JOURNAL OF CLINICAL MICROBIOLOGY, Oct. 2000, p. 3544–3549 Vol. 38, No. 10
0095-1137/00/$04.00+0

Multicenter Evaluation of AMPLICOR and Automated COBAS AMPLICOR CT/NG Tests for *Neisseria gonorrhoeae*

DAVID H. MARTIN,[1,2]* CATHY CAMMARATA,[1,2] BARBARA VAN DER POL,[3] ROBERT B. JONES,[3] THOMAS C. QUINN,[4,5] CHARLOTTE A. GAYDOS,[5] KIMBERLY CROTCHFELT,[5] JULIUS SCHACHTER,[6] JEANNE MONCADA,[6] D. JUNGKIND,[7] BUFFY TURNER,[8] AND CYNTHIA PEYTON[8]

Louisiana State University[1] and City of New Orleans' Delgado Clinic,[2] New Orleans, Louisiana; Indiana University School of Medicine, Indianapolis, Indiana[3]; National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland[4]; Johns Hopkins University, Baltimore, Maryland[5]; University of California—San Francisco, San Francisco, California[6]; Thomas Jefferson University Hospital, Pittsburgh, Pennsylvania[7]; and University of Texas Medical Branch, Galveston, Texas[8]

Received 3 March 2000/Returned for modification 20 May 2000/Accepted 15 July 2000

The fully automated COBAS AMPLICOR CT/NG and semiautomated AMPLICOR CT/NG tests were evaluated in a multicenter trial for their ability to detect *Neisseria gonorrhoeae* infections. Test performance compared to that of culturing was evaluated for 2,192 matched endocervical swab and urine specimens obtained from women and for 1,981 matched urethral swab and urine specimens obtained from men. Culture-negative, PCR-positive specimens that tested positive in a confirmatory PCR test for an alternative target sequence within the *N. gonorrhoeae* 16S rRNA gene were considered to be true positives. The overall prevalences of gonorrhea were 6.6% in women and 20.1% in men. The COBAS AMPLICOR and AMPLICOR formats yielded concordant results for 98.8% of the specimens and exhibited virtually identical sensitivities and specificities. The results that follow are for the COBAS AMPLICOR format. With the infected patient as the reference standard, the resolved sensitivities of PCR were 92.4% for endocervical swab specimens and 64.8% for female urine specimens. There were no significant differences in these rates between women with and without symptoms. Among symptomatic men, COBAS AMPLICOR sensitivities were 94.1% for urine and 98.1% for urethral swabs; for asymptomatic men, the results were 42.3 and 73.1%, respectively. In comparison, the sensitivities of culturing were 84.8% for endocervical specimens, 92.7% for symptomatic male urethral specimens, and only 46.2% for urethral specimens obtained from asymptomatic men. When PCR results were analyzed as if only a single test had been performed on a single specimen type, the resolved sensitivity was always higher. The resolved specificities of PCR were 99.5% for endocervical swab specimens, 99.8% for female urine specimens, 98.9% for male urethral swab specimens, and 99.9% for male urine specimens. The internal control revealed that 2.1% of specimens were inhibitory when initially tested. Nevertheless, valid results were obtained for 99.2% of specimens because 60.0% of the inhibitory specimens were not inhibitory when a second aliquot was tested. The COBAS AMPLICOR CT/NG test for *N. gonorrhoeae* exhibited high sensitivity and specificity with urethral swab and urine specimens from men and endocervical swab specimens from women and thus is well suited for diagnosing and screening for *N. gonorrhoeae* infection.

Approximately 62 million new *Neisseria gonorrhoeae* infections occur annually worldwide, making gonorrhea a major public health problem (WHO Office of HIV/AIDS and Sexually Transmitted Diseases, Sexually Transmitted Diseases Fact Sheet, http://www.who.int/asd/knowledge/facsheet.html). Accurate diagnosis is critical for preventing the serious consequences of long-term infection, such as pelvic inflammatory disease, and for controlling the spread of infection. Better tests for screening high-risk groups, such as inner-city adolescent girls, is one approach to controlling this problem (2, 6).

Culturing on selective media, the current "gold standard" for the diagnosis of *N. gonorrhoeae* infection, can achieve a sensitivity of 80 to 95% when performed under optimal conditions (9). Poor specimen storage and transport conditions can, however, cause false-negative results. Another limitation of culturing is that it requires invasively collected endocervical or urethral swab specimens. Recent studies have shown that a nucleic acid amplification-based test can achieve high sensitivity and specificity for the detection of *N. gonorrhoeae* (4, 8, 10, 12, 16). This test can be performed on self-collected urine, vaginal, and vulval specimens (8, 20; A. Stary and B. Hartman, Program Abstr. 12th Meet. Int. Soc. Sex. Transm. Dis. Res., p. 110, 1997). Compared to culturing, a nucleic acid amplification-based test should be less affected by transport conditions, since organism viability is not necessary.

Ideally, high-risk populations such as adolescents should be screened for both *N. gonorrhoeae* and *Chlamydia trachomatis*, since populations with a high prevalence for one infection often exhibit a high prevalence for the other (1, 2). Roche Molecular Systems has developed a multiplex PCR-based test for *C. trachomatis* and *N. gonorrhoeae* that allows simultaneous amplification of both targets. It is available in two formats, the fully automated COBAS AMPLICOR CT/NG and the semiautomated AMPLICOR CT/NG microwell plate format tests.

These tests exhibit high sensitivity for *C. trachomatis* with both invasive urethral and endocervical swabs and noninvasive urine specimens (7, 13, 14, 17, 19). In this paper, we present the

* Corresponding author. Mailing address: Department of Internal Medicine, LSU Medical Center, 1542 Tulane Ave., New Orleans, LA 70118. Phone: (504) 568-5031. Fax: (504) 568-6752. E-mail: dhmartin@lsumc.edu.

results of the first multicenter study evaluating these tests for *N. gonorrhoeae* with endocervical swab specimens, male urethral swab specimens, and matched female and male urine specimens. The results of testing of the same specimens for *C. trachomatis* are presented elsewhere (18).

MATERIALS AND METHODS

Patient population. In Baltimore and San Francisco, specimens were collected from consecutive, consenting individuals visiting sexually transmitted disease clinics or family-planning centers. In Indianapolis, Philadelphia, New Orleans, and Galveston, specimens were collected from consecutive, consenting individuals attending sexually transmitted disease clinics. Exclusion criteria were insufficient volume of any specimen, mishandling or inappropriate storage of any specimen, receiving antibiotic therapy within 3 weeks prior to specimen collection, having urinated within 2 h prior to sample collection, or having undergone a hysterectomy.

Patients were categorized as symptomatic if they presented with one or more of the following: dysuria, urogenital bleeding, pelvic or genital pain, vaginal or urethral discharge, genital lesions or warts, genital itching or rash, or urethritis. Patients not exhibiting any of these symptoms were classified as asymptomatic.

Specimen collection and storage. Two endocervical swab specimens from each woman and two urethral swab specimens from each man were collected by standard procedures. The first swab was used for gonococcal culturing. The second swab was inoculated into 2SP, M-4 (Microtest, Inc., Atlanta, Ga.), or Bartel's chlamydial (Intracel Corp., Issaquah, Wash.) transport medium for chlamydial cell culturing and PCR testing. These specimens were stored at 2 to 8°C and were transported to the laboratory within 24 to 72 h of collection. The specimens were vortexed with the swab still in the tube, cell cultures were inoculated, and an aliquot of each specimen was transferred to a new tube, which was stored at 2 to 8°C for up to 7 days postcollection and then processed for PCR testing. At the same time, four additional aliquots of each swab specimen were stored at −70°C for use in discrepant analysis.

Ten to 50 ml of first-catch urine was also collected from each subject. Female urine specimens were collected either before or after swab collection. Male urine specimens were collected after the urethral swab specimens had been obtained. Urine specimens were stored at room temperature and transported to the laboratory within 24 h or were stored at 2 to 8°C if not transported within 24 h of collection. Upon arrival at the laboratory, a 500-μl aliquot was stored at 2 to 8°C for up to 7 days from the time of collection until it was processed for PCR testing. Four additional 500-μl aliquots were stored at −20°C for use in discrepant analysis.

***N. gonorrhoeae* culturing.** The swab collected for *N. gonorrhoeae* culturing was processed according to each laboratory's standard procedure. Gram-negative diplococci were confirmed as *N. gonorrhoeae* by glucose utilization profiles (Louisiana State University; Thomas Jefferson University Hospital; University of California, San Francisco) or antibody reactivity (Syva Microtrak at Indiana University, Gonocheck at Johns Hopkins University, Gonogen II at University of Texas Medical Branch at Galveston).

PCR testing. Each specimen was processed and tested in both the AMPLICOR and the COBAS AMPLICOR tests as previously described (18). For each processed specimen, the *C. trachomatis*, *N. gonorrhoeae*, and internal control (IC) target DNAs were simultaneously amplified in a single reaction that contained two primer pairs, one specific for *C. trachomatis* and the IC and one specific for *N. gonorrhoeae*. The resulting amplification products were detected separately by hybridization to microwell plates or to magnetic microparticles coated with *N. gonorrhoeae*-, *C. trachomatis*-, and IC-specific oligonucleotide probes.

Interpretation of results. Specimens yielding *N. gonorrhoeae* signals above the positive cutoff (optical density [OD] of 3.5) were interpreted as positive, regardless of the IC result. Specimens yielding *N. gonorrhoeae* signals below the negative cutoff (OD of 0.2) were interpreted as negative, provided that the IC signal was above the assigned cutoff (OD of 0.2). Specimens yielding signals below the negative cutoff for both *N. gonorrhoeae* and the IC were interpreted as inhibitory. Inhibitory specimens were retested by processing another aliquot of the original specimen. The repeat test results were classified using the above criteria. Repeatedly inhibitory specimens were excluded from the sensitivity and specificity calculations.

All specimens with an *N. gonorrhoeae* signal of $0.2 \leq \text{OD} < 3.5$ were interpreted as equivocal, regardless of the IC result. Analyses performed after the study was completed indicated that this large equivocal zone was required to achieve optimal sensitivity and specificity. We were able to classify each equivocal specimen as positive or negative by retesting frozen aliquots in duplicate. Analysis of the retest data demonstrated that optimal performance was obtained by using the following algorithm. Initially equivocal specimens were interpreted as positive if at least two of the three tests (including the initial test) yielded *N. gonorrhoeae* signals of ≥2.0 OD units. Initially equivocal specimens were interpreted as negative if fewer than two tests yielded *N. gonorrhoeae* signals of ≥2.0 OD units, provided that any repeat test that yielded an *N. gonorrhoeae* signal of <0.2 OD unit had an IC signal of ≥0.2. Initially equivocal specimens were interpreted as invalid if one or both repeat tests yielded an *N. gonorrhoeae* signal of <0.2 OD unit and an IC signal of <0.2 OD unit.

Resolution of discrepant results. Specimens that were positive by PCR but negative by culturing were resolved by performing a PCR assay for an alternative target DNA sequence, a portion of the *N. gonorrhoeae* 16S rRNA gene (S. Y. Lu, S. Y. Kao, S. Silver, A. Purohit, M. Longiaru, and T. J. White, Program Abstr. 91st Gen. Meet. Am. Soc. Microbiol. 1991, p. 361, 1991). If the specimen was negative in the 16S rRNA test, the other specimen type was also tested in the 16S rRNA test, to provide evidence that the patient was infected; this test was performed regardless of whether the second specimen type had originally tested positive for *N. gonorrhoeae*.

Calculation of test performance. Sensitivity and specificity for each specimen in each format were calculated two ways. The main analysis was done using the infected patient as the standard of comparison. Culture results and the results of all four PCR tests (swab and urine specimens, each tested by AMPLICOR and COBAS AMPLICOR) were used to define infection status (18). A patient was considered infected if the culture was positive or if at least one of the four PCR tests was positive and either the swab or the urine specimen was positive in the 16S rRNA test. The second analytic approach evaluated results on a sample basis to show the performance that would have been obtained if only one specimen type had been tested in one assay format (18). For each specimen type tested in each format, infection status was defined using culturing and only the PCR result for the specimen type and test format under consideration. A negative PCR result in a culture-negative patient who was PCR positive for a different specimen or format was considered true negative in this analysis. A positive PCR result was considered true positive if the patient was culture positive or either the swab or the urine specimen was positive in the 16S rRNA test.

RESULTS

Patient population. A total of 1,110 asymptomatic women, 1,155 symptomatic women, 721 asymptomatic men, and 1,291 symptomatic men were included in the study. Of these, 37 asymptomatic women, 36 symptomatic women, 7 asymptomatic men, and 24 symptomatic men (total, 104) were missing results for at least one specimen type in at least one test format, either because a test was not performed ($n = 7$) or because a specimen was repeatedly inhibitory ($n = 97$). Thus, 1,073 asymptomatic women, 1,119 symptomatic women, 714 asymptomatic men, and 1,267 symptomatic men had valid results for both specimen types (swab and urine) in both test formats (COBAS AMPLICOR and AMPLICOR), for a total of four valid PCR results.

Initially, we evaluated test performance for all subjects. We then performed the same analyses for the subset of subjects who had four valid PCR results. The two analyses yielded virtually identical results for prevalence, sensitivity, specificity, and concordance between the COBAS AMPLICOR and AMPLICOR formats. In this paper, we present the data for the subset of subjects who had four valid PCR results.

Concordance between test formats. Matched swab and urine specimens obtained from 4,173 patients yielded valid test results in both the COBAS AMPLICOR and the AMPLICOR formats, for a total of 8,346 test results in each test format. The AMPLICOR and COBAS AMPLICOR results were 98.8% concordant. Consistent with this high degree of concordance, the two test formats exhibited virtually identical performance characteristics (data not shown). Thus, for simplicity of presentation, the remainder of this paper will focus on results obtained with the COBAS AMPLICOR format. Nevertheless, results obtained with the AMPLICOR format were used to identify all infected patients for calculating performance on a patient basis (see Materials and Methods).

Frequency of inhibition. Overall, 2.1% of specimens inhibited amplification of the IC in the COBAS AMPLICOR test, with female urine and symptomatic male urine specimens having the highest inhibition rates (Table 1). Inhibition was eliminated for 59.7% (105 of 176) of inhibitory specimens when another aliquot of the specimen was processed and tested within 7 days (Table 1). The frequency of successful retesting was lower for endocervical swab specimens (30.2%) (Table 1).

Thirty-two initially inhibitory specimens yielded positive results when retested; 29 of these were from infected individuals,

TABLE 1. Frequency of inhibition in the COBAS AMPLICOR CT/NG test when assaying for *N. gonorrhoeae*

Group	Specimen	% Inhibitory (no. inhibitory/total no.)	No. with the indicated result after retesting:		
			Positive	Negative	Inhibitory
All women	Endocervical	1.9 (43[a]/2,238)	2[b]	11	29
	Urine	3.4 (77[c]/2,256)	1	52[d]	22
Asymptomatic men	Urethral	0.7 (5/704)	0	2	3
	urine	0.4 (3/719)	1	1	1
Symptomatic men	Urethral	0.2 (3[a]/1,242)	1[b]	0	1
	Urine	3.5 (45[c]/1,287)	27	7	9
All	All	2.1 (176/8,446)	32	73	65

[a] One inhibitory specimen was not retested.
[b] All three represent false-positive results.
[c] Two inhibitory specimens were not retested.
[d] One specimen was from an infected patient and thus represents a false-negative result.

and most of these were from symptomatic men (Table 1). These 29 specimens would have been classified as false negative had the IC not been used.

Results for *N. gonorrhoeae* in women. Initially, we evaluated test performance separately for asymptomatic and symptomatic women. Because these two groups yielded very similar results (data not shown), we present the combined results here. Test performance was calculated for the 2,192 women who had valid results for both specimen types in both test formats.

N. gonorrhoeae infection was detected in 145 women (5.4%), 123 of whom were found positive by culturing (Table 2). For the 22 culture-negative, infected women, at least one specimen type gave at least one PCR-positive result that was confirmed by PCR for an alternative target sequence within the *N. gonorrhoeae* 16S rRNA gene (Table 2). For each specimen type, the fraction of true-positive COBAS AMPLICOR results was higher in culture-positive women than in infected, culture-negative women (Table 2). PCR detected *N. gonorrhoeae* in the swab specimen more frequently than in the urine specimen (Table 2). Among the 2,047 symptomatic uninfected women, at least one specimen type gave a positive result for 15 women; these positive results represent false positives, since none were confirmed by the 16S rRNA PCR (Table 2). The sensitivity and specificity were calculated from these results (Table 3). Test performance was similar in all six laboratories (data not shown).

Results for *N. gonorrhoeae* in men. Test performance was calculated for the 714 asymptomatic and 1,267 symptomatic men who had valid results for both specimen types in both test formats. *N. gonorrhoeae* infection was detected in 26 (3.6%) of the asymptomatic men, only 12 of whom were found positive by culturing, and in 372 (29.4%) of the symptomatic men, 345 of whom were found positive by culturing (Table 2). The remaining infected men were classified as infected by PCR criteria only (Table 2). For each specimen type, the fraction of true-positive COBAS AMPLICOR results was higher in culture-positive men than in infected, culture-negative men (Table 2). PCR detected *N. gonorrhoeae* in the swab specimen more frequently than in the urine specimen (Table 2). Both specimen types gave negative COBAS AMPLICOR results for 681 of the 688 asymptomatic and 883 of the 895 symptomatic uninfected men (Table 2). At least one specimen type gave a false-positive result for the remaining men. The sensitivity and

TABLE 2. Results of *N. gonorrhoeae* testing

Category	COBAS result for the following specimens:		No. of specimens			
	Swab	Urine	All F	Asym M	Sym M	All
Culture positive	+	+	80	8	329	417
	+	−	38	2	14	54
	−	+	2	1	2	5
	−	−	3	1	0	4
16S rRNA positive, culture negative	+	+	9	1	16	26
	+	−	7	8	6	21
	−	+	3	1	3	7
	−[b]	−[b]	3	4	2	9
Culture negative, 16S rRNA negative	+	+	1	1	0	2
	+	−	10	6	11	27
	−	+	4	0	1	5
	−[c]	−[c]	12	7	21	40
Culture negative	−	−	2,020	674	862	3,556
Total			2,192	714	1,267	4,173

[a] F, female; M, male; Asym, asymptomatic; Sym, symptomatic.
[b] Specimens were interpreted as COBAS AMPLICOR false negative because they had yielded positive results in the AMPLICOR that were confirmed by a 16S rRNA test.
[c] Specimens were interpreted as COBAS AMPLICOR true negatives. They were subjected to 16S rRNA testing because they had yielded positive results in the AMPLICOR test format. The AMPLICOR results were interpreted as false positives.

TABLE 3. Sensitivity and specificity for the detection of *N. gonorrhoeae* by COBAS AMPLICOR calculated on a patient basis

Group (no. of specimens)	Specimen	%		
		Prevalence	Sensitivity	Specificity
All women (2,192)	Endocervical	6.6	92.4	99.5
	Urine		64.8	99.8
Asymptomatic men (714)	Urethral	3.6	73.1	99.0
	Urine		42.3	99.9
Symptomatic men (1,267)	Urethral	29.4	98.1	98.8
	Urine		94.1	99.9

TABLE 4. Sensitivity and specificity for the detection of *N. gonorrhoeae* by COBAS AMPLICOR calculated on a sample basis

Group (no. of specimens)	Specimen	%		
		Prevalence	Sensitivity	Specificity
All women (2,192)	Endocervical	6.3	96.4	99.5
	Urine	6.2	69.6	99.8
Asymptomatic men (714)	Urethral	2.9	90.5	99.0
	Urine	2.0	78.6	99.9
Symptomatic men (1,267)	Urethral	29.0	99.5	98.8
	Urine	28.7	96.2	99.9

specificity were calculated from these results (Table 3). For both specimen types, COBAS AMPLICOR exhibited a higher sensitivity for symptomatic men than for asymptomatic men.

Effect of IC on test sensitivity. Using the IC substantially enhanced test sensitivity for *N. gonorrhoeae* in urine specimens from symptomatic men. Thirty-six of the 45 initially inhibitory urine specimens were obtained from symptomatic infected men. Twenty-seven of the 36 initially inhibitory, infected specimens, 26 from culture-positive men and 1 from a culture-negative man, were found to be noninhibitory and positive when retested and interpreted as true positive; these would have been interpreted as false negative ($n = 26$) or true negative ($n = 1$) had the IC not been used. The other nine initially inhibitory specimens from infected men were found to be inhibitory when retested ($n = 7$) or were not retested ($n = 2$) and thus were excluded from performance calculations; they would have been interpreted as false negative had the IC not been used. Given these observations, sensitivity for urine from symptomatic men would have been reduced from 94.1% (350 of 372) to 85.0% (323 of 380) had the IC not been used.

Using the IC had little effect on test sensitivity with other specimen types, where only 5 of 131 initially inhibitory specimens were obtained from infected individuals. Two of these five inhibitory specimens were found positive when retested. If the IC had not been used, the sensitivities for endocervical swab, female urine, and asymptomatic male urine specimens would have been only 0.6, 1.1, and 3.8 percentage points lower, respectively; the sensitivity for urethral swab specimens from both symptomatic and asymptomatic men was unchanged.

Per-specimen performance calculations. Certain infected, culture-negative patients yielded negative COBAS AMPLICOR results for one specimen type. Had this specimen been the only one tested, the infection would have gone undetected and the result would have been classified as true negative instead of false negative. Consequently, the resulting sensitivity would have been higher had only one specimen type been tested only in the COBAS AMPLICOR format (Table 4). Especially noteworthy is the increase in sensitivity for asymptomatic men and the corresponding lower apparent prevalence (Tables 3 and 4).

Prevalence of infection in different populations. The overall prevalence of infection varied between sites (Table 5). The prevalence of infection was lower in asymptomatic women than in symptomatic women at three sites but higher at the other three sites. At all six sites, the prevalence was lower in asymptomatic men than in symptomatic men. The combination of five tests—culturing, COBAS AMPLICOR and AMPLICOR performed with swab specimens, and COBAS AMPLICOR and AMPLICOR performed with urine specimens—yielded overall prevalences that were substantially higher than the prevalences determined by culturing (Table 5).

DISCUSSION

The results of this study demonstrate that the COBAS AMPLICOR and AMPLICOR CT/NG tests exhibited excellent sensitivities (92.4 to 98.1%) and specificities (98.8 to 99.9%) for *N. gonorrhoeae* in endocervical swab specimens from women and urethral swab and urine specimens from symptomatic men. For each of these specimen types, COBAS AMPLICOR was more sensitive than culturing (84.8% in women and

TABLE 5. Sensitivity of culturing and prevalence of infection at each test site

Group	Site[a]	Asymptomatic				Symptomatic			
		No. of specimens	%			No. of specimens	%		
			Culture sensitivity	Prevalence			Culture sensitivity	Prevalence	
				Culturing	Overall			Culturing	Overall
Women	JHU	67	75.0	4.5	6.0	205	74.3	12.7	17.1
	LSU	135	100.0	0.7	0.7	62	66.7	3.2	4.8
	UTMB	24	50.0	8.3	16.7	112	85.7	5.4	6.3
	IU	205	90.0	17.6	19.5	270	91.2	11.5	12.6
	UCSF	227	100.0	0.9	0.9	236	100.0	2.1	2.1
	TJUH	415	85.7	1.4	1.7	234	100.0	1.3	1.3
	All	1,073	86.2	4.7	5.4	1,119	83.9	6.5	7.8
Men	JHU	140	7.7[b]	0.7	9.3	439	85.7	28.7	33.5
	LSU	164	60.0	1.8	3.0	31	85.7	38.7	45.2
	UTMB	60	100.0	5.0	5.0	100	100.0	33.0	33.0
	IU	290	100.0	1.7	1.7	517	98.1	30.4	30.9
	UCSF	60	NA[c]	<1.6	<1.6	180	94.4	9.4	10.0
	All	714	46.2	1.7	3.6	1,267	92.7	27.2	29.4

[a] JHU, Johns Hopkins University; LSU, Louisiana State University; UTMB, University of Texas Medical Branch at Galveston; IU, Indiana University; UCSF, University of California, San Francisco; TJUH, Thomas Jefferson University Hospital.

[b] COBAS AMPLICOR exhibited low sensitivity for asymptomatic men at JHU: 69.2% (9 of 13) and 15.4% (2 of 13) for urethral swab and urine specimens, respectively. Excluding JHU data, the overall sensitivities were 84.6% (11 of 13) for culturing and 76.9% (10 of 13) and 69.2% (9 of 13) for COBAS AMPLICOR with urethral swab and urine specimens, respectively.

[c] NA, none of the 60 individuals was infected with *N. gonorrhoeae*.

92.7% in symptomatic men). In contrast, COBAS AMPLICOR performed with female urine specimens was less sensitive than endocervical specimen culturing.

For both swab and urine specimens from asymptomatic men, COBAS AMPLICOR sensitivity was dramatically lower than that obtained for symptomatic men. Similarly, culturing was less sensitive for asymptomatic men. The sensitivity of all methods was influenced by exceptionally poor performance at one site, which contributed one-half of the asymptomatic male infections (see footnote *b* of Table 5). However, when this site was excluded, all methods still were less sensitive for asymptomatic men than for symptomatic men (see footnote *b* of Table 5). While COBAS AMPLICOR performed with asymptomatic male urine specimens was somewhat less sensitive than culturing, noninvasive urine testing would probably detect more infections than culturing, as few asymptomatic men would consent to collection of a urethral swab specimen.

These data raise the question of whether any test is appropriate for testing of asymptomatic men. The low sensitivities of PCR and culturing probably reflect the low organism burden in infected men who remain symptom free. When there are relatively few organisms, it is more likely that an infected individual will test negative by culturing and will have positive PCR results for only one specimen type. Thus, only a subset of infected individuals is detected as infected by any one test alone.

Sensitivity for urine specimens was considerably lower than sensitivity for the corresponding swab specimens in women and asymptomatic infected men. This was probably due to partial loss during urine specimen processing of the already limited amount of target DNA. *N. gonorrhoeae* cells are relatively fragile and thus are prone to lysis during transport, storage, and incubation with urine wash buffer, releasing target DNA that would not be recovered during centrifugation. In contrast, because swab specimens are not centrifuged, target DNA from both intact and disrupted cells is retained by the processing procedure. The high sensitivity obtained with urine from symptomatic men probably reflects the high organism burden, which minimizes the impact of the loss of a portion of the target DNA.

Although test sensitivity and prevalence of infection differed substantially in symptomatic and asymptomatic men, these parameters were very similar in asymptomatic and symptomatic women. This lack of a difference probably reflects the fact that symptoms in women are not specific for *N. gonorrhoeae* infection.

Based on a review of the literature, the COBAS AMPLICOR test performed comparably to the Abbott LCx *N. gonorrhoeae* test for male specimens and female endocervical specimens. The published LCx studies calculated performance as if only one specimen type from each patient had been tested by the LCx. Compared to COBAS AMPLICOR performance calculated on a per-specimen basis, the LCx exhibited very similar sensitivities for female endocervical swabs (3, 4, 10), male urethral swabs (3, 4), and male urine (3). In contrast, LCx sensitivity was approximately 20% higher for female urine (16, 20). Both tests exhibited greater than 99% specificity.

A broad equivocal zone, from 0.2 to 3.5 A_{660} units, was required to achieve the high specificity reported here because the COBAS AMPLICOR CT/NG test for *N. gonorrhoeae* cross-reacts with certain strains of the nonpathogenic *Neisseria* species *N. subflava* and *N. cinerea* (5; M. Rosenstraus, Roche Molecular Systems, personal communication). These nonpathogenic species are part of the normal flora in the throats of most humans and are rarely present in urogenital specimens (11). Specimens containing cross-reactive *Neisseria* strains will give signals above the negative cutoff of 0.2 A_{660} unit. In this study, approximately 80% of such specimens gave signals below 3.5 A_{660} units because the target was present at a very low concentration and because it hybridized inefficiently to the capture probe due to minor DNA sequence differences in the probe region (B. Van Der Pol et al., unpublished data). Most of these false-positive specimens retested negative or gave two out of three results below the final cutoff of 2.0 A_{660} units. Consequently, retesting of specimens in the large equivocal zone reduced the frequency of false positives from 1 to 2% to 0.2 to 0.4%. In contrast, only 20% of *N. gonorrhoeae*-infected specimens gave signals below 3.5 A_{660} units. Most of the few true positives that did have to be retested gave two out of three results above the final cutoff of 2.0 A_{660} units. Thus, the use of an expanded equivocal zone substantially increased test specificity without impacting sensitivity (Van Der Pol et al., unpublished).

The COBAS AMPLICOR and AMPLICOR tests include an IC to ensure the integrity of negative results and to maximize test sensitivity by monitoring amplification in specimens yielding negative test results. The conversion of specimens from inhibitory to noninhibitory upon retesting could indicate that inhibitors were labile, nonuniformly distributed, or present at low concentrations (15). Regardless of the mechanism, the absence of inhibition during retesting enables a laboratory to generate additional valid test results without having to collect a second specimen. Retesting of endocervical swab specimens may not be productive, as the proportion of valid results was only 30% and false positives were observed. Using the IC substantially increased test sensitivity for asymptomatic male urine and symptomatic male urine specimens. The IC enabled us to determine that the frequency of inhibition in this study ranged from <1% for male urethral specimens to 3.5% for symptomatic male urine specimens.

In summary, both the COBAS AMPLICOR and the AMPLICOR CT/NG tests exhibited high sensitivities and specificities for *N. gonorrhoeae* with urogenital swab specimens from both women and men and symptomatic male urine specimens. Performance with urine specimens collected from women and asymptomatic men was not as good. Elsewhere, we demonstrate that these tests can be used to simultaneously screen for *C. trachomatis* infections (18). Thus, the COBAS AMPLICOR and AMPLICOR tests make it possible to screen for both pathogens in symptomatic male urine, symptomatic male urethral swab, or female endocervical swab specimens by processing and amplifying a specimen once.

ACKNOWLEDGMENTS

We thank Maurice Rosenstraus for manuscript review, James F. Kelly for database management, James Williams and Laura Brandenburg for laboratory assistance, and the many clinicians at each site for assistance with enrolling patients.

This work was supported by Roche Molecular Systems.

REFERENCES

1. **Batteiger, B. E., J. Fraiz, W. J. Newhall, B. P. Katz, and R. B. Jones.** 1989. Association of recurrent chlamydial infection with gonorrhea. J. Infect. Dis. **159:**661–669.
2. **Burstein, G. R., G. Waterfield, A. Joffe, J. M. Zenilman, T. C. Quinn, and C. A. Gaydos.** 1998. Screening for gonorrhea and chlamydia by DNA amplification in adolescents attending middle school health centers. Sex. Transm. Dis. **25:**395–402.
3. **Carroll, K. C., W. E. Aldeen, M. Morrison, R. Anderson, D. Lee, and S. Mottice.** 1998. Evaluation of the Abbott LCx ligase chain reaction assay for detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in urine and genital swab specimens from a sexually transmitted disease population. J. Clin. Microbiol. **36:**1630–1633.
4. **Ching, S., 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.
5. **Farrell, D.** 1999. Evaluation of AMPLICOR *Neisseria gonorrhoeae* PCR using *cppB* nested PCR and 16S rRNA PCR. J. Clin. Microbiol. **37:**386–390.
6. **Fox, K. K., W. L. Whittington, W. C. Levine, J. S. Moran, A. A. Zaidi, and A. K. Nakashima.** 1998. Gonorrhea in the United States, 1981–1996: demographic and geographic trends. Sex. Transm. Dis. **25:**386–393.
7. **Goessens, W. H. F., J. W. Mouton, W. I. van der Meijden, S. Deelen, T. H. van Rijsoort-Vos, N. Lemmens-den Toom, H. A. Verbrugh, and R. Verkooyen.** 1997. Comparison of three commercially available amplification assays, AMP CT, LCx, and COBAS AMPLICOR, for detection of *Chlamydia trachomatis* in first-void urine. J. Clin. Microbiol. **35:**2628–2633.
8. **Hook, E. W., III, S. F. Ching, J. Stephens, K. F. Hardy, K. R. Smith, and H. H. Lee.** 1997. Diagnosis of *Neisseria gonorrhoeae* infections in women by using the ligase chain reaction on patient-obtained vaginal swabs. J. Clin. Microbiol. **35:**2129–2132.
9. **Hook, E. W., III, and H. H. Handsfield.** 1990. Gonococcal infections in the adult, p. 149–165. *In* K. Holmes, et al. (ed.), Sexually transmitted diseases. McGraw-Hill Book Co., New York, N.Y.
10. **Kehl, S. C., K. Georgakas, G. R. Swain, G. Sedmak, S. Gradus, A. Singh, and S. Foldy.** 1998. Evaluation of the Abbott LCx assay for detection of *Neisseria gonorrhoeae* in endocervical swab specimens from females. J. Clin. Microbiol. **36:**3549–3551.
11. **Knapp, J., and R. J. Rice.** 1995. *Neisseria* and *Branhamella*, p. 324–340. *In* P. R. Murray, et al. (ed.), Manual of clinical microbiology, 6th ed. ASM Press, Washington, D.C.
12. **Palladino, S., J. W. Pearman, I. D. Kay, D. W. Smith, G. B. Harnett, M. Woods, L. Marshall, and J. McCloskey.** 1999. Diagnosis of Chlamydia trachomatis and Neisseria gonorrhoeae genitourinary infections in males by the Amplicor PCR assay of urine. Diagn. Microbiol. Infect. Dis. **33:**141–146.
13. **Pasternack, R., P. Vourinen, T. Pitkajarvi, M. Koskela, and A. Miettinen.** 1997. Comparison of manual AMPLICOR PCR, COBAS AMPLICOR PCR, and LCx assays for detection of *Chlamydia trachomatis* infection in women by using urine specimens. J. Clin. Microbiol. **35:**402–405.
14. **Puolakkainen, M., E. Hiltunen-Back, T. Reunala, S. Suhonen, P. Lähteenmäki, M. Lehtinen, and J. Paavonen.** 1997. Comparison of performance of two commercially available tests, a PCR assay and a ligase chain reaction test, in detection of urogenital *Chlamydia trachomatis* infection. J. Clin. Microbiol. **35:**2628–2633.
15. **Rosenstraus, M., Z. Wang, S. Y. Chang, D. DeBonville, and J. P. Spadoro.** 1998. An internal control for routine diagnostic PCR: design, properties, and effect on clinical performance. J. Clin. Microbiol. **36:**191–197.
16. **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.
17. **Steingrímsson, O., K. Jonsdottir, J. H. Olafsson, S. M. Karlsson, R. Palsdottir, and S. Davidsson.** 1998. Comparison of Roche COBAS AMPLICOR and Abbott LCx for the rapid detection of *Chlamydia trachomatis* in specimens from high risk patients. Sex. Transm. Dis. **25:**44–48.
18. **Van Der Pol, B., T. C. Quinn, C. A. Gaydos, K. Crotchfelt, J. Schachter, J. Moncada, D. Jungkind, D. H. Martin, B. Turner, C. Peyton, and R. B. Jones.** 2000. Multicenter evaluation of the AMPLICOR and automated COBAS AMPLICOR CT/NG tests for detection of *Chlamydia trachomatis*. J. Clin. Microbiol. **38:**1105–1112.
19. **Vincelette, J., J. Schirm, M. Bogard, A. M. Bourgault, D. S. Luijt, A. Bianchi, P. C. van Voorst, A. Butcher, and M. Rosenstraus.** 1999. Multi-center evaluation of the fully automated COBAS AMPLICOR PCR test for the detection of *Chlamydia trachomatis* in urogenital specimens. J. Clin. Microbiol. **37:**74–80.
20. **Xu, K., V. Glanton, S. R. Johnson, C. Beck-Sague, V. Bhullar, D. H. Candal, K. S. Pettus, C. E. Farshy, and C. M. Black.** 1998. Detection of *Neisseria gonorrhoeae* infection by ligase chain reaction testing of urine among adolescent women with and without *Chlamydia trachomatis* infection. Sex. Transm. Dis. **25:**533–538.