

Comparison of the PACE 2 Assay, Two Amplification Assays, and Clearview EIA for Detection of *Chlamydia trachomatis* in Female Endocervical and Urine Specimens

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Screening for sexually transmitted diseases (STDs) in a greater proportion of sexually active patients has become an accepted protocol by most health care providers. The purpose of this study was to compare the current test methods for detection of *Chlamydia trachomatis* used at the University of South Alabama, the PACE 2 assay (Gen-Probe) and the Clearview EIA (Wampole Laboratories), with two amplification technologies, the AMP CT (Gen-Probe) and LCx (Abbott) assays. In addition, a number of demographic parameters were ascertained by asking questions at the time of examination as well as for health care provider concerns and preferences. One urine and four endocervical swab specimens were collected in random order from 787 female patients attending one of four obstetrics-gynecology clinics. Eighty-seven percent of patients had no STD-related symptoms. Patients were considered positive for *C. trachomatis* if three or more assays (swab and/or urine) were positive. Abbott and Gen-Probe confirmed discrepant results by alternate amplified assays. A total of 66 true-positive specimens were detected by use of the combination of endocervical swabs and urine specimens. After discrepant analysis, sensitivities for endocervical swab specimens for the EIA and the PACE 2, LCx, and AMP CT assays were 50, 81, 97, and 100%, respectively. Sensitivities for the LCx and AMP CT assays with urine specimens were 98 and 81%, respectively. The prevalence of *C. trachomatis* was 8.4%, as determined by amplification technology. Overall, the amplification technologies were the most sensitive methods with either swab (AMP CT assay) or urine (LCx assay) specimens. The PACE 2 assay offered the advantage of a simpler and less expensive assay with acceptable sensitivity. The clearview CT EIA, while yielding a rapid in-office result, had unacceptably low sensitivity. The wide variation in performance with amplification assays with urine specimens as reported in both this study and the literature obviates the need to clarify optimal parameters for this specimen type.

In 1996, the most recent year for which figures are available, *Chlamydia trachomatis* and *Neisseria gonorrhoeae* were the two most common sexually transmitted diseases (STDs) reported to the Centers for Disease Control and Prevention (CDC) (11). CDC estimates that more than 4 million new cases of chlamydia and 800,000 new cases of gonorrhea occur every year (17). These numbers likely represent an underestimation of the true prevalence given that these infections may be asymptomatic in women up to 75% of the time for *C. trachomatis* and 50% of the time for *N. gonorrhoeae* (6, 34). Men with *C. trachomatis* infection may also be asymptomatic up to 50% of the time (6). Sequelae of untreated infection are variable. Pelvic inflammatory disease, infertility, ectopic (tubal) pregnancy, increased risk of human immunodeficiency virus infection, chronic pelvic pain, and neonatal disease are all well documented (6, 17, 34). The annual cost for the treatment of complications related to *C. trachomatis* infection is in the millions (6, 12, 34). Thus, CDC is emphasizing the need for a sensitive, specific, and convenient means of diagnosing *C. trachomatis* infection in sexually active persons. In addition, a heightened educational initiative for both physicians and patients to increase the rate of screening for *C. trachomatis* is being encouraged (12, 13).

Classic detection of these pathogens has been by cell culture for *C. trachomatis* and Gram staining with culture on selective

medium for *N. gonorrhoeae*. These detection methods have relied on the collection of one or more endocervical swab specimens as part of pelvic examination for women and urethral swab specimen collection for men and are very susceptible to adequate collection and transport. Currently, nonculture, nonamplification assays such as enzyme immunoassays (EIAs) and probe hybridization assays (Gen-Probe PACE 2 assay) have become the most widely used screening tests. While their costs are reasonable, the sensitivities of these assays are less than those of amplification assays. Recently, multiple amplification assays that allow increased sensitivity and increased specimen stability compared with those of culture methods have become available. An additional advantage of these U.S. Food and Drug Administration-approved assays is that the specimens used for the assay, urine, in addition to swab specimens, can be obtained by noninvasive means. The disadvantages of these amplification methods are cost, lack of confirmatory assays for specimens with positive test results, and increased technical manipulations.

Few published studies on direct comparisons of amplification assays and the hybridization probe test exist. The purpose of this study was to evaluate two amplification methods, ligase chain reaction (LCx assay; Abbott Laboratories, Abbott Park, Ill.) and transcription-mediated amplification (TMA; AMP CT assay; Gen-Probe, San Diego, Calif.), versus the standard test methods used at the University of South Alabama Medical Center (USAMC) (PACE 2 assay [Gen-Probe] for *C. trachomatis* and *N. gonorrhoeae*, Clearview EIA [Wampole Laboratories, Cranbury, N.J.] for *C. trachomatis* and culture for *N. gonorrhoeae* with samples from a population with a moderate

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prevalence of STDs. A second goal of the study was to assess the various test methods in relation to patient demographics, health care provider and laboratory preferences, and overall resources available for testing to encourage increased screening for STDs.

MATERIALS AND METHODS

Study population. A total of 787 consecutive females who attended one of four outpatient obstetrics-gynecology (OB/GYN) clinics from June 1997 to November 1997 at the University of South Alabama were included in this study. Clinics included the Center Street Clinic, Student Health Clinic, Stanton Road Clinic, and the private and Health Maintenance Organization Health Services campus clinics. Demographic information was obtained by the clinic staff for each patient, which included patient age, reason(s) for examination, patient complaints and clinical assessment, antibiotic use within the previous 21 days, and laboratory findings within the previous 21 days. The general population had low to moderate prevalences of *C. trachomatis* and *N. gonorrhoeae* infections (3.0 to 6.5%).

Specimen collection, transport, and processing. Four endocervical swab specimens and a urine specimen were collected from each patient according to package insert instructions for the assays tested. The four-swab specimen collection kits included one for Gen-Probe's PACE 2 assay and one for the AMP CT assay (Gen-Probe), one for the LCx assays (Abbott Laboratories) for *C. trachomatis* and *N. gonorrhoeae*, and one for *N. gonorrhoeae* culture (Starplex Scientific, Ontario, Canada). For a subset of patients ($n = 68$) the Clearview EIA (Wampole Laboratories) was also performed. For these patients, only one Gen-Probe assay swab was collected and was used for the AMP CT assay. The PACE 2 assay was performed with the remainder of the specimen in this specimen collection tube. To avoid sampling bias, equal numbers of the swab collection kits were labeled 1 through 4, packaged, and distributed to the clinics. Specimen collection packets were randomly picked for patient sampling. For urine specimens, the patient must not have urinated 2 h prior to specimen collection. Urine specimens were always collected before swab specimens were collected. Urine specimens were refrigerated after collection. The clinic staff performed the Clearview EIA on-site by their usual protocol. The other swabs and urine specimens were transported in a container with cold packs to the Microbiology Laboratory of USAMC on the same day. Most swab and urine specimens were stored at 4°C for 1 to 3 days prior to processing; the exception was swabs for *N. gonorrhoeae* culture, which were set up for culture upon arrival in the laboratory. Prior to any testing, an aliquot of the urine specimen was removed, placed in a sterile tube, and stored at -20°C. These aliquots were used for the discrepant analysis performed by the manufacturers. For swab specimens, the presence of blood and/or mucus was recorded.

***C. trachomatis* detection.** Five tests were performed for *C. trachomatis* detection. The swab specimens were tested by the PACE 2 and/or Clearview assay, the AMP CT assay, and the LCx assay. The urine specimens were tested by the AMP CT and LCx assays. The assays were performed according to the instructions of the manufacturers and have been described in detail previously (7, 13, 14, 28). A brief description of each assay is presented here.

(i) Clearview EIA. The Clearview EIA was the test used by USAMC OB/GYN clinics at the time of the study. This is a rapid EIA with visual result interpretation in result and control windows. Chlamydial antigen is extracted from the specimen by heating the swabs at 80°C for 10 to 12 min in the extraction buffer. After the swab is discarded the sample is cooled at room temperature for 5 min. The extracted antigen is then added to the sample window containing a latex-labeled murine monoclonal antibody directed against chlamydial lipopolysaccharide. The results are read after 15 min. If the *C. trachomatis* antigen is present, the extract-latex complex would migrate to the result window, be captured by another immobilized antichlamydial antibody to form a sandwich, and produce a line in the result window. The excess latex-labeled antibody would migrate to the control window, complex with immobilized rabbit anti-mouse antibody, and form a line there. The presence of a line in the result window is interpreted as a positive result.

(ii) Gen-Probe PACE 2 assay. The PACE 2 assay has been used at the USAMC Microbiology Laboratory only to screen patients attending the emergency clinic for *C. trachomatis* and *N. gonorrhoeae* infection. In this DNA-rRNA hybridization assay, the acridinium ester-labeled DNA probe is hybridized to a specific sequence of chlamydial 16S rRNA. The test results are expressed in relative light units (RLU). Three negative reference controls and one positive control were included in each run. In the present study, the cutoff for a positive assay was the mean for the negative reference controls plus 200 RLU. If the difference between the sample RLU and the mean for the negative controls was between 0 and 200 RLU, the specimen was retested in duplicate.

(iii) Gen-Probe AMP CT assay. The Gen-Probe AMP CT TMA system amplifies a specific chlamydial 23S rRNA target via DNA intermediates. The amplified RNA product (amplicon) is detected by hybridization with a complementary acridinium ester-labeled DNA probe. For urine specimens, 1.5 ml was pipetted into a microcentrifuge tube. The tubes were incubated at 37°C for 10 or 20 min and were then centrifuged at 10,000 × *g* for 5 min. The supernatants were forcefully decanted, and the remaining liquid was blotted. The pellet was resuspended in 200 μl of specimen dilution buffer. Prepared urine specimens were

used for amplification within 2 h as instructed in the package insert. For swab specimens, the swabs remained in the transport tube. Swab specimens were first centrifuged at 400 × *g* for 5 min. The specimen processing reagent was then added to the tubes and the tubes were incubated at 60°C for 10 min, after which 20 μl was transferred to another tube containing specimen dilution buffer. The remainder of the assay was performed according to the manufacturer's directions. After amplification and hybridization, the tubes were read by a Gen-Probe LEADER50 luminometer, with the results expressed in RLU. Specimens yielding >500,000 RLU were considered positive, specimens yielding <40,000 RLU were considered negative, and specimens with ≥40,000 but ≤500,000 RLU were considered equivocal and were retested in duplicate. Specimens yielding ≥50,000 RLU on retesting were considered positive. One negative control and one positive control were included in each run of up to 48 specimens.

(iv) LCx assay for *C. trachomatis*. In the Abbott LCx assay, the target is the multicopy cryptic plasmid found in all serovars of *C. trachomatis*. For urine specimens, 1.0 ml was pipetted into a microcentrifuge tube, the tube was centrifuged at 10,000 × *g* for 15 min, and the supernatant was discarded. The pellet was then resuspended in 1 ml of LCx assay urine specimen resuspension buffer, and the suspension was heated at 97°C for 15 min and then cooled at room temperature for 15 min. Swab specimens were heated and cooled in the same way as the urine specimens. After cooling, the swabs were discarded. One hundred microliters of the processed urine or swab specimen was added to a unit-dose tube containing the LCx assay amplification mixture. The remainder of the assay was performed according to the manufacturer's directions. Two negative and two calibrator vials were included in each run of up to 20 specimens. The results were expressed as a sample rate/cutoff value (S/CO) ratio. The cutoff value was the mean rate for the LCx assay calibrator duplicates multiplied by 0.45. An S/CO ratio of ≥1.00 was considered positive, and an S/CO ratio of <0.80 was considered negative. An S/CO ratio of 0.80 to 0.99 was considered equivocal and the sample was retested in duplicate. An S/CO ratio of ≥1.00 on retesting was considered positive, and one of <1.00 was considered negative.

Discrepant analysis. When there was a discrepancy in any of the five test results (PACE 2 assay, AMP CT assay with swab and urine specimens, and LCx assay with swab and urine specimens), the test(s) was repeated in our laboratory in duplicate. Repeat testing of samples with discrepant Clearview EIA results was not performed. The manufacturers performed further discrepant analysis. An alternative TMA assay targeting the 16S rRNA of *C. trachomatis* was used by Gen-Probe for discrepant analysis. In addition to the same LCx assay procedure, Abbott used an alternative LCx assay targeting the major outer membrane protein of *C. trachomatis* for discrepant analysis. It should be noted that discrepant analysis of a sample with a positive result by an alternate amplification assay only confirms that the target was present in the sample. The presence of the target does not clarify if contamination of a specimen occurred at the site of collection.

Definition of true positive, false positive, and false negative. If three or more of the five tests were positive, the specimen was considered to be a true positive and the negative tests for the specimen were considered false negatives. A false-positive result was assigned if only one of the five test results was positive and was not confirmed by any alternate assay. The exception to the above was when only the urine specimen was positive and all swab specimens were negative. The urine was considered a true positive if both the TMA and LCx assays with urine were positive and/or the manufacturers' alternate assays were positive. No other combinations of results, i.e., two of five tests were positive and the three others were negative, occurred.

RESULTS

Testing for *C. trachomatis*. A total of 787 endocervical swab specimen sets were received for the study, and for 747 of these specimens, companion urine specimens were submitted for testing. The majority of specimens were tested by all of the assays being compared with the exception of Clearview EIA, by which only 68 swab specimens were tested. Results for the specimen sets that were not tested by at least three assays were excluded from the data analysis. This occurred with two sets.

Comparison of Clearview EIA with LCx and AMP CT assays with swab specimens. The Clearview EIA was performed with 68 of the endocervical specimen sets, of which 65 were also tested by both the LCx and the AMP CT assays. In addition, 19 of them were tested by the PACE 2 assay. The total number of confirmed positive patients (see Materials and Methods for definition of true positive) among the patients whose specimens were tested by EIA was 10. Both the LCx and the AMP CT assays detected infection in all 10 of the positive patients, while the Clearview EIA detected infection in only 5 patients. The PACE 2 assay detected infection in 7 of the 10 positive patients. It should be noted that all 10 of these patients were

TABLE 1. Comparison of EIA and LCx and AMP CT assays for detection of *C. trachomatis* in 65 endocervical swab specimens

Result by the following assay ^a :			No. of specimens
Clearview EIA	Abbott LCx	Gen-Probe AMP CT	
Pos	Pos	Pos	5
Neg	Pos	Pos	5
Neg	Neg	Neg	55

^a Pos, positive; Neg, negative. By the Clearview EIA and the LCx and AMP CT assays, 5 (50%), 10 (100%), and 10 (100%) of the specimens were positive, respectively (percentages were calculated as [number of positive specimens detected/total number of positive specimens] × 100).

confirmed to be positive for *C. trachomatis* by testing of their urine specimens also. The remaining 55 specimens were negative by all tests. Table 1 provides the results for the 65 specimens tested by EIA and the LCx and AMP CT assays.

Comparison of PACE 2, LCx, and AMP CT assays with swab specimens. Of the 787 swab specimen sets received, 738 sets were tested by the three assays, the PACE 2, LCx, and AMP CT assays. There were 62 confirmed positive cases of infection in these 738 sets. The PACE 2 assay detected infection in 50 (80.6%) of the 62 sets of positives specimens, the LCx assay detected infection in 60 (96.8%) of the specimens, and the AMP CT assay detected infection in all 62 (100%) of the specimens. Discrepant analysis by an alternate amplification method was not performed for PACE 2 assay-negative, amplification-positive specimens by Gen-Probe, although this may have helped answer any question about specimens that were false positive by the amplification assay. One of the two AMP CT assay-positive, LCx assay-negative specimens was found by Abbott to be negative on retesting. For the other apparent false-negative specimen, the undiluted sample tested negative but the sample diluted 1:2 was positive, as determined by Abbott. These two swab specimens were considered false negative by the LCx assay because the AMP CT and LCx assay results with urine were also positive. The S/CO ratios for these two specimens were 0.23 and 0.04, respectively. By each of the three assays one specimen had a false-positive result. Table 2 compares the results for the 738 specimen sets.

For the PACE 2 assay, the RLU for the 11 false-negative specimens ranged from 41 to 532, with an average of 217. Only the specimen with 532 RLU would have fallen within the criteria for retesting. The average RLU for samples positive by the PACE 2 assay was 20,520.

Comparison of LCx and AMP CT assays with urine specimens. A total of 747 urine specimens were tested for *C. trachomatis*. Sixty-two specimens were confirmed to be positive; of these, the LCx assay detected 61 (98.4%) and the AMP CT assay detected 50 (80.6%). The one LCx assay-negative, AMP CT assay-positive specimen was positive on retesting and was considered to be false negative by the LCx assay. Swab specimens from this set of specimens were also positive. Six of the 12 LCx assay-positive, AMP CT assay-negative urine specimens tested negative by an alternative AMP CT assay performed by Gen-Probe. Swab specimens from these six sets of specimens tested positive for *C. trachomatis* by both the LCx assay and TMA. The other six LCx assay-positive, AMP CT assay-negative urine specimens tested positive by an alternate amplification assay performed by Gen-Probe. Swab specimens from four of these specimen sets were positive. The results for urine specimens are presented in Table 3. The false-negative LCx assay result was clearly negative, with an S/CO ratio of 0.03, compared to the average S/CO ratio for positivity of 2.94.

TABLE 2. Comparison of PACE 2, LCx, and AMP CT assays for detection of *C. trachomatis* in 738 endocervical swab specimens^a

Result by the following assay ^b :			No. of specimens
Gen-Probe PACE 2	Abbott LCx	Gen-Probe AMP CT	
Pos	Pos	Pos	50
Neg	Pos	Pos	10
Neg	Neg	Pos	2
Neg	Neg	Neg	673

^a There were three false-positive specimens, one by each assay, for which data are not presented.

^b Pos, positive; Neg, negative. By the PACE 2, LCx, and AMP CT assays, 50 (80.6%), 60 (96.8%), and 62 (100%) of the specimens were positive, respectively (percentages were calculated as described in footnote a of Table 1).

The range of RLU for urine specimens with false-negative results by TMA was from 4,113 to 21,260, and the average was 9,164 RLU. The average RLU for urine specimens with positive results by TMA was 2,617,508.

Comparison of results for swab and urine specimens. When the results obtained with swab and urine specimens were combined, there were 66 confirmed positive cases of *C. trachomatis* infection. Sixty-two of the infections were detected with swab specimens only, and four were detected with urine specimens only. For 58 of the 62 swab specimens that were positive, the corresponding urine specimens were also positive. For three of the positive swab specimens, a corresponding urine specimen was not available for testing. For one swab specimen that was positive for *C. trachomatis*, the corresponding urine specimen was negative. Among the 62 urine specimens that were positive for *C. trachomatis* by all assays, the corresponding swab specimens were negative for 4 specimens. Thus, approximately 6% of *C. trachomatis* infections were detected only with urine.

Performance characteristics of EIA, and PACE 2, LCx, and AMP CT assays. The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of each assay are listed in Table 4. For swab specimens, the sensitivities of EIA and the PACE 2, LCx, and AMP CT assays were 50, 80.6, 96.8, and 100%, respectively. Specificities with swab specimens were 100% for EIA and 99.9% for the PACE 2, LCx, and AMP CT assays. PPVs for these assays were 100% (EIA), 98.0% (PACE 2 assay), 98.4% (LCx assay), and 98.4% (AMP CT assay). NPVs for these assays were 91.7% (EIA), 98.3% (PACE 2 assay), 99.7% (LCx assay), and 100% (AMP CT assay). For urine specimens, the sensitivity, specificity, PPV, and NPV were 98.4, 100, 100, and 99.9%, respectively, for the LCx assay and 80.6, 100, 100, and 98.2%, respectively, for the AMP CT assay.

Association between test results for *C. trachomatis* infection and specimen appearance. No differences were seen between

TABLE 3. Comparison of LCx and AMP CT assays for detection of *C. trachomatis* in 747 urine specimens

Result by the following assay ^a :		No. of specimens
Abbott LCx	Gen-Probe AMP CT	
Pos	Pos	49
Pos	Neg	12
Neg	Pos	1
Neg	Neg	685

^a Pos, positive; Neg, negative. By the LCx and AMP CT assays, 61 (98.4%) and 50 (80.6%) of specimens were positive, respectively (percentages were calculated as described in footnote a of Table 1).

TABLE 4. Performance characteristics of EIA and PACE 2, LCx, and AMP CT assays for detection of *C. trachomatis*

Specimen and assay	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Endocervical swabs				
Clearview EIA	50 (5/10) ^a	100 (55/55)	100 (5/5)	91.7 (55/60)
Gen-Probe PACE 2	80.6 (50/62)	99.9 (675/676)	98.0 (50/51)	98.3 (675/687)
Abbott LCx	96.8 (60/62)	99.9 (675/676)	98.4 (60/61)	99.7 (675/677)
Gen-Probe AMP CT	100 (62/62)	99.9 (675/676)	98.4 (62/63)	100 (677/677)
Urine				
Abbot LCx	98.4 (61/62)	100 (685/685)	100 (61/61)	99.9 (685/686)
Gen-Probe AMP T	80.6 (50/62)	100 (685/685)	100 (50/50)	98.2 (685/697)

^a Values in parentheses are number of specimens positive by the assay/total number of specimens tested.

the appearance (presence of blood) of the specimens in relation to either positive patients and/or inaccurate results. Of the 787 swab specimens evaluated, 131 (16.6%) were bloody, and 25 (3.2% of all specimens) of those 131 specimens were grossly bloody. None of these 131 swabs were false positive or false negative for *C. trachomatis* by the PACE 2, LCx, or AMP CT assay.

DISCUSSION

Nonculture, nonamplification methods such as EIAs and probe hybridization assays (PACE 2 and Gen-Probe assays) have become the most widely used screening tests for the detection of *C. trachomatis* infection in recent years (4). The three nucleic acid (NA) amplification assays approved by the U.S. Food and Drug Administration, PCR, ligase chain reaction (LCx assay), and TMA (AMP CT assay), are gaining wider use because they have been shown to be more sensitive than nonamplification tests and culture (5, 8, 14, 15, 35, 41). Additionally, these amplification assays offer an additional advantage in that urine can be used as an alternative specimen for testing. A number of reports comparing the performance of these NA amplification assays to each other, cell culture, or nonamplification assays have been published, and the data have been summarized (6). However, few reports on same-specimen comparison of the PACE 2 test and amplification methods have been published, and to date, only one report has compared the AMP CT and LCx assays with both endocervical swab and urine specimens (39). In addition, most of the early reports of amplification assays have been of studies performed with populations with a high prevalence of STDs and not in routine-care OB/GYN clinic settings.

In the present study, NA amplification assays, the Abbott LCx assay and the Gen-Probe AMP CT assay, were compared to Clearview EIA and the Gen-Probe PACE 2 assay for the detection of *C. trachomatis* in female endocervical swab specimens. The LCx and AMP CT assays were also compared for the detection of *C. trachomatis* in urine specimens.

The prevalence of *C. trachomatis* infection was 8.4% in the population studied. The average age of the patients was 25.6 years (age range, 13 to 60 years). The primary reasons for attending the clinics were prenatal care (39.4%) and routine annual examination (22.7%). The percentage of patients with no symptoms consistent with STDs by patient complaint was 87% for patients both positive and negative for *C. trachomatis* infection. A total of 66 true-positive infections were detected. For 738 endocervical swab specimens, the NA amplification assays had the best sensitivities (LCx assay, 96.8%; AMP CT assay, 100%). The Clearview EIA had the lowest sensitivity (50%), and the PACE 2 assay had a sensitivity of 80.6%. For the 747 urine specimens tested, the sensitivities of the LCx and

AMP CT assays were 98.4 and 80.6%, respectively. All assays had specificities of $\geq 99.9\%$. PPVs were high for all assays ($\geq 98.4\%$). NPVs ranged from 91.7 to 100% (Table 4).

Factors affecting the sensitivities of assays for the detection of *C. trachomatis* have been discussed in previous reports and reviews (1, 6, 15, 24, 29, 37, 41). These include the prevalence of *C. trachomatis* infection in the study population, discrepant analysis, the choice of "gold standard" or reference method, and swab-to-swab variability. The presence of possible inhibitors in the specimens can also affect the NA amplification assay sensitivities for the LCx and AMP CT assays (3, 21–23, 33, 41). Whereas culture for *C. trachomatis* has previously been the comparative standard, currently, the accepted standard of comparison for assays for the detection of *C. trachomatis* is an amplification assay, and more recently, two amplification assays (39) were used for discrepant analysis. All of these factors must be taken into consideration when comparing data from the present study to previously published data.

Previous studies that compared the Clearview EIA to the PACE 2 assay, cell culture, or "home-brew" PCR reported Clearview EIA sensitivities that ranged from 62 to 95% (2, 7, 30, 37, 40). The difference in the sensitivity (50%) reported in this study is most likely due to the comparison of the EIA with an amplification assay. The reference standard in previous studies had been culture. In addition, one study of the Clearview EIA that yielded a high sensitivity was with a population with a high prevalence of STDs (17.5%) and symptomatic patients (2). One study with asymptomatic patients had a Clearview EIA sensitivity of 62% (37). Another study showed a PPV of 79% due to the large number of false-positive results secondary to problems with test interpretation (40). False-positive results by EIAs due to possible cross-reactivity with other bacteria are also a concern. While false-positive results did not occur in the present study, the sensitivity of the EIA was extremely poor. These results warrant careful consideration for use of any of the new EIAs for the detection of *C. trachomatis* now available and designed for use in physicians' offices.

In the present study, the sensitivity of the LCx assay (96.8%) is similar to those (88 to 98%) from other studies (8, 18, 22, 28, 31, 35, 41) with endocervical swab specimens. For the AMP CT assay, the 100% sensitivity seen in our study was also reported in three other studies (15, 20, 31). The sensitivity of the PACE 2 assay with cervical swab specimens in the present study was 80.6% and falls within the wide range of reported sensitivities of this assay. Thus, this study supports other data that amplification assays consistently perform better than nonamplification assays for the detection of *C. trachomatis* infections.

However, the data from studies comparing the PACE 2 assay with amplification assays are conflicting. At one extreme are PACE 2 assay sensitivities of 48% (25), 63% (19), and 65%

(5) with genital swab specimens when the PACE 2 assay was compared to PCR or the LCx assay, or both, with either urine or swab specimens. At the opposite extreme are reports of PACE 2 assay sensitivities of 83% (31), 90% (32), 93% (29), and 95% (38) compared to amplification assay results. It is important that in the latter studies the definitive comparative standard was an amplification assay and not culture. In one report the sensitivity of the PACE 2 assay (90%) was better than Amplicor PCR (88%) (32). Why this wide range of variability in PACE 2 test performance compared to amplification assay performance continues to exist is unclear. For populations with a lower prevalence of STDs the variability between the PACE 2 and amplification assays is not as great (29, 31, 38). Likewise, the selection of patients can affect assay results. In early reports of amplification assays, most studies reported results for both patients with a high prevalence of STDs and selected symptomatic patients. For studies that test a more generic group, such as patients seeking routine OB/GYN care, as in this study, the differences between amplification assay and PACE 2 assay results seem to be less pronounced (18, 29, 31, 38). In both of these scenarios the small number of specimens with positive results or low prevalence may make statistically significant differences harder to attain. In the present study, the prevalence of *C. trachomatis* infection was moderate, and the results are in the middle of the reported range for the PACE 2 assay. Other variables that affect direct probe sensitivity are the specimen collectors and subsequent specimen adequacy, as well as the technical ability of the laboratory. One recent study showed that 50% of endocervical swab specimens had insufficient endocervical cells for testing for *C. trachomatis* and subsequently a lower reported prevalence of *C. trachomatis* infection (31a). In the present study, the specimen collectors were all OB/GYN nurse practitioners and the PACE 2 assay had been in use for a period of 3 years; <0.5% of the specimens required retesting. The possibility of false-positive results with amplification technology is a concern. In a low-prevalence population, a sudden increase in positive results when switching from PACE to an amplification test should be carefully evaluated. Contamination of patient specimens could occur at the site of collection or in the laboratory. Current amplification assays have no way of clarifying this potential problem.

Results from studies on amplification testing with urine specimens are also variable and have presented some interesting information in terms of the pathogenesis of *C. trachomatis*. For the LCx assay, sensitivities have ranged from 79 to 96% compared to the results of culture and/or amplification techniques (3, 8, 14, 22, 24, 39, 41). Reported sensitivities by the AMP CT assay with urine specimens have been 76 to 97% compared with the results of culture and/or amplification (4, 20, 24, 33, 39). In the present study, the sensitivity of the LCx assay with urine is the highest reported to date, 98.4%. The AMP CT assay sensitivity of 80.6% with urine specimens was slightly lower than others have reported to date. Again, variability in the urine assay results may be attributed to a host of factors. Inhibitors, especially from pregnant patients, have been reported (22). However, in the present study, this was not the case. Adequate collection of a first-void urine specimen and subsequent transport of the specimen could also contribute to assay variability. In the present study, patients' urine specimens were collected before swab specimens were collected, subsequently put into refrigerators, and shipped in cooler packs to optimize the transport of the specimen. Finally, the increased manipulations required for both the AMP CT and the LCx assays with urine specimens could present some technical difficulties in performance of the assays. This has

been reported previously (23). In the present study, retesting of urine specimens in triplicate for a series of patients showed intrarun variability for both TMA and the LCx assay. Overall, the variable sensitivity of amplification tests with urine reported for an assay from any one manufacturer is disturbing and warrants further evaluation.

While urine specimen collection offers possible advantages for increased screening for *C. trachomatis* infection for both sexes, there has been some concern about using only urine for screening for *C. trachomatis* infection in women. Urine testing alone has been estimated to miss 6 to 30% of women with cervical infections (16). In the present study, there were four urine-positive, swab-negative specimens (6.1% of positive cases), indicating that some *C. trachomatis* infections may be detected with urine only due to urethral infection. Patients who are urine positive and swab negative for *C. trachomatis* have been reported in previous studies (36, 39). Other investigators have expressed concern about other conditions that may be missed (i.e., human papillomavirus and trichomonas infections) if a cervical examination is not performed (13a).

Overall, the amplification assays (the LCx and AMP CT assays) performed better than the Clearview EIA and the PACE 2 assay for the detection of *C. trachomatis* infections. However, many factors need to be considered in choosing a test for the routine diagnosis of *C. trachomatis* infection. In addition to test performance, cost, health care provider preferences, laboratory considerations, and level of reimbursement are concerns (10). Several recent articles have discussed in detail the performance, cost-effectiveness, advantages, and disadvantages of using different assays for screening for and verification of *C. trachomatis* infections (6, 10, 16, 26). In addition, the recent recommendations to screen sexually active teens every 6 months calls for health care providers to optimize their ability to screen for *C. trachomatis* infection (9).

At USAMC, the OB/GYN clinics have chosen the PACE 2 test as the routine screening test for the detection of *C. trachomatis* and *N. gonorrhoeae*. There were many reasons for this decision. While the PACE 2 assay was not the most sensitive assay, it was an improvement over the EIA. In addition, the PACE 2 assay addressed two significant concerns, namely, cost to the patient and specimen stability. Surprisingly, urine was not an analyte that the clinics wanted to use, and thus, use of this specimen would not have resulted in an increase in the rate of screening for STDs. A pelvic examination for all of their patients was standard practice because many of them were pregnant. Therefore, swab specimens were routinely obtained and it was not a significant inconvenience to obtain such specimens. The clinics also wanted a test that would allow testing for both *C. trachomatis* and *N. gonorrhoeae*, when needed. For the laboratory, this decision was acceptable because the PACE 2 assay had an improved sensitivity for the detection of *C. trachomatis* and allowed the laboratory to increase the volume for a test already being performed. Reimbursement issues are extremely complicated, but in institutions where the majority of patients are enrolled in a capitated payment system or are self-paying, routine amplification testing is the most expensive option. By using the PACE 2 assay, the clinics have increased screening for *C. trachomatis* infection by 20%, which was the primary desired outcome.

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