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Detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* by Ligase Chain Reaction-Based Assays with Clinical Specimens from Various Sites: Implications for Diagnostic Testing and Screening

M. BUIMER,[1] G. J. J. VAN DOORNUM,[1]* S. CHING,[2] P. G. H. PEERBOOMS,[1] P. K. PLIER,[2] D. RAM,[1] AND H. H. LEE[2]

Department of Public Health, Municipal Health Service of Amsterdam, Amsterdam, The Netherlands,[1] and Abbott Laboratories, Abbott Park, Illinois[2]

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Ligase chain reaction (LCR)-based tests for the diagnosis of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* infections in men and women attending a sexually transmitted disease clinic were evaluated. LCR testing of urethral swab and urine specimens from men and cervical swab and urine specimens from women was compared with culture of male urethral swabs and female cervical and urethral swabs, respectively. An expanded "gold standard" was defined as a positive culture or at least one specimen confirmed to be positive by LCR testing. The prevalence of *C. trachomatis* infection as detected by cell culture was 7.0% among 614 men and 5.0% among 602 women. By LCR, these values increased to 11.4 and 9.9% with urethral swabs and urine, respectively, for men and 9.6 and 9.1% with cervical swabs and urine, respectively, for women. Relative to the expanded gold standard, the sensitivity of cell culture with male urethral swabs or female cervical swabs was 57.3 and 45.5%, respectively, compared with corresponding values of 93.3 and 87.9% for LCR. The sensitivity of LCR with urine specimens was 77.3 and 78.8% for men and women, respectively. The prevalence of *N. gonorrhoeae* infection as detected by culture was 5.9% among 220 men and 2.9% among 383 women. The corresponding values were 8.2 and 5.5%, respectively, by LCR testing of swabs. Prevalence values by LCR testing of urine were 7.3% for men and 2.9% for women. The sensitivity of culture was 72.2% for men and 50.0% for women. The sensitivities of LCR were 100% with male urethral swabs, 95.4% with female cervical swabs, 88.9% with male urine, and 50.0% with female urine. These results indicate that the LCR-based assays represent a major improvement in *C. trachomatis* and *N. gonorrhoeae* diagnostics. The sensitivity of testing of urethral or cervical swabs by LCR was markedly greater than that by culture. The sensitivity of testing female or male urine specimens was equal to or greater than that of culturing cervical or urethral specimens. LCR testing of urine specimens may prove useful for screening for *C. trachomatis*.

Recent advances in molecular biology have allowed for the development of sensitive diagnostic assays based on the detection of specific nucleic acid sequences in clinical specimens. Furthermore, the high degrees of sensitivity of such tests allow the use of alternative specimen types in which the abundance of microorganisms is below the limit of detection of more traditional assays. Thus, recent studies have shown that diagnostic assays based on PCR or ligase chain reaction (LCR) can detect *Chlamydia trachomatis* infection with male urine and urethral specimens (3, 5, 9, 16, 21, 23). Besides endocervical specimens, female urine can be also be used for the detection of *C. trachomatis* by PCR- or LCR-based assays (1, 2, 4, 13, 16, 18, 19, 21, 23). An LCR-based assay for the detection of *Neisseria gonorrhoeae* in female urine specimens has also been described (6, 24). The use of these highly sensitive tests has prompted the adoption of an expanded "gold standard" to compare the assay results obtained with specimens collected from different sites of infection (15, 18). We assayed urethral and cervical swabs and urine specimens for both *C. trachomatis* and *N. gonorrhoeae* by LCR and compared the results with those obtained by swab culture in an attempt to evaluate the value of these LCR tests for diagnostic and screening applications.

* Corresponding author. Mailing address: Laboratory of Public Health, Municipal Health Service of Amsterdam, Nieuwe Achtergracht 100, 1018 WT Amsterdam, The Netherlands. Phone: (31) (20) 555 5293. Fax: (31) (20) 555 5533.

MATERIALS AND METHODS

Study population. From October 1993 through January 1994 and from June 1994 through August 1994, consecutive new patients attending the sexually transmitted disease (STD) clinic of the Municipal Health Service in Amsterdam were included in the evaluation of the LCR-based assay for *C. trachomatis*. The LCR-based *N. gonorrhoeae* assay was evaluated with samples from patients attending the clinic from June 1994 through August 1994. All patients underwent a physical examination and filled out a questionnaire concerning STDs and the use of antibiotics in the 2 weeks prior to the visit. Complaints, symptoms, and signs of discharge, dysuria, vaginal bleeding, cervical friability, and abdominal pain were recorded. The number of polymorphonuclear leukocytes and gram-negative diplococci lying within polymorphonuclear leukocytes seen in Gram stains of urethral or cervical exudate or urine were also recorded.

Specimen collection and processing. Cervical and urethral swabs were collected from women for the detection of *C. trachomatis* and *N. gonorrhoeae* by culture. The initial order of collection of specimens was as follows: (i) urethral and cervical swab specimens for culture of *N. gonorrhoeae* were obtained together, (ii) urethral and cervical swabs for culture of *C. trachomatis* were obtained separately, and (iii) a cervical swab specimen was obtained for detection of *C. trachomatis* and *N. gonorrhoeae* by LCR-based assays. Halfway through the study, the order of specimen collection was reversed. Female urethral swabs were not tested routinely by LCR.

Separate urethral swabs were obtained from men for culture of *N. gonorrhoeae* and *C. trachomatis*. An urethral swab specimen was obtained for the LCR assays after swabs for culture were taken. Halfway through the study, the order of specimen collection was reversed.

Urine samples were also collected from both men and women for the LCR-based assays after the physical examination was performed and the swab specimens were collected.

The swabs and transport medium for the LCR assays were provided by Abbott Laboratories, North Chicago, Ill. Specimens for the LCR assay were transported to the laboratory at ambient temperature and were stored at 4 to 6°C for up to 5 days or at −20°C for longer intervals prior to processing.

Cotton swabs on an aluminum shaft were used to collect specimens for *C. trachomatis* culture. The swabs were placed in containers with 2 ml of 4SP transport medium comprising 0.4 M sucrose-phosphate buffer (pH 7.2), 10% fetal bovine serum, and antibiotics (nystatin [25,000 IU/liter], streptomycin [100 mg/liter], and vancomycin [100 mg/liter]). The vials were transported daily at 4°C to the laboratory and were stored at −70°C before cell culture, which was performed three times a week by a standard laboratory protocol.

Culture of *N. gonorrhoeae* was initiated at the STD clinic by direct inoculation of modified Thayer-Martin plates. The culture plates were placed immediately at 37°C in candle jars with moist wads of cotton, and the jars were transported to the laboratory twice daily and were further incubated at 37°C. Before the male urethral and female cervical specimens for culture were obtained, a smear was prepared and Gram stained with aqueous fuchsin counterstain.

First-catch urine (10 ml) was collected at least 2 h after the last urination. The urine specimens were transported at ambient temperature and were stored at 4 to 6°C for up to 5 days or at −20°C for longer intervals prior to processing.

Tissue culture of *C. trachomatis.* Chlamydia culture was performed with HeLa 229 cells grown in flat-bottom tubes (diameter, 10 mm). The frozen specimens were thawed in a water bath at 37°C and were mixed by swirling to resuspend any settled material. Cell monolayers that had been pretreated with DEAE-dextran (30 μg/ml) in Hanks-Earle balanced salt solution were inoculated with 300-μl portions of each specimen. The tubes were then centrifuged at 2,000 × *g* for 60 min at 32°C, and the supernatants were replaced with 1 ml of minimal essential medium-Hanks-Earle supplemented with 10% fetal bovine serum, cycloheximide (20 μg/ml), streptomycin (100 mg/liter), and vancomycin (100 mg/liter). After incubation at 34°C for 48 to 72 h, the monolayers were fixed and stained with a fluorescein-labelled genus-specific monoclonal antibody (bioMérieux, Marcy l'Etoile, France), and the number of inclusions was counted.

Culture of *N. gonorrhoeae.* After incubation for 48 h the plates were examined and suspected colonies were subjected to Gram staining with safranin counterstain and the cytochrome oxidase test with tetramethyl-*p*-phenylene-diamine-dihydrochloride, 1% solution, as the reagent. Oxidative utilization of glucose (positive) and maltose (negative), the *o*-nitrophenyl-β-D-galactosidase test (negative) as a substitute for lactose degradation, the γ-glutamyl-aminopeptidase test (negative), and growth on nutrient agar without blood (negative) were used as confirmatory tests.

LCR. The LCR amplification methods have been described previously (6–8, 11, 14, 17). Male urethral and female cervical samples were collected in transport medium. The specimens were heated at 97°C for 15 min, and after cooling, the swabs were expressed and discarded and 100 μl of the remaining solution was added to a unit-dose tube containing 100 μl of the LCR mixture. The LCR mixture contained thermostable DNA ligase and DNA polymerase, deoxyribonucleoside triphosphates, and the labelled probes in buffer. The probes for *C. trachomatis* were derived from the endogenous plasmid, and those for *N. gonorrhoeae* were directed against the multicopy *Opa* genes (6, 20). Two positive controls, two negative controls, and two calibrators were included in each test run. Amplification was performed for 40 cycles of incubation for 1 s at 97°C, 1 s at 55°C, and 50 s at 62°C. A portion (100 μl) of the amplified product was transferred to a reaction cell which was then placed in the automatic analyzer for detection by a microparticle enzyme immunoassay.

Urine specimens were briefly mixed by swirling to resuspend any settled material, after which 1 ml was removed and centrifuged for 15 min at 13,000 × *g*. The resulting pellet was resuspended in 1 ml of urine resuspension buffer, and the mixture was heated at 95 to 100°C for 15 min. After cooling to ambient temperature, the samples were tested by LCR as described above.

Discrepancy analysis. Discrepant results for *C. trachomatis* were resolved by repeating the plasmid-LCR test of the original urine, male urethral, or female cervical specimen, as well as by additional testing of the original specimen by LCR with probes specific for the major outer membrane protein (MOMP) gene (14). The latter test was performed at Abbott Laboratories.

The results for specimens culture negative and LCR positive for *N. gonorrhoeae* were also resolved by repeating the original LCR assay with the original specimen and by LCR assay with probes specific for other targets (protein 1 and pilin genes) (24). The latter test was also performed at Abbott Laboratories.

Definition of confirmed cases. A confirmed case of *C. trachomatis* infection was defined as an individual with a positive chlamydial cell culture or at least one specimen positive by the initial LCR and by the confirmatory MOMP-LCR. A confirmed case of *N. gonorrhoeae* infection was defined as an individual with a positive gonococcal culture or at least one specimen positive by both the initial LCR and the confirmatory LCR with different probes.

Statistical analysis. Categorical variables were analyzed by the chi-square test with Epi Info (12). A P value of <0.05 was considered statistically significant.

RESULTS

Testing for *C. trachomatis.* A total of 614 men and 602 women participated in the study for *C. trachomatis*. The mean age was 36.8 years (range, 17 to 69 years) for the men and 31.2 years (range, 17 to 65 years) for the women.

TABLE 1. Comparison of LCR and cell culture assays for *C. trachomatis* in urine and urethral swab specimens collected from 614 men attending an STD clinic

Specimen and LCR result	No. of men				
	Cell culture (urethra)			MOMP-LCR	
	Positive	Negative	Total	Positive	Negative
Urethral swab					
Positive	40	30	70	70[a]	0
Negative	3	541	544	3	541
Total	43	571	614	73	541
Urine					
Positive	37	24	61	58[b]	3
Negative	6	547	553	6	547
Total	43	571	614	64	550

[a] Includes 14 participants with only urethral swab specimen testing LCR positive.
[b] Includes two participants with only urine specimen testing LCR positive.

The prevalence of *C. trachomatis* infection detected by cell culture was 7.0% (43 of 614) for men (Table 1) and 5.0% (30 of 602) for women (Table 2). The number of participants with positive test results increased when the plasmid-LCR assay was used. For the men, 11.4% (70 of 614) of the urethral swabs and 9.9% (61 of 614) of the urine samples were positive by the plasmid-LCR. For three men whose cultures contained a small number of inclusions (one to three), their urethral swabs tested negative by the plasmid-LCR; the urine sample of one of these three men tested positive by the plasmid-LCR. For six male participants with positive results by culture of urethral swab specimens, their urine specimens tested negative by the plasmid-LCR; the urethral swabs of three of these six men tested positive by the plasmid-LCR.

For the women, 9.6% (58 of 602) of the cervical swabs and 9.1% (55 of 602) of the urine samples tested positive by the plasmid-LCR. Both the cervical swab and urine specimens from one woman, who had a low-count positive cervical culture (one to three inclusions), tested negative by the plasmid-LCR. For four additional women whose cervical swabs were positive

TABLE 2. Comparison of LCR and cell culture assays for *C. trachomatis* in urine and cervical swab specimens collected from 602 women attending an STD clinic

Specimen and LCR result	No. of women				
	Cell culture (cervix)			MOMP-LCR	
	Positive	Negative	Total	Positive	Negative
Cervical swab					
Positive	29	29	58	58[a]	0
Negative	1	543	544	1	543
Total	30	572	602	59	543
Urine					
Positive	26	29	55	49[b]	6
Negative	4	543	547	4	543
Total	30	572	602	53	549

[a] Includes 13 participants with only cervical swab specimen testing LCR positive.
[b] Includes seven participants with only urine specimen testing LCR positive.

TABLE 3. Evaluation of LCR and cell culture assays for *C. trachomatis* relative to the number of confirmed cases of infection among 614 men and 602 women

Gender and assay	Specimen	Sensitivity[a] (%)	Specificity[a] (%)	Positive predictive value[b] (%)	Negative predictive value[b] (%)
Males					
Culture	Urethral swab	57.3 (43/75)	100 (539/539)	100 (43/43)	94.4 (539/571)
LCR	Urethral swab	93.3 (70/75)	100 (539/539)	100 (70/70)	99.1 (539/544)
LCR	Urine	77.3 (58/75)	99.4 (536/539)	95.1 (58/61)	96.9 (536/553)
Females					
Culture	Cervical swab	45.5 (30/66)	100 (536/536)	100 (30/30)	93.7 (536/572)
LCR	Cervical swab	87.9 (58/66)	100 (536/536)	100 (58/58)	98.5 (536/544)
LCR	Urine	78.8 (52/66)	99.4 (533/536)	94.5 (52/55)	97.4 (533/547)

[a] Values in parentheses are number of specimens with test results of assay in question/number of specimens with results according to gold standard.
[b] Values in parentheses are number of specimens with test results confirmed according to gold standard/total number of specimens with results of assay in question.

by both culture and LCR, their urine samples tested negative by the plasmid-LCR.

All male plasmid-LCR-positive urethral swabs were confirmed to be positive by the MOMP-LCR, as were 95% (58 of 61) of the male plasmid-LCR-positive urine samples. For women, all 58 cervical swabs that tested positive by the plasmid-LCR were confirmed to be positive by the MOMP-LCR, and 89.1% (49 of 55) of the plasmid-LCR-positive urine specimens were confirmed to be positive by the MOMP-LCR.

The total number of confirmed positive cases (i.e., individuals with a positive cell culture or with at least one specimen positive by both the plasmid-LCR and the MOMP-LCR) was 141, resulting in a prevalence of 12.2% (75 of 614) for men and 10.1% (66 of 602) for women. Culture and LCR test characteristics relative to the expanded gold standard for confirmed cases are presented in Table 3. The sensitivities of cell culture were 57.3% (43 of 75) and 45.5% (30 of 66) for men and women, respectively. Testing of male urethral swab specimens and female cervical swab specimens by the plasmid-LCR had sensitivities of 93.3% (70 of 75) and 87.9% (58 of 66), respectively. The sensitivities of testing male and female urine specimens by the plasmid-LCR were 77.3% (58 of 75) and 78.8% (52 of 66), respectively. It must be noted that three of the six plasmid-LCR-positive female urine specimens that were not confirmed by the less sensitive MOMP-LCR were obtained from patients with confirmed cases of infection on the basis of the results obtained with cervical specimens. For men the sensitivity of the plasmid-LCR for urethral swabs and urine specimens was significantly greater than that of culture of the urethral swab specimens ($P < 0.001$ and $P = 0.01$, respectively), and the LCR test of urethral swabs was significantly more sensitive than LCR testing of urine ($P = 0.005$). For women the sensitivity of plasmid-LCR testing of cervical swabs or urine differed significantly from that of cell culture ($P < 0.001$). The difference between the sensitivity of LCR testing of the cervical swab and female urine was not significant. The specificities of the plasmid-LCR assay were 100% with male urethral and female cervical swabs and 99.4% with male (536 of 539) and female (533 of 536) urine specimens.

Of the 75 confirmed cases of infection in men, for 56 (74.7%) men both urethral and urine specimens tested positive by LCR, for 14 men (18.7%) only urethral swabs were LCR positive, and for 2 (2.7%) participants only urine specimens were LCR positive.

Of the 66 confirmed cases of infection in women, for 45 (68.2%) women both cervical swab and urine specimens tested positive by LCR. For 13 (19.7%) women only the cervical swab was LCR positive, and for 7 (10.6%) women only the urine specimen was LCR positive. The order of collection of the specimens did not influence the results of the various tests for both *C. trachomatis* and *N. gonorrhoeae* in either women or men.

Testing for *N. gonorrhoeae*. A total of 220 men and 383 women participated in the study for *N. gonorrhoeae*. When tested by culture, the prevalence of *N. gonorrhoeae* infection was 5.9% (13 of 220) in men (Table 4) and 2.9% (11 of 383) in women (Table 5). Testing of male urethral and female cervical swabs by LCR yielded prevalence rates of 8.2% (18 of 220) and 5.5% (21 of 383), respectively, whereas LCR testing of urine samples identified prevalence rates of 7.3% (16 of 220) for men and 2.9% (11 of 383) for women. All five male urethral samples with discrepant results and the 11 female cervical swab specimens with discrepant results were confirmed to be positive when they were tested by LCR with different probes. The LCR-positive urine samples (three male and one female) with discrepant results were also confirmed to be positive. The prevalence rates of *N. gonorrhoeae* infections in men and women determined by the gold standard test were 8.2 and 5.7%, respectively.

The performances of the various specimen and test combinations were evaluated relative to that of the expanded gold standard (Table 6). Culture of male urethral swabs had a sensitivity of 72.2% (13 of 18). The LCR assay had a sensitivity of 100% (18 of 18) for male urethral swabs and 88.9% (16 of 18) for male urine specimens. The LCR assay for urethral swab

TABLE 4. Comparison of LCR and culture assays for *N. gonorrhoeae* in urine and urethral swab specimens collected from 220 men attending an STD clinic

Specimen and LCR result	No. of men				
	Culture (urethra)			Confirmatory LCR	
	Positive	Negative	Total	Positive	Negative
Urethral swab					
Positive	13	5	18	18	0
Negative	0	202	202	0	202
Total	13	207	220	18	202
Urine					
Positive	13	3	16	16	0
Negative	0	204	204	0	204
Total	13	207	220	16	204

TABLE 5. Comparison of LCR and culture assays for *N. gonorrhoeae* in urine and cervical swab specimens collected from 383 women attending an STD clinic

Specimen and LCR result	No. of women				
	Culture (cervix)			Confirmatory LCR	
	Positive	Negative	Total	Positive	Negative
Cervical swab					
Positive	10	11	21	21	0
Negative	1[a]	361	362	1	361
Total	11	372	383	22	361
Urine					
Positive	10	1	11	11[b]	0
Negative	1	371	372	1	371
Total	11	372	383	12	371

[a] Repeat LCR positive.
[b] Includes seven participants with only urine specimen testing LCR positive.

specimens performed statistically significantly better than culturing of gonococci ($P = 0.02$).

The sensitivity of culture of cervical swab specimens was 50% (11 of 22), identical to that of the LCR assay with female urine specimens. With cervical swabs the LCR assay had a sensitivity of 95.4% (21 of 22). The sensitivity of the LCR assay for cervical specimens was significantly greater than that of culture for gonococci or the LCR test for urine specimens ($P < 0.001$). The specificity of each of the various specimen and test combinations was 100%.

Association between test results for the presence of *C. trachomatis* or *N. gonorrhoeae* and complaints or symptoms. Of the 602 female participants in the study for *C. trachomatis*, 207 reported complaints of vaginal discharge, and 23 (11.1%) of these 207 women were confirmed to be positive for *C. trachomatis* infection. Of the 395 women who did not report vaginal discharge, 43 (10.9%) were confirmed to have *C. trachomatis* infection. In other words, 34.8% (23 of 66) of the females with confirmed cases of infection were symptomatic and 65.2% (43 of 66) were asymptomatic. In contrast to the results for women, 22% (40 of 182) of the men with complaints of urethral discharge or dysuria were confirmed to have cases of *C. trachomatis* infection, whereas only 8.1% (35 of 430) of the men who did not report urethral discharge were confirmed to be infected (data for two men with complaints in the group with nonconfirmed cases of infection were missing). Thus, 53.3% (40 of 75) of the males with confirmed cases of infection were symptomatic and 46.7% (35 of 75) were asymptomatic. The percentage of men reporting complaints was statistically significantly greater for individuals confirmed to be infected than for uninfected individuals (53.3 versus 35.8%; $P < 0.001$). Signs of urethral discharge on physical examination or an increased number of leukocytes in Gram stains of the urethral discharge were also significantly greater for men with confirmed *C. trachomatis* infection than for uninfected men (77.3 versus 35.1%; $P < 0.001$).

Fifteen of the 18 (83.3%) men with confirmed *N. gonorrhoeae* infection presented with complaints of urethral discharge, which was significantly greater ($P = 0.03$) than the 11 of the 22 (50%) women who were confirmed to be infected and who complained of vaginal discharge. The percentage of men with complaints of urethral discharge was significantly greater for men with confirmed gonococcal infection than for uninfected individuals (83.3 versus 27.6%; $P < 0.001$).

Coinfection with *C. trachomatis* and *N. gonorrhoeae*. On evaluating the rate of coinfection with *C. trachomatis* and *N. gonorrhoeae*, 16.7% (3 of 18) of men and 18.2% (4 of 22) of women diagnosed with gonorrhoea also appeared to be infected with *C. trachomatis.*

Association between Gram staining and *N. gonorrhoeae* test results. Only 3 of the 18 men with confirmed gonococcal infection had no leukocytes in Gram stains of the urethral swab specimens, 2 men had between 2 and 10 leukocytes, and the remaining 13 men had more than 10 leukocytes. For 15 males with confirmed cases of infection, Gram stains presented gram-negative diplocci, resulting in a sensitivity of 83.3% for Gram staining of specimens from men.

Of the 22 women diagnosed with gonorrhoea, 6 had no leukocytes in Gram stains of the cervical or urethral swab specimens, 2 had between 2 and 10 leukocytes, and 14 women had more than 10 leukocytes.

DISCUSSION

The present study has demonstrated the value of testing individuals attending an STD clinic by LCR-based assays for the presence of *C. trachomatis* or *N. gonorrhoeae*. Male urethral and female cervical swabs, as well as both male and female urine specimens, were tested. In previous studies testing of urethral or cervical swabs or urine specimens by the plasmid-LCR assay for *C. trachomatis* was compared with culture or enzyme immunoassays of male urethral or female cervical swabs and confirmatory LCR analysis of the original swab specimen (2, 9, 10, 18, 23). The criteria for the confirmation of

TABLE 6. Evaluation of LCR and culture assays for *N. gonorrhoeae* relative to the number of confirmed cases of infection among 220 men and 383 women

Gender and assay	Specimen	Sensitivity (%)[a]	Specificity (%)[a]	Positive predictive value (%)[b]	Negative predictive value (%)[b]
Males					
Culture	Urethral swab	72.2 (13/18)	100 (202/202)	100 (13/13)	97.6 (202/207)
LCR	Urethral swab	100 (18/18)	100 (202/202)	100 (18/18)	100 (202/202)
LCR	Urine	88.9 (16/18)	100 (202/202)	100 (16/16)	99.0 (202/204)
Females					
Culture	Cervical swab	50.0 (11/22)	100 (361/361)	100 (11/11)	97.0 (361/372)
LCR	Cervical swab	95.4 (21/22)	100 (361/361)	100 (21/21)	99.7 (361/362)
LCR	Urine	50.0 (11/22)	100 (361/361)	100 (11/11)	97.0 (361/372)

[a] Values in parentheses are number of specimens with test results of assay in question/total number of specimens with results according to gold standard.
[b] Values in parentheses are number of specimens with test results confirmed according to gold standard/total number of specimens with results of assay in question.

infection were extended in the present study to include confirmatory LCR-based testing and the combination of results for specimens obtained from various sites. We thus included the confirmed results of the plasmid-LCR test for male urethral or female cervical swab specimens in combination with those for urine specimens in order to represent the true situation for the patient. Thus, the percentage of individuals with confirmed infections was greater and the sensitivity of each specific test-specimen combination appeared lower than those in previous studies. The results of the present study are consistent with those of our previous study comparing LCR-based testing and culture for the detection of *C. trachomatis* (25). Our current data also agree with those of Schachter et al. (22) showing that the sensitivity of LCR testing of female urine compared with cell culture of cervical and urethral swabs was 85.9%.

The sensitivity of the plasmid-LCR assay for *C. trachomatis* applied to male urethral swabs was 93.3%, and this approach proved to be the most sensitive way to demonstrate genital chlamydial infection in men. The sensitivity of the plasmid-LCR with male urine (77.3%) was markedly higher than that of cell culture with male urethral swabs (57.3%). For women, the LCR assay for *C. trachomatis* applied to cervical swab specimens was the most sensitive assay-specimen combination, with a sensitivity of 87.9%, which was followed by the LCR-urine combination, with a sensitivity of 78.8%. Cell culture of cervical swab specimens showed the lowest level of sensitivity (45.5%). The sensitivity of the *N. gonorrhoeae* LCR-based assay with male urethral swabs was 100%; the sensitivity of the LCR assay with male urine specimens (88.9%) was greater than that of culture by direct inoculation of male urethral swabs (72.2%). The sensitivity of the *N. gonorrhoeae* LCR-based assay with cervical swab specimens was 95.4%. In contrast to the superior performance of the LCR assay for *C. trachomatis* with urine specimens, the sensitivity of the *N. gonorrhoeae* LCR assay with female urine specimens was similar to that of culture with cervical swabs and was only 50%. In an earlier study in which LCR testing of female urine for *N. gonorrhoeae* was compared with culture of urogenital swabs, the sensitivities of the two assays were 94.6 and 96.4%, respectively (24). However, that study did not mention the rate of symptomatic participants. The prevalence of *N. gonorrhoeae* infection of 19.1% in that study indicates that the number of symptomatic patients might be high in contrast to the low rates found in our study, in which 50% of the patients with confirmed cases of infection were asymptomatic. It is conceivable that in asymptomatic patients the bacterial load is low, and as a consequence contamination of urine does occur less often.

It must be noted that although the culture results for female urethral swab specimens were not a part of the study, for 15 of the 30 cervical culture-positive women, a urethral specimen was also positive by culture, and for 4 women the urethral swab was culture positive while the corresponding cervical swab was culture negative. All four women were confirmed to be positive on the basis of the LCR results.

The presence of *C. trachomatis* DNA in female urine specimens might result from contamination of urine by vaginal secretions or from infection of the female urethra. To distinguish between these possibilities, we tested by plasmid-LCR the urethral specimens that were obtained for cell culture from women with confirmed *C. trachomatis* infections. Urethral specimens from 56 of the 66 females with confirmed cases of infection were still available. For 41 (73%) of these 56 women, both the cervical swab and the urethral swab tested positive by LCR, indicating infection at both sites; consequently, the urine specimens from 36 (88%) of these 41 women were also LCR positive. For 9 (16%) of the 56 women, the cervical but not the urethral specimen tested positive by LCR, indicating that the cervix was the only site of infection. The fact that corresponding urine specimens from five of the nine women were also LCR positive likely reflects specimen contamination. For the remaining 6 (11%) of the 56 women, the urethral swab but not the cervical swab tested positive by LCR, indicating that the urethra was the sole site of infection; the urine specimens from all six of these women were also LCR positive. These latter six cases of infection would have been missed by collecting only a cervical swab. Three of these six women reported complaints of abdominal discomfort or vaginal discharge.

Three male and six female urine specimens positive for *C. trachomatis* by plasmid-LCR could not be confirmed to be positive by the less sensitive MOMP-LCR. However, three of the six female urine samples were obtained from women who were confirmed to be positive on the basis of the results for other specimens. Besides the possibility of sampling error or false positivity, this finding can be explained by infection higher in the urethra or vesical bladder accompanied by a small number of chlamydial bacteria in the first-voided urine.

Our data might be applied to the choice of a specimen-test combination for screening programs for *C. trachomatis* outside of STD clinics. The success of a screening program will depend on several factors, including participation rate and the availability of diagnostic and treatment facilities. Although male urethral or female cervical swab specimens can be collected from selected populations such as people attending an STD clinic, this form of examination is too unpleasant to be widely acceptable for screening the general population (26). The plasmid-LCR assay allows for the use of urine as a reliable and readily obtainable specimen for the detection of *C. trachomatis* infection in both women and men, and consequently, its availability should increase the rate of participation in screening programs. The higher level of sensitivity of the LCR assay with urine than that of culture with urethral or cervical swabs should also increase the rate of detection of both symptomatic and asymptomatic *C. trachomatis* infections in both women and men.

Our data indicate that for individual diagnosis of *C. trachomatis* or *N. gonorrhoeae* infection, LCR testing of urethral swabs from men or cervical swabs from women is the best single approach for detecting the most cases of infection. An additional advantage is the possibility of detecting the presence of both *C. trachomatis* and *N. gonorrhoeae* in the same sample. With regard to the diagnosis of *C. trachomatis* infection, additional testing of a urine specimen by LCR may increase the number of infected individuals who are detected by ~10%, but it will be associated with increased testing costs.

REFERENCES

1. **Bass, C. A., D. L. Jungkind, N. S. Silverman, and J. M. Bondi.** 1993. Clinical evaluation of a new polymerase chain reaction assay for detection of *Chlamydia trachomatis* in endocervical specimens. J. Clin. Microbiol. **31:**2648–2653.
2. **Bassiri, M., H. Y. Hu, M. A. Domeika, J. Burczak, L.-O. Svensson, H. H. Lee, and P.-A. Mårdh.** 1995. Detection of *Chlamydia trachomatis* in urine specimens from women by ligase chain reaction. J. Clin. Microbiol. **33:**898–900.
3. **Bauwens, J. E., A. Clark, M. J. Loeffelholz, S. A. Herman, and W. E. Stamm.** 1993. Diagnosis of *Chlamydia trachomatis* urethritis in men by polymerase chain reaction assay of first-catch urine. J. Clin. Microbiol. **31:**3013–3016.
4. **Bauwens, J. E., A. Clark, and W. E. Stamm.** 1993. Diagnosis of *Chlamydia trachomatis* endocervical infections by a commercial polymerase chain reaction assay. J. Clin. Microbiol. **31:**3023–3027.
5. **Bianchi, A., C. Scieux, N. Brunat, D. Vexiau, M. Kermanach, P. Pezin, M. Janier, P. Morel, and P. H. Lagrange.** 1994. An evaluation of the polymerase chain reaction Amplicor *Chlamydia trachomatis* in male urine and female urogenital specimens. Sex. Transm. Dis. **21:**196–200.
6. **Birkenmeyer, L., and A. S. Armstrong.** 1992. Preliminary evaluation of the ligase chain reaction for specific detection of *Neisseria gonorrhoeae*. J. Clin. Microbiol. **30:**3089–3094.
7. **Burczak, J. D., S. F. Ching, H. Y. Hu, and H. H. Lee.** 1995. Ligase chain

reaction for the detection of infectious agents, p. 315–328. *In* D. Wiebrauk and D. H. Farkas (ed.), Molecular methods for virus detection. Academic Press, Inc., New York.
8. **Carrino, J. J., and H. H. Lee.** 1995. Nucleic acid amplification methods. J. Microbiol. Methods **23:**3–20.
9. **Chernesky, M. A., D. Jang, H. Lee, J. D. Burczak, H. Hu, J. Sellors, S. J. Tomazic-Allen, and J. B. Mahony.** 1994. Diagnosis of *Chlamydia trachomatis* infections in men and women by testing first-void urine by ligase chain reaction. J. Clin. Microbiol. **32:**2682–2685.
10. **Chernesky, M. A., H. Lee, J. Schachter, J. D. Burczak, W. E. Stamm, W. M. McCormack, and T. C. Quinn.** 1994. Diagnosis of *Chlamydia trachomatis* urethral infection in symptomatic and asymptomatic men by testing first-void urine in a ligase chain reaction assay. J. Infect. Dis. **170:**1308–1311.
11. **Ching, S., H. Lee, E. W. Hook III, M. R. Jacobs, and J. Zenilman.** 1995. Ligase chain reaction for the detection of *Neisseria gonorrhoeae* in urogenital swabs. J. Clin. Microbiol. **33:**3111–3114.
12. **Dean, A. D., J. A. Dean, J. H. Burton, and R. C. Dicker.** 1990. Epi Info, version 5: a word processing, database, and statistics program for epidemiology on microcomputers. Centers for Disease Control, Atlanta.
13. **De Barbeyrac, B., I. Pellet, B. Dutihl, C. Bébéar, B. Dumon, M. Géniaux, and C. Bébéar.** 1994. Evaluation of the *Amplicor Chlamydia trachomatis* test versus culture in genital samples in various prevalence populations. Genitourin. Med. **70:**162–166.
14. **Dille, B. J., C. C. Butzen, and L. G. Birkenmeyer.** 1993. Amplification of *Chlamydia trachomatis* DNA by ligase chain reaction. J. Clin. Microbiol. **31:**729–731.
15. **Jang, D., J. W. Sellors, J. Mahony, L. Pickard, and M. A. Chernesky.** 1992. Effects of broadening the gold standard on the performance of a cheminoluminometric immunoassay to detect *Chlamydia trachomatis* antigens in centrifuged first void urine and urethral swab samples from men. Sex. Transm. Dis. **19:**315–319.
16. **Jaschek, G., C. A. Gaydos, L. E. Welsh, and T. C. Quinn.** 1993. Direct detection of *Chlamydia trachomatis* in urine specimens from symptomatic and asymptomatic men by using a rapid polymerase chain reaction assay. J. Clin. Microbiol. **31:**1209–1212.
17. **Laffler, T. G., J. J. Carrino, and R. L. Marshall.** 1993. The ligase chain reaction in DNA-based diagnosis. Ann. Biol. Clin. **50:**821–826.
18. **Lee, H. H., M. A. Chernesky, J. Schachter, J. D. Burczak, W. W. Andrews, S. Muldoon, G. Leckie, and W. E. Stamm.** 1995. Diagnosis of *Chlamydia trachomatis* genitourinary infection in women by ligase chain reaction of urine. Lancet **345:**213–216.
19. **Loeffelholz, M. J., C. A. Lewinski, S. R. Silver, A. P. Purohit, S. A. Herman, D. A. Buonagurio, and E. A. Dragon.** 1992. Detection of *Chlamydia trachomatis* in endocervical specimens by polymerase chain reaction. J. Clin. Microbiol. **30:**2847–2851.
20. **Meyer, T. F., C. P. Gibbs, and R. Hass.** 1990. Variation and control of protein expression in Neisseria. Annu. Rev. Microbiol. **44:**451–477.
21. **Ossewaarde, J. M., M. Rieffe, M. Rozenberg-Arska, P. M. Ossenkoppele, R. P. Nawrocki, and A. M. van Loon.** 1992. Development and clinical evaluation of polymerase chain reaction test for detection of *Chlamydia trachomatis*. J. Clin. Microbiol. **30:**2122–2128.
22. **Schachter, J., J. Moncada, R. Whidden, H. Shaw, G. Bolan, J. D. Burczak, and H. H. Lee.** 1995. Noninvasive tests for diagnosis of *Chlamydia trachomatis* infection: application of ligase chain reaction to first-catch urine specimens of women. J. Infect. Dis. **172:**1411–1414.
23. **Schachter, J., W. E. Stamm, T. C. Quinn, W. W. Andrews, J. D. Burczak, and H. H. Lee.** 1994. Ligase chain reaction to detect *Chlamydia trachomatis* infection of the cervix. J. Clin. Microbiol. **32:**2540–2543.
24. **Smith, K. R., S. Ching, H. Lee, Y. Ohhashi, H.-Y. Hu, H. C. Fisher III, and E. W. Hook III.** 1995. Evaluation of ligase chain reaction for use with urine for identification of *Neisseria gonorrhoeae* in females attending a sexually transmitted disease clinic. J. Clin. Microbiol. **33:**455–457.
25. **Van Doornum, G. J. J., M. Buimer, M. Prins, C. M. J. Henquet, R. A. Coutinho, P. K. Plier, S. Tomazic-Allen, H. Hu, and H. Lee.** 1995. Detection of *Chlamydia trachomatis* infection by ligase chain reaction in female and male urine samples. J. Clin. Microbiol. **33:**2042–2047.
26. **Wilson, J. M. G., and G. Jungner.** 1968. Principles and practice of screening for disease. Public Health Papers, vol. 34. World Health Organization, Geneva.