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Duration of Persistence of Gonococcal DNA Detected by Ligase Chain Reaction in Men and Women following Recommended Therapy for Uncomplicated Gonorrhea

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***Neisseria gonorrhoeae* infection remains relatively common in the United States, representing a public health challenge. Ligase chain reaction (LCR) is both highly sensitive and specific for the detection of *N. gonorrhoeae* in urine and patient-obtained vaginal swab specimens. Because of the LCR test's exquisite sensitivity, it may potentially detect DNA from nonviable organisms following effective therapy, leading to false-positive test results and unnecessary additional treatment. The purpose of the present study was to determine the duration that gonococcal DNA is detectable by LCR following therapy for uncomplicated gonococcal infection. One hundred thirty men and women between the ages of 16 and 50 years presenting to a sexually transmitted disease clinic with urogenital gonorrhea were enrolled. After the standard history was taken and a genital examination was done, the patients were asked to submit either a urine specimen (men) or a urine specimen plus a self-obtained vaginal swab specimen (women) for *N. gonorrhoeae* testing by LCR at the initial visit and each day during the study period. At enrollment, patients were treated with single doses of ofloxacin, cefixime, or ceftriaxone. The median time to a negative urine LCR test result was 1 day for the men (mean, 1.6 ± 0.14 days) and 2 days for the women (mean, 1.7 ± 0.19 days). Among the women the clearance time was significantly longer for vaginal specimens (mean, 2.8 ± 0.30 days) than for urine specimens (mean, 1.7 ± 0.11 days). Irrespective of patient gender and specimen type, gonococcal DNA can be expected to be absent from urogenital specimens within 2 weeks following successful therapy.**

Gonorrhea is the second most common reportable infectious disease in the United States, and its control represents a continuing public health challenge. In 2000, over 350,000 cases of gonorrhea were reported, a 21% increase over the number of reported cases since 1997 and a sustained reversal of the downward trend in the prevalence of gonococcal infections reported in the United States from 1977 to 1997 (2). At the same time, over the past decade powerful new tools for the diagnosis of gonorrhea have become available. Among these are nucleic acid amplification tests which permit the use of specimens obtained by simplified specimen collection methods (i.e., voided urine specimens or, for women, patient-collected vaginal swab specimens) without any compromise in the sensitivity of detection (4, 7, 8, 11–13). Because nucleic acid amplification tests do not require living organisms for the detection of infection, concern has also been expressed about the potential for residual nucleic acids from nonviable organisms to persist in the genital tract, giving rise to false-positive test results if patients are retested too soon following successful therapy. Support for these concerns comes from studies demonstrating the persistence of *Chlamydia trachomatis* nucleic acids for periods as long as 2 to 3 weeks following therapy (1, 5, 14). No similar studies have been performed to describe the clearance of *Neisseria gonorrhoeae* nucleic acids from genital specimens following therapy. To address these needs we evaluated the clearance of *N. gonorrhoeae* following treatment from urine and patient-collected vaginal swab specimens collected daily by a commercially available ligase chain reaction (LCR) assay.

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MATERIALS AND METHODS

Study population. The study was conducted at the Jefferson County Department of Public Health Sexually Transmitted Disease (STD) Clinic in Birmingham, Ala. Men and women (age range, 16 to 50 years) presenting to the clinic with urogenital gonorrhea between September 1998 and January 2001 were invited to participate in the study. For this study a patient was considered to have gonococcal infection if gram-negative intracellular diplococci were present on Gram staining of genital secretions or a culture of urethral or cervical specimens was previously positive for *N. gonorrhoeae*. Patients with complicated gonococcal infection (pelvic inflammatory disease, epididymitis, disseminated gonococcal infection) or isolated pharyngeal or rectal gonorrhea were excluded. Patients with known *C. trachomatis* infection or exposure, allergy to the recommended therapy for gonorrhea, or a history of antibiotic use within the 3 weeks preceding study enrollment and pregnant or lactating women were also excluded. The study protocol was reviewed and approved by the Institutional Review Boards for Protection of Human Subjects of the University of Alabama at Birmingham and the Jefferson County Department of Health.

Laboratory specimens. After informed consent was obtained, patients meeting the enrollment criteria underwent a directed history and genitourinary examination. Urethral specimens (men) or cervical swab specimens (women) were collected for testing for gonorrhea by culture on Thayer-Martin agar or Gonostat (Sierra Diagnostics, Sonora, Calif.) and for testing for *C. trachomatis* by cell culture techniques. Cultures for *N. gonorrhoeae* and *C. trachomatis* and Gonostat testing were performed as described previously (9, 10, 13). Patients were then asked to submit either a urine specimen alone (men) or a urine specimen plus a self-obtained vaginal swab specimen (women) for testing for *N. gonorrhoeae* by

TABLE 1. Characteristics of study population by gender[a]

Characteristic	Men (n = 87)	Women (n = 43)	P
Age (yr [mean ± SD])	26.5 ± 7.3	22.8 ± 5.7	0.004
No. (%) of subjects			
Race			
African American	86 (97.7)	38 (88.4)	0.02
White	1 (1.1)	5 (11.6)	
Marital status			
Single	77 (88.5)	40 (93.0)	0.66
Married	7 (8.1)	3 (7.0)	
Divorced or other	3 (3.4)	0 (0.0)	
Reason for visit			
Symptoms	79 (90.8)	10 (23.3)	<0.001
Contact	1 (1.1)	12 (27.9)	
Positive laboratory test	1 (1.2)	14 (32.6)	
Other	6 (6.9)	7 (16.2)	
Symptoms			
Discharge	76 (87.4)	9 (20.9)	<0.001
Dysuria	52 (59.8)	1 (2.3)	<0.001
Treatment for gonorrhea within the last year			
Yes	37 (42.5)	7 (16.3)	0.003
No	50 (57.5)	36 (83.7)	
Treated for other STDs			
Yes	21 (24.1)	22 (51.2)	0.002
No	66 (75.8)	21 (48.8)	
With the following no. of partners within the last 6 mo:			
1	23 (26.4)	23 (53.5)	0.29
2	28 (32.2)	11 (25.6)	
3 or more	36 (41.4)	9 (20.9)	
With a new partner within the last 30 days			
No	58 (66.7)	37 (86.1)	0.02
Yes	29 (33.3)	6 (13.9)	
Number of days with discharge (mean ± SE)	3.5 ± 4.0	6.9 ± 4.6	0.02

[a] A total of 130 patients were evaluated.

LCR at the initial visit and each day during the study period. LCR tests were performed according to the instructions of the manufacturer.

At the time of study enrollment, each participant was treated with a single dose of ceftriaxone, cefixime, or ofloxacin, according to the STD treatment guidelines of the Centers for Disease Control and Prevention (3). Participants were asked to return to the clinic for follow-up visits every other day for up to 21 days or until two consecutive negative LCR results were obtained for specimens from each site sampled. On the interim days, patients collected their own specimens in prelabeled specimen containers which were stored in a refrigerator until the return clinic visit. Repeat cultures of urethral specimens (men) or cervical swab specimens (women) for gonococci were obtained from participants between days 5 and 7.

Questionnaire survey data. Initial baseline data including patient demographic characteristics, symptom duration (if symptoms were present), and risk behaviors (condom use, the number of sexual partners in the last 6 months, whether the patient had new sexual partners, and a history of prior STDs) were obtained. At each subsequent follow-up visit, one of two designated study nurses (J.S. or A.H.) obtained information on symptom resolution, interval sexual exposures, and partner treatment. If symptoms persisted, a brief genital examination was performed to document the presence of physical signs, and further laboratory testing was performed as indicated.

Patients were advised to refer all sexual partners within the past 60 days or, if they had not been sexually active for greater than 60 days, their last sexual partner for treatment. Doxycycline was given (for *C. trachomatis* infection) at 100 mg orally twice a day at the time of a positive chlamydia culture or at the last follow-up visit. Participants were reimbursed $20 at each visit and $30 at the final visit for their time and trouble.

Statistical analysis. Categorical data were compared by the chi-square test or Fisher's exact test where appropriate. Continuous variables were compared by Student's t test. Gonococcal clearance data were analyzed by using Kaplan-Meier curves. Time to clearance (in days) was calculated from the first treatment day for all patients. The event, consistent clearance of gonococcal nucleic acids, was defined as two successive negative gonococcal LCR tests. The data for patients who did not experience the event were censored. Patients who had an episode defined as one or more negative tests followed by a positive test were defined as intermittently shedding gonococcal nucleic acids. Depending on the pattern of shedding, participants with intermittent shedding may or may not have eventually met the study's working definition of having cleared gonococcal nucleic acids (two successive negative gonococcal LCR tests). Nucleic acid clearance was compared for participants with and without intermittent shedding by gender, site of specimen (for women, urine versus vaginal swab specimens), and history of treatment for STDs within the last year (yes versus no) by the log-rank test. Clearance times are reported as means ± standard errors (SEs) and medians. A P value of <0.05 was deemed statistically significant.

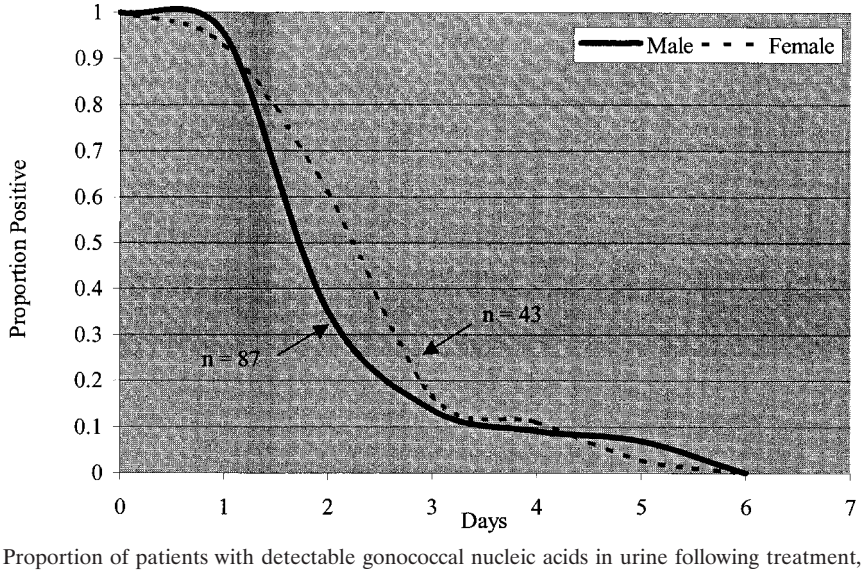

FIG. 1. Proportion of patients with detectable gonococcal nucleic acids in urine following treatment, by gender.

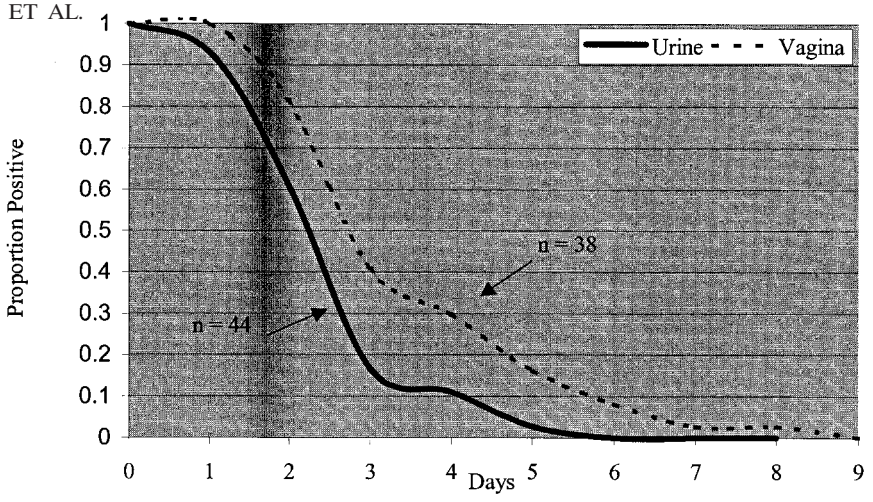

FIG. 2. Proportion of women with detectable gonococcal nucleic acids following treatment, by site.

RESULTS

Descriptive data evaluation. One hundred sixty-nine patients were enrolled in the study, and 39 (23%) of these were lost to follow-up or their data were censored. Therefore, the present analysis is based on 130 evaluable individuals. The characteristics of the group not included in the analysis did not differ significantly from those who completed the study (data not shown). The majority of the patients were African American (95%) and male (66%) (Table 1). The reason for presentation differed significantly by gender, with 91% of men presenting for care due to symptoms (discharge, dysuria) but only 23% of women presenting for care due to symptoms. Women more commonly presented to an STD clinic for treatment for gonorrhea not treated after a previous positive test result (33% for women versus 1% for males). Over half (51.5%) of the participants had been treated for an STD within the past 12 months. The number of reported sex partners within the last 6 months ranged from 1 to 12, with the majority of participants reporting a single partner. Within the last 30 days, 27% reported having a new partner, with males being significantly more likely to report a recent new partner than females (P = 0.02). Ninety-five percent of patients received a single dose of ofloxacin on the day of enrollment, 4% received cefixime, and less than 1% (1 patient) received ceftriaxone.

Clearance analysis. LCR tests and culture for *N. gonorrhoeae* were positive for all patients at the time of study enrollment. The median time to a negative urine LCR test was 1 day for men (mean, 1.6 ± 0.14 days) and 2 days for women (mean, 1.8 ± 0.19 days) (P = 0.19) (Fig. 1). Among the women, the clearance time was significantly longer for vaginal specimens (mean= 2.8 ± 0.30 days) than for urine specimens (mean, 1.7 ± 0.11 days) (P = 0.008) (Fig. 2). Urine LCR tests were negative for over 90% of the patients by day 5 following therapy. The only factor associated with accelerated clearance of gonococcal nucleic acids was a history of treatment for STDs within the last 12 months. Patients who had a treatable STD within the last year had a significantly shorter clearance time than those who did not (means, 1.3 ± 0.08 and 1.9 ± 0.2 days, respectively [P = 0.005]).

Overall, 18% of the population (15% of the men and 25% of the women) experienced intermittent shedding at some point

TABLE 2. Intermittent shedding patterns for male participants (days 0 to 21)

Subject and test	Result on day[a]												
	0	1	2	3	4	5	6	7	8	9	10	11	12
Male 01													
LCR, urine	+	−	−	−	−	−	+	−	−				
Culture	+								−				
Male 02													
LCR, urine	+	+	−	+	−								
Culture	+												
Male 03													
LCR, urine	+	+	−	−	−	+	−	NS	−				
Culture	+								−				
Male 04													
LCR, urine	+	−	+	−	−	−	−	−	−	−			
Culture	+								−				
Male 05													
LCR, urine	+	−	−	−	−	−	−	+					
Culture	+							−					
Male 06													
LCR, urine	+	−	−	−	+								
Culture	+				−								
Male 07													
LCR, urine	+	+	−	−	−	−	+	−	−	−	−	−	−
Culture	+							−					
Male 08													
LCR, urine	+	+	NS	−	−	−	−	NS	+				
Culture	+								−				
Male 09													
LCR, urine	+	−	+	+	−	−	−	−	−				
Culture	+								−				
Male 10													
LCR, urine	+	NS	−	−	−	−	−	−	+	−			
Culture	+									−			
Male 11													
LCR, urine	+	+	−	+	NS	+	−	−					
Culture	+						−						
Male 12													
LCR, urine	+	−	−	−	+	−	−	−					
Culture	+							−					
Male 13													
LCR, urine	+	−	+	−	−	−							
Culture	+					−							

[a] +, positive; −, negative; NS, no sample.

TABLE 3. Intermittent shedding patterns for female participants (days 0 to 21)

Subject and test	Result on day[a]:																					
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Female 01																						
LCR, urine	−	−	−	−	−	−	−	−														
LCR, vagina[b]	+	+	−	+	−	−	−	−														
Culture	+							−														
Female 02																						
LCR, urine	+	+	+	NS	−	−	−	−	−	−												
LCR, vagina	+	NS	+	NS	−	−	−	+	+	+												
Culture	+				−																	
Female 03																						
LCR, urine	+	−	−	−	−	−	−	−														
LCR, vagina	+	−	−	+	−	−	−	−														
Culture	+							−														
Female 04																						
LCR, urine	+	+	−	+	−	−	−	−	−	−												
LCR, vagina	+	+	−	+	+	−	−	−	−	−												
Culture	+									−												
Female 05																						
LCR, urine	+	+	−	+	−	−	−	−	NS	−	−	−										
LCR, vagina	+	+	+	−	−	−	−	−	NS	−	−	−										
Culture	+							−														
Female 06																						
LCR, urine	+	−	−	−	−	+	−	−	−	NS												
LCR, vagina	+	+	−	−	−	−	−	−	−	−												
Culture	+						−															
Female 07																						
LCR, urine	−	−	−	−	−	−	−	−	−													
LCR, vagina	+	−	−	−	−	−	−	+	+													
Culture	+								−													
Female 08																						
LCR, urine	+	+	−	−	−	−	−	−	−	−	−	NS	NS	NS	−	−	−	−	−	−	−	−
LCR, vagina	+	+	+	−	−	−	−	−	−	−	−	NS	NS	NS	+	−	−	−	−	+	−	−
Culture	+							−														
Female 09																						
LCR, urine	+	+	−	−	−	−	−	−	−	−												
LCR, vagina	+	+	−	+	−	−	−	−	−	−												
Culture	+							−														
Female 10																						
LCR, urine	+	+	−	+	−	NS	NS	−														
LCR, vagina	+	+	+	+	−	NS	NS	−														
Culture	+							−														
Female 11																						
LCR, urine	−	−	−	−	−	−	−	−														
LCR, vagina	+	−	−	−	+	−	−	+														
Culture	+							−														

[a] +, positive; −, negative; NS, no sample.
[b] Vagina refers to a vaginal swab specimen.

during the follow-up period. Among the women, intermittent shedding of gonococcal nucleic acids was detected in urine from four women and vaginal specimens from eight women (intermittent shedding was noted in both vaginal and urine specimens from one woman). Tables 2 and 3 demonstrate the intermittent shedding patterns and follow-up gonorrhea culture results for each individual by gender. Cultures or Gonostat tests were negative for all 21 participants with intermittent shedding (88% of the 24 participants with intermittent shedding) for whom a culture for gonorrhea or Gonostat testing was performed on the same day or within several days of the transiently positive LCR test (Tables 2 and 3). The characteristics of participants with intermittent shedding are compared to those of the remainder of the participants in Table 4. Pa-

TABLE 4. Characteristics of study population by shedding status[a]

Characteristic	Normal (n = 106)	Intermittent (n = 24)	P
Age (yr [mean ± SD])	25.6 ± 7.0	23.3 ± 6.6	0.13
No. (%) of subjects			
Gender			
Male	73 (69.2)	13 (54.2)	0.23
Female	33 (30.8)	11 (45.8)	
Race			
African American	101 (95.3)	23 (95.8)	1.0
White	5 (4.7)	1 (4.2)	
Marital status			
Single	95 (89.6)	22 (91.7)	0.87
Married	8 (7.6)	2 (8.3)	
Divorced or other	3 (2.8)	0 (0.0)	
Reason for Visit			
Symptoms	75 (70.8)	14 (58.3)	0.26
Contact	11 (10.4)	2 (8.3)	
Positive lab test	12 (11.3)	3 (12.6)	
Other	8 (7.7)	5 (20.8)	
Symptoms			
Discharge	72 (67.9)	13 (54.2)	0.06
Dysuria	41 (38.7)	12 (50.0)	0.05
STD treated within the last year			
Yes	60 (56.6)	7 (29.2)	0.02
No	46 (43.4)	17 (70.8)	
Treatment for gonorrhea within the last year	39 (36.8)	5 (20.8)	0.16
Other STDs	40 (37.7)	3 (12.5)	
With the following of partners within the last 6 mo:			
1	36 (34.0)	10 (41.7)	0.67
2	34 (33.1)	5 (20.8)	
3 or more	35 (32.9)	9 (37.5)	
New partner within the last 30 days			
No	76 (71.7)	19 (79.2)	0.61
Yes	30 (28.3)	5 (20.8)	
Treatment at enrollment date			
Ofloxacin	101 (95.3)	21 (87.5)	0.16
Ceftriaxone	1 (1.0)	0 (0.0)	1.0
Cefixime	4 (3.8)	2 (8.3)	0.31
No. of days with discharge (mean ± SD)	3.9 ± 4.5	4.0 ± 2.2	0.92

[a] A total of 130 patients were evaluated.

tients treated for an STD within the last year were less likely to shed intermittently than patients with no recent treatable STD. As might be expected, the patients who experienced intermittent shedding tended to have longer clearance times than those who did not. The 24 patients with intermittent shedding had an overall mean clearance time of 2.2 days, whereas those who did not have intermittent shedding had an overall mean clearance time of 1.4 days (P = 0.03). A multivariable Cox regression model of predictors of clearance for urine specimens showed that the only predictor of a faster clearance time was a history of a treatable STD within the last 12 months (hazard ratio, 1.5; 95% confidence interval, 1.0, 2.2), which was of borderline significance (P = 0.07).

TABLE 5. Percent urine LCR tests reactive following gonorrhea treatment, by gender

Gender and LCR test result	% Urine LCR tests reactive on day:					
	1	2	3	4	5	6+
Male						
+	95.4	35.3	13.8	9.2	6.9	0
−	4.6	64.7	86.2	90.8	93.1	100
Female						
+	93.0	61.1	16.7	11.1	2.8	0
−	7.0	38.9	83.3	88.9	97.2	100

DISCUSSION

The present treatments for uncomplicated gonorrhea recommended by the Centers for Disease Control and Prevention are highly efficacious, yielding 97 to 100% cure rates (3) and rendering cultures of urine, urethral swab, and semen specimens from men with gonococcal urethritis negative within 24 h of therapy (6). Because of the exquisite sensitivity of nucleic acid amplification tests such as LCR, DNA from nonviable organisms could be detected following effective therapy, leading to a false-positive result and unnecessary additional treatment. This phenomenon has been reported in several studies evaluating the persistence of *C. trachomatis* nucleic acids by PCR or LCR following effective treatment (1, 5, 14). For instance, Workowski and colleagues (14) documented that 15% (3 of 20) of their female population continued to shed chlamydial DNA in cervical specimens for 1 week following completion of successful treatment with doxycycline. Similarly, detectable chlamydial nucleic acids were demonstrated in urine specimens tested by LCR and PCR for up to 2 weeks after adequate therapy in a population of female high school students. These findings are consistent with those from a smaller study of men and women who were treated with a single dose of azithromycin and who submitted urine specimens daily for testing for chlamydia by PCR and transcription-mediated amplification (1, 5). Collectively, these findings have led to the recommendation to refrain from using urine nucleic acid amplification tests for test of cure within the first 3 weeks following therapy for *C. trachomatis* (3).

This study documents the rapidity with which gonococcal nucleic acids are cleared from urine and vaginal swab specimens for LCR following single-dose therapy for uncomplicated gonorrhea. Survival analysis demonstrated that 91% of urine specimens from men and 89% of urine specimens from women were cleared of nucleic acids by day 4, and all urine specimens were cleared by day 6 posttreatment (Table 5). Furthermore, gender did not affect the time to consistently negative results for urine specimens.

Interestingly, 18% of the study population shed gonococcal nucleic acids intermittently during follow-up. Day-to-day variations in sampling and the high sensitivity of the LCR test are likely explanations for this observation, given that 88% (21 of 24) of intermittent shedders had a documented negative cul-

ture result for gonorrhea on the same day or within several days of the positive test result.

Our study has several limitations which should be acknowledged. Logistical considerations and participant preferences precluded collection of cervical swab specimens for LCR testing following treatment. In addition, we were unable to directly supervise specimen collection in the clinic every day. Although the containers were labeled with the correct date before they were sent home with each participant, it is possible that on weekends, when more than one container was sent home, participants may have confused the dates. If the samples were not consecutive, this could account for a few of the episodes of intermittent shedding; however, this is not consistent with the patterns of intermittent shedding that we most often observed, in which a series of days with negative tests would be followed by a single positive LCR test result.

In conclusion, on the basis of the data presented here, which demonstrate the clearance of nucleic acids from all urine and vaginal swab specimens by posttreatment days 6 and 9, respectively, follow-up testing for gonococci by LCR would best be performed on day 10 following treatment or beyond. While the fact that intermittent shedding occurred in a minority of the population may raise concerns regarding the potential for false-positive test results when LCR is used for test of cure soon after treatment, we believe that in most instances this concern is offset by the general increased sensitivity of LCR as well as the ease of specimen collection in the setting of a follow-up examination (i.e., when a pelvic examination is less likely to be necessary) should test-of-cure testing be performed.

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