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Ligase Chain Reaction for Detection of *Neisseria gonorrhoeae* in Urogenital Swabs

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The ligase chain reaction (LCR) is an in vitro nucleic acid amplification technique that exponentially amplifies targeted DNA sequences. In a multicenter study, we evaluated the use of a 4-h LCR-based assay for the diagnosis of *Neisseria gonorrhoeae* infection of the cervix and male urethra. The LCR results were compared with those of culture for *N. gonorrhoeae* by using selective media. This assay amplifies target sequences within the *N. gonorrhoeae* opacity gene. Discordant LCR-positive and culture-negative specimens were further evaluated by testing by another LCR assay which used *N. gonorrhoeae*-specific pilin probe sets. A total of 1,539 female endocervical specimens and 808 male urethral swab specimens were evaluated in the study. An expanded "gold standard" was defined to include all culture-positive as well as culture-negative, confirmed LCR-positive specimens. After resolution of discrepant samples, the sensitivities of the *N. gonorrhoeae* LCR assays for the female and male specimens were 97.3 and 98.5%, respectively, with specificities of 99.6 and 99.8%, respectively. Resolved culture sensitivities were 83.9 and 96.5% for the female and male specimens, respectively. The LCR assay for gonorrhea is a rapid, highly sensitive nonculture method for detecting gonococcal infection of the cervix and male urethra.

Sexually transmitted infections caused by *Neisseria gonorrhoeae* are a worldwide health problem. In 1993, 439,673 cases of gonorrhea were reported in the United States (3, 4), but most experts believe that this is an underestimate of the true incidence. In men, acute urethritis is the predominant clinical sign, although a substantial proportion of infections are asymptomatic (17). Salpingitis occurs in 10 to 20% of acutely infected women (12). Salpingitis can result in infertility and ectopic pregnancy (8). For these reasons, public health policy often includes provision for screening for *N. gonorrhoeae* infection.

The current standard for the microbiological diagnosis of gonorrhea is culture on selective medium (7). Culture sensitivity is believed to be 80 to 95%, with false-negative results attributed to poor specimen storage, transport problems, and inhibition of growth by the components of selective media (12). A variety of nonculture diagnostic test alternatives such as direct immunofluorescence assay, enzyme immunoassay, and DNA hybridization have been developed, and these tests are 72 to 100% sensitive compared with culture (5, 10, 13–15, 18, 20). DNA amplification techniques, as recently applied to chlamydia, offer the promise of eliminating transport and specimen collection issues which are believed to affect test sensitivity in the field setting (6). We describe the performance of a ligase chain reaction (LCR) DNA amplification assay for the detection of *N. gonorrhoeae* in female endocervical and male urethral swabs.

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MATERIALS AND METHODS

Study populations and specimens. Specimens were obtained from patients evaluated at three geographically dispersed sites in the United States: the sexually transmitted disease clinics at the Jefferson County Department of Health in Birmingham, Ala., and the Baltimore City Health Department, and the obstetrics and gynecology clinic at University Hospitals of Cleveland and Case Western Reserve University, Cleveland, Ohio. Only women were enrolled at the Cleveland site. Studies were conducted under protocols approved by the institutional review boards of the respective institutions. Female patients were eligible for enrollment in the study if they were sexually active and did not use barrier forms of contraceptives. All males attending sexually transmitted disease clinics were eligible for the study. Individuals were excluded if they had used antibiotics within the 2 weeks before specimen collection. The results of culture and the LCR assay were not correlated until the completion of both tests.

Specimen collection and processing. The specimen collection kits for the study included two sterile swabs (one larger than the other) and a transport tube containing the transport buffer [5 mM *N*-(2-hydroxyethyl) piperazine-*N*′-(3-propanesulfonic acid) (K^+ salt) (pH 7.8), 60 mM $MgCl_2$, 0.1% NaN_3]. For the collection of endocervical specimens, the larger-tipped swab was used to remove endocervical mucus; this was followed by the insertion of the smaller-tipped swab into the cervix, which was then rotated for 15 to 30 s. The smaller swab was then used to immediately inoculate a culture medium and was then placed in the *N. gonorrhoeae* LCR assay transport tube. The shaft of the swab was broken at a score mark, and the tube was sealed. Similarly, for male urethral specimens, the smaller-tipped swab was inserted 2 to 4 cm into the urethra and was rotated for 3 to 5 s. The swab was used to prepare a specimen for Gram staining and to directly inoculate a culture plate. It was then placed in the *N. gonorrhoeae* LCR assay transport tube as described above for the specimens from female patients. Specimens were immediately stored at 2 to 8°C for up to 4 days or at −20°C until they were processed.

Bacteriologic culture. For culture, modified Thayer-Martin (MTM) plates were routinely warmed to room temperature before use. The specimens used to culture for *N. gonorrhoeae* were collected by using the swabs provided as part of the LCR specimen transport kit and were directly inoculated onto MTM medium. Cultures were examined following overnight incubation at 34 to 36°C under 3 to 10% CO_2 and, if they were negative on initial incubation, were examined again following an additional 24 and 48 h of incubation. Typical colonies containing gram-negative diplococci and giving a positive oxidase reac-

tion were presumptively identified as *N. gonorrhoeae*. The identities of presumptive *N. gonorrhoeae* isolates were confirmed by using carbohydrate degradation, fluorescent-antibody staining, or GenProbe DNA hybridization assays according to local laboratory protocols (7).

***N. gonorrhoeae* LCR assay.** The *N. gonorrhoeae* LCR assay was developed by Abbott Laboratories (2). The assay amplification kit uses four probes complementary to a 48-bp region of the gonococcal opacity 1 (*opa-1*) gene (GenBank accession name, NGOOPC B) (21). This gene sequence is repeated up to 11 times in the *N. gonorrhoeae* genome. The chosen sequence is specific for *N. gonorrhoeae*, as described previously (1a). To reduce possible laboratory contamination, specimen processing was performed in a biohood in one area of the laboratories (area 1), while amplification and detection of amplified products were carried out in a geographically separate part of the laboratory (area 2). For processing, specimens were placed in a heat block at 95 to 100°C for 15 min. After heating, 100 μl of each processed specimen was then added to the unit dose assay tubes containing 100 μl of the LCR mixture (thermostable DNA ligase, thermostable DNA polymerase, NAD, magnesium, deoxynucleoside triphosphate, and oligonucleotide probes in a pH 7.8 buffer). The assay tubes were then moved to area 2 of the laboratory for DNA amplification. In the amplification step, two pairs of probes hybridize to the target sequence of DNA, and there is a gap of a few nucleotides between the probes. Polymerase acts to fill in this gap with nucleotides in the LCR mixture, and then ligase can covalently join the pair of probes to form an amplified product. During thermal cycling, the temperature is raised above the melting point of the hybridized amplification product, causing it to dissociate from the original target sequence. Lowering of the temperature allows more of the oligonucleotide probes to hybridize to the targets that have become available. The thermal cycle continues in this manner until a sufficient number of target amplification products have accumulated in the LCR mixture. For each amplification run, positive and negative controls and a calibrator provided in the amplification kit were included. Positive controls consisted of DNA corresponding to 210 CFU of *N. gonorrhoeae* ATCC 27631 in a background of 330 ng of human placental DNA (Sigma) per assay. The calibrator corresponded to DNA from 45 CFU from the same *N. gonorrhoeae* strain. Negative controls contained 330 ng of human placental DNA only. Amplification was performed for 40 cycles of 97°C for 1 s, 55°C for 1 s, and 62°C for 50 s in a Perkin-Elmer model 480 thermocycler. The amplified products were detected immunochemically with an IMx (Abbott Laboratories) instrument. The detection step requires linkage of two hapten-labeled oligonucleotide probes (probes A and B) through an oligonucleotide chain generated by the amplification step (19). The IMx instrument performs an automated microparticle enzyme immunoassay (MEIA) (9). In the first step of the MEIA, both ligated and nonligated oligonucleotides labeled with hapten A are captured by microparticles coated with anti-hapten A antibodies. Nonligated probes labeled with hapten B are removed by a subsequent wash step. Oligonucleotide chains that were ligated are tagged with both haptens A and B and are therefore linked to the solid phase. Alkaline phosphatase-conjugated antibodies to hapten B are then added, after which the particles are washed thoroughly. In the presence of 4-methylumbelliferyl phosphate, alkaline phosphatase generates a fluorescent molecule, 4-methylumbelliferone. The rate of fluorescence emission is detected by the IMx analyzer. Controls were included with each LCR assay run to ensure that the values were within the expected limits. Patient samples recorded as positive have values equal to or greater than 0.25 times the average of the two values for the calibrator for that run. After detection, all sample containers are automatically subjected to chemical inactivation of the amplicon, further reducing the risk of contamination. The chemical inactivation step uses a two-component reagent system (a chelated metal complex and an oxidizing agent) to degrade the amplified DNA molecules.

Resolution of discrepant samples. To resolve discrepant culture-negative, LCR-positive specimens, another LCR assay with the pilin probe set specific for *N. gonorrhoeae* was used (16). The pilin probe set is specific for *N. gonorrhoeae*, as described previously (1a). The specificity of the pilin LCR assay for *N. gonorrhoeae* was greater than 99% when it was further evaluated with clinical specimens (data not shown). The pilin LCR assay procedure was similar to the *N. gonorrhoeae* LCR assay procedure as described above, except that thermocycling was for 53 cycles of 85°C for 30 s and 60°C for 1 min and 100 μl of each sample diluted 1:1 with *N. gonorrhoeae* LCR assay transport buffer was assayed. Specimens that gave duplicate IMx signals above 100 counts per second per second were confirmed as *N. gonorrhoeae* LCR assay positive.

To resolve the results for specimens that were culture positive but *N. gonorrhoeae* LCR assay negative, samples were diluted with specimen transport buffer and were retested by the *N. gonorrhoeae* LCR assay. The bacterial isolates, when available, were also heated in specimen transport buffer and were tested by the *N. gonorrhoeae* LCR assay.

RESULTS

A total of 1,539 female endocervical specimens and 808 male urethral specimens were examined. The results of the *N. gonorrhoeae* LCR assay and culture were concordant for 1,513 female endocervical and 794 male urethral specimens. The 26 endocervical and 14 male urethral specimens which gave discordant results included 23 endocervical and 10 male urethral specimens that were LCR positive and culture negative and 3 endocervical and 4 male urethral specimens that were LCR negative and culture positive. Thus, before the resolution of the results for specimens with discrepant results, for the endocervical specimens, the initial sensitivity of the *N. gonorrhoeae* LCR assay compared with that of culture was 96.8% (91 of 94 specimens) and the specificity was 98.4% (1,422 of 1,445 specimens). Similarly, for male urethral specimens, the initial sensitivity of the *N. gonorrhoeae* LCR assay compared with that of culture was 98.4% (247 of 251 specimens) and the specificity was 98.2% (547 of 557 specimens).

The discordant LCR-positive, culture-negative specimens were further tested by the pilin LCR assay as described in the Materials and Methods. For the 23 endocervical LCR-positive, culture-negative specimens, 18 were confirmed to be positive and the results for 5 specimens were not confirmed. For the 10 male urethral LCR-positive, culture-negative specimens, 9 were confirmed to be positive (eight were pilin LCR positive and one matched urine specimens was confirmed to be positive) and the result for 1 specimen was not confirmed. For the three endocervical and four male urethral LCR-negative, culture-positive specimens, *N. gonorrhoeae* LCR assays of diluted samples or the bacterial isolates from these specimens yielded positive results.

After the resolution analysis for the endocervical specimens, the sensitivity of the *N. gonorrhoeae* LCR assay was 97.3% and the sensitivity of culture was 83.9% (Table 1). The resolved specificity of the *N. gonorrhoeae* LCR assay was 99.6%. Culture specificity was always taken as 100%. The positive and negative predictive values of the *N. gonorrhoeae* LCR assay for endocervical swabs were 95.6 and 99.8%, respectively.

After the resolution analysis for male urethral specimens, the sensitivity of the *N. gonorrhoeae* LCR assay was 98.5% and the sensitivity of culture was 96.5% (Table 2). The specificity of the *N. gonorrhoeae* LCR assay was also high (99.8%). The positive and negative predictive values of the *N. gonorrhoeae* LCR assay were 99.6 and 99.3%, respectively.

DISCUSSION

In the present study, the sensitivities of the *N. gonorrhoeae* LCR assay were higher than those of culture for all groups after resolution of specimens with discrepant results. The difference in the sensitivities of the two assays was particularly great for endocervical swab specimens (Table 1). The possible reasons for this difference in sensitivities between LCR and culture include sampling errors, inhibition of growth by components of the selective culture medium, and the loss of viability during specimen transport or storage before the initiation of culture. Nonamplified probe assays require *N. gonorrhoeae* loads of 10^3 to 10^4 CFU/ml (14), while the detection limit for the *N. gonorrhoeae* LCR assay is between 10 and 100 CFU/ml (1). A PCR-based *N. gonorrhoeae* assay showed a sensitivity of 100% and a specificity of 88.9% relative to those of culture in one study of 52 female cervical and male urethral swab specimens (11); these values are similar to those obtained by the *N. gonorrhoeae* LCR assay in the present study.

Because of the high degrees of sensitivity and specificity of the *N. gonorrhoeae* LCR assay, the separation of signal values-to-cutoff values between specimen populations with positive and negative results is relatively clear. After analyzing the signal value-to-cutoff value frequency distribution for all of the clinical specimens, 88.6% of the positive female endocervical

TABLE 1. Performance of the *N. gonorrhoeae* LCR assay for the detection of *N. gonorrhoeae* in cervical specimens[a]

Site	Assay	Prevalence (%)	Sensitivity (%)	Specificity (%)	Positive (%)[b] *PV*	Negative (%)[b] *PV*
Cleveland	LCR	2.6 (26/991)[c]	96.2 (25/26)	99.7 (962/965)	89.3 (25/28)	99.9 (962/963)
	Culture[d]	1.9 (19/991)	73.1 (19/26)			
Baltimore	LCR	17.6 (38/216)	100 (38/38)	99.4 (177/178)	97.4 (38/39)	100 (177/177)
	Culture[d]	16.2 (35/216)	92.1 (35/38)			
Birmingham	LCR	14.5 (48/332)	95.8 (46/48)	99.6 (283/284)	97.9 (46/47)	99.3 (283.285)
	Culture[d]	12.0 (40/3,323)	83.3 (40/48)			
Total[e]	LCR	7.3 (112/1,539)	97.3 (109/112)	99.6 (1,422/1,427)	95.6 (109/114)	99.8 (1,422/1,425)
	Culture[d]	6.1 (94/1,539)	83.9 (94/112)			

[a] True positives include culture-positive and resolved LCR-positive and culture-negative specimens.
[b] PV, predictive value..
[c] Values in parentheses are number of specimens with the indicated result/total number of specimens tested.
[d] Culture specificity is assumed to be 100%.
[e] Compared with culture only, the total sensitivity, specificity, positive predictive, and negative predictive value are 96.8, 98.4, 79.8, and 99.8%, respectively.

specimens and 96.5% of the positive male urethral specimens had a signal value-to-cutoff value greater than 3.0. For the specimen population with negative results, the mean signal value-to-cutoff value is 9.8 standard variations away from the cutoff value. Only a few positive specimens (4.4% female and 1.6% male) were in the equivocal zone (between 1.0 and 1.5 signal value to cutoff value).

Our results afford insight into the sensitivity of the culture technique for the detection of *N. gonorrhoeae*. Culture was highly sensitive for the detection of infection in males. However, infections in only 83.9% of the endocervical specimens were diagnosed by culture (Table 1). Interestingly, no statistically meaningful associations were observed between symptoms and the sensitivities of the *N. gonorrhoeae* LCR assay and culture. Nevertheless, these results suggest that standard culture techniques underdiagnose gonorrhea in women by 16%, or in nearly one of six women.

For the discordant LCR-negative, culture-positive specimens, *N. gonorrhoeae* LCR assays of diluted samples or the bacterial isolates from these specimens yielded positive results, indicating the presence of the target sequence. The initial failure of the *N. gonorrhoeae* LCR assay for these specimens may have been attributable to the presence of an interfering substance, a low biologic loading, or procedural errors.

Most (21 of 27) of the confirmed *N. gonorrhoeae* LCR assay-positive, culture-negative specimens were from symptomatic patients (15 of 21) or from individuals who reported sexual contact with *N. gonorrhoeae*-infected partners (6 of 21). These data further support evidence that the underdiagnosis was due to culture failures. The increased sensitivity of the *N. gonorrhoeae* LCR assay compared with that of culture for such individuals has important public health ramifications, because the identification of infected men and women not detected by culture in screening programs should result in better control of infection. In the long term, such improved screening techniques could markedly reduce health care expenditures. Our data also indicate that the LCR assay is highly specific (between 99.4 and 100% in men and women) and thus may be useful in screening even populations with a low prevalence of infection.

The *N. gonorrhoeae* LCR assay requires substantially less time than culture for the definitive laboratory diagnosis of *N. gonorrhoeae* infection. This advantage is further reinforced by the ease of performance of the assay; the technician is required to perform only four hands-on steps. Chemical inactivation of the amplified material also decreases the risk of false-positive results attributable to contamination. Moreover, the processed sample for this assay is identical to that used for the *Chlamydia trachomatis* LCR assay (6, 19); thus, the same swab could be used to test for both organisms. The performance of the *N. gonorrhoeae* LCR assay described in this report warrants further evaluation, for example, of the ways in which the *N. gonorrhoeae* LCR test can be cost-effectively used in gonorrhea control programs.

TABLE 2. Performance of the *N. gonorrhoeae* LCR assay for the detection of *N. gonorrhoeae* in male urethral specimens[a]

Site	Assay	Prevalence (%)	Sensitivity (%)	Specificity (%)	Positive PV (%)[b]	Negative PV (%)[b]
Baltimore	LCR	33.7 (105/312)[c]	99.0 (104/105)	100 (207/207)	100 (104/104)	99.5 (207/208)
	Culture[d]	32.7 (102/312)	97.1 (102/105)			
Birmingham	LCR	31.3 (155/496)	98.1 (152/155)	99.7 (340/341)	99.3 (152/153)	99.1 (340/343)
	Culture[d]	30.0 (149/496)	96.1 (149/155)			
Total[e]	LCR	32.2 (260/808)	98.5 (256/260)	99.8 (547/548)	99.6 (256/257)	99.3 (547/551)
	Culture[d]	31.1 (251/808)	96.5 (251/260)			

[a] True positives include culture-positive and resolved LCR-positive and culture-negative specimens.
[b] PV, predictive value.
[c] Values in parentheses are number of specimens with the indicated result/total number of specimens tested.
[d] Culture specificity is assumed to be 100%.
[e] Compared with culture only, the total sensitivity, specificity, positive predictive value, and negative predictive values are 98.4, 98.2, 96.1, and 99.3%, respectively.

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REFERENCES

1. **Abbott Laboratories.** Data on file.
1a. **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.
2. **Burczak, J. D., S. F. Ching, H. Y. Hu, and H. Lee.** 1995. Ligase chain reaction for the detection of infectious agents, p. 315–327. *In* D. Wiebrauk and D. H. Farkas (ed.), Molecular methods for virus detection. Academic Press, Inc., New York.
3. **Centers for Disease Control and Prevention.** 1993. Sexually Transmitted Disease Surveillance 1992. Division of STD/HIV Prevention, Centers for Disease Control, and Prevention, Atlanta.
4. **Centers for Disease Control and Prevention.** 1994. Summary of notifiable diseases—United States 1993. Morbid. Mortal. Weekly Rep. **42:**3.
5. **Chapin-Robertson, K.** 1993. Use of molecular diagnostics in sexually transmitted diseases. Critical assessment. Diagn. Microbiol. Infect. Dis. **16:**173–184.
6. **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.
7. **Ehret, J. M., F. N. Judson, and J. W. Biddle.** 1984. Gonorrhea. *In* B. B. Wentworth and F. N. Judson (ed.), Laboratory methods for the diagnosis of sexually transmitted diseases, p. 43–79. American Public Health Association, Washington, D.C.
8. **Eschenbach, D. A., T. M. Buchanan, H. M. Pollock, P. S. Forsyth, E. R. Alexander, J. Lin, S. Wang, B. B. Wentworth, W. M. McCormack, and K. K. Holmes.** 1975. Polymicrobial etiology of acute pelvic inflammatory disease. N. Engl. J. Med. **293:**166–171.
9. **Fiore, M., J. Mitchell, T. Doan, R. Nelson, G. Winter, C. Grandone, K. Zeng, R. Haraden, J. Smith, K. Harris, J. Lesczynski, D. Berry, D. Safford, G. Barnes, A. Scholnick, and K. Ludington.** 1988. The Abbott IMx automated benchtop immunochemistry analyzer system. Clin. Chem. **34:**1726–1732.
10. **Grato, P. A., and M. R. Franz.** 1989. Evaluation of a prototype DNA probe test for the noncultural diagnosis of gonorrhea. J. Clin. Microbiol. **27:**632–635.
11. **Ho, B. S. W., W. G. Feng, B. K. C. Wong, and S. I. Eggleston.** 1992. Polymerase chain reaction for the detection of *Neisseria gonorrhoeae* in clinical samples. J. Clin. Pathol. **45:**439–442.
12. **Hook, E. W., III, and H. H. Handefield.** 1990. Gonococcal infections in the adult, p. 149–165. *In* K. Holmes et al. (ed.), Sexually transmitted diseases. McGraw-Hill Book Co., New York.
13. **Ison, C. A., K. McClean, and J. Gedney.** 1985. Evaluation of a direct immunofluorescence test for diagnosing gonorrhea. J. Clin. Pathol. **38:**1142–1145.
14. **Lewis, J. S., O. Fakile, E. Foss, G. Legarza, A. Lesky, K. Lowe, and D. Powning.** 1993. Direct DNA probe assay for *Neisseria gonorrhoeae* in pharyngeal and rectal specimens. J. Clin. Microbiol. **31:**2783–2785.
15. **Limberger, R. J., R. Biega, A. Evancole, L. McCarthy, L. Slivienski, and M. Kirkwood.** 1992. Evaluation of culture and the GenProbe PACE assay for detection of *Neisseria gonorrhoeae* and *Chlamydia trachomatis* in endocervical specimens transported to a state health laboratory. J. Clin. Microbiol. **30:**1162–1166.
16. **Meyer, T. F., E. Billyard, R. Haas, S. Storzbach, and M. So.** 1984. Pilus genes of *Neisseria gonorrhoeae*: chromosomal organization and DNA sequence. Proc. Natl. Acad. Sci. USA **81:**6110–6114.
17. **Pelouze, P. S.** 1941. Gonorrhea in the male and female. The W. B. Saunders Co., Philadelphia.
18. **Schachter, J., W. M. McCormack, R. F. Smith, R. M. Parks, R. Bailey, and A. C. Ohlin.** 1984. Enzyme immunoassay for diagnosis of gonorrhea. J. Clin. Microbiol. **19:**57–59.
19. **Schachter, J., W. E. Stamm, T. C. Quinn, W. W. Andrews, and J. D. Burczak.** 1994. Ligase chain reaction to detect *Chlamydia trachomatis* infection of the cervix. J. Clin. Microbiol. **32:**2540–2543.
20. **Stamm, W. A.** 1986. Diagnosis of *Neisseria gonorrhoeae* and *Chlamydia trachomatis* infections using antigen detection methods. Diagn. Microbiol. Infect. Dis. **4:**93s–99s.
21. **Stern, A., M. Brown, P. Nickel, and T. F. Meyer.** 1986. Opacity genes in *Neisseria gonorrhoeae*. Cell **47:**61–71.