Comparison between the LCx Probe System and the COBAS AMPLICOR System for Detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* Infections in Patients Attending a Clinic for Treatment of Sexually Transmitted Diseases in Amsterdam, The Netherlands

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Two assays for the detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* were compared: the LCx Probe system (the LCx system; Abbott Diagnostic Laboratories, North Chicago, Ill.) and the COBAS AMPLICOR *C. trachomatis*/*N. gonorrhoeae* system (the COBAS AMPLICOR system; Roche Diagnostic Systems, Branchburg, N.J.). Endocervical swab specimens, male urethral swab specimens, and female and male urine specimens were collected from 503 female and 498 male visitors attending a sexually transmitted diseases clinic in Amsterdam, The Netherlands. Prevalences for *C. trachomatis* were 12.5% (63 of 503) and 10.0% (50 of 498) in females and males, respectively. The prevalences for *N. gonorrhoeae* were 1.2% (6 of 503) and 4.2% (21 of 498) in females and males, respectively. Both assays showed high values for sensitivity and specificity with regard to the detection of *C. trachomatis* in endocervical swab specimens, male urethral swab specimens, and female and male urine specimens. The sensitivities for the LCx system were 92.1, 90.0, 88.9, and 94.0% for each type of specimen, respectively; and the sensitivities for the COBAS AMPLICOR system were 96.8, 98.0, 82.5, and 92.0% for each type of specimen, respectively. Specificities ranged between 98.4 and 100%. The sensitivity of the LCx system for the detection of *N. gonorrhoeae* was 100% for female cervical swab and urine specimens and male urethral swab specimens, while for male urine specimens the sensitivity was 95.2%; the specificity was 100% for all types of specimens. For the detection of *N. gonorrhoeae* by the COBAS AMPLICOR assay, the sensitivity for female cervical swab and male urethral swab specimens was 100%, that for female urine specimens was 66.7%, and that for male urine specimens was 95.2%. However, the predictive values of a positive test for female cervical swab specimens and urine specimens were 31.6 and 36.4%, respectively. Sequence analysis of the amplimers obtained by an in-house 16S rRNA PCR of the solely COBAS AMPLICOR system-positive swab specimens revealed neither *N. gonorrhoeae* nor other *Neisseria* spp. The COBAS AMPLICOR assay was considered not suitable for screening for infections with *N. gonorrhoeae*. If this assay is used for detection of *N. gonorrhoeae*, confirmation of positive results by a reliable test is mandatory.

Visitors attending clinics for sexually transmitted diseases (STDs) are considered to be at high risk for infection with *Chlamydia trachomatis* and *Neisseria gonorrhoeae*, and both infections pose major public health problems.

C. trachomatis is the causative agent of urethritis and epididymitis in men. In women it can cause cervicitis, pelvic inflammatory disease, infertility, and ectopic pregnancy, whereas in both sexes it may additionally be the cause of lymphogranuloma venereum, infant pneumonia, and infant conjunctivitis. Infection with *C. trachomatis* in men is usually symptomatic, whereas in women it can go unnoticed. Asymptomatic infections can also lead to sterility if untreated.

Infection with *N. gonorrhoeae* causes gonorrhoea in both men and women. Although the symptoms are more intense in men than in women, the latter are at risk of development of chronic pelvic pain, ectopic pregnancy, and infertility. Un-

treated individuals serve as reservoirs for the transmission of the infection to their sexual partners, so it is of importance to screen high-risk populations for both *C. trachomatis* and *N. gonorrhoeae* infections.

Both sexually transmissible pathogens are detectable by swabbing the urethra and/or the cervix (for women) and performing either a culture and/or a nucleic acid amplification assay (3, 5, 6, 10). The detection of the pathogens in urine samples by amplification assays is an improvement because most patients can collect samples themselves. A noninvasive technique for the collection of a specimen is an advantage for both women and men. Urine specimens have been shown to be suitable for the screening of large numbers of individuals in prevalence studies and the identification of chlamydial infections in order to prevent sequelae and transmission to sexual partners (5). A test that can be used for the simultaneous detection of both pathogens in one specimen would be desirable.

In the prospective study described here we compared the performances of two commercially available amplification assays for the duplex testing for both pathogens with one spec-

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imen: the LCx probe system (the LCx system; Abbott Diagnostic Laboratories, North Chicago, Ill.), which uses two separate assays specific for *C. trachomatis* and *N. gonorrhoeae* in the same specimen, and the COBAS AMPLICOR *C. trachomatis*/*N. gonorrhoeae* system (the COBAS AMPLICOR system; Roche Diagnostic Systems, Branchburg, N.J.), a PCR, in which *C. trachomatis* and *N. gonorrhoeae* DNAs are amplified in a multiplex setting and detected in separate steps. Both assays have different logistics, going from the specimen sampling via nucleic acid isolation and amplification to the detection of the amplimers and registration of the results.

Adhering strictly to the protocols of the manufacturers, we performed a study among visitors of an STD clinic in Amsterdam, The Netherlands. The aim was to assess and compare the sensitivity and specificity of detection of both *C. trachomatis* and *N. gonorrhoeae* in urethral swab specimens from men, endocervical swab specimens from women, and urine specimens from both sexes. Also, the ease of use and the possible additional value of using an internal control (IC; COBAS AMPLICOR system) were assessed. An important goal was to test the feasibility of using urine samples instead of swab specimens. Swabs are still used as "gold standard" test specimens, but recently, a number of studies have shown that urine can also successfully be used for the detection of *C. trachomatis* (3, 5, 7). However, we found in our laboratory that the sensitivity of detection of *C. trachomatis* and *N. gonorrhoeae* in urine specimens was lower relative to the sensitivity of detection of these pathogens in endocervical or male urethral swab specimens (3). In the present study the latest test formats for the LCx system (Abbott Diagnostic Laboratories) and the COBAS AMPLICOR PCR were used.

MATERIALS AND METHODS

Study population. Visitors who were younger than 30 years of age and who were attending a clinic for STDs were included in the study after giving informed consent to participate. The goal was to include 500 men and 500 women, with the expectation of about 60 *C. trachomatis*- and/or 30 *N. gonorrhoeae*-positive patients. Patients were included regardless of clinical symptoms. They underwent a physical examination and they filled out a questionnaire concerning STDs and the use of antibiotics. The study period was September 1998 to November 1998.

Specimen collection. The order of collection of swabs for the LCx and the COBAS AMPLICOR systems was reversed at the time point when 250 men and 250 women had been included in the study. More men than women visited the clinic in a certain time span. At the time that 250 men were included, only women were included in the study until 250 women had also donated samples before the inclusion of male visitors was restarted. Endocervical and urethral cotton swab specimens from women and urethral swab specimens from men were routinely collected for culture of *N. gonorrhoeae*. Then, urethral swab specimens from men and endocervical swab specimens from women were collected separately for the LCx and COBAS AMPLICOR system. The specimens were transported in Abbott Transport Medium and the AMPLICOR STD specimen collection kit, respectively. Participants were asked to collect first-catch urine after not having voided for at least 2 h. Urine samples were always collected after the swab specimens had been taken. The samples were sent to the Regional Laboratory of Public Health, Amsterdam, within 6 h of collection.

Culture of *N. gonorrhoeae*. At the STD clinic, GC-lect plates were directly inoculated and immediately placed at 37°C in candle jars. They were thus transported to the laboratory within 6 h. After incubation for 48 h the plates were examined, and suspected colonies were subjected to Gram staining with aqueous fuchsin counterstain and a cytochrome oxidase test. As confirmatory tests, oxidative utilization of glucose (positive) and maltose (negative), the *o*-nitrophenyl- β -D-galactosidase test (negative) as a substitute for lactose degradation, the γ -glutamyl-aminopeptidase test (negative), and growth on nutrient agar without blood (negative) were used.

LCx system analysis. The swabs and transport medium (1 ml) in tubes were provided by Abbott Diagnostic Laboratories. The isolation of bacterial DNA in the case of the swab specimens was by heating the tubes at 95°C for 15 min, and 100 μ l was used for amplification by ligase chain reaction. The urine samples were briefly mixed by swirling, and 1 ml was taken and centrifuged at $13,000 \times g$ for 15 min. The pellet was dissolved in 1 ml of resuspension buffer, of which 100 μ l was used as input in an amplification reaction (equivalent to 0.1 ml of urine). Amplification and detection were carried out as prescribed by the manufacturer (Abbott Diagnostic Laboratories) and has been published before in detail (3). Each run for either *C. trachomatis* or *N. gonorrhoeae* consisted of 48 samples, including two positive samples and two negative samples. Samples that had been analyzed were stored at -20°C for possible discrepancy analysis.

COBAS AMPLICOR PCR analysis. After obtaining the endocervical or urethral swab specimens from the patients, the swabs were eluted by vigorously agitating the AMPLICOR STM tube for 15 s and were then discarded. Elution for longer than 15 s proved to be inhibitory for the PCR. Before nucleic acid isolation, 1 ml of specimen diluent containing Mg^{2+} was added and the tubes were incubated for at least 16 h (overnight) at 2 to 8°C. The longer this incubation, the better the results with the internal control proved to be. Urine samples (0.5 ml) were pretreated by adding 0.5 ml of urine wash buffer and incubating the mixture for 15 min at 37°C. After centrifugation at $12,500 \times g$ for 5 min and removal of the supernatant, a volume of 250 μ l of lysis buffer (Roche Diagnostic Systems) was added and the mixture was thoroughly mixed to dissolve the pellet. After incubation for 15 min, 250 μ l of specimen diluent was added to the lysate. Then, it was again centrifuged at $12,500 \times g$ for 10 min to pellet possible inhibitory substances. A total of 100 μ l from the supernatant, corresponding to 0.1 ml of urine, was used in the amplification reaction. Amplification and detection in the automated COBAS AMPLICOR system were performed as recommended by the manufacturer, with a multiplex amplification for *C. trachomatis*, *N. gonorrhoeae*, and the IC (which is a plasmid containing the primer sequences used for the amplification of *C. trachomatis*), and separate detection steps with probes for *C. trachomatis*, *N. gonorrhoeae*, and the IC. The added IC should be positive for a valid amplification result, or the *C. trachomatis* and/or *N. gonorrhoeae* reaction should be positive. In case of a negative result for all three amplifications (IC, *C. trachomatis*, and *N. gonorrhoeae*), the amplification and detection were repeated once. Runs were performed in so-called A rings, which can hold 12 samples each. In each run of two A rings (24 reactions), one positive sample and one negative sample were included as controls. The positive control for *N. gonorrhoeae* serves as the negative control for *C. trachomatis*, and vice versa. The COBAS AMPLICOR system was instructed with AMPLILINK, a software program running under Windows 95, which simplifies the test runs, and the results were registered by using this program. Samples that had been analyzed were stored at -20°C for possible discrepancy analysis.

PCR of the *Neisseria* 16S rRNA gene and sequence analysis. PCR amplifications were performed in an Omnigene thermal cycler (Hybaid Ltd., Teddington, United Kingdom). DNA amplification was done in 25- μ l reaction volumes. For the amplification of *Neisseria* DNA, each reaction mixture contained 10 pmol of forward primer 16S8FEvar1 (CGGAATTCCAGAGTTTGATCMTGGCTCAG) and reverse primer 16SNeiss1 (TTAAGTAACCGTCTGCGC) and 10 μ l of HotStar master mix (Qiagen, Hilden, Germany). A 25- μ l overlay of paraffin oil was added to the tubes, followed by the addition of 5 μ l of the clinical sample. After an activation step of 15 min at 95°C, a touchdown PCR program was used: two cycles of 20 s at 95°C, 60 s at 66°C, 60 s at 72°C, and then two cycles with conditions identical to the previous cycles, but with an annealing temperature of 64°C. During two subsequent cycle sets the annealing temperature was lowered by 2°C until it reached 56°C. Then, an additional 40 cycles each of 20 s at 94°C, 60 s at 57°C, and 60 s at 72°C followed the touchdown program. The PCR was ended by an extra incubation for 7 min at 72°C.

The PCR products were purified with Qiaquick PCR purification kits (Qiagen). For DNA sequencing reactions the fluorescence-labeled dideoxynucleotide technology was used (Perkin-Elmer, Applied Biosystems Division, Nieuwerkerk a/d IJssel, The Netherlands). Sequencing fragments were separated, and data were collected on an ABI 3700 automated DNA sequencer (Perkin-Elmer, Applied Biosystems Division). The sequences were compared with the 16S rRNA gene sequences in the GenBank database by using the Blast program (1).

Discrepancy analysis. All samples (two swab specimens and one urine sample per patient) were tested once initially by both test systems as described above. This resulted for *C. trachomatis* in four test results per patient and for *N. gonorrhoeae* in five test results (including the swab culture for *N. gonorrhoeae*) per patient. Samples from patients who had four concordant results (all positive or all negative) were not retested. If a sample gave results that were discordant for the two test assays, both assays were repeated. Samples with discrepant results for *N. gonorrhoeae* that were solitary and repeatedly positive by only one

assay, even though the other specimens from that participant were negative for *N. gonorrhoeae*, were further studied at the National Institute of Public Health and the Environment by 16S rRNA gene amplification analysis.

Definition of truly infected patients. Patients were considered to be infected with *C. trachomatis* if at least three of four test results were positive, when the two swab tests were positive, or when the two urine tests were positive. Initially indeterminate results with regard to this definition were obtained when a swab specimen and a concomitant urine sample were each positive by only one test. In each of these cases the results of repeat testing were taken into account to determine the truly infected patients, and in all cases it was possible to determine whether the patient was truly infected or not (resolved patient status).

For *N. gonorrhoeae* infection, basically the same rules for the amplification assays were used to determine the resolved patient status. For each patient on *N. gonorrhoeae* culture result was also available. This did not change the resolved patient status as determined by the amplification tests.

Statistical analysis. The tests used are indicated in the text. The McNemar test was used to compare the results of the LCx and the COBAS AMPLICOR systems, and the chi-square test was used to compare the sensitivities and specificities of both assays.

RESULTS

Detection of *C. trachomatis* and *N. gonorrhoeae* using LCx and COBAS AMPLICOR systems. Samples from a total of 1,030 patients arrived at the laboratory, and these samples were tested immediately. For 29 patients (5 men and 24 women) only swab specimens and no urine samples were received. This group did not differ from the total group in clinical aspects. The data for these patients were excluded from further analysis to assert a uniform criterion for determination of the infection status of the patient.

Paired swab and urine samples were available for 498 men and 503 women. The results of first amplification testing for *C. trachomatis* and *N. gonorrhoeae* DNAs in samples with the LCx and COBAS AMPLICOR systems are summarized in Table 1. For those specimens which gave an invalid result or a negative result for the IC and a negative result for *N. gonorrhoeae* and *C. trachomatis* on the first amplification, the result obtained upon retesting was taken. Specimens with invalid results for the IC upon retesting and negative results for both *C. trachomatis* and *N. gonorrhoeae* were considered COBAS AMPLICOR test negative. Combinations of positive and negative test results other than those shown in Table 1 did not occur for any of the patients.

IC of the COBAS AMPLICOR system. The COBAS AMPLICOR test results should be considered valid only if the result for the IC was positive. The use of this IC is optional but was added to study possible inhibition in the amplification reaction. If the result for the IC was negative and the reactions for *C. trachomatis* and *N. gonorrhoeae* were also negative, all three reactions were repeated once. The result for the IC was initially negative for 40 urine specimens (3.9%) and 201 swab specimens (20.0%), irrespective of sex or in which period of the study the specimen was collected. After repeat testing, 32 urine specimens became IC positive and eight urine samples remained IC negative, one of which became *C. trachomatis* positive. A total of 115 swab samples became IC positive, and 86 swab samples remained IC negative on repeat testing, of which 3 swab samples turned out to be *C. trachomatis* and *N. gonorrhoeae* positive, 15 became *C. trachomatis* positive, and 4 were *N. gonorrhoeae* positive. Thus, 64 (6.4%) swab specimens remained after retesting negative for *C. trachomatis*, *N. gonorrhoeae* and the IC; 59 of the 64 specimens were obtained from

TABLE 1. Detection of *C. trachomatis* and *N. gonorrhoeae* DNAs with the LCx and COBAS AMPLICOR systems after initial testing^a

Organism and sex	Specimen	Result with the LCx system	No. of specimens with the following result with the COBAS AMPLICOR system:		
			Positive	Negative	Total
<i>C. trachomatis</i>					
Women	Swab	Positive	58	3	61
		Negative	7	435	442
		Total	65	438	503
	Urine	Positive	49	11	60
		Negative	4	439	443
		Total	53	450	503
Men	Swab	Positive	45	2	47
		Negative	7	444	451
		Total	52	446	498
	Urine	Positive	44	9	53
		Negative	2	443	445
		Total	46	452	498
<i>N. gonorrhoeae</i>					
Women	Swab	Positive	6	0	6
		Negative	13	484	497
		Total	19	484	503
	Urine	Positive	4	2	6
		Negative	7	490	497
		Total	11	492	503
Men	Swab	Positive	21	0	21
		Negative	4	473	477
		Total	25	473	498
	Urine	Positive	19	1	20
		Negative	4	474	478
		Total	23	475	498

^a For specimens with COBAS AMPLICOR negative or invalid results for the IC, *C. trachomatis*, and *N. gonorrhoeae*, all three assays were repeated. The results of the repeat tests are included here.

men and 5 were obtained from women. Of the seven urine samples (0.7%) that remained negative by all three tests, three were collected from women and four were collected from men. These 7 urine specimens and 64 swab samples were designated to be negative for *C. trachomatis* and *N. gonorrhoeae* with the COBAS AMPLICOR system. This seems permitted, as the LCx test results for the latter samples were also negative.

Resolved patient status for *C. trachomatis* and *N. gonorrhoeae*. To summarize the pathogen detection rates in the swab specimens and the urine specimens relative to the resolved patient status, the data are presented in Table 2 by assay used. The prevalences for *C. trachomatis* in women and men were 12.5% (63 of 503) and 10.0% (50 of 498), respectively. The prevalences for *N. gonorrhoeae* in women and men were 1.2% (6 of 503) and 4.2% (21 of 498), respectively.

For the detection of *N. gonorrhoeae* differences between the two assay systems were seen for the female endocervical and

TABLE 2. Initial test results for detection of *C. trachomatis* and *N. gonorrhoeae* DNAs in swab and urine samples with the LCx and COBAS AMPLICOR systems^a

Organism	Result with the:				Culture result	No. of patients		Resolved patient status ^b
	LCx system		COBAS			Women	Men	
	Swab	Urine	Swab	Urine				
<i>C. trachomatis</i>	+	+	+	+		44	40	Pos
	+	+	+	−		7	2	Pos
	+	−	+	+		3	2	Pos
	−	+	+	+		3	3	Pos
	+	−	+	−		4	1	Pos
	−	+	−	+		2	1	Pos
	+	+	−	−		1	0	Neg
	−	−	+	+		1	0	Neg
	−	+	+	−		0	1	Pos
	−	−	+	−		3	3	Neg
	−	+	−	−		3	6	Neg
	+	−	−	−		2	2	Neg
	−	−	−	−		430	437	Neg
						503	498	Total
<i>N. gonorrhoeae</i>	+	+	+	+	+	2	16	Pos
	+	+	+	+	−	2	3	Pos
	+	+	+	−	+	1	1	Pos
	+	+	+	−	−	1	0	Pos
	+	−	+	+	+	0	1	Pos
	−	−	+	+	−	2	1	Neg
	−	−	−	+	−	5	2	Neg
	−	−	+	−	−	11	3	Neg
	−	−	−	−	−	479	471	Neg
					503	498	Total	

^a For specimens with negative results for the IC, *C. trachomatis*, and *N. gonorrhoeae* with the COBAS AMPLICOR system, all three assays were repeated.

^b For *C. trachomatis*, resolved patient status is according to repeat testing and definition as mentioned in the text. For *N. gonorrhoeae*, resolved patient status is according to discrepancy analysis including repeat testing and definition as described in the text. Pos, positive; Neg, negative.

male urethral swab specimen that were positive for *N. gonorrhoeae* with the COBAS AMPLICOR system only ($n = 11$ and $n = 3$, respectively), while the corresponding specimens from these patients were *N. gonorrhoeae* negative with the LCx system and by culture. Retesting of these swabs with both the LCx and COBAS AMPLICOR systems resulted in seven swab specimens negative with both systems, whereas the other seven remained COBAS AMPLICOR system positive and LCx system negative. These specimens were further analyzed at the National Institute of Health and the Environment by a *Neisseria* genus-specific 16S rRNA PCR. Aside from these specimens, 10 cervical swab specimens from women negative for *N. gonorrhoeae* with both the LCx and the COBAS AMPLICOR systems and 4 cervical swab specimens from women positive for *N. gonorrhoeae* with both the LCx and the COBAS AMPLICOR systems were analyzed. The specimens positive for *N. gonorrhoeae* with only the COBAS AMPLICOR system were also positive by this PCR. Sequencing of the amplicons from this PCR revealed neither *N. gonorrhoeae* nor other *Neisseria* spp. but did reveal sequences belonging to members of the *Lactobacillus* group, while the sequences of the positive control were of the expected *N. gonorrhoeae* origin. This indicates that a large amount of lactobacillus DNA can lead to false priming of the *Neisseria* genus-specific primer. In another experiment the specimens that were positive and negative for *N. gonorrhoeae* with the LCx and COBAS AMPLICOR systems, respectively, were tested. Three of the four specimens positive for *N. gonorrhoeae* with the two systems yielded suffi-

cient DNA for sequencing. Two of the three sequenced PCR products were of *N. gonorrhoeae* origin, and one specimen that was weakly positive on a gel yielded a sequence that was not of *Neisseria* origin. Of the 10 specimens negative for *N. gonorrhoeae* with the LCx and COBAS AMPLICOR systems, 3 specimens gave sufficient PCR product to be sequenced. These sequences did also not belong to *Neisseria* spp. but were closely related to an exotic bacterium called *Megasphaera elsdenii*.

Three participants had both swab specimens and urine which were positive for *N. gonorrhoeae* with the COBAS AMPLICOR system only. Repeat testing of the six specimens (three swab and three urine specimens) with the COBAS AMPLICOR and LCx systems yielded five (three swab and two urine specimens) with positive results with the COBAS AMPLICOR system that could not be confirmed by testing by the 16S rRNA PCR at the National Institute of Health and the Environment. Other discrepant results concerned five urine samples that were positive for *N. gonorrhoeae* only with the COBAS AMPLICOR system but that were negative by all other tests. Repeat testing showed that all five were negative for *N. gonorrhoeae* with the COBAS AMPLICOR system.

Statistical testing revealed that the LCx and COBAS AMPLICOR systems did not differ significantly in performance for the detection of *C. trachomatis* ($P > 0.05$ by the McNemar test). However, for testing for *N. gonorrhoeae* in female swab and female urine specimens, there was a significant difference ($P < 0.05$). Taking into account the results of the discrepancy analysis for the assessment of truly infected

patients, the sensitivity, specificity, positive predictive value, and negative predictive value could be calculated. These percentages are shown in Table 3.

Detection of *C. trachomatis* and *N. gonorrhoeae* in different specimens: swab versus urine samples. For both test formats the sensitivity for the detection of *C. trachomatis* was somewhat lower for female urine specimens than for cervical swab specimens (Table 3). The sensitivity of the COBAS AMPLICOR system with male urine specimens was also less sensitive than that with male urethral swab specimens. No statistically significant difference was found between the results for cervical or urethral swab samples and urine samples with respect to specificity and predictive values of positive or negative tests.

With regard to *N. gonorrhoeae*, only the sensitivity of the COBAS AMPLICOR assay with urine samples was lower in comparison with that with cervical swab specimens (Table 3). The predictive value of a positive test result for *N. gonorrhoeae* with the COBAS AMPLICOR system was remarkably low for female cervical swab and urine specimens.

DISCUSSION

The main results of the present study were that the sensitivities of both assays did not differ significantly for the detection of *C. trachomatis*, irrespective of the type of specimen used. Furthermore, urine samples instead of male urethral or female endocervical swab specimens appeared to be reliable specimens for the detection of *C. trachomatis* by both assays for men and women. However, the sensitivity of the COBAS AMPLICOR system for the detection of *N. gonorrhoeae* in urine specimens obtained from women was significantly lower than that of the LCx system. The specificity and the predictive value of a positive test result with COBAS AMPLICOR system for the detection of *N. gonorrhoeae* in female cervical swab and urine specimens was an even greater problem, as in 17 female and male swab specimens negative for *N. gonorrhoeae* with the LCx system, an initial COBAS AMPLICOR system signal indicating positivity for *N. gonorrhoeae* was found. After repeat testing by both assays, the COBAS AMPLICOR system remained positive for 10 of 17 swab specimens, whereas all 17 remained negative with the LCx system. These specimens were also tested by a *Neisseria* genus-specific 16S rRNA PCR at the National Institute of Health and the Environment. By sequencing of the amplimers of this in-house 16S rRNA PCR, these specimens could not be confirmed to contain *N. gonorrhoeae*-specific DNA, but by sequence analysis the amplimers turned out to be *Lactobacillus* in origin. The 16S rRNA PCR with the 4 specimens positive for *N. gonorrhoeae* with both the LCx and the COBAS AMPLICOR systems and the 10 specimens negative for *N. gonorrhoeae* with both systems was carried out later, and the results might have been influenced by the repeated freezing and thawing of these specimens. The results for the specimens that were negative for *N. gonorrhoeae* with the LCx and COBAS AMPLICOR systems demonstrate the potential errors in the amplification process of the 16S rRNA PCR with *Neisseria* genus-specific primers.

As reported by Farrell (4), the COBAS AMPLICOR test did yield a high proportion of false-positive results for specimens selected from a population with a high prevalence (4). In that study, the false-positivity rate was caused by the presence of

Neisseria subflava. However, strains of *Lactobacillus* were not included in that study to assess the sensitivities and specificities of the PCR methods. As advocated by Farrell (4), our study was a local evaluation of both commercially available nucleic acid amplification-based diagnostic tests. On the basis of the results of this local evaluation, we think that the positive results obtained with the COBAS AMPLICOR system were not caused by cross-reacting *Neisseria* species but were caused by the presence of a *Lactobacillus* species. In contrast to the population in the study of Farrell (4), the specimens in the present study were collected from an STD clinic population with a low prevalence of *N. gonorrhoeae* infection. The predictive value of a positive result for *N. gonorrhoeae* by the COBAS AMPLICOR test was especially low for endocervical and female urine specimens, to such an extent that this test is not suitable for screening. If this test is used for the detection of *N. gonorrhoeae*, a reliable confirmatory test is mandatory.

The possible cause of the different results of both assays for the detection of *N. gonorrhoeae* might be the choice of primers. In the LCx system the primers are derived from the multi-copy *opa* genes (2, 8). The primers used in the COBAS AMPLICOR system are directed against the cytosine DNA methyltransferase gene of *N. gonorrhoeae* (9a). As we expected that false-positive test results would probably be the result of the presence of species other than *N. gonorrhoeae* species, we had chosen a *Neisseria* genus-specific 16S rRNA primer set in order to be able to sequence the amplimers.

Reproducibility and inhibitory problems have been described previously for nucleic acid amplification tests (6, 7, 9, 11, 13). In the present study a semiautomated format was used and fewer problems with reproducibility could be expected (7, 9, 13). After repeat testing of inhibitory urine specimens and swab specimens, the frequency of inhibition decreased appreciably, from 3.9 to 0.7% and from 20 to 6.4%, respectively. Repeat testing of specimens with discrepant results gave the same result with the COBAS AMPLICOR system for 61% of the specimens and the same result with the LCx system for 75% of the specimens. The reproducibility for the COBAS AMPLICOR system was higher with regard to *C. trachomatis* (71%) than *N. gonorrhoeae* (47%), whereas this was the other way around for the LCx system (57% for *C. trachomatis* and 100% for *N. gonorrhoeae*).

The use of the IC in the COBAS AMPLICOR system is advocated as a tool to get reliable negative results. In our study the initial frequency of inhibition for urine specimens was lower than that for swab specimens collected in the prescribed transport buffer solution: 3.9% for urine specimens versus 20.0% for swabs specimens. This was in contrast to a prevalence of inhibitory specimens of 0.9% for the endocervical swab specimens and 6.7% for urine specimens from women (overall, 2.9%), as reported by Vincelette et al. (13), or an overall frequency of inhibition of 2.4%, as reported by Van der Pol et al. (12). However, in both studies a chlamydial culture transport medium (2SP, SGP, or M-4) was used instead of the specimen transport buffer supplied by Roche Diagnostic systems. In our opinion, the initial high rate of inhibition for endocervical swab and male urethral swab specimens in our study was mainly caused by the specimen transport buffer itself. This buffer solution contains inhibitors such as sodium dodecyl sulfate that must be neutralized by incubation with specimen

TABLE 3. Evaluation of *C. trachomatis* and *N. gonorrhoeae* detection with the LCx and COBAS Amplicor systems using infected patient status as the reference standard^a

Organism and system	Sex	Sample	Sensitivity ^b	% (95% CI)	Specificity ^b	% (95% CI)	PPV ^b	% (95% CI)	NPV ^b	% (95% CI)
<i>C. trachomatis</i>										
LCx system	Women	Swab	58/63	92.1 (81.7–97.0)	437/440	99.3 (97.8–99.8)	58/61	95.1 (85.4–98.7)	437/445	98.2 (97.2–99.6)
		Urine	56/63	88.9 (77.8–95.0)	436/440	99.1 (97.5–99.7)	56/60	93.3 (83.3–97.8)	436/443	98.4 (96.6–99.3)
	Men	Swab	45/50	90.0 (77.4–96.3)	446/448	99.6 (98.2–99.9)	45/47	95.7 (84.3–99.3)	446/451	98.9 (97.3–99.6)
		Urine	47/50	94.0 (82.5–98.4)	441/448	98.4 (96.7–99.3)	47/54	87.0 (74.5–94.2)	441/444	99.3 (97.9–99.8)
COBAS AMPLICOR system										
	Women	Swab	61/63	96.8 (88.0–99.4)	436/440	99.1 (97.5–99.7)	61/65	93.8 (84.2–98.0)	436/438	99.5 (98.2–99.9)
		Urine	52/63	82.5 (70.5–90.6)	439/440	99.8 (98.5–100.0)	52/53	98.1 (88.6–99.9)	439/450	97.6 (95.5–98.7)
	Men	Swab	49/50	98.0 (88.8–99.9)	444/448	99.1 (97.6–99.7)	49/53	92.5 (80.9–97.6)	444/445	99.8 (98.6–100.0)
		Urine	46/50	92.0 (79.9–97.4)	448/448	100 (98.9–100.0)	46/46	100.0 (90.4–100.0)	448/452	99.1 (97.6–99.7)
<i>N. gonorrhoeae</i>										
LCx system	Women	Swab	6/6	100.0 (51.7–100.0)	497/497	100.0 (99.0–100.0)	6/6	100.0 (51.7–100.0)	497/497	100.0 (99.0–100.0)
		Urine	6/6	100.0 (51.7–100.0)	497/497	100.0 (99.0–100.0)	6/6	100.0 (51.7–100.0)	497/497	100.0 (99.0–100.0)
	Men	Swab	21/21	100.0 (80.8–100.0)	477/477	100.0 (99.0–100.0)	21/21	100.0 (80.8–100.0)	477/477	100.0 (99.0–100.0)
		Urine	20/21	95.2 (74.1–99.8)	477/477	100.0 (99.0–100.0)	20/20	100.0 (80.8–100.0)	477/478	99.8 (98.7–100.0)
COBAS AMPLICOR system										
	Women	Swab	6/6	100.0 (51.7–100.0)	484/497	97.4 (95.5–98.5)	6/19	31.6 ^c (13.6–56.5)	484/484	100.0 (99.0–100.0)
		Urine	4/6	66.7 ^c (24.1–94.0)	490/497	98.6 (97.0–99.4)	4/11	36.4 ^c (12.4–68.4)	490/492	99.6 (98.4–99.9)
	Men	Swab	21/21	100.0 (80.8–100.0)	473/477	99.2 (97.7–99.7)	21/25	84.0 (63.1–94.7)	473/473	100.0 (99.0–100.0)
		Urine	20/21	95.2 (74.1–99.8)	474/477	99.4 (98.0–99.8)	20/23	87.0 (65.3–96.6)	474/475	99.8 (98.6–100.0)

^a Abbreviations: CI, confidence interval; PPV, positive predictive value; NPV, negative predictive value.^b Data represent number of specimens positive or negative for the indicator/total number of specimens positive or negative according to infected patient status.^c $P < 0.05$.

diluent for 10 min, as stated in the package insert. However, incubation for at least 16 h (overnight) instead of 10 min yielded much better results. Another explanation related to the specimen transport buffer might be that inhibitory patient material is eluted by the sodium dodecyl sulfate when the swab is not removed within 15 s. This inhibitory material must be unstable, irrespective of the temperature, as the rate of inhibition drops also when the samples are stored at 4 to 8°C. The nature of these postulated inhibitory substances is unknown, to the best of our knowledge. Inhibition rates for urine specimens with the COBAS AMPLICOR system did not differ significantly, in agreement with reports by others (6, 7, 13). As shown by Mahony et al. (7), the detection of *C. trachomatis* in urine specimens was inhibited somewhat less for the LCx system than for the COBAS AMPLICOR system, especially when urine from pregnant women was used. It was remarkable that the majority (59 of 64 [92%]) of the swab specimens that remained negative for *C. trachomatis*, *N. gonorrhoeae*, and the IC were collected from men. These specimens were also negative for *C. trachomatis* and *N. gonorrhoeae* with the LCx system. A hypothetical male inhibitory substance is, in our opinion, not a possible explanation. It might be possible that collection of several swab specimens from men one after the other does not allow sufficient time for removal of each swab after 15 s from the transport tube.

We did not exclude the 64 swab specimens and the 7 urine specimens that remained negative by all amplification assays, including the IC, for the calculation of the test characteristics. Calculation of these values after exclusion of these samples yielded exactly the same sensitivity and predictive value of a positive test result, as all samples belonged to the subgroup of samples negative by all amplifications and also for culture of *No. gonorrhoeae*, while the corresponding samples were also negative. The specificity and predictive value of a negative test result changed only slightly or not at all (data not shown).

In the present study, the sensitivity of the LCx system for the detection of *C. trachomatis* in urine specimens was somewhat higher than that of the COBAS AMPLICOR system, whereas for swab specimens the reverse was the case. The differences were statistically not significant.

Since the COBAS AMPLICOR and LCx systems did not differ significantly for the detection of *C. trachomatis* in men and women, it was possible to test whether the specimen has an influence on the detection of *C. trachomatis* DNA. When looking at the sensitivity, 119 positive endocervical swab specimens and 94 positive male urethral swab specimens were recorded, whereas 108 positive female urine specimens and 93 positive male urine specimens were recorded, for a total of 2,002 (LCx system plus COBAS AMPLICOR system) amplification test results; these figures are not significantly different ($P > 0.05$ by the Fisher exact test).

The sample preparation for the LCx system was less time-consuming than that for the COBAS AMPLICOR system, both for urine samples and for swab specimens. The preparation of one run of 24 swab specimens for the COBAS AMPLICOR system takes 45 min, and that for 24 urine specimens takes 90

min. One technician can process 24 swab specimens for the LCx system in 30 min and 24 urine specimens for the LCx system in 60 min. The amplification and detection step for 24 specimens with the COBAS AMPLICOR system take 3.5 to 4 h, while one technician needs the same time for 24 or 48 specimens assayed with the LCx system.

We concluded that the performances of both the COBAS AMPLICOR system and the Abbott LCx system were equivalent for the detection of *C. trachomatis*. However, for the detection of *N. gonorrhoeae* the results of the COBAS AMPLICOR assay are false positive too often for it to be a suitable test for use with endocervical swab specimens or urine samples obtained from women.

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REFERENCES

- Altschul, S. F., T. L. Madden, A. A. Schäffer, J. Zhang, Z. Zhang, W. Miller, and D. J. Lipman. 1997. Gapped BLAST and PSI-Blast: a new generation of protein database search programs. *Nucleic Acids Res.* **25**:3389–3402.
- Birkenmeyer, L., and A. S. Armstrong. 1992. Preliminary evaluation of the ligase chain reaction for specific detection of *Neisseria gonorrhoeae*. *J. Clin. Microbiol.* **30**:3089–3094.
- Buimer, M., G. J. J. van Doornum, S. Ching, P. G. H. Peerbooms, P. K. Plier, D. Ram, and H. H. Lee. 1996. Detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* by ligase chain reaction-based assays with clinical specimens from various sites: implications for diagnostic testing and screening. *J. Clin. Microbiol.* **34**:2395–2400.
- Farrell, D. J. 1999. Evaluation of AMPLICOR *Neisseria gonorrhoeae* PCR using *cppB* nested PCR and 16S rRNA PCR. *J. Clin. Microbiol.* **37**:386–390.
- Gaydos, C. A., M. R. Howell, B. Pare, K. L. Clark, D. A. Ellis, R. M. Hendrix, J. C. Gaydos, K. T. McKee, and T. C. Quinn. 1998. *Chlamydia trachomatis* infections in female military recruits. *N. Engl. J. Med.* **339**:739–744.
- 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. P. Verkooyen. 1997. Comparison of three commercially available amplification assays, AMP CT, LCx, and COBAS AMPLICOR, for the detection of *Chlamydia trachomatis* in first-void urine. *J. Clin. Microbiol.* **35**:2628–2633.
- Mahony, J., S. Chong, D. Jang, K. Luinstra, M. Faught, D. Dalby, J. Sellers, and M. Chernesky. 1998. Urine specimens from pregnant and nonpregnant women inhibitory to amplification of *Chlamydia trachomatis* nucleic acid by PCR, ligase chain reaction, and transcription-mediated amplification: identification of urinary substances associated with inhibition and removal of inhibitory substances. *J. Clin. Microbiol.* **36**:3122–3126.
- Meyer, T. F., C. P. Gibbs, and R. Hass. 1990. Variation and control of protein expression in *Neisseria*. *Annu. Rev. Microbiol.* **44**:451–477.
- Peterson, E. M., V. Darrow, J. Blanding, S. Aarnaes, and L. M. De la Maza. 1997. Reproducibility with the AMPLICOR PCR *Chlamydia trachomatis* test. *J. Clin. Microbiol.* **35**:957–959.
- Purohit, A. P., and S. B. Silver. August 1996. U.S. Patent 5550040.
- Stary, A., Shan-Fun Ching, L. Teodorowicz, and H. Lee. 1997. Comparison of ligase chain reaction and culture for detection of *Neisseria gonorrhoeae* in genital and extragenital specimens. *J. Clin. Microbiol.* **35**:239–242.
- Toye, B., W. Woods, M. Bobrowska, and M. Ramotar. 1998. Inhibition of PCR in genital and urine specimens submitted for *Chlamydia trachomatis* testing. *J. Clin. Microbiol.* **36**:2356–2358.
- 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 the detection of *Chlamydia trachomatis*. *J. Clin. Microbiol.* **38**:1105–1112.
- Vincent, J., J. Schirm, M. Bogard, A.-M. Bourgault, D. S. Luijck, A. Bianchi, P. C. Van Voorst Vader, A. Butcher, and M. Rosenstrauss. 1999. Multicenter evaluation of the fully automated COBAS AMPLICOR PCR test for detection of *Chlamydia trachomatis* in urogenital specimens. *J. Clin. Microbiol.* **37**:74–80.