

Determination of *Chlamydia trachomatis* Prevalence in an Asymptomatic Screening Population: Performances of the LCx and COBAS Amplicor Tests with Urine Specimens

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This study determined the performances of the LCx (Abbott) and COBAS Amplicor (Roche) tests with urine specimens for the detection of *Chlamydia trachomatis* in an asymptomatic screening population. Randomly selected women and men (age range, 15 to 40 years) registered in 20 general practices in Amsterdam, The Netherlands, were invited to participate in this study. Urine specimens ($n = 2,906$; 1,138 specimens from men and 1,717 specimens from women) were tested for *C. trachomatis* by the COBAS Amplicor (Roche) and LCx (Abbott) tests. Samples which were positive by only one assay were subjected to discrepant analyses by a third assay (in-house plasmid PCR). By the LCx assay *C. trachomatis* DNA was detected in urine specimens from 46 of 1,717 women and 29 of 1,138 men, while the COBAS Amplicor detected *C. trachomatis* DNA in 52 and 35 specimens, respectively. When comparing the LCx and COBAS Amplicor tests, 32 test results (20 for women and 12 for men) were discrepant. After discrepant analyses the following sensitivities, specificities, and positive predictive values were found for the LCx and COBAS Amplicor tests: 78.6 versus 98.8%, 99.7 versus 99.9%, and 88.0 versus 95.4%, respectively. No prominent differences were found between men and women with regard to the test performances. After discrepant analyses the overall prevalences of *C. trachomatis* in women and men were 3.0 and 2.8%, respectively. For both women and men the prevalence in the younger age groups was higher than that in the older age groups. In conclusion, the COBAS Amplicor tests shows better diagnostic characteristics than the LCx assay for the detection of *C. trachomatis* in urine specimens from an asymptomatic screening population. In this asymptomatic population the overall prevalence of *C. trachomatis* was 2.9%.

Many urogenital *Chlamydia trachomatis* infections run an asymptomatic course and therefore remain undetected and subsequently untreated. This may result in severe sequelae like pelvic inflammatory disease, tubal scarring, ectopic pregnancy, and tubal infertility (14). Recently, DNA amplification techniques (Amplicor PCR [Roche Diagnostic Systems, Basel, Switzerland] [13, 30] and ligase chain reaction [Abbott Laboratories, North Chicago, Ill.] [6, 15]) and RNA amplification techniques AMPLIFIED Chlamydia Trachomatis Assay [AMP-CT; Gen-Probe] [5, 7] and nucleic acid sequence-based amplification [Organon Teknika] [17, 18]) have been successfully introduced for the diagnosis of *C. trachomatis* infections. They have the advantage of higher sensitivities and specificities compared to those of conventional techniques (3, 25, 26, 29). However, the use of cervical and urethral swabs for screening for *C. trachomatis* infection in an asymptomatic population will result in low participation rates. Therefore, the reliable detection of *C. trachomatis* in urine specimens from both men and women by amplification methods (5, 10, 29) is a major breakthrough. The sensitivities of these assays with urine specimens are higher than those of enzyme immunoassay, direct immunofluorescence assay, and cell culture performed with urethral swabs for men (3, 10, 26, 34). In addition, amplification methods had comparable sensitivities with urine specimens and the corresponding cervical scrapes and urethral swabs from a pop-

ulation with sexually transmitted diseases, making the use of urine specimens a valuable tool for the early detection of *C. trachomatis* infections (23, 34). The two most widely used commercial *C. trachomatis* detection systems are the LCx (Abbott) and the COBAS Amplicor (Roche) tests. These are primarily used for the detection of symptomatic *C. trachomatis* infections in diagnostic settings. It has been suggested that screening programs for the detection of asymptomatic *C. trachomatis* infections should be developed (12, 20, 27, 28). On the basis of the high sensitivities and specificities of the amplification assays and the noninvasive means by which urine specimens can be obtained, these screening programs must be designed for use with urine specimens.

The aim of this study was to compare the performances of the LCx and COBAS Amplicor tests with urine specimens for the detection of *C. trachomatis* in an asymptomatic screening population in Amsterdam, The Netherlands. In addition, discrepant analysis has been performed, and the value of a second confirmatory assay has been investigated. Finally, the prevalences of *C. trachomatis* were established in men and women in different age categories.

MATERIALS AND METHODS

Clinical specimens. Urine specimens ($n = 2,906$; 1,138 specimens from men and 1,771 specimens from women) were obtained from randomly selected women and men (age range, 15 to 40 years) who were registered in 20 general practices in Amsterdam and who were invited to participate in this study. Participants were requested to send both a questionnaire and a 20-ml, first-void, first-stream urine specimen by mail (19). Written instructions emphasized the collection of the first void and the first stream of urine. Urine specimens were well mixed, and 0.5 ml was used for the COBAS Amplicor test, 1 ml was used for the LCx assay, and a 5-ml pellet was used for DNA isolation followed by the in-house PCR (see *C. trachomatis* testing).

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TABLE 1. Inhibition in urine specimens as determined with the internal control included in the COBAS Amplicor assay

| Age range (yr) | Males | | Females | |
|----------------|---|----------------|---|-----|
| | No. of samples with inhibition/total no. of samples | % ^a | No. of samples with inhibition/total no. of samples | % |
| 11-20 | 2/79 | 2.5 | 8/128 | 6.3 |
| 21-25 | 9/157 | 5.7 | 26/284 | 9.2 |
| 25-30 | 7/272 | 2.5 | 32/466 | 6.9 |
| 31-35 | 16/339 | 4.7 | 40/453 | 8.8 |
| 36-40 | 11/288 | 3.8 | 30/386 | 7.8 |
| 11-40 | 45/1,138 | 4.0 | 136/1,717 | 7.9 |

^a Percentage of samples with inhibition relative to the number of persons in the specific age group.

C. trachomatis testing. (i) **COBAS Amplicor PCR.** The COBAS Amplicor PCR was performed according to the instructions of the manufacturer (Roche). In brief, 0.5 ml of wash buffer was added to 0.5 ml of a well-mixed urine specimen in a 1.5-ml tube. The mixture was incubated for 15 min at 37°C, followed by centrifugation at 14,000 rpm (Eppendorf centrifuge model 5415C; Merck, Amsterdam, The Netherlands) for 5 min at room temperature. The supernatant was removed with a 1-ml aerosol-barrier tip to ensure removal of all urine supernatant. The urine pellets were frozen at -20°C for no longer than a week. Subsequently, the pellet was resuspended in 0.25 ml of lysis buffer, vortexed, and incubated for 15 min at room temperature. An equal volume (0.25 ml) of specimen diluent was added immediately after incubation. The tubes were vortexed thoroughly and centrifuged at 14,000 × g for 10 min. Fifty microliters of the supernatant was transferred to the amplification tubes containing 50 µl of amplification mixture. The internal control used to monitor inhibition is introduced into each amplification reaction (in the amplification mixture) and is coamplified with the possible *C. trachomatis* target DNA from the clinical specimen. AM-PIPERASE is incorporated to prevent carryover contamination through the enzyme uracil-N-glycosylase. Urine-buffer solutions were stored at -80°C after testing.

(ii) **LCx test.** The LCx test was performed according to the instructions of the manufacturer (Abbott). Briefly, 1 ml of urine was pipetted in a 1.5-ml tube and the tube was centrifuged at 14,000 rpm (Eppendorf centrifuge model 5415C; Merck) for 15 min. Urine pellets were frozen at -20°C for no longer than a week. Subsequently, the pellet was resuspended in 1 ml of urine resuspension buffer and the tubes were placed in a heating block (95 to 100°C) for 15 min. The samples were tested by the LCx test with 100 µl of the processed urine transferred to individual LCx unit-dose tubes containing 100 µl of the LCx assay mixture for amplification. The LCx system chemically inactivates all specimens at the end of each run to prevent carryover contamination. Urine-buffer solutions were stored at -80°C after testing.

(iii) **In-house PCR.** A filter tube-based isolation method (High Pure PCR Template Preparation Kit; Boehringer Mannheim, Mannheim, Germany) was used to isolate the DNA from the 5-ml urine pellet. A human β-globin PCR was performed to assess DNA quality and possible inhibition as described previously (19). An in-house PCR was performed to detect *C. trachomatis* plasmid DNA as described previously (17), followed by Southern blot hybridization with a specific ³²P-end-labelled internal oligonucleotide probe (17).

Discrepancy analysis. Samples identified as *C. trachomatis* positive or *C. trachomatis* negative by both the LCx and the COBAS Amplicor tests were defined as true positives and true negatives, respectively. For samples which had discrepant results after LCx and COBAS Amplicor testing, the original stored urine sample was subjected to an in-house PCR to detect *C. trachomatis* plasmid DNA (17). Samples which were either *C. trachomatis* positive or *C. trachomatis* negative in two of three assays (LCx test, COBAS Amplicor test, and in-house PCR) were defined as true positives or true negatives, respectively.

The sensitivity, specificity, positive predictive value, and the negative predictive value were calculated as described previously (11).

RESULTS

LCx and COBAS Amplicor test results. By the LCx assay *C. trachomatis* DNA was detected in 46 of 1,717 urine specimens from women and 29 of 1,138 urine specimens from men, while the COBAS Amplicor test detected *C. trachomatis* in 52 and 35 of these urine specimens, respectively.

An internal control was included in the COBAS Amplicor test to monitor inhibition (the LCx test does not provide an internal control). The results are shown in Table 1. Inhibition

was found in 136 (7.9%) of the urine specimens from women, while significantly less inhibition was found in urine specimens from men (45 samples [4.0%]; $P < 0.001$ by chi-square test). In all age groups the inhibition in urine specimens from women was roughly twice as much as that in urine specimens from men. In urine specimens from both women and men, the inhibition was highest in the group consisting of those ages 21 to 25 years (5.7 and 9.2%, respectively), but no age-dependent association was found for inhibition. The greatest difference in inhibition between urine specimens from women and men was found for those ages 26 to 30 years (2.5 versus 6.9%, respectively).

Discrepancy analyses. Since the COBAS Amplicor system includes an internal control for the monitoring of inhibition, contrary to the LCx test, the discrepancy analysis was performed with all samples (including those with inhibition) (see also Discussion).

For all samples with discrepant results (no inhibition was observed in these samples, as assessed by the internal control of the Amplicor assay), DNA was isolated from the original urine specimens and positivity for *C. trachomatis* was determined by an in-house PCR (17). By using serial dilutions of *C. trachomatis* serovar L2, the in-house test was 10 to 100 times more sensitive than the commercial assays, resulting in a detection limit of 1 inclusion-forming unit for the COBAS Amplicor and the LCx tests (identical sensitivity, as confirmed by others (26, 33) and 0.1 to 0.01 inclusion-forming units for the in-house PCR, which is in agreement with sensitivities found previously (17).

When comparing the results of the LCx and COBAS Amplicor tests, 32 test results (20 for women and 12 for men) were discrepant. The samples with discrepant results were defined as true positive if the in-house PCR was positive (two of three tests were positive) and were defined as true negative if the in-house PCR was negative (two of three tests were negative). The results are shown in Table 2. By using the definitions for true positive and true negative, LCx and COBAS Amplicor test performances such as sensitivity and specificity were calculated for the women, the men, and the total population. Results are shown in Table 3. The specificities of both tests for both sexes were greater than 99.5%. The most prominent differences between the LCx and COBAS Amplicor assays for the detection of *C. trachomatis* in this asymptomatic population are the sensitivity (78.6 versus 98.8% for women and men, respectively) and the positive predictive value (88.0 versus 95.4% for women and men, respectively).

Confirmation by either COBAS Amplicor or LCx assay. Since in an actual screening setting confirmation of the results for positive patients by a second independent test is not possible, the value of retesting (by either the COBAS Amplicor or the LCx test) with the same buffer-sample solution as a con-

TABLE 2. Discrepancy analysis for samples with discrepant results by COBAS Amplicor and LCx tests based on the in-house PCR as a third test

| LCx test ^a | COBAS Amplicor test | No. of samples | No. of samples with the following results after discrepancy analysis: | |
|-----------------------|---------------------|----------------|---|---------------|
| | | | True positive | True negative |
| - | + | 22 | 18 | 4 |
| + | - | 10 | 1 | 9 |

^a The LCx assay is a ligase chain reaction.

TABLE 3. Performances of LCx and COBAS Amplicor assays for detection of asymptomatic *C. trachomatis* infections in urine specimens^a

| Subject and test | Sensitivity (%) | Specificity (%) | Positive predictive value (%) | Negative predictive value (%) |
|----------------------|-----------------|-----------------|-------------------------------|-------------------------------|
| Women | | | | |
| LCx | 76.9 | 99.6 | 87.0 | 99.3 |
| COBAS Amplicor | 98.1 | 99.9 | 98.1 | 99.9 |
| Men | | | | |
| LCx | 81.3 | 99.7 | 90.0 | 99.5 |
| COBAS Amplicor | 100 | 99.7 | 91.4 | 100 |
| Women and men | | | | |
| LCx | 78.6 | 99.7 | 88.0 | 99.4 |
| COBAS Amplicor | 98.8 | 99.9 | 95.4 | 99.9 |

^a Prevalence of *C. trachomatis* infection, 2.9%.

firmatory test was investigated for the samples with discrepant results.

Upon retesting, 2 of 32 samples were LCx test negative, COBAS Amplicor test positive, and true positive; both the second LCx test and the second COBAS Amplicor test indicated positivity for *C. trachomatis*. Nine of 32 samples were LCx test positive, COBAS Amplicor test negative, and true negative; both the second COBAS Amplicor test and the second LCx test were negative. The mean LCx test value for these nine samples with discrepant results by the first LCx assay was 904 (range, 572.2 to 1,801), while a strong positive LCx signal was about 2,000. Of the remaining 20 samples with discrepant results, retesting showed that the first and second COBAS Amplicor tests were positive, while the first and second LCx tests were negative and comprised samples with four true-negative results. Finally, for one sample the LCx test was positive both times, whereas the COBAS Amplicor test was negative both times. As determined by the in-house PCR, this sample was determined to be true positive.

***C. trachomatis* prevalences.** The resulting prevalences of *C. trachomatis* in urine specimens from men and women (different age groups and the total population) as determined by the LCx test, the COBAS Amplicor test, and the rate of true positivity after discrepancy analysis are shown in Table 4. The true prevalences of *C. trachomatis* in this asymptomatic screening population were 2.8% for males and 3.0% for females. A clear age-related *C. trachomatis* prevalence (true positives) was

found in both young men and women, who had higher *C. trachomatis* prevalences than the older subjects: for men ages 11 to 25 years versus men ages 26 to 40 years, 3.8% (9 of 236) and 2.5% (23 of 902), respectively; for women ages 11 to 25 years versus women ages 26 to 40 years, 3.6% (15 of 412) and 2.8% (37 of 1305), respectively. The highest prevalence was found in young women ages 11 to 20 years (7.0% [9 of 128]). For men the prevalence in the youngest age group (ages 11 to 20 years) was relatively low (2.5%), most likely due to the low number of male participants included in the study ($n = 79$).

When comparing the *C. trachomatis* prevalences as determined either by the LCx assay or the by COBAS Amplicor test (Table 4) with the true *C. trachomatis* prevalences, the most prominent difference was the lack of detection of *C. trachomatis* in 5 of 11 *C. trachomatis*-positive subjects ages 11 to 20 years by the LCx assay.

DISCUSSION

The present study is the first study to have compared the performances of the COBAS Amplicor and the LCx tests for the detection of *C. trachomatis* with first-void urine specimens from both men and women who were part of an asymptomatic screening population. This study showed that the COBAS Amplicor assay performed better for the detection of *C. trachomatis* in urine specimens from asymptomatic men and women. After discrepancy analysis the true *C. trachomatis* prevalence appeared to be 2.9%.

When the overall *C. trachomatis* prevalences for both men (2.8%) and women (3.0%) were compared, no prominent differences were found in this asymptomatic infected population. However, for both women and men the prevalences in the younger age groups were higher than those in the older age groups (Table 3). This observation is in agreement with those of other studies that have compared *C. trachomatis* prevalences and age (9). When analyzing the samples with discrepant results with respect to age, the most prominent difference between the LCx and the COBAS Amplicor assays was the lack of detection of 5 of 11 *C. trachomatis*-positive subjects in those ages 11 to 20 years by the LCx test. The prevalences in the older age group (ages 30 to 40 years) were still 2.0 and 2.3% for men and women, respectively. This strongly indicates that the use of young age alone as a selection criterion for screening would result in the lack of detection of a considerable number of the *C. trachomatis* infections in this population. Up to now only two studies dealing with *C. trachomatis* prevalences in asymptomatic populations have been published: In asymptom-

TABLE 4. *C. trachomatis* prevalences in men and women as determined by the COBAS Amplicor and LCx tests and after discrepancy analysis

| Age | Males | | | | Females | | | |
|-------|-----------------------|--|----------------|-------------------|----------|--|----------------|----------|
| | <i>n</i> ^a | No. (%) <i>C. trachomatis</i> positive | | | <i>n</i> | No. (%) <i>C. trachomatis</i> positive | | |
| | | LCx | COBAS Amplicor | True ^b | | LCx | COBAS Amplicor | True |
| 11–15 | 14 | 0 (0) | 0 (0) | 0 (0) | 20 | 1 (5.0) | 2 (10) | 2 (10) |
| 16–20 | 65 | 1 (1.5) | 3 (4.6) | 2 (3.1) | 108 | 4 (3.7) | 6 (5.6) | 7 (6.5) |
| 21–25 | 157 | 5 (3.2) | 7 (4.4) | 7 (4.4) | 284 | 8 (2.8) | 7 (2.5) | 6 (2.1) |
| 26–30 | 275 | 12 (4.4) | 12 (4.4) | 10 (3.6) | 466 | 11 (2.4) | 15 (3.2) | 15 (3.2) |
| 31–35 | 339 | 7 (2.1) | 9 (2.7) | 9 (2.7) | 453 | 14 (3.1) | 12 (2.6) | 12 (2.6) |
| 35–40 | 288 | 4 (4.1) | 4 (4.1) | 4 (4.1) | 386 | 8 (2.1) | 10 (2.6) | 10 (2.6) |
| 11–40 | 1,138 | 29 (2.5) | 35 (3.1) | 32 (2.8) | 1,717 | 46 (2.7) | 52 (3.0) | 52 (3.0) |

^a *n*, number of persons and urine specimens.

^b True, positive for *C. trachomatis* after discrepancy analysis.

atic military women (9) and male recruits (29), prevalences of 7.1 and 4.1%, respectively, were reported. These prevalences were higher than those found in our study (2.9%), most likely due to the differences in the study groups investigated, i.e., age and sexual behavior.

The intratest differences between urine samples from asymptomatic infected women and men in relation to the performance of either the LCx or the COBAS Amplicor assay were trivial. The greatest differences between the LCx and the COBAS Amplicor assays were found in the sensitivities (78.8 versus 98.8%) and the positive predictive values (88.2 versus 95.5%) (Table 2). With regard to the positive predictive value, all nine false-positive samples in the LCx assay were true negative after retesting by either the LCx or the COBAS Amplicor assay. Also, in a nested *omp1* PCR (which is as sensitive as the in-house plasmid PCR) used for *C. trachomatis* serovar determinations (in progress), these samples were also *C. trachomatis* negative (data not shown). This indicates that after retesting of all *C. trachomatis*-positive specimens the positive predictive value of the LCx assay would be 100%.

The urine specimens in our study are mailed specimens that were obtained in the home. This approach has been reported before (1, 21) and has been validated with respect to DNA degradation and reliable *C. trachomatis* DNA detection (19). The sensitivities and specificities found in this study are comparable to those found by others. In general, it was found that the sensitivity of the LCx assay with specimens from populations with sexually transmitted diseases (symptomatic *C. trachomatis* infections) is slightly lower than the COBAS Amplicor assay with urine specimens. However, only two studies (9, 29) addressed true asymptomatic populations. In one of those studies (29) the sensitivity of the COBAS Amplicor was reported to be 61.2%, whereas the sensitivity of the LCx assay was 93.1%. However, after freezing and thawing the number of *C. trachomatis*-positive specimens was equal by both assays, but the unexpected high inhibition percentage of 33% for the *C. trachomatis*-positive specimens remained unexplained. The differences in sensitivity reported in the literature (2, 3, 8–10, 22–24, 26, 30) for different assays and urine specimens versus other clinical samples are most likely due to the presence of inhibitors in urine specimens (4, 23, 30, 34), the type of clinical specimen used (urine versus cervical specimen) (23, 30, 31, 34), the patient infection status (4, 8, 23, 34), and the definition of the "gold standard."

The LCx assay detected fewer positive specimens in our study, even though we used twice the processed urine volume compared to the volume used for the COBAS Amplicor assay (100 versus 50 μ l). Since in the COBAS Amplicor test protocol smaller pellets are generated, due to the urine washing step, compared to the size of the pellets generated by the LCx test protocol, more inhibitors could be introduced in the LCx assay. However, it cannot be excluded that LCx assay-specific inhibitors were present since no internal control is present in this assay. In this study the inhibition, as defined by the internal control of the COBAS Amplicor assay, was 4.0% for urine specimens from men and 7.9% for urine specimens from women. These percentages are comparable to those reported by others for urine specimens (10, 23, 24, 30, 32, 34). A recent study by Mahony et al. (16) indicated that the prevalence of nucleic acid amplification inhibitors in urine from women is different for each technology (PCR, ligase chain reaction, and transcription-mediated amplification; range, 2.6 to 7.5%), that this prevalence may be predicted by the presence of urinary factors, and that storage and dilution remove most of the inhibitors. It would be valuable to know the performances of the LCx test, the COBAS Amplicor test, and cell culture for

cervical and urethral swab specimens compared to those for the corresponding urine specimens. However, this invasive means of sample collection was less "friendly" for these asymptomatic men and women and would subsequently lead to low participation rates. As has been reported previously by several groups, cell culture is less sensitive (40 to 80% [8, 10, 22, 23, 26, 34]) than the LCx and the COBAS Amplicor tests with cervical and urethral swab specimens (80% to 100% (2, 3, 23, 30, 34)). Therefore, the use of amplification assays (LCx and COBAS Amplicor assays) with urine specimens is the best way to detect *C. trachomatis* in asymptomatic populations, even though the *C. trachomatis* prevalence found might be a little underrepresented in women.

In conclusion, this study showed that for *C. trachomatis* screening programs with urine specimens from asymptomatic populations, the COBAS Amplicor system outperforms the LCx assay. Furthermore, although the overall true *C. trachomatis* prevalence was 2.9%, a clear age dependency was found for both men and women, with higher prevalences in the younger age groups.

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