

## Diagnosis of *Neisseria gonorrhoeae* Infections in Women by Using the Ligase Chain Reaction on Patient-Obtained Vaginal Swabs

EDWARD W. HOOK III,<sup>1,2\*</sup> SHAN FUN CHING,<sup>3</sup> JOAN STEPHENS,<sup>2</sup>  
KIM F. HARDY,<sup>1</sup> KIM R. SMITH,<sup>1</sup> AND HELEN H. LEE<sup>4</sup>

*University of Alabama at Birmingham<sup>1</sup> and Jefferson County Department of Health,<sup>2</sup>  
Birmingham, Alabama; Abbott Laboratories, Abbott Park, Illinois<sup>3</sup>;  
and University of Cambridge, Cambridge, England<sup>4</sup>*

Received 21 October 1996/Returned for modification 7 January 1997/Accepted 1 May 1997

**The increased sensitivities of nucleic acid amplification tests such as ligase chain reaction (LCR) have the potential to simplify specimen collection for gonorrhea diagnosis. In this study patients took their own vaginal swab specimens for gonorrhea culture and LCR testing. Immediately following specimen collection by patients, a trained clinician obtained endocervical swab specimens for the same tests. By using LCR to diagnose gonorrhea, 54 (17.5%) of 309 patients had positive tests. Forty-five patients with positive cervical LCR tests also had positive vaginal LCR tests; for one patient, only a cervical LCR specimen was positive, and for eight patients, only vaginal specimens were positive. For specimens from patients whose gonorrhea cultures were positive, all vaginal swab specimens were positive by LCR and 42 (91%) of 46 cervical swab specimens were positive by LCR. LCR-positive specimens from eight patients with negative cultures (four with positive vaginal specimens only, one with a positive cervical specimen only, and three with positive vaginal and cervical specimens) were further evaluated with unrelated probe sets for gonococcal pilin B. Following resolution of the discrepancies between culture-negative and LCR-positive specimens, a diagnosis of gonorrhea could be confirmed for 52 of 54 patients with positive LCR tests. LCR testing with vaginal swabs was 100% sensitive and 99.6% specific and had a positive predictive value of 98.1% and a negative predictive value of 100%. In this study LCR testing of vaginal swab specimens obtained by patients themselves was significantly more sensitive for gonorrhea diagnosis of women than cervical LCR or culture (100% versus 84.6% for cervical LCR or culture; Mantel-Haenszel chi-square test result, 8.58;  $P = 0.003$ ).**

Despite declines in incidence rates over the past 20 years, *Neisseria gonorrhoeae* infections remain common and their complications and sequelae continue to be among the leading preventable causes of tubal factor infertility and ectopic pregnancy. In recent years, the epidemiology of gonorrhea in the United States has changed, with infection being increasingly concentrated in groups of individuals who may not regularly utilize traditional sources of health care. As a result, efforts to sustain the continued decline of gonorrhea have begun to emphasize outreach to provide diagnosis and management for infected individuals encountered at sites other than clinics and physician offices. Such sites include school-based clinics, adolescent detention centers, community centers, etc.

Until recently, however, accurate diagnosis of gonorrhea in women could be achieved only when pelvic examination could be carried out to permit speculum-guided specimen collection (3). Nucleic acid amplification tests, such as ligase chain reaction (LCR) and PCR assays, have been shown to accurately diagnose *N. gonorrhoeae* and *Chlamydia trachomatis* with voided urine as well as swab specimens collected during pelvic examination (1–3, 6–10). These observations suggested to us that the high sensitivities of these assays have the potential to greatly simplify specimen collection for gonorrhea, particularly in settings where it is difficult to conduct pelvic examinations for specimen collection. To further explore this possibility, we conducted a study to evaluate the utility of patient-obtained

vaginal swab specimens for culture and LCR assays for gonorrhea diagnosis, comparing their performance to that of the same tests performed with swab specimens collected by a trained clinician during a pelvic examination.

### MATERIALS AND METHODS

**Specimen collection and processing.** Three hundred nine women attending the Jefferson County Department of Health Sexually Transmitted Diseases (STDs) Clinic in Birmingham, Ala., were evaluated for gonorrhea and coexistent chlamydia infections in the following manner. First, each patient was asked to sequentially obtain three vaginal swab specimens by inserting swabs about 2.5 inches or as far as was comfortable into the vagina, rotating them, and removing them. The requested length for insertion was demonstrated by the clinician with another swab. Following specimen collection, swabs were handed to a clinician, who used one swab to directly inoculate modified Thayer-Martin medium for gonorrhea culture and one swab to inoculate *C. trachomatis* transport medium and who placed the third vaginal swab specimen into a separate snap-top tube containing the LCR transport buffer (LCx; Abbott Laboratories, Abbott Park, Ill.) for subsequent LCR testing. The order of specimen collection for patient-obtained swabs was varied according to a preassigned randomization code.

Following specimen collection by participants, each woman was then examined by a trained clinician who obtained endocervical swab specimens for gonorrhea culture, chlamydia culture, and LCR testing. Immediately following inoculation, gonorrhea cultures were placed in a 5% CO<sub>2</sub> environment and incubated at 35°C. *C. trachomatis* transport media and LCR specimens (two swabs, consisting of a vaginal specimen collected by the patient and an endocervical specimen collected by the clinician, for each type of test) were kept at 4°C for no more than 18 h until they could be transported to a laboratory, where swabs were stored frozen at –20°C until LCR testing. The results of chlamydia cultures and LCR tests performed as part of this study are published separately (6).

**Gonorrhea culture methods.** Specimens for *N. gonorrhoeae* culture were obtained with the Dacron-tip swabs provided as part of the LCR specimen collection kit and were directly inoculated onto modified Thayer-Martin medium. Cultures were examined following overnight incubation, and if they were negative on initial evaluation, they were examined again following an additional 24 and 48 h of incubation. Typical colonies containing gram-negative diplococci and giving a positive oxidase reaction were presumptively identified as *N. gonorrhoeae*. The identities of presumptive *N. gonorrhoeae* colonies were then con-

\* Corresponding author. Mailing address: Division of Infectious Diseases, University of Alabama at Birmingham, 1900 University Blvd., 229 Tinsley Harrison Tower, Birmingham, AL 35294-0006. Phone: (205) 934-5191. Fax: (205) 934-5155. E-mail: ehook@uabid.dom.uab.edu.

firmed with fluorescein-conjugated monoclonal antibodies (MicroTrak; Syva Corp., Palo Alto, Calif.) (3, 9).

**DNA amplification.** The *N. gonorrhoeae* LCR assay was performed according to the manufacturer's instructions (3, 7). For each DNA amplification run, 12 controls and up to 36 patient samples were prepared. Clinical specimens were brought to room temperature and then heated in a dry bath at 95 to 100°C for 15 min. After cooling to room temperature, 100 µl was added to the manufacturer's unit dose reagent tube containing a mixture of four oligonucleotide probes, a thermostable ligase, and DNA polymerase. Controls (positive, negative, and calibration) were prepared for thermocycler processing by addition of 100 µl of a magnesium activator supplied by the manufacturer, vortexed, and transferred to appropriately labeled unit dose tubes. Specimens and controls were then immediately placed into the thermocycler and run for 40 cycles over a period of 2 h. Each cycle consisted of the following temperatures and durations: 97°C for 1 s, 55°C for 1 s, and 62°C for 50 s. After thermocycler processing, samples were held at 4°C until they were tested for detection of amplicons.

Amplicon detection was performed by a microparticle-based enzyme immunoassay with the LCx instrumentation to detect a fluorescent reaction. Amplicons containing hapten-labeled, ligated probes were captured by antibody-coated microparticles held by a filter. Addition of substrate to the conjugate enzyme catalyzed a fluorescent reaction, which was detected by the LCx instrument and recorded as a numerical value. For amplicon detection, samples were brought to room temperature and then centrifuged at 13,000 rpm briefly to eliminate condensation around the cap and 100 µl was then transferred to separate LCR cells. Eighteen patient samples and six control samples were included in each LCR run. Patient samples with values at least 0.25 the average of two calibration samples for the run were recorded as positive.

**Resolution of culture-LCR discrepancies.** For patients whose cervical culture results failed to agree with the results of LCR testing, efforts to resolve discrepancy were carried out as follows. For culture-negative specimens (from both sites) which were LCR positive, the LCR assay was repeated at Abbott Laboratories with a different, unrelated probe set directed against other *N. gonorrhoeae* targets (gonococcal pilin B) (3, 9). All confirmatory LCR testing was done in a blind fashion in specimen runs which also included at least the same number of known negative specimens.

**Analysis.** For calculation of test performance, patients with true-positive infections were defined as patients with positive cultures for *N. gonorrhoeae* from either site (vaginal or cervical) or patients with negative cultures and positive LCR tests which could be confirmed by a second LCR test with different, unrelated probe sets. Uninfected patients were defined as those with negative cultures and negative LCR tests of specimens from both vaginal and cervical sites. False-positive LCR results were defined as positive LCR tests on specimens taken from either body site which could not be confirmed by culture or a positive result in the second, alternative LCR test.

Following resolution of discrepant results, the sensitivity, specificity, and predictive values (positive and negative) for each type of specimen were calculated based on the results of the resolution process described above.

## RESULTS

**Patient population.** Patients who volunteered to participate in this study were typical of women visiting the Jefferson County Department of Health STDs Clinic. Participants tended to be young (median age, 25 years; range, 14 to 56 years), African-American (270 [87.4%]) residents of Birmingham, Ala. On the day of enrollment, self-reported reasons for visiting the clinic were for symptom evaluation ( $n = 175$ ), because the women were sexual partners of men with recent STD diagnoses ( $n = 87$ ), because the women were referred from other health care providers or had tested positive ( $n = 22$ ), or for other reasons, including STD screening (25). Seventy-one percent (218) of participants reported genitourinary symptoms at the time of clinic presentation. Other STDs were also relatively common in the study population: 39 (12.6%) women had positive cervical cultures for *C. trachomatis*, and 49 (15.9%) had trichomonas visualized by wet-mount microscopy.

**Gonorrhea culture.** A total of 46 (14.9%) patients had positive cultures for *N. gonorrhoeae* (Table 1). Endocervical swab cultures were positive for 44 (14.2%) women, of whom 28 also had positive vaginal cultures. For two additional patients, self-obtained vaginal swab specimens were culture positive while cervical cultures were not. Overall, vaginal swab cultures for *N. gonorrhoeae* were positive for 30 (65.2%) of 46 participants with positive *N. gonorrhoeae* cultures.

TABLE 1. Results of testing for gonorrhea by culture and LCR with patient-obtained and clinician-obtained specimens

No. of samples (total = 309)	Result by indicated test with specimen type:			
	Vaginal		Cervical	
	Culture ( $n = 30$ [9.79%])	LCR ( $n = 53$ [17.2%])	Culture ( $n = 44$ [14.2%])	LCR ( $n = 46$ [14.9%])
255	–	–	–	–
27	+	+	+	+
15	–	+	+	+
2	+	+	–	–
1	+	+	+	–
1	–	+	+	–
4 <sup>a,b</sup>	–	+	–	–
3 <sup>a,c</sup>	–	+	–	+
1 <sup>a,d</sup>	–	–	–	+

<sup>a</sup> These samples produced discrepant results (they were LCR positive but no positive cultures were obtained from vaginal or endocervical specimens). These eight samples were evaluated with different LCR probe sets targeted to pilin B. Six of the eight specimens were also positive when they were tested with the second gonococcal DNA target site; hence, these samples were reclassified as true positives.

<sup>b</sup> Four of the four samples were confirmed positive on the basis of pilin B LCR.

<sup>c</sup> Two of the three samples were resolved as positive by pilin B LCR (true-positive LCR), and one of the three samples was nonreactive by pilin B LCR with both vaginal and cervical specimens (false-positive LCR).

<sup>d</sup> This sample was not confirmed as gonococcal and was negative by pilin B LCR (false-positive LCR).

**LCR detection of *N. gonorrhoeae*.** Overall, 54 patients had positive *N. gonorrhoeae* test results; of these, 53 (98.1%) patients had positive vaginal LCR tests while 46 (85.2%) had positive cervical LCR tests. Following resolution of discrepancies between results of culture and LCR tests (8 patients were culture negative and LCR positive at one or more sites) by gonococcal pilin B LCR, 52 patients were classified as having confirmed positive LCR tests. The two LCR test results classified as false positive were from women with positive endocervical swabs; one culture-negative patient had positive LCR test results from both endocervical and vaginal specimens, and one patient had only an LCR-positive endocervical swab specimen. Of 52 patients with confirmed positive LCR test results, the infections of all (100%) were detected by LCR performed on patient-obtained vaginal swabs and those of 44 (84.6%) were detected with clinician-obtained endocervical swab specimens. Of the 52 participants with confirmed positive LCR test results, 44 (84.6%) had positive swabs from both sites and 8 had positive swabs only from the vaginal site.

With a positive culture from either site or a positive LCR assay result confirmed by a second LCR assay using primers for the pilin B gene as the standard for comparison, the performance of culture and LCR performed with either patient-obtained vaginal swabs or clinician-obtained endocervical swabs was calculated (Table 2). By using this standard for comparison, the sensitivity of culture for *N. gonorrhoeae* with modified Thayer-Martin medium for vaginal and endocervical specimens was 57.7 and 84.6%, respectively. The sensitivity of LCR performed on endocervical swabs (84.6%) was similar to that of endocervical culture (84.6%), while the sensitivity of LCR for gonorrhea diagnosis using patient-obtained vaginal swabs was 100% ( $P = 0.003$ , Mantel-Haenszel chi-square test).

## DISCUSSION

*N. gonorrhoeae* is an exclusively human pathogen which is often spread to others by individuals who are unaware of their

TABLE 2. Performance of testing of patient- and clinician-obtained swab specimens for detection of *N. gonorrhoeae* in women

Assay and specimen source	% of sensitive samples (no. of sensitive samples/ total no. of samples)	% of specific samples (no. of specific samples/ total no. of samples)	Positive predictive value (%)	Negative predictive value (%)
<b>Culture</b>				
Patient-obtained vaginal swabs	57.7 (30/52)	100 (257/257)	100	92.1
Clinician-obtained endocervical swabs	84.6 (44/57)	100 (257/257)	100	97
<b>LCR</b>				
Patient-obtained vaginal swabs	100 (52/52)	99.6 (256/257)	98.1	100
Clinician-obtained endocervical swabs	84.6 (44/52)	99.2 (255/257)	95.7	97

infections (5). In the mid-1960s, development of selective media for isolation of *N. gonorrhoeae* facilitated diagnosis of infection occurring at mucosal surfaces and provided the tools for expanded and more effective gonorrhea screening (5). In part due to improved test performance, as well as a national screening program, since 1978 the number of reported cases of gonorrhea in the United States has declined by more than 50% (4). These declines have been less dramatic, however, for the young, for racial minorities, and in the southeastern United States (4). For groups for whom gonorrhea rates remain highest, access to health care, including screening for STDs such as gonorrhea, is often limited.

Until recently, optimal screening for bacterial STDs in women has required performance of pelvic examination and endocervical specimen collection. With the development of sensitive PCR- and LCR-based tests for detection of *C. trachomatis* and *N. gonorrhoeae*, alternate clinical specimens, such as voided urine, have been found to perform as well as swab specimens for STD diagnosis (7, 9). In the laboratory, however, preparation of urine specimens requires additional processing steps (aliquoting and centrifugation), and urine specimens are often bulky and difficult to transport. In this study we found that while the sensitivity of culture of contemporaneously obtained vaginal swabs for gonorrhea was only 57.7%, the sensitivity of LCR assays using vaginal swabs obtained by patients was 100%. In addition, the specificity of LCR for detection of *N. gonorrhoeae* in this study, whether the assay was performed on endocervical swab specimens or patient-obtained vaginal swabs, exceeded 99%. Thus, when these results are considered with the results of several prior studies (2, 7, 9), it appears that clinicians using LCR can utilize either clinician-obtained endocervical swabs, patient-obtained vaginal swabs, or urine as specimens for gonorrhea diagnosis, according to whichever specimen might be most readily obtained, without compromising the accuracy of test results.

In this study we found that clinician-obtained endocervical swabs for both culture and for LCR testing could be used to detect 84.6% of infections in groups of women for whom the prevalence of gonorrhea is relatively high. Testing with patient-obtained vaginal swabs was significantly more sensitive, detecting 100% of gonococcal infections. The reasons for this possibly superior performance are unclear. A small number of women with gonorrhea are infected at the urethra but do not appear to be infected at the cervix (5, 9). It is possible that testing of intravaginal specimens may have detected some of these infections. In addition, it is possible that LCR detected nonviable gonococci or gonococcal nucleic acids present in vaginal secretions. Alternatively, while LCR is equivalent to culture of a single endocervical swab for gonorrhea detection, there may be other cervical factors which compromise optimal detection of *N. gonorrhoeae* by LCR. Finally, the detection of gonorrhea with endocervical swabs (irrespective of whether by

culture or LCR) is potentially limited by issues related to specimen adequacy that concern women in whom the cervix is difficult to visualize or women for whom pelvic examinations are uncomfortable and thus difficult to perform or for other reasons which might otherwise compromise specimen quality. In contrast, collection of an intravaginal swab is a virtually painless, easy-to-perform test which, as indicated in this study, provides a high-quality specimen for detection of infection by tests such as LCR.

The cost differential between amplified nucleic acid detection tests and culture may limit the application of LCR for gonorrhea diagnosis in settings where pelvic examinations are already being performed. At this time the cost of LCR tests for gonorrhea diagnosis is three- to fourfold greater than that of culture. Nonetheless, for evaluation of high-risk women such as adolescents, women with prior STDs, incarcerated women, etc., the opportunity to provide accurate diagnostic screening for *N. gonorrhoeae* without the necessity of performing pelvic examinations provides an opportunity to broaden gonorrhea screening and to improve efforts to detect otherwise asymptomatic infections, thereby reducing risks for both complications to the infected individual and transmission of infection to sexual partners.

In summary, this study expands previous evaluations describing the high sensitivities and specificities of LCR tests for detection of *N. gonorrhoeae* infections to include the ability of these tests to accurately detect infections with vaginal specimens obtained by patients themselves rather than with endocervical swabs or urine obtained by a clinician. The ability to use patient-obtained specimens for accurate detection of *N. gonorrhoeae* provides the opportunity to expand screening to individuals who might not otherwise receive gonorrhea screening, thereby potentially augmenting our ongoing efforts to control the infection.

#### ACKNOWLEDGMENTS

We thank P. Joy Gibson for her secretarial assistance with manuscript preparation and Charity Richey for statistical consultation.

#### REFERENCES

1. Bauwens, J. E., A. M. 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.
2. Buimer, M., and G. J. J. Van Doornum. 1996. 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. *J. Clin. Microbiol.* **34**:2394-2400.
3. Ching, S. F., H. H. Lee, E. W. Hook III, M. R. Jacobs, and J. Zenilman. 1995. Ligase chain reaction for detection of *Neisseria gonorrhoeae* in urogenital swabs. *J. Clin. Microbiol.* **33**:3111-3114.
4. Division of STD Prevention, Centers for Disease Control and Prevention. 1995. Sexually transmitted disease surveillance, 1994. Public Health Service,

- U.S. Department of Health and Human Services, Atlanta, Ga.
5. **Hook, E. W., III, and H. H. Handsfield.** 1990. Gonococcal infections in the adult, p. 149–165. *In* K. K. Holmes et al. (ed.), Sexually transmitted diseases. McGraw-Hill, New York, N.Y.
  6. **Hook, E. W., III, K. Smith, C. Mullen, J. Stephens, L. Rinehardt, M. S. Pate, and H. H. Lee.** 1997. Diagnosis of genitourinary *Chlamydia trachomatis* infections by using the ligase chain reaction on patient-obtained vaginal swabs. *J. Clin. Microbiol.* **35**:2133–2135.
  7. **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.
  8. **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.
  9. **Smith, K. R., S. Ching, H. Lee, Y. Ohhashi, H.-Y. Hu, H. Fisher, 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.
  10. **Viscidi, R. P., L. Bobo, E. W. Hook III, and T. C. Quinn.** 1993. Transmission of *Chlamydia trachomatis* among sex partners assessed by polymerase chain reaction. *J. Infect. Dis.* **168**:488–492.