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Multicenter Evaluation of the AMPLICOR and Automated COBAS AMPLICOR CT/NG Tests for Detection of *Chlamydia trachomatis*

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The fully automated COBAS AMPLICOR CT/NG and semiautomated AMPLICOR CT/NG tests were evaluated in a multicenter trial for the ability to detect *Chlamydia trachomatis* infections. Test performance compared to that of culture was evaluated for 2,236 matched endocervical swab and urine specimens obtained from women and for 1,940 matched urethral swab and urine specimens obtained from men. Culture-negative, PCR-positive specimens that tested positive in a direct fluorescent-antibody test or in a confirmatory PCR test for an alternative target sequence were resolved as true positives. The overall prevalences of chlamydia were 2.4% in women and 7.2% in men. The COBAS AMPLICOR and AMPLICOR formats yielded concordant results for 98.1% of the specimens. With the infected patient as the reference standard, the resolved sensitivities of COBAS AMPLICOR were 89.7% for endocervical swab specimens, 89.2% for female urine specimens, 88.6% for male urethral swab specimens, and 90.3% for male urine specimens. When 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 99.4% for endocervical swab specimens, 99.0% for female urine specimens, 98.7% for male urethral swab specimens, and 98.4% for male urine specimens. The internal control revealed that 2.4% of the specimens were inhibitory when initially tested. Nevertheless, valid results were obtained for 98.6% of the specimens because 59.1% of the inhibitory specimens were not inhibitory when a second aliquot was tested. The COBAS AMPLICOR and AMPLICOR CT/NG tests for *C. trachomatis* exhibited equally high sensitivity and specificity with both urogenital swab and urine specimens and thus are well suited for screening for *C. trachomatis* infection.

According to the World Health Organization, approximately 89 million new *Chlamydia trachomatis* infections occur annually worldwide (http://www.who.ch/programmes/asd/facsheet.htm). Chlamydia screening programs decrease the prevalence of chlamydia infection (6, 24) and reduce the incidence of pelvic inflammatory disease (30). Economic modeling studies suggest that the savings resulting from the prevention of long-term sequelae more than compensate for the cost of screening for and treating infections (18).

Diagnosis remains a challenge because *C. trachomatis* infections are often asymptomatic, especially in women. Recent studies have shown that nucleic acid amplification-based tests are ideally suited for screening. Unlike conventional tests, they exhibit high sensitivity and specificity for the detection of *C. trachomatis* in noninvasively collected specimens (3, 11, 14, 17, 28, 31, 35). Testing of such samples should encourage asymptomatic individuals in at-risk populations to undergo screening and should reduce the costs associated with specimen collection.

Ideally, high-risk populations such as adolescents should be screened for both *C. trachomatis* and *Neisseria gonorrhoeae*.

Studies indicate that up to 52% of women and 22% of men with gonorrhea are coinfected with *C. trachomatis* (2, 5). With current commercial amplification assays, specimens must be processed and amplified separately to test for both pathogens. A test method that can detect both pathogens by performance of a single amplification on a processed specimen should reduce the cost of screening.

Roche Molecular Systems has developed a multiplex PCR-based test for *C. trachomatis* and *N. gonorrhoeae* that is available in two formats. The fully automated COBAS AMPLICOR CT/NG test is performed on the COBAS AMPLICOR analyzer, an integrated unit that automatically amplifies RNA and DNA targets and detects the resulting amplicon (20). In the semiautomated AMPLICOR CT/NG test, amplified products are detected in an enzyme-linked immunosorbent assay-like format on microwell plates (22). Both CT/NG tests use a master mix containing two pairs of primer oligonucleotides, one specific for *C. trachomatis* and a second specific for *N. gonorrhoeae*, to simultaneously amplify both organisms in a single processed specimen (11). The master mix also contains an internal control (IC) DNA that monitors amplification in each clinical specimen. The IC contains primer binding regions identical to those of the *C. trachomatis* target sequence, a randomized internal sequence similar in length and base composition to the *C. trachomatis* target sequence, and a unique

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probe binding region that differentiates the IC from amplified *C. trachomatis* target nucleic acid (29). The *C. trachomatis*, *N. gonorrhoeae*, and IC amplification products are detected in separate reaction mixtures using separate, target- and IC-specific, oligonucleotide capture probes.

In this paper, we present results of a multicenter study evaluating the performance of the fully automated COBAS AMPLICOR CT/NG test and the AMPLICOR CT/NG (microwell plate format) test for *C. trachomatis* on endocervical swab specimens, male urethral swab specimens, and matched female and male urine specimens. The results of testing of the same specimens for *N. gonorrhoeae* will be presented elsewhere.

MATERIALS AND METHODS

Patient population. In Indianapolis, Philadelphia, New Orleans, and Galveston, specimens were collected from consecutive, consenting individuals attending sexually transmitted disease clinics. In Baltimore and San Francisco, specimens were collected from consecutive, consenting individuals visiting sexually transmitted disease clinics or family planning centers. Exclusion criteria were insufficient volume of any specimen, mishandling or inappropriate storage of any specimen, antibiotic therapy within 3 weeks prior to specimen collection, urination within 2 h prior to sample collection, and 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, urethritis, or positive Gram stain for gram-negative diplococci. Patients not exhibiting any of these signs or 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 culture. The second swab was inoculated into SPG, 2SP, or M-4 (Microtest, Inc., Snellville, Ga.) chlamydial transport medium for chlamydial cell culture and PCR testing. These specimens were stored at 2 to 8°C and were transported to the laboratory within 24 h of collection. An aliquot of each swab specimen was stored at 2 to 8°C for up to 7 days postcollection and processed for PCR testing. 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 prior to or after swab collection. Male urine specimens were collected after the urethral swab specimens. Urine specimens were held at room temperature for up to 24 h or at 2 to 8°C for longer periods and transported to the laboratory within 72 h postcollection. 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.

Chlamydia cell culture processing and interpretation. An aliquot of each swab specimen was transferred for chlamydia culture onto cycloheximide-treated Mc-Coy cells (Indiana University [IU], Johns Hopkins University [JHU], Louisiana State University [LSU], University of California-San Francisco [UCSF], and Thomas Jefferson University Hospital [TJUH]) or BGMK cells (University of Texas Medical Branch at Galveston [UTMB]) in accordance with each laboratory's standard procedure. The aliquot for culture was stored at −70°C if cultures were not initiated within 24 h of specimen collection. Chlamydial inclusions were detected by immunofluorescence using monoclonal antibodies specific for the major outer membrane protein (MicroTrak; Syva Co., San Jose, Calif.) or lipopolysaccharide (Kalestad, Chaska, Minn.).

PCR testing. Each specimen was processed and subjected to both the AMPLICOR and COBAS AMPLICOR tests exactly as described in the manufacturer's package inserts. For each processed specimen, the *C. trachomatis*, *N. gonorrhoeae*, and IC target DNAs were simultaneously amplified in a single reaction mixture that contained two primer pairs, one pair specific for *C. trachomatis* and one pair specific for *N. gonorrhoeae*. The resulting amplification products were captured separately and detected colorimetrically by hybridization to microwell plates (AMPLICOR format) (11) or to magnetic microparticles (COBAS AMPLICOR format) coated with *N. gonorrhoeae*-, *C. trachomatis*-, and IC-specific oligonucleotide probes. The COBAS AMPLICOR analyzer automatically performed all of the amplification, hybridization, and detection steps (12, 20). In the AMPLICOR format, amplification was performed with a GeneAmp PCR System 9600 thermal cycler (Perkin-Elmer, Norwalk, Conn.) and hybridization and detection were performed manually (22).

Interpretation of results. Specimens yielding *C. trachomatis* signals above the positive cutoff (optical density [OD] of 0.8) were interpreted as positive, regardless of the IC result. Specimens yielding *C. trachomatis* 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 cutoff values for both *C. trachomatis* and IC were interpreted as inhibitory. Inhibitory specimens were retested by processing of a frozen aliquot of the

original specimen. The repeat test results were classified using the above criteria. Repeatedly inhibitory specimens were excluded form the sensitivity and specificity calculations.

Specimens yielding *C. trachomatis* results between the negative and positive cutoffs ($0.2 \le OD < 0.8$) were considered equivocal, regardless of the IC signal. Equivocal results were resolved by retesting of the processed specimen in duplicate. These specimens were interpreted as positive if at least one of the two repeat tests yielded a *C. trachomatis* OD of ≥0.2. These specimens were interpreted as negative if the two repeat tests yielded *C. trachomatis* signals of <0.2 OD, provided that the IC signals were above the assigned cutoff. If the two repeat tests yielded a *C. trachomatis* OD of <0.2 and the IC signal was below the assigned cutoff for either of the duplicate repeat tests, the specimen was interpreted as inhibitory and was excluded from sensitivity and specificity calculations.

Resolution of discordant results. Specimens that were positive by PCR but negative by culture were resolved by direct fluorescent-antibody (DFA) (Micro-Trak) testing on the transport medium sediment obtained following centrifugation. If the DFA test was negative, the specimen was tested with a PCR assay for an alternative target DNA sequence, a portion of the major outer membrane protein (MOMP) gene (13). If the specimen was negative in the MOMP test, the other specimen type was also tested for MOMP to provide evidence that the patient was infected; this test was performed regardless of whether the second specimen type had originally tested positive for *C. trachomatis*.

Calculation of test performance. Sensitivity and specificity were calculated in two ways. Calculations on a patient basis used the culture results, the results of all four PCR tests (swab and urine specimens, each tested by AMPLICOR and COBAS AMPLICOR), and results of DFA and MOMP testing, when necessary, to ascertain infected patients. A patient was considered infected if the culture was positive or if any one of the four PCR tests was positive and either the swab was positive by DFA testing or the swab or urine specimen was positive by the MOMP test. The second calculation 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. For each specimen type tested in each format, infected patients were ascertained using culture and only the PCR result for the specimen type and test format under consideration; patients who were PCR positive only for a different specimen or format were considered uninfected in this analysis. A patient was considered infected if (i) the culture was positive or (ii) the PCR test under consideration was positive and either the swab specimen was positive by DFA or any specimen was positive in the MOMP test.

RESULTS

Patient population. Totals of 1,134 asymptomatic women, 1,173 symptomatic women, 723 asymptomatic men, and 1,298 symptomatic men were enrolled in the study. Of these, seven asymptomatic women, four symptomatic women, and two symptomatic men were excluded because culture results were not available. For another 29 asymptomatic women, 31 symptomatic women, 13 asymptomatic men, and 66 symptomatic men (total = 139), results were missing for at least one specimen type in at least one test format, either because a test was not performed ($n = 31$) or because a specimen was repeatedly inhibitory ($n = 108$) in either the COBAS AMPLICOR or the AMPLICOR test. Thus, there were valid results for 1,098 asymptomatic women, 1,138 symptomatic women, 710 asymptomatic men, and 1,230 symptomatic men for both specimen types (swab and urine) and both test formats (COBAS AMPLICOR and AMPLICOR), for a total of four valid PCR results.

Initially, we evaluated test performance for all subjects whose culture results were valid. We then performed the same analyses for the subset of subjects for whom four PCR results were valid. 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 for whom four PCR results were valid.

Concordance between test formats. Matched swab and urine specimens obtained from 4,176 patients yielded valid test results in both the COBAS AMPLICOR and AMPLICOR formats, for a total of 8,352 test results in each test format. The AMPLICOR and COBAS AMPLICOR results were 98.1% concordant (98.4 and 97.8% for swab and urine specimens, respectively; Table 1). The number of COBAS AMPLICOR-positive, AMPLICOR-negative specimens was virtually identical to the

TABLE 1. Concordance between the COBAS AMPLICOR and AMPLICOR test formats

Specimen(s) and COBAS result	No. of AMPLICOR results	
	Positive	Negative
Swab		
Positive	493	33
Negative	32	3,618
Urine		
Positive	496	46
Negative	46	3,588
All		
Positive	989	79
Negative	78	7,206

number of COBAS AMPLICOR-negative, AMPLICOR-positive specimens. 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 test format. Nevertheless, results obtained with the AMPLICOR microwell plate format were used to identify patients.

Frequency of inhibition. Overall, 203 (2.4%) of 8,618 specimens inhibited amplification of the IC in the COBAS AMPLICOR test (Table 2). Endocervical swab, asymptomatic male urethral swab, and asymptomatic male urine specimens exhibited the lowest inhibition rates (≤1%; Table 2). Symptomatic male urethral swabs had a slightly higher inhibition rate (2%), and female urine and symptomatic male urine specimens had the highest inhibition rates (3 to 5%).

Overall, 59.1% (120 of 203) of inhibitory specimens were not inhibitory when another aliquot of the specimen was processed and tested in the COBAS AMPLICOR format (Table 1). The conversion of specimens from inhibitory to noninhibitory could indicate that inhibitors were labile, nonuniformly distributed, or present at a low concentration (29). Regardless of the mechanism, the absence of inhibition during retesting enables the laboratory to generate valid test results without having to collect a second specimen.

Eleven initially inhibitory specimens yielded positive results when retested; seven of these were from infected individuals

(Table 2). These seven male urine specimens would have been classified as false negative had the IC not been used.

Results for *C. trachomatis* in women. Test performance was calculated from the 1,098 asymptomatic and 1,138 symptomatic women whose results were valid for both specimen types in both test formats. *C. trachomatis* infection was detected in 95 (8.7%) of the asymptomatic women, 69 of whom were positive by culture, and 108 (9.5%) of the symptomatic women, 86 of whom were positive by culture (Table 3). For the 26 asymptomatic and 22 symptomatic, culture-negative, infected women, at least one specimen type gave a positive result in at least one PCR test format; the PCR results were confirmed by DFA testing or by PCR testing for an alternative target sequence within the *C. trachomatis* MOMP gene (Table 3). *C. trachomatis* was detected only in the swab specimen (i.e., the urine specimen was negative in both PCR tests) for 11 of the 95 asymptomatic and 5 of the 108 symptomatic, infected women. *C. trachomatis* was detected only in the urine specimen (i.e., the swab specimen was negative by culture and both PCR tests) for a similar number of women: nine asymptomatic and four symptomatic, infected women.

Both specimen types gave negative COBAS AMPLICOR results for 994 of the 1,003 asymptomatic and 1,007 of the 1,030 symptomatic, uninfected women (Table 3). At least one specimen type gave a positive COBAS AMPLICOR result for the remaining 9 asymptomatic and 23 symptomatic, uninfected women; these positive results represent false positives, since none were confirmed by DFA or MOMP PCR testing (Table 3).

(i) Endocervical swabs. COBAS AMPLICOR performed on endocervical swab specimens yielded positive results for 97.1% (67 of 69) of asymptomatic and 95.3% (82 of 86) of symptomatic, culture-positive women (Table 3). COBAS AMPLICOR performed on endocervical swab specimens also yielded positive results for 61.5% (16 of 26) of asymptomatic and 77.3% (17 of 22) of symptomatic, infected, culture-negative women (Table 3).

The sensitivity, specificity, and positive and negative predictive values were calculated from these results (Table 4). COBAS AMPLICOR performance on endocervical swab specimens was slightly more sensitive for symptomatic women (91.7%) than for asymptomatic women (87.4%). COBAS AMPLICOR performed on endocervical swab specimens exhibited virtually identical specificities (99.5 versus 99.2%) for both asymptomatic and symptomatic women. The PCR test performed simi-

TABLE 2. Frequency of inhibition in the COBAS AMPLICOR CT/NG test when assaying for *C. trachomatis*

Sex	Symptom category	Specimen(s)	% Inhibitory (no. inhibitory/total)	No. of retest results		
				Positive	Negative	Inhibitory
Female	Asymptomatic	Endocervical	1.0 (11[a]/1,126)	1[b]	7	2
		Urine	3.7 (42/1,124)	0	28	14
	Symptomatic	Endocervical	0.9 (10/1,169)	0	3	6
		Urine	3.3 (38/1,166)	0	26	12
Male	Asymptomatic	Urethral	0.8 (6/723)	0	2	4
		Urine	0.3 (2/723)	0	1	1
	Symptomatic	Urethral	1.9 (24[a]/1,296)	2[b]	7	14
		Urine	5.4 (70/1,291)	8[c]	35[d]	27
		All	2.4 (203/8,618)	11	109	80

[a] One inhibitory specimen that gave a result in the grey zone when retested was not included in the retest results.
[b] All were false-positive results.
[c] Seven were true-positive results, and one was a false-positive result.
[d] Thirty-four were true-negative results, and one was a false-negative result.

TABLE 3. Results of *C. trachomatis* testing

Category	COBAS result		No. of specimens[b]				
	Swab	Urine	Asym F	Sym F	Asym M	Sym M	All
Culture positive	+	+	62	77	66	150	355
	+	−	5	5	6	13	29
	−	+	1	2	0	0	3
	−	−	1	2	1	6	10
DFA or MOMP positive, culture negative	+	+	11	14	10	59	94
	+	−	5	3	0	6	14
	−	+	9	5	6	25	45
	−[a]	−[a]	1	0	0	2	3
Culture negative, DFA negative, MOMP negative	+	+	0	1	2	2	5
	+	−	5	7	6	11	29
	−	+	4	15	8	13	40
Culture negative	−	−	994	1,007	605	943	3,549
Total			1,098	1,138	710	1,230	4,176

[a] Specimens were interpreted as COBAS AMPLICOR false negatives because they had yielded positive results in the AMPLICOR CT/NG test that were confirmed by DFA or MOMP testing.
[b] Asym, asymptomatic; Sym, symptomatic; F, female; M, male.

larly in all six laboratories (data not shown), while there was considerable variation in culture sensitivity (see Table 6) among laboratories.

None of the 21 initially inhibitory endocervical swab specimens (11 asymptomatic and 10 symptomatic; Table 2) were obtained from infected women. Thus, the sensitivity with endocervical swab specimens was not affected by use of the IC.

(ii) Female urine specimens. COBAS AMPLICOR performed on urine specimens yielded positive results for 91.3% (63 of 69) of asymptomatic and 91.9% (79 of 86) of symptomatic, culture-positive women (Table 3). PCR performed on urine specimens also yielded positive results for 76.9% (20 of 26) of asymptomatic and 86.4% (19 of 22) of symptomatic, infected, culture-negative women (Table 3).

The sensitivity, specificity, and positive and negative predictive values were calculated from these results (Table 4). COBAS AMPLICOR performed on urine specimens was slightly more sensitive for symptomatic women (90.7%) than for asymptomatic women (87.4%). COBAS AMPLICOR performed on urine specimens exhibited similar specificities (99.6 versus 98.4%) for both asymptomatic and symptomatic women. The

test performed similarly in all six laboratories (data not shown).

Two of the 70 initially inhibitory urine samples were obtained from asymptomatic, infected (1 culture positive, PCR positive and 1 culture negative, PCR positive) women. These specimens were inhibitory when retested and were thus excluded from performance calculations. If the IC had not been used, these specimens would have been scored as false negative. Use of the IC had no impact on sensitivity for urine from symptomatic women.

Results for *C. trachomatis* in men. Test performance was calculated from the 710 asymptomatic and 1,230 symptomatic men whose results were valid for both specimen types in both test formats. *C. trachomatis* infection was detected in 89 (12.5%) of the asymptomatic men, 73 of whom were positive by culture, and 261 (21.2%) of the symptomatic men, 169 of whom were positive by culture (Table 3). In the remaining 16 asymptomatic and 92 symptomatic, infected men, at least one specimen type gave a positive result in at least one PCR test format; the PCR results were confirmed by DFA testing or by PCR testing for an alternative target sequence within the *C.*

TABLE 4. Sensitivity, specificity, and positive and negative predictive values for the detection of *C. trachomatis* by COBAS AMPLICOR calculated on a patient basis

Sex and symptom category	Specimen	n	% Prevalence	% Sensitivity	% Specificity	% PPV[a]	% NPV[b]
Female							
Asymptomatic	Endocervical	1,098	8.7	87.4	99.5	94.3	98.8
	Urine			87.4	99.6	95.4	98.8
Symptomatic	Endocervical	1,138	9.5	91.7	99.2	92.5	99.1
	Urine			90.7	98.4	86.0	99.0
Male							
Asymptomatic	Urethral	710	12.5	92.1	98.7	91.1	98.9
	Urine			92.1	98.4	89.1	98.9
Symptomatic	Urethral	1,230	21.2	87.4	98.7	94.6	96.7
	Urine			89.7	98.5	94.0	97.2

[a] PPV, positive predictive value.
[b] NPV, negative predictive value.

TABLE 5. Sensitivity, specificity, and positive and negative predictive values for the detection of *C. trachomatis* by COBAS AMPLICOR calculated on a sample basis

Sex and symptom category	Specimen	*n*	% Prevalence	% Sensitivity	% Specificity	% PPV[a]	% NPV[b]
Female							
Asymptomatic	Endocervical	1,098	7.7	97.6	99.5	94.3	99.8
	Urine		8.1	93.3	99.6	95.4	99.4
Symptomatic	Endocervical	1,138	9.1	96.1	99.2	92.5	99.6
	Urine		9.2	93.3	98.5	86.0	99.3
Male							
Asymptomatic	Urethral	710	11.7	98.8	98.7	91.1	99.8
	Urine		12.5	92.1	98.4	89.1	98.9
Symptomatic	Urethral	1,230	19.0	97.4	98.7	94.6	99.4
	Urine		20.6	92.5	98.5	94.0	98.1

[a] PPV, positive predictive value.
[b] NPV, negative predictive value.

trachomatis MOMP gene (Table 3). *C. trachomatis* was detected only in the swab specimens of 6 of the 89 asymptomatic and 19 of the 261 symptomatic, infected men. *C. trachomatis* was detected only in the urine specimens of similar numbers of men: 6 asymptomatic and 25 symptomatic, infected men.

Both specimen types gave negative COBAS AMPLICOR results for 605 of the 621 asymptomatic and 943 of the 969 symptomatic, uninfected men (Table 3). At least one specimen type gave a positive COBAS AMPLICOR result for the remaining 16 asymptomatic and 26 symptomatic, uninfected men; these positive results represent false positives, since none were confirmed by DFA or MOMP PCR testing (Table 3).

(i) Urethral swabs. COBAS AMPLICOR performed on urethral swab specimens yielded positive results for 98.6% (72 of 73) of asymptomatic and 96.4% (163 of 169) of symptomatic, culture-positive men (Table 3). COBAS AMPLICOR performed on urethral swab specimens also yielded positive results for 62.5% (10 of 16) of asymptomatic and 70.7% (65 of 92) of symptomatic, infected, culture-negative men (Table 3).

The sensitivity, specificity, and positive and negative predictive values were calculated from these results (Table 4). COBAS AMPLICOR performed on urethral swab specimens was slightly more sensitive for asymptomatic men (92.1%) than for symptomatic men (87.4%). COBAS AMPLICOR performed on urethral swab specimens exhibited the same specificity (98.7%) for both asymptomatic and symptomatic men. The test performed similarly in the five laboratories that tested male specimens (data not shown).

One of the 30 initially inhibitory urethral swab specimens (6 asymptomatic and 24 symptomatic; Table 2) was obtained from a symptomatic, infected (culture-negative, urine PCR-positive) man. This specimen was inhibitory when retested and was thus excluded from performance calculations. If the IC had not been used, this specimen would have been scored as false negative. Use of the IC had no impact on sensitivity for urethral swab specimens from asymptomatic men.

(ii) Urine specimens. COBAS AMPLICOR performed on urine specimens yielded positive results for 90.4% (66 of 73) of asymptomatic and 88.8% (150 of 169) of symptomatic, culture-positive men (Table 3). COBAS AMPLICOR performed on urine specimens also yielded positive results for 100.0% (16 of 16) of asymptomatic and 91.3% (84 of 92) of symptomatic, infected, culture-negative men (Table 3).

The sensitivity, specificity, and positive and negative predictive values were calculated from these results (Table 4). COBAS AMPLICOR performed on urine specimens was slightly more sensitive for asymptomatic men (92.1%) than for symptomatic men (89.7%). COBAS AMPLICOR performed on urine specimens exhibited virtually identical specificities (98.4 versus 98.5%) for both asymptomatic and symptomatic men. The test performed similarly in four of the five laboratories (data not shown). The sensitivity of COBAS AMPLICOR performed on urine specimens tested at one site, however, was significantly lower than that observed at the other four sites. The combined sensitivity for asymptomatic and symptomatic males at this site was 74.4% (67 of 90), compared to 95.8% (249 of 260) for the other four sites.

Thirteen of the 72 initially inhibitory urine specimens (2 asymptomatic and 70 symptomatic; Table 2) were obtained from symptomatic, infected (seven culture-positive, urethral PCR-positive and six culture-negative, urethral PCR-positive) men. One initially inhibitory, culture-positive specimen was negative when retested and was scored as false negative, regardless of whether the IC was used. Five (1 culture positive and 4 culture-negative, urethral PCR positive) of the 13 initially inhibitory specimens were inhibitory when retested and were thus excluded from performance calculations. Seven (five culture positive and two culture negative, urethral PCR positive) of the initially inhibitory specimens that yielded positive results when retested were interpreted as true positive when the IC was used but would have been interpreted as false negative if the IC had not been used. Thus, 12 additional specimens would have been scored as false negative without the IC, thereby lowering sensitivity for urine from symptomatic men from 89.7% (234 of 261) to 85.3% (227 of 266). Use of the IC had no impact on sensitivity for urine specimens from asymptomatic men.

Individual specimen performance calculations. Certain infected, culture-negative patients yielded negative COBAS AMPLICOR results for one specimen type; they were identified as positive because the other specimen type was COBAS AMPLICOR positive or because at least one specimen type was positive in the AMPLICOR format. If only one test had been performed, only those COBAS AMPLICOR-negative specimens obtained from culture-positive patients would have been interpreted as false negative. Consequently, the resulting sensitivities would have ranged from 92.1 to 98.8% if only one specimen type had been tested in the COBAS AMPLICOR format only (Table 5).

Detection of *C. trachomatis* in specimens coinfected with *N. gonorrhoeae*. A total of 3.7% (159 of 4,315) of the individuals tested were coinfected. The rates of *C. trachomatis* coinfection

TABLE 6. Sensitivity of culture and prevalence of infection at each test site

Sex and site(s)	Asymptomatic				Symptomatic			
	n	Culture sensitivity (%)	% Prevalence		n	Culture sensitivity (%)	% Prevalence	
			Culture	Overall			Culture	Overall
Female								
JHU	68	33.3	2.9	8.8	208	79.2	9.1	11.5
LSU	132	90.0	6.8	7.6	59	87.5	11.9	13.6
UTMB	26	33.3	7.7	23.1	117	57.9	9.4	16.2
IU	212	80.0	15.1	18.9	269	83.9	9.7	11.5
UCSF	243	83.3	2.1	2.5	246	83.3	4.1	4.9
TJUH	417	70.4	4.6	6.5	239	92.9	5.4	5.9
All	1,098	72.6	6.3	8.7	1,138	79.6	7.6	9.5
Male								
JHU	137	75.0	6.6	8.8	421	69.2	12.8	18.5
LSU	164	93.9	18.9	20.1	29	72.7	27.6	37.9
UTMB	59	20.0	1.7	8.5	93	9.1	2.2	23.7
IU	289	82.9	10.0	12.1	505	69.6	15.8	22.8
UCSF	61	75.0	4.9	6.6	182	71.4	13.7	19.2
All	710	82.0	10.3	12.5	1,230	64.8	13.7	21.2

among *N. gonorrhoeae*-infected individuals were 27.6% (16 of 58) and 36.3% (33 of 91), respectively, for asymptomatic and symptomatic women and 18.5% (5 of 27) and 27.1% (105 of 388), respectively, for asymptomatic and symptomatic men.

COBAS AMPLICOR detected *C. trachomatis* in 82.9% (131 of 158; 1 specimen was inhibitory) of the swab specimens tested and 87.6% (134 of 153; 6 specimens were inhibitory) of the urine specimens tested from individuals coinfected with *N. gonorrhoeae*. The sensitivity for *C. trachomatis* in coinfected individuals was similar to that observed in all individuals, with the exception of urethral swab specimens obtained from symptomatic men, where the sensitivity was 76.9% (80 of 104) for coinfected men, compared to 87.4% (228 of 261) for all of the men.

Prevalence of infection in different populations. The overall prevalence of infection varied between sites, ranging from 2.5 to 23.1% in asymptomatic women and from 4.9 to 16.2% in symptomatic women (Table 6). The prevalence of infection was lower in asymptomatic women than in symptomatic women at three sites but higher at the other three sites. Similarly, the prevalence of infection ranged from 6.6 to 20.1% in asymptomatic men and from 18.5 to 37.9% in symptomatic men (Table 6). At all five sites, the prevalence was lower in asymptomatic men than in symptomatic men. The combination of five tests—culture, COBAS AMPLICOR and AMPLICOR performed on swab specimens, and COBAS AMPLICOR and AMPLICOR performed on urine specimens—yielded overall prevalences that were 38.1, 25.0, 21.4, and 54.7% higher than the prevalences determined by culture for asymptomatic women, symptomatic women, asymptomatic men, and symptomatic men, respectively.

DISCUSSION

The results of this study demonstrate that the COBAS AMPLICOR and AMPLICOR CT/NG (data not shown) tests exhibit excellent sensitivity and specificity for *C. trachomatis*. In both men and women, the COBAS AMPLICOR test exhibited essentially the same sensitivity for urine and urogenital swab specimens. Sensitivity was somewhat higher in symptomatic than in asymptomatic women and in asymptomatic than in symptomatic men. When COBAS AMPLICOR test results were compared to patients defined as infected, the test sensitivities

for the individual specimen types ranged from 87.4 to 92.1%. In contrast, the sensitivity of culture was only 64.8 to 82.0%. Thus, PCR performed on any one specimen type detected approximately 10 to 20% more infections than cell culture. The specificity of the COBAS AMPLICOR test ranged from 98.4 to 99.6%, and the positive and negative predictive values ranged from 86.0 to 95.4% and from 96.7 to 99.1%, respectively. Similar results were obtained with the COBAS AMPLICOR test in two other studies in which both swab and urine specimens were tested and PCR results were compared to the infected patient standard (14, 35).

Somewhat higher sensitivities (92 to 94%) were reported in two studies in which urine specimens only were tested by CO-BAS AMPLICOR (27, 32). Other recent studies have also demonstrated that the estimate of sensitivity for each specimen type is lower when multiple specimens from each patient are tested and the infected patient is used as the "gold standard" (8, 14). Testing of multiple specimens detects a greater number of infections but, predictably, has lower sensitivity compared to single-specimen testing, because some patients yield positive results for only one specimen type. This reduction in sensitivity may introduce a bias into the analysis of test performance, but that bias equally affects all of the tests considered, including culture sensitivity. In the present study, depending on the patient group, 4.3 to 11.6% of infected patients yielded positive results for urogenital swab specimens only and 3.7 to 9.5% of infected subjects yielded positive results for urine specimens only.

To compare the performance achieved in this study to that obtained in earlier studies, we also calculated performance as if only one specimen from each patient had been tested. Test sensitivity ranged from 92.1 to 98.8%, and specificity ranged from 98.4 to 99.6%. Thus, the results of the multiplex COBAS AMPLICOR and AMPLICOR CT/NG (data not shown) tests match those obtained with other semiautomated amplification-based tests that detect only *C. trachomatis* (7, 14, 21), including the AMPLICOR *C. trachomatis* test (3, 7, 28, 36).

The multiplex COBAS AMPLICOR test was able to amplify and detect *C. trachomatis* DNA in the presence of *N. gonorrhoeae* DNA. Overall, 27.0% of *C. trachomatis*-infected patients were coinfected with *N. gonorrhoeae*. The sensitivity of COBAS AMPLICOR for *C. trachomatis* was similar whether

or not coinfection was present for all specimen types, except urethral swab specimens from symptomatic men, in which case the sensitivity was lower for coinfected specimens. For this reason, urine is the preferred specimen type for testing of symptomatic men.

Each study site adhered to its standard protocol for the collection, transportation, and inoculation of tissue culture. Variations in technique, such as the use of shell vials versus microtiter plates, were not controlled for, since they reflected the protocol of each laboratory. Therefore, the results reported here are representative of the results routinely available from these laboratories. The sensitivity of culture exhibited considerable variation between clinical test sites. Most likely, this reflects a combination of differences in skill between the clinicians collecting the specimens, differences in the transport and storage conditions, and variability in laboratory expertise. In contrast, the sensitivity of PCR varied little between sites. This suggests that the sensitivity of PCR is much less dependent on specimen collection, transport, and laboratory technique, which represents another advantage of PCR over culture.

The COBAS AMPLICOR and AMPLICOR tests include an IC to ensure the integrity of negative results and maximize test sensitivity by monitoring amplification in specimens yielding negative test results. In the COBAS AMPLICOR test format, the cost of using the IC can be minimized by programming the analyzer to detect the IC only in those specimens that test negative for *C. trachomatis*. Use of the IC increased test sensitivity by 1.8, 0.4, and 4.3% for asymptomatic female urine, symptomatic male urethral swab, and symptomatic male urine specimens, respectively. The IC enabled us to definitively determine that the frequency of inhibition in this study ranged from <1% in endocervical specimens to 5% in urine specimens from symptomatic males. Similar inhibition rates have recently been reported (23, 33). It is difficult to directly compare the frequencies of inhibition for different amplification technologies because the commercially available ligase chain reaction (LCx) and transcription-mediated amplification (TMA) *C. trachomatis* tests lack an IC, making it impossible to assess inhibition in reference test-negative specimens. One study overcame this problem by comparing inhibition in specimens that had been seeded with a small, defined quantity of *C. trachomatis* DNA (23). This study showed that the AMPLICOR CT/NG and LCx *C. trachomatis* tests had similar inhibition rates but that the inhibition rate for the TMA test was 2.5-fold higher (23). In published clinical evaluations, the frequency of inhibitory, positive specimens can be estimated from the frequency of reference test-positive, amplification test-negative specimens that give positive results when retested (29). This type of analysis suggests that the inhibition rates in positive specimens are 1 to 30% for the LCx *C. trachomatis* test (1, 4, 8–10, 19, 26, 34) and approximately 8% for the TMA *C. trachomatis* test (25). The inhibition rate for all specimens is probably higher, since weak inhibition may go undetected in positive specimens that contain relatively high target concentrations (29). Indeed, the IC results in this study demonstrated that the inhibition frequency (i.e., the proportion of specimens giving negative results for both *C. trachomatis* and the IC) was higher for culture-negative specimens (2.5%; 195 of 7,732) than for culture-positive specimens (0.9%; 8 of 886).

The combined performance of two PCR tests on two specimens from each patient detected 98.0% (389 of 397) of the culture-positive individuals. This high detection rate, coupled with the use of the IC to eliminate false-negative results due to inhibition, makes it likely that we detected the vast majority of culture-negative infections, thus minimizing any bias that

might be introduced as a result of discrepant analysis (16). The use of discrepant analysis is further justified because any bias it introduces is less than the bias that results from comparing a test to a less sensitive gold standard without resolving discrepancies (15). In addition, the existence of infections that were missed by both PCR and culture would not change the conclusion that PCR performed on a single specimen type detects 10 to 20% more infections than culture because the sensitivity of both methods would be reduced proportionately.

In summary, both the COBAS AMPLICOR and AMPLICOR CT/NG tests exhibited equally high sensitivities and specificities for *C. trachomatis* with both urogenital swab and urine specimens and are thus well suited for screening for *C. trachomatis* in male and female urine specimens. Elsewhere, we will demonstrate that these tests can be used to simultaneously screen for *N. gonorrhoeae* infections (D. H. Martin et al., unpublished data). Thus, the COBAS AMPLICOR and AMPLICOR tests make it possible to screen for both pathogens by processing and amplifying a specimen once.

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