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Multicenter Evaluation of the Fully Automated COBAS AMPLICOR PCR Test for Detection of *Chlamydia trachomatis* in Urogenital Specimens

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The fully automated COBAS AMPLICOR CT/NG test for the detection of *Chlamydia trachomatis* was evaluated in a multicenter trial. Test performance was evaluated for 2,014 endocervical swab and 1,278 urine specimens obtained from women and for 373 urethral swab and 254 urine specimens obtained from men. Culture served as the reference test. Culture-negative, COBAS AMPLICOR-positive specimens that tested positive in a confirmatory PCR test for an alternative target sequence within the *C. trachomatis* major outer membrane protein gene were resolved as true positives. The overall prevalence of chlamydia was 4.3% in cervical swabs and 11.0% in urethral swabs from men. When the results for each specimen type were considered separately, the resolved sensitivities were 96.5% (83 of 86) for endocervical swab specimens, 95.1% (39 of 41) for urine specimens from women, 100.0% (41 of 41) for urethral swab specimens from men, and 94.4% (17 of 18) for urine specimens from men; the resolved specificities were 99.4% (1,912 of 1,924) for endocervical swab specimens, 99.8% (1,204 of 1,207) for urine specimens from women, 98.5% (325 of 330) for urethral swab specimens from men, and 100.0% (236 of 236) for urine specimens from men. For the subset of patients from whom both swab and urine specimens were collected, the combined results for both specimen types were used to identify all infected patients. Using these combined reslts as criteria, the resolved sensitivities for the COBAS AMPLICOR test were 82.6% (38 of 46) for endocervical swab specimens, 84.4% (38 of 45) for urine specimens from women, 84.2% (16 of 19) for urethral swab specimens from men, and 89.5% (17 of 19) for urine specimens from men. In comparison, the sensitivity of culture was only 56.5% (26 of 46) for endocervical specimens and 63.2% (12 of 19) for urethral specimens from men. The internal control provided in the COBAS AMPLICOR test revealed that 2.9% of specimens were inhibitory when they were initially tested. Nevertheless, valid results were obtained for 99.1% of specimens because 68.7% of the inhibitory specimens were not inhibitory when a second aliquot of the original sample was tested. Two additional COBAS AMPLICOR-positive specimens were detected by retesting inhibitory specimens. The COBAS AMPLICOR CT/NG test for the detection of *C. trachomatis* exhibited equally high sensitivities and specificities with both urogenital swab and urine specimens and, thus, is well-suited for use in screening.

With an estimated 89 million new cases occurring annually worldwide (36), *Chlamydia trachomatis* infections are a major public health problem. Untreated *C. trachomatis* infections can cause pelvic inflammatory disease, cervicitis, urethritis, infertility, ectopic pregnancy, epididymitis, infant pneumonia, and infant conjunctivitis (for a review, see reference 4). Untreated individuals serve as a reservoir for the transmission of infections to their sexual partners. Chlamydia screening programs have been shown to decrease the prevalence of chlamydia infection (5, 21) and reduce the incidence of pelvic inflammatory disease (31). Economic modeling studies based on decision-tree analysis suggest that screening programs are cost-effective; the savings resulting from the prevention of long-term sequelae more than compensate for the cost of screening patients for infections and treating infections (20, 24).

Diagnosis remains a challenge because *C. trachomatis* infections are asymptomatic in up to 80% of infected women and 50% of infected men. Culture is relatively insensitive (for a review, see reference 4). Antigen-based tests such as enzyme immunoassay have limited sensitivities and specificities (for a review, see reference 4). Furthermore, asymptomatic individuals are reluctant to seek medical attention, especially because of the discomfort associated with collecting a swab specimen from the endocervix or urethra. Recent studies have shown that nucleic acid amplification-based tests are ideally suited for screening because they exhibit high sensitivities and specificities for the detection of *C. trachomatis* in noninvasively collected urine specimens (1, 2, 6, 7, 9, 11, 14, 18, 23, 25–28, 30, 33). The availability of urine testing should encourage asymptomatic individuals in at-risk populations to undergo screening and should reduce the costs associated with specimen collection.

The availability of a fully automated test method will also

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reduce the cost of screening. Roche Molecular Systems has developed a PCR-based test for the detection of *C. trachomatis* that is performed on the COBAS AMPLICOR system, an integrated unit that automatically amplifies RNA or DNA targets and that detects the resulting amplicon (12, 16). The COBAS AMPLICOR CT/NG test for *C. trachomatis* uses a master mixture containing one pair of primer oligonucleotides specific for *C. trachomatis* DNA and a second pair specific for *Neisseria gonorrhoeae* DNA to simultaneously amplify both organisms in a single processed specimen (11). The master mixture also contains an internal control (IC) DNA that monitors the amplification for each clinical specimen. The IC contains primer binding regions identical to those of the *C. trachomatis* target sequence, a randomized internal sequence with a length and base composition similar to those of the target sequence, and a unique probe binding region that differentiates the IC from the amplified target nucleic acid (29). The *C. trachomatis*, *N. gonorrhoeae*, and IC amplification products can be detected separately in the same amplified specimen with different target- and IC-specific oligonucleotide capture probes.

Here, we describe the results of a multicenter evaluation of the COBAS AMPLICOR CT/NG test for the detection of *C. trachomatis*. Test performance was evaluated for endocervical swab specimens, urethral swab specimens from men, and urine specimens from women and men.

MATERIALS AND METHODS

Patient population. In Montreal, Quebec, Canada, specimens were collected from individuals visiting general practitioners and family-planning clinics. In Groningen, The Netherlands, specimens were collected from individuals visiting general practitioners, dermatologists, gynecologists, sexually transmitted disease clinics, and family-planning centers. In Meaux, France, specimens were obtained from men and women consulting the outpatient clinic of Institut Alfred Fournier, Paris, France. Most of the individuals studied in Groningen and Meaux were symptomatic. Information on symptomatology was not available for individuals from Montreal.

Specimen collection and processing. Endocervical swab specimens and urethral swab specimens from men were collected by standard procedures and inoculated into 2SP transport medium (0.2 M sucrose–0.02 M phosphate). These specimens were stored at 2 to 8°C until they were transported to the laboratory; all specimens were transported within 24 h. An aliquot of the specimen was used for *Chlamydia* culture on cycloheximide-treated McCoy cells (Montreal and Groningen) or cycloheximide-treated HeLa 229 cells (Institut Fournier). Chlamydial inclusions were detected by immunofluorescence with monoclonal antibodies specific for the major outer membrane protein (MOMP; Micro Trak; Syva Co.). When a specimen was toxic for cell culture, a passage was performed. If the toxicity persisted the specimen was excluded from analysis. A second aliquot was processed and tested with the COBAS AMPLICOR system. A 100-μl sample of specimen was mixed with 100 μl of CT/NG Lysis Buffer, and the mixture was incubated for 10 min at room temperature. The resulting mixture was combined with 200 μl of CT/NG Specimen Diluent, and this mixture was incubated for an additional 10 min at room temperature.

A total of 10 to 50 ml of first-catch urine was also collected from each subject. The urine was shipped to the laboratory at room temperature and was stored at 2 to 8°C for up to 7 days (Montreal and Meaux) or at −20°C (Groningen) until it was processed and tested with the COBAS AMPLICOR system. A 500-μl sample of urine was combined with 500 μl of CT/NG Urine Wash Buffer, and the mixture was incubated at 37°C for 15 min. The mixture was then centrifuged at 12,500 × *g* for 5 min. The supernatant was discarded and the pellet was resuspended in 250 μl of CT/NG Lysis Buffer. After a 15-min incubation at room temperature, 250 μl of CT/NG Specimen Diluent was added to the lysate. The specimens were centrifuged at 12,500 × *g* for 10 min, and the resulting supernatant was tested.

Amplification and detection. A 50-μl sample of processed specimen was added to 50 μl of Master Mix containing IC and was amplified by using the thermal cycler onboard the COBAS AMPLICOR system. The thermal cycling conditions were automatically performed by the COBAS AMPLICOR system (12, 16). Upon the completion of amplification, the COBAS AMPLICOR system automatically denatured the amplified product, hybridized the amplicon to target-specific oligonucleotides bound to magnetic microparticles, and colorimetrically detected the captured amplicon by using an avidin-horseradish peroxidase complex (12, 16). The *C. trachomatis* target and the IC were detected in separate reactions with separate *C. trachomatis*- and IC-specific oligonucleotide capture probes.

TABLE 1. Frequency of inhibition for culture-positive and culture-negative specimens

Specimen source and type	Culture-positive specimens			Culture-negative specimens		
	Total no.	No. (%) inhibitory	No. retest valid[a]	Total no.	No. (%) inhibitory	No. retest valid[a]
Females						
Swab	49	1 (2.0)	1	1,965	17 (0.9)	13
Urine	26	0 (0.0)	NA[b]	1,252	84 (6.7)	54
Males						
Swab	27	0 (0.0)	NA	346	9 (2.6)	7
Urine	12	0 (0.0)	NA	242	4 (1.7)	4
All	114	1 (0.9)	1	3,805	114 (3.0)	78

[a] Number of specimens that were *C. trachomatis* positive or *C. trachomatis* negative and IC positive when they were retested.
[b] NA, not applicable.

Interpretation of results. Specimens yielding *C. trachomatis* signals above the test cutoff were interpreted as positive, regardless of the IC result. Specimens yielding *C. trachomatis* signals below the test cutoff were interpreted as negative, provided that the IC signal was above the assigned cutoff. Specimens with signals below the cutoffs for both the *C. trachomatis* and IC signals were interpreted as inhibitory. Specimens with inhibitory activity were retested by processing another aliquot of the original specimen. The repeat test results were classified by the criteria presented above.

Sensitivity and specificity were calculated by comparing PCR results to the resolved results because culture is not 100% sensitive. Results for specimens with discrepant results (positive by PCR but negative by culture) were resolved by performing PCR for an alternative target DNA sequence, a portion of the MOMP gene (13). The results for the specimens were resolved as being positive for infection if the culture was positive or if the specimen was PCR positive for both the primary and the alternative targets.

Sensitivity and specificity were calculated in two ways. The calculation on a sample basis showed the results that would have been obtained if only one specimen type had been tested by PCR. Culture-negative infections were identified by using the PCR results for only one specimen type (swab or urine). Samples were classified as positive for infection if the reference test was positive or if the specimen type being evaluated was PCR positive for both the primary and the alternative targets.

The calculation on a patient basis considered the results for both the swab and urine specimens to identify all infected patients. This analysis was performed for the subset of patients from whom both specimen types were collected. Culture-negative infections were identified by using PCR results for both the swab and urine specimens from a single patient. Patients were classified as positive for infection if the culture was positive or if either specimen type was PCR positive for both the primary and the alternative targets.

RESULTS

Frequency of inhibition. Only 1 of 114 specimens (0.9%) obtained from culture-positive patients was inhibitory when it was initially tested; this specimen yielded a true-positive result when it was retested (Table 1). The frequency of inhibition was somewhat higher for specimens from culture-negative patients, ranging from 0.9% for endocervical swab specimens to 6.7% for urine specimens from women (Table 1). The frequency of inhibition did not vary substantially between laboratories.

Approximately 68.7% (79 of 115) of inhibitory specimens were not inhibitory when another aliquot of the specimen was processed and tested (Table 1). The frequency of successful retesting did not vary between specimen types or between laboratories. 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.

Performance with specimens from women. A total of 2,014 endocervical swab specimens were evaluated, 49 (2.4%) of

TABLE 2. Comparison of COBAS AMPLICOR and culture results[a]

Specimen source and type	PCR result	No. of specimens from the following locations with the indicated culture result[b]							
		Meaux		Montreal		Groningen		All sites	
		+	−	+	−	+	−	+	−
Females									
Swab	+	1	0	16	11 (7)	29[c]	38 (30)	46[c]	49 (37)
	−	0	176	1	782	2	954	3	1,912
	Inhibitory[d]	0	1	0	1	0	2	0	4
Urine	+	1	1 (0)	15	7 (5)	8	10[e] (10)	24	18[e] (15)
	−	0	163	1	743	1	298	2	1,204
	Inhibitory	0	2	0	28	0	0	0	30
Males									
Swab	+	3	1 (0)	4	1 (0)	20	17 (14)	27	19 (14)
	−	0	26	0	110	0	189	0	325
	Inhibitory	0	0	0	1	0	1	0	2
Urine	+	3	0	4	1 (1)	4	5 (5)	11	6 (6)
	−	0	28	0	110	1	98	1	236
	Inhibitory	0	0	0	0	0	0	0	0

[a] Symbols: +, positive result; −, negative result.
[b] Numbers in parentheses indicates numbers of COBAS AMPLICOR-positive, culture-negative specimens that were confirmed to be true positive by MOMP PCR.
[c] Includes one specimen that was inhibitory when it was initially tested but positive when it was retested.
[d] Specimens that were COBAS AMPLICOR negative and IC negative for *C. trachomatis* in both initial and repeat tests.
[e] Includes one specimen that was inhibitory when it was initially tested but positive when it was retested and was confirmed to be true positive by MOMP PCR.

which were positive for *C. trachomatis* by culture. The COBAS AMPLICOR test yielded positive results for 46 of the 49 endocervical swab specimens obtained from culture-positive women (Table 2). One of these 46 positive specimens was inhibitory when it was initially tested and would have been interpreted as false negative if the IC had not been used. An additional 49 specimens obtained from culture-negative women generated positive results by the COBAS AMPLICOR test. Thirty-seven of these 49 specimens were confirmed to be positive by performing PCR for an alternative target sequence within the *C. trachomatis* MOMP gene (Table 2). Thus, 12 specimens yielded false-positive COBAS AMPLICOR test results. When performed with endocervical swab specimens, the prevalence of chlamydia was 4.3% (86 of 2,014), the resolved sensitivity and specificity of the COBAS AMPLICOR test were 96.5 and 99.4%, respectively, and the corresponding positive and negative predictive values were 87.4 and 99.8%, respectively (Table 3). Test performances were similar in all three laboratories.

To assess the impact of using the IC, we also calculated the test sensitivity and specificity by ignoring the IC results and interpreting all PCR results as positive or negative on the basis of the initial test result. If the IC had not been used, the sensitivity would have decreased to 95.3% and the specificity would not have changed (data not shown).

A total of 1,278 urine specimens were evaluated; 26 (2.0%) of these were obtained from women who were positive by culture of endocervical swab specimens. The COBAS AMPLICOR test yielded positive results for 24 of the 26 urine specimens obtained from culture-positive women (Table 2). An additional 18 specimens obtained from culture-negative women generated positive results by the COBAS AMPLICOR test. Fifteen of these 18 specimens were confirmed to be positive by performing the MOMP PCR (Table 2); 1 of these specimens confirmed to be positive was inhibitory when it was initially tested and would have been incorrectly interpreted as negative if the IC had not been used. Thus, three specimens

yielded false-positive COBAS AMPLICOR test results. When performed with urine specimens from women, the resolved sensitivity and specificity of the COBAS AMPLICOR test were 95.1 and 99.8%, respectively, and the corresponding positive and negative predictive values were 92.9 and 99.8%, respectively (Table 3). Test performances were similar in all three laboratories. If the IC had not been used, the sensitivity would have decreased to 95.0% and the specificity would not have changed (data not shown).

Because two COBAS AMPLICOR tests were performed for each patient, we were able to identify infections that would not have been detected had we tested only a single specimen. Matched urine and endocervical swab specimens were available for 1,253 women, 46 of whom were infected with *C. trachomatis*. Twenty-six of the 46 infections were detected by culture (Table 4). The COBAS AMPLICOR test was positive for 25 of the endocervical swab specimens and 24 of the urine specimens from culture-positive patients (the false-negative swab specimen and one of the two false-negative urine specimens came from the same patient). The COBAS AMPLICOR test detected 20 infections in culture-negative patients. For seven of these 20 infections, both the endocervical swab and urine specimens were positive by the COBAS AMPLICOR test. For 6 of the 20 infected patients, only the endocervical swab specimen was positive, and for the remaining 7 patients only the urine specimen was positive. Consequently, when the results for the infected patient were used as the "gold standard," the resolved sensitivities were 56.5% for endocervical swab specimen culture and 82.6% for endocervical swab specimens and 84.4% for urine specimens by the COBAS AMPLICOR test (Table 4).

Performance with specimens from men. A total of 373 urethral swab specimens were evaluated; 27 (7.2%) of these were positive for *C. trachomatis* by culture. The COBAS AMPLICOR test yielded positive results for all 27 urethral swab specimens obtained from culture-positive men (Table 2). An additional 19 specimens obtained from culture-negative men

TABLE 3. Resolved sensitivity, specificity, and positive and negative predictive values of the COBAS AMPLICOR test calculated separately for each specimen[a]

Specimen source and type	Site	Sensitivity (%)[b]	Specificity (%)[c]	PPV (%)[d]	NPV (%)[e]
Females					
Swab	Meaux	100.0 (1/1)	100.0 (176/176)	100.0 (1/1)	100.0 (176/176)
	Montreal	95.8 (23/24)	99.5 (782/786)	85.2 (23/27)	99.9 (782/783)
	Groningen	96.7 (59/61)	99.2 (954/962)	88.1 (59/67)	99.8 (954/956)
	All	96.5 (83/86)	99.4 (1912/1924)	87.4 (83/95)	99.8 (1912/1915)
Urine	Meaux	100.0 (1/1)	99.4 (163/164)	50.0 (1/2)	100.0 (163/163)
	Montreal	95.2 (20/21)	99.7 (743/745)	90.9 (20/22)	99.9 (743/744)
	Groningen	94.7 (18/19)	100.0 (298/298)	100.0 (18/18)	99.7 (298/299)
	All	95.1 (39/41)	99.8 (1204/1207)	92.9 (39/42)	99.8 (1204/1206)
Males					
Swab	Meaux	100.0 (3/3)	96.3 (26/27)	75.0 (3/4)	100.0 (26/26)
	Montreal	100.0 (4/4)	99.1 (110/111)	80.0 (4/5)	100.0 (110/110)
	Groningen	100.0 (34/34)	98.4 (189/192)	91.9 (34/37)	100.0 (189/189)
	All	100.0 (41/41)	98.5 (325/330)	89.1 (41/46)	100.0 (325/325)
Urine	Meaux	100.0 (3/3)	100.0 (28/28)	100.0 (3/3)	100.0 (28/28)
	Montreal	100.0 (5/5)	100.0 (110/110)	100.0 (5/5)	100.0 (110/110)
	Groningen	90.0 (9/10)	100.0 (98/98)	100.0 (9/9)	99.0 (98/99)
	All	94.4 (17/18)	100.0 (236/236)	100.0 (17/17)	99.6 (236/237)

[a] Sensitivity and specificity were calculated by taking the IC results into account. Results for all specimens that were initially COBAS AMPLICOR negative and IC negative for *C. trachomatis* were interpreted as positive, negative, or inhibitory on the basis of the result of the repeat COBAS AMPLICOR test. PPV, positive predictive value; NPV, negative predictive value.
[b] Values in parentheses are true positive/(true positive + false negative).
[c] Values in parentheses are true negative/(true negative + false positive).
[d] Values in parentheses are true positive/(true positive + false positive).
[e] Values in parentheses are true negative/(true negative + false negative).

generated positive results by the COBAS AMPLICOR test. Fourteen of these 19 specimens were confirmed to be positive by the MOMP PCR (Table 2). Thus, five specimens yielded false-positive COBAS AMPLICOR test results. When performed with urethral swab specimens from men, the prevalence of chlamydia was 11.0% (41 of 373), the resolved sensitivity and specificity of the COBAS AMPLICOR test were 100.0 and 98.5%, respectively, and the corresponding positive and negative predictive values were 89.1 and 100.0%, respectively (Table 3). The same sensitivity and specificity would have been obtained had the IC not been used because none of the

positive specimens gave negative IC results when they were initially tested. Test performances were similar in all three laboratories.

A total of 254 urine specimens were evaluated; 12 (4.7%) of these were obtained from men who were positive by culture of urethral swab specimens. The COBAS AMPLICOR test yielded positive results for 11 of the 12 urine specimens obtained from culture-positive men (Table 2). An additional six specimens obtained from culture-negative men generated positive results by the COBAS AMPLICOR test, and all of these were confirmed to be positive by performing the MOMP PCR

TABLE 4. Resolved sensitivity of the COBAS AMPLICOR test and culture calculated on the basis of patient infection status[a]

Sex	Site	No. of patients[a]	No. infected[b]	Sensitivity (% [no. positive])[c]		
				Culture	PCR of swabs	PCR of urine
Female	Meaux	160	1	100.0 (1)	100.0 (1)	100.0 (1)
	Montreal	794	26	61.5 (16)	84.6 (22)	80.0 (20)[d]
	Groningen	299	19	47.4 (9)	78.9 (15)	89.5 (17)
	All	1,253	46	56.5 (26)	82.6 (38)	84.4 (38)[d]
Male	Meaux	30	3	100.0 (3)	100.0 (3)	100.0 (3)
	Montreal	115	5	80.0 (4)	80.0 (4)	100.0 (5)
	Groningen	106	11	45.5 (5)	81.8 (9)	81.8 (9)
	All	251	19	63.2 (12)	84.2 (16)	89.5 (17)

[a] The total number of patients with matched urine and swab specimens.
[b] Patients were considered infected if culture was positive or if either the swab or the urine specimen was positive by the COBAS AMPLICOR test and was confirmed to be positive by the MOMP PCR.
[c] Sensitivity was calculated by interpreting the results for all specimens initially COBAS AMPLICOR negative and IC negative for *C. trachomatis* as positive, negative, or inhibitory on the basis of the result of the repeat COBAS AMPLICOR test.
[d] The urine from one infected patient was repeatedly inhibitory to the PCR. Data for this patient were excluded when calculating the sensitivity of the PCR with urine but were included when calculating the sensitivity of culture and PCR with swabs.

(Table 2). Thus, there were no false-positive COBAS AMPLI-COR test results. When performed with urine specimens from men, the sensitivity and specificity of the COBAS AMPLICOR test were 94.4 and 100.0%, respectively, and the corresponding positive and negative predictive values were 100.0 and 99.6%, respectively (Table 3). The same sensitivity and specificity would have been obtained had the IC not been used. Test performances were similar in all three laboratories.

Matched urine and urethral swab specimens were available for 251 men, 19 of whom were infected with *C. trachomatis*. Twelve of the 19 infections were detected by culture (Table 4). The COBAS AMPLICOR test was positive for all 12 urethral swab specimens and 11 of the urine specimens from culture-positive patients. The COBAS AMPLICOR test detected infections in seven culture-negative patients. For three of these seven patients, both the urethral swab and urine specimens were positive by the COBAS AMPLICOR test, for one of these seven infected patients, only the urethral swab specimen was positive, and for the remaining three patients only the urine specimen was positive. Consequently, when the results for the infected patient were used as the gold standard, the resolved sensitivities were 63.2% for urethral swab specimen culture and 84.2% for urethral swab specimens and 89.5% for urine specimens by the COBAS AMPLICOR test (Table 4).

DISCUSSION

The results of this study demonstrate that the COBAS AM-PLICOR CT/NG test for the detection of *C. trachomatis* exhibited excellent sensitivity and specificity. Our data do not allow us to contrast the performance of the test among symptomatic and asymptomatic subjects. A transport medium other than 2SP may result in a different performance. When performance was calculated as if only one specimen from each patient had been tested, the sensitivity ranged from 94.4 to 100.0%, the specificity ranged from 98.5 to 100.0%, and the positive and negative predictive values ranged from 89.1 to 100.0% and 99.6 to 100.0%, respectively. While the manuscript was being prepared, two other studies reported similar results for the COBAS AMPLICOR test (14, 26). These values are similar to those reported for the nonautomated AMPLICOR *C. trachomatis* test (2, 6, 25–28, 30, 35) and other amplification-based *C. trachomatis* tests (1, 6, 7, 9, 14, 18, 23, 25, 26, 30, 33). Virtually identical performance was observed for urine and endocervical swab specimens from women. Similarly, the test performed equally well with urethral swab specimens from men and urine specimens from men. Thus, the COBAS AM-PLICOR CT/NG test for the detection of *C. trachomatis* is well-suited for the screening of noninvasively collected specimens.

When two specimens were evaluated from each patient, for some patients positive results were obtained for only one specimen type. Thus, the total number of infections detected was greater than the number that would have been detected if only a single specimen had been tested. As a result, the estimate of test sensitivity for each specimen type was more stringent, but also more realistic, than it would have been in the absence of a test result for the second specimen. Actually, the combination of testing of both urine and swab specimens by PCR increased the rate of detection of *C. trachomatis* infection by 17% (64 versus 54 when only swab specimens were tested or 64 versus 55 when only urine specimens were tested). Other recent studies (8, 14) have also demonstrated that the estimate of the sensitivity of each test is lower when multiple tests are performed for each patient to identify all infected patients (i.e., the results for the infected patient served as the gold standard).

When COBAS AMPLICOR test results were compared to the results for the infected patient used as a gold standard, the test sensitivities for the individual specimen types ranged from 82.6 to 89.5%. In contrast, the sensitivities of culture were only 56.5 to 63.2%. Thus, the COBAS AMPLICOR CT/NG test for *C. trachomatis* performed with any one specimen type detected approximately 40% more infections than cell culture (Table 4). Enhancement of the rate of detection appears even greater (50 to 80%) when the rate is estimated from the results for all specimens (Table 2). The low sensitivity of culture has been reported previously (7). Delay between specimen collection and initiation of culture may have contributed to the low culture sensitivity in our study. Because a loss of sensitivity during transport is unavoidable in most clinical settings, amplification tests that do not depend on maintenance of specimen viability will offer a large improvement in sensitivity over that of culture for routine testing.

When evaluating the positive predictive value of a test, one must take disease prevalence into account. Even a test with a sensitivity of 95% and a specificity of 99.5% will yield a positive predictive value of below 90% for a population with a prevalence of less than 4.5%. The positive predictive values of the COBAS AMPLICOR CT/NG Test for the detection of *C. trachomatis* with swab and urine specimens from women and swab and urine specimens from men were 87.4, 92.9, 89.1, and 100.0%, respectively. When specimens with false-positive results at one site (Groningen) were retested, nearly all of them became negative, increasing the positive predictive value to >99%. Given this observation, laboratories that test populations with a low prevalence of *C. trachomatis* infection may want to evaluate whether positive predictive value could be significantly enhanced by retesting all positive specimens.

Use of the IC increased the test sensitivity for urine and swab specimens from women because two initially inhibitory specimens were found to be true positive upon retesting. Numerous studies have demonstrated that a small but significant proportion of clinical specimens contain substances that inhibit PCR, the ligase chain reaction, transcription-mediated amplification (TMA), and nucleic acid sequence-based amplification (1–3, 6, 8–10, 15, 17, 19, 22, 25, 32–35). Use of the IC enabled us to determine definitively that the overall frequency of inhibition for the COBAS AMPLICOR CT/NG test for *C. trachomatis* was 2.9% (115 of 3,919) and that female urine specimens from women were somewhat more inhibitory than endocervical swab specimens and urethral swab and urine specimens from men. We cannot directly compare the frequencies of inhibition for different amplification technologies because the commercially available LCx assay and TMA tests for *C. trachomatis* lack an IC, making it impossible to assess inhibition for reference test-negative specimens. The frequency of inhibitory, positive specimens can be estimated from the number of reference test-positive, amplification test-negative specimens that give positive results when they are retested (29). This analysis indicates that the inhibition rates for positive specimens were 1 to 30% for the LCx assay for *C. trachomatis* (1, 3, 8–10, 15, 26, 33) and 8% for the TMA for *C. trachomatis* (25). The inhibition rate for all specimens is probably higher since weak inhibition may go undetected for positive specimens that contain relatively high concentrations of target (29). Indeed, the results for the IC obtained in this study demonstrated that the observed frequency of inhibition was higher for culture-negative specimens than for culture-positive specimens.

Use of the IC ensures the integrity of negative results and maximizes test sensitivity by monitoring amplification for specimens with negative test results for *C. trachomatis*. The added cost of using the IC corresponds to the cost for reagents, but

the workload is almost identical whether or not the IC is used with the COBAS AMPLICOR system. This cost can be minimized by programming the system to detect the IC only for those specimens that test negative for *C. trachomatis* (12). When inhibition is found to be rare, the IC may be omitted or used selectively, for instance, to monitor proficiency as part of a quality control program or to validate the results for specimens more likely to be inhibitory.

The COBAS AMPLICOR system improves laboratory productivity by fully automating amplification and detection. After laboratory personnel process clinical specimens and load the samples onto the COBAS AMPLICOR system, they are free to perform other tasks. One technician can process 24 swab specimens in 30 min or 24 urine specimens in 1 h. Results for these specimens are available approximately 5 h after the technician starts the COBAS AMPLICOR system. While these specimens are being amplified, the technician can process a second set of specimens, which can be loaded into the system and amplified while any amplicon in the first set are being detected. Results for the second set of specimens are available approximately 7 h after the start of the first run. A third set of specimens can be processed while the first two sets are being tested and can be loaded into the system at the end of the workday. The system will run unattended overnight, and the results will be available the next morning. Thus, a single operator performing 3 to 4 h of hands-on work can test 96 specimens in a single workday.

In summary, the fully automated COBAS AMPLICOR CT/NG test for the detection of *C. trachomatis* exhibited high sensitivity and specificity with both urogenital swab and urine specimens and used an IC to ensure the integrity of negative results. Because the test can detect *C. trachomatis* in noninvasively collected urine specimens from men and women without sacrificing sensitivity, it is well-suited for use in screening.

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