-specimen variation caused an apparent decrease in sensitivity when calculations were per patient rather than per specimen. When calculated on a per-patient basis, the sensitivity of PCR for gonorrhea was 88.3% for urine specimens from men and 94.8% for urethral swab specimens from men. Again, these lowered sensitivities would be expected when sample variability and sampling from alternate sites are taken into account. PCR for *N. gonorrhoeae* was 90.0% sensitive for urine specimens from women and 100% sensitive for endocervical swab specimens. In the analysis of the matched urine and endocervical specimens from women for gonorrhea, the combination of screening both samples increased the sensitivity to 100%. However, because of sample variability, the apparent sensitivity of PCR performed on urine specimens from women was 78.3% when calculated on a per-patient basis. For endocervical swab specimens, the sensitivity of PCR on a per-specimen or per-patient analysis remained the same at 100%.

This study evaluated a combination PCR assay that simultaneously amplifies and detects both chlamydia and gonorrhea in a single specimen. To our knowledge this is the first report in which the performance of a combination chlamydia-gonorrhea test is compared by using both genitourinary swabs and urine specimens. In analyzing samples by using matched genitourinary swabs and urine specimens, it is important to note the cause of the lowered sensitivities for any one specimen type, e.g., sample variability and dual site sampling. Although sampling of multiple sites results in the best yield of identifying active infection (i.e., the highest sensitivity), it is also impractical and not cost-effective. The results indicate that this duplex test was more sensitive than both chlamydia and gonorrhea culture alone. Furthermore, sensitivity was equivalent for urine and swab specimens. Thus, this assay provides a cost-effective way to screen for the two major STD pathogens in noninvasively collected urine specimens.

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