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Comparison of Performance and Cost-Effectiveness of Direct Fluorescent-Antibody, Ligase Chain Reaction, and PCR Assays for Verification of Chlamydial Enzyme Immunoassay Results for Populations with a Low to Moderate Prevalence of *Chlamydia trachomatis* Infection

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Many laboratories use a commercial enzyme immunoassay (EIA) with verification testing to diagnose *Chlamydia trachomatis* infections in an effort to contain costs. This study was designed to compare the performance and cost-effectiveness of direct fluorescent-antibody assay (DFA), commercial PCR, and ligase chain reaction (LCR) for the verification of EIA results. Cervical specimens were screened by EIA. DFA, PCR, and LCR were compared as verification tests for EIA-reactive specimens and negative greyzone (NGZ) specimens at 50% below the cutoff value. These samples were also tested by in-house PCR, which was used in the analysis of verification results. A total of 477 (7%) of 6,571 samples were reactive or within the NGZ. EIA results with verification by DFA testing (EIA/DFA results) agreed with 93% of the true results compared with 97% for EIA/PCR results for one set of 242 samples; there was 97% agreement with true results for EIA/DFA results versus 95% for EIA/LCR results for another set of 235 samples. Ten samples were false positive by LCR. Time and costs were equivalent for EIA with the DFA, PCR, or LCR as the verification test but were two- to threefold greater for PCR or LCR alone than for EIA with verification. Since it is important to balance cost containment with public health objectives, DFA, PCR, and LCR as EIA verification tests for cervical samples offer acceptable sensitivities and specificities at reasonable cost for low- to moderate-risk populations and therefore can be extended to a broader spectrum of at-risk populations.

The high prevalence of *Chlamydia trachomatis* urogenital infections in the developed world is well documented (5, 31). The estimated $4.2 billion annual cost of chlamydial sexually transmitted diseases (STDs) is expected to increase to $10 billion by the year 2000 (33). These soaring costs and the fact that chlamydial infections among both men and women are frequently asymptomatic have prompted the need for broad-based screening programs (5). Indeed, in a recent study by Scholes et al. (26), screening for chlamydial cervical infections among asymptomatic women reduced the rate of pelvic inflammatory disease by 60%. However, cost-efficient, technically straightforward, and highly sensitive and specific assays have not been available for broad-based screening.

Since the early 1980s, new technologies have been developed for the detection of *C. trachomatis*. Recently, nucleic acid amplification methods based on commercial PCR and the ligase chain reaction (LCR) have been reported to offer improved performance over culture (1–3, 10, 11, 13, 23, 28) and nonculture chlamydia test methods such as direct fluorescent-antibody assays (DFA) and enzyme immunoassays (EIA) (16, 21, 30). For example, the amplification tests have sensitivities of up to 100% (16, 21, 30) compared with EIA with DFA verification where the sensitivities are 88 to 96.1%. The specificities of both tests are close to 100% (7). However, the higher reagent and labor costs associated with PCR and LCR may limit their usefulness for broad-based screening for chlamydial STDs.

With the advent of managed care and shrinking public health care budgets, cost containment and cost-effectiveness have become critical factors for public health programs. Thus, the debate over STD screening centers on whether the new, higher-cost technologies that are more sensitive should replace lower-cost, automated assays, such as EIA, that have less sensitivity but similar specificity for low- to moderate-prevalence populations. The development of a broad-based chlamydial screening program must also consider patient acceptability of testing and the anatomic site for sampling. There is considerable support in the literature for the use of amplification tests for noninvasive screening for chlamydia using urine samples (25). This approach is an important alternative to urethral swabs as it can increase patient compliance with testing. Further, urine screening detects some cervical infections. However, this screening approach would still miss 6 to 30% of women who have cervical but not urethral infections (14, 18, 22, 25, 27). Thus, the controversy remains as to how to best screen for cervical chlamydial infections.

A standard chlamydial test algorithm for cervical infections is EIA screening followed by DFA verification of reactive specimens (EIA/DFA testing). Some laboratories, including ours, have identified additional true-positive specimens by extending DFA verification testing to all specimens with EIA results below but close to the assay cutoff value (i.e., specimens within a "negative greyzone" [NGZ]) (5, 7, 15, 16). This algo-

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rithm has been shown to provide improved sensitivity and a high degree of specificity at an acceptable cost (6, 7, 17, 21, 30).

To determine the best method for *C. trachomatis* screening of cervical specimens in two cost-sensitive and low- to moderate-prevalence community settings, we investigated whether there were significant performance and cost differences among DFA, PCR, and LCR as verification tests for EIA that would justify the additional costs of the amplification methods.

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MATERIALS AND METHODS

Study sites and population. Specimens for routine *C. trachomatis* testing were collected at two sites. Site 1 was the San Joaquin County Regional Public Health Laboratory, Stockton, Calif., which serves a culturally diverse population of 650,000. The chlamydia testing volume is ~3,000 specimens per month. Site 1 contributed 1,768 endocervical specimens for the PCR study (part of group 1 samples) and 1,313 specimens for the LCR study (part of group 2 samples).

Site 2 was the Diagnostic Laboratory, Auckland, New Zealand, which provides centralized testing for the medical practitioners and family planning clinics serving a culturally diverse population of 1,000,000. The chlamydia testing volume is ~5,000 specimens per month. Site 2 contributed 2,005 and 1,485 endocervical specimens for the PCR (remainder of group 1 samples) and LCR (remainder of group 2 samples) studies, respectively.

The populations at both sites are similar and include both symptomatic and asymptomatic women. The populations have similar distributions of low- and moderate-risk patients for chlamydial STDs.

Specimen collection. At site 1, endocervical specimens were collected by standard techniques from consecutively seen females age 14 to 39 years attending family planning clinics. The mean age was 20 years, and the median range was 20 to 30 years. Briefly, cervical mucus was removed prior to insertion of a cotton swab into the endocervical canal. The cotton swab was immediately placed in the EIA specimen vial supplied by the manufacturer (Behring Diagnostics, San Jose, Calif.). The EIA specimen vial contains 200 μl of buffer. The samples were stored at 4°C prior to processing, which occurred within 6 days of receipt of the sample.

At site 2, endocervical specimens were collected from females age 12 to >50 years attending community and family planning clinics in Auckland, New Zealand. The mean age was 25 years and the median range was 26 to 34 years. Endocervical samples were collected as described above and stored at 4°C prior to processing within 6 days of receipt of the samples.

Only one sample was obtained from each patient. This sample, referred to as the EIA remnant sample, was aliquoted for use in each of the assays: EIA, DFA, commercial PCR and LCR, and in-house PCR. The EIA remnant sample is defined as the original swab sample placed in the EIA specimen vial with 1 ml of specimen treatment buffer (as suggested and supplied by the manufacturer). The EIA remnant sample used for DFA was stored at 4°C and processed within 24 h of obtaining the EIA results; the remnant for the remaining three tests was stored at −20°C until use (see below). Thus, aliquots of the same sample were used for each assay.

Diagnostic tests. The cervical specimens were routinely processed for *C. trachomatis* detection by automated EIA with verification testing by DFA at each site. PCR (group 1) and LCR (group 2) and analysis of discrepant results were also done on all specimens above the NGZ, defined as 50% below the cutoff value.

Each specimen was tested by automated MicroTrack II Chlamydia EIA (Behring Diagnostics) within 6 days of receipt according to the manufacturer's instructions. Briefly, the instructions recommend that 1 ml of specimen treatment buffer (supplied by the manufacturer) be added to the swab since it absorbs the original 200 μl of buffer in the EIA vial. The swab was removed, and 100 μl was used in the EIA. Specimens with absorbance values below the NGZ were considered negative and were not tested further. Specimens with absorbances above the 50% cutoff (all reactive and NGZ specimens) underwent further testing.

For DFA, the remaining 900 μl of the EIA remnant sample was centrifuged and half of the resulting pellet was used to verify each reactive and NGZ sample within 24 h of the EIA. The remaining pellet and supernatant were subsequently used for the commercial amplification tests and in-house PCR (see below). The *C. trachomatis* major outer membrane protein (MOMP)-specific monoclonal antibody (MicroTrak Direct Specimen Kit; Behring Diagnostics) was used in this assay. Seven samples and three controls were tested at the same time in batches. These tests were done in the blind. The slides were prepared according to the manufacturer's instructions and examined for chlamydial elementary bodies (EB) by fluorescence microscopy at ×1,000 with oil immersion. A specimen was considered positive if ≥3 EB were identified.

The remaining aliquots of EIA remnant samples with absorbances above 50% NGZ were used for commercial PCR and LCR and analyses of discrepant samples in a blinded fashion where there was no knowledge of the DFA results. Group 1 specimens (n = 242; from sites 1 and 2) were prepared as described by Østergaard and Møller (21) for commercial PCR (Amplicor PCR; Roche Diagnostics Systems, Inc., Branchburg, N.J.). Briefly, 50 μl of the EIA remnant was added to 200 μl of urine resuspension buffer from the Roche STD urine resuspension kit and the mixture was vortexed and incubated at room temperature for 1 h. One milliliter of urine diluant from the Roche STD urine resuspension kit was added, the specimen was vortexed and incubated at room temperature for 10 min, and 50 μl was then used in the commercial PCR assay according to the manufacturer's instructions.

Group 2 specimens (n = 235; from sites 1 and 2) were tested by commercial LCR (LCx Probe; Abbott Laboratories, Abbott Park, Ill.) by the following technique. First, 50 μl of EIA remnant sample was added to 1 ml of urine specimen resuspension buffer (Abbott Laboratories) and the mixture was vortexed and incubated at 98°C for 15 min. Then, 100 μl of this sample was processed by LCR according to the manufacturer's instructions.

Discrepancy resolution. All samples that were EIA positive or above the NGZ were subjected to in-house PCR with primers that were specific for *omp1*, FII, and BII (8). The technician was not told of the DFA and commercial PCR and LCR results for any samples. Briefly, 50 μl of the EIA specimen remnant was centrifuged at 14,000 × g for 15 min. The pellet was washed with 1× phosphate-buffered saline and resuspended in 10 μl of TE (Tris-Cl [pH 8.3], 0.1 mM EDTA) and 10 μl of 40 mM dithiothreitol. After incubation at 98°C for 10 min, 5 μl was used in a PCR volume of 100 μ; the thermocycling profile and PCR product verification were performed as previously described (9). Two negative and two positive control samples were randomly included in each PCR run; none of the negative controls were positive by in-house PCR. In the event that the sample was negative, 1 μl from the first PCR product was reamplified in a nested PCR with primers MF21 and MB4 as previously described (9). The nested PCR was repeated once to verify a negative sample. To decrease contamination, barrier pipette tips and four different rooms were used for reagent preparation, setup of PCR, running of PCR in a thermocycler, and gel electrophoresis.

Inhibitors that would have affected the commercial PCR and LCR results may have been present in the EIA remnant samples. This issue was addressed by spiking both 20 EIA specimen vials (containing 1 ml of specimen treatment buffer supplied by the manufacturer) and 20 EIA sample remnants that were negative by EIA with 1, 5, 10, 50, and 100 *C. trachomatis* serovar E EB obtained by titration. For example, four of the EIA specimen vials and four of the EIA remnant samples that were negative by EIA were each spiked with one EB, four of each were spiked with five EB, etc. Thus, a total of 40 spiked samples were tested for inhibitors. The 20 EIA remnant samples that were negative by EIA had been tested by DFA, commercial LCR and PCR, and in-house PCR and had been found to be negative by these tests. The 40 spiked samples were subjected to in-house PCR as described above. In addition, LCR was done on the same spiked samples.

Performance and cost analysis. The reagent costs for testing 92 specimens on one EIA microwell plate were recorded at site 1. The cost for one plate of 92 specimens was $176.00. Overhead costs were kept fixed. Labor costs for these tests were calculated based on the pay scales in effect for laboratory personnel in California in 1996 ($23.75 per h, fully burdened, including employee benefits). The reagent costs were based on costs for a clinical laboratory that would have a testing volume similar to those of the laboratories described here. Hands-on time for each test was calculated from commencement of handling the specimens and controls in the laboratory to the final absorbance results. An average of seven specimens per EIA plate required verification. The verification tests with appropriate controls were used for determining hands-on test time.

The commercial PCR and LCR kit costs were calculated based on the pricing available to public health laboratories in the United States and are referred to here as public health costs: $4.78 per sample for PCR and $5.15 per sample for LCR. The kit costs are higher for non-public health laboratories, but a discount is given for moderate- and high-volume laboratories. These costs are referred to here as non-public health costs and are $9.00 per sample for PCR and $9.25 per sample for LCR, not including collection kit costs. The hands-on test times for EIA, DFA, and commercial PCR and LCR were recorded on three separate occasions; the shortest times were used in the labor cost calculations. This was done to ensure that the technician was operating at optimal efficiency, as might be the case in a high-volume laboratory. The labor costs were added to the kit costs.

Data analysis. A sample was considered true positive if it was positive by any two tests (DFA, PCR, LCR, and in-house PCR). A sample was considered true negative if the sample was negative by all tests or positive by only one test whose result could not be verified by another test. Additionally, for the purpose of this study, all samples with absorbances below the NGZ limit were considered true negatives.

The number of false negatives is defined as the number of individuals for whom the test is negative but who actually have the disease. The number of false positives is defined as the number of individuals for whom the test is positive but who do not have the disease. The number of true negatives is defined as the number of individuals for whom the test is negative and who do not have the disease. The number of true positives is defined as the number of individuals for whom the test is positive and who actually have the disease. Sensitivity is defined as $a/(a + c)$ where a is the number of individuals for whom the test is positive and

TABLE 1. Comparison of DFA with commercial PCR (group 1) as EIA verification tests[a]

DFA result	No. of samples with indicated EIA and PCR results				Total
	EIA reactive (n = 134)		EIA NGZ (n = 108)		
	PCR +	PCR −	PCR +	PCR −	
+	102	7	9	1	119
−	6	19	12	86	123
Total	108	26	21	87	242

[a] The NGZ was defined as extending to 50% below the cutoff value. +, positive result; −, negative result.

who actually have the disease and c is the number of individuals for whom the test is negative but who actually have the disease. Specificity is defined as $d/(b + d)$, where d is the number of individuals for whom the test is negative and who do not have the disease and b is the number of individuals for whom the test is positive but who do not have the disease. The positive predictive value is defined as $a/(a + b)$. The negative predictive value is defined as $d/(c + d)$.

The fraction of true results is defined as the total number of true-positive and true-negative results divided by the total number of samples for a given test. The concordance rate is defined as the number of samples for which the results of two tests agree divided by the total number of samples that have been tested by both tests.

RESULTS

Of 6,571 specimens from sites 1 and 2, 6,094 had absorbances below the 50% NGZ and were assumed to be true negatives; 477 (7%) had absorbances above the 50% NGZ. These specimens underwent further testing.

The results of DFA verification and commercial PCR testing of 242 of these specimens (group 1) are shown in Table 1. There were 109 EIA-reactive, DFA-positive samples, with an additional 10 samples in the NGZ identified as positive. All 18 DFA-negative, commercial PCR-positive specimens were positive by in-house PCR, as were all 8 DFA-positive, commercial PCR-negative samples. Thus, the overall prevalence of chlamydial infections after discrepancy resolution was 3.63% (137/3,773). All 242 samples were tested by in-house PCR, and the remaining 105 EIA-reactive and NGZ samples were negative by this assay.

Table 2 is a summary of the results of DFA verification and commercial LCR testing of 235 specimens (group 2). Ninety-six samples were EIA reactive and DFA positive, and an additional seven were identified among the NGZ samples. The one DFA-positive, LCR-negative sample was positive by in-house PCR. Of the 17 samples that were DFA negative and LCR positive, 7 were positive by in-house PCR. Thus, there were 10 false positives; these samples were in the NGZ. The prevalence rate for chlamydial infections was 3.9% (110/2,798) after discrepancy resolution. All 235 samples were tested by in-house PCR, and the remaining 115 EIA-reactive and NGZ samples were negative by this assay.

No inhibitors were detected in the spiked samples for the in-house PCR. The commercial LCR and in-house PCR results were positive for each of the spiked samples.

Table 3 summarizes the performance of EIA/DFA versus that of the commercial PCR and LCR verification tests for true-negative and true-positive results. The specificities and positive predictive values for the DFA and commercial PCR were each 100%.

Table 4 shows the analysis of labor, kit, and material costs for each test. DFA verification with controls added 1 h of labor and ~$20 in reagent costs per plate. Using commercial PCR and LCR as verification tests was slightly more expensive. For commercial PCR and LCR as distinct tests (not as verification tests), the costs included the collection and reagent kits required to run 92 samples with appropriate controls.

The EIA/PCR and EIA/LCR assays were essentially equivalent to EIA/DFA in terms of time and cost, whereas the commercial PCR and LCR assays, if used alone on all samples, were twofold more expensive than EIA/DFA and took two to three times longer to do. The commercial PCR and LCR assays at the non-public health cost rate were 3.7 times more expensive than EIA/DFA.

DISCUSSION

Reducing the incidence and prevalence of chlamydial STDs and their sequelae has become an important public health goal. Most investigators agree that broad-based screening programs are needed to achieve these goals. Yet there is no consensus regarding the most appropriate surveillance strategy and diagnostic test(s) to use. This decision is further complicated by the lack of low-cost, highly sensitive diagnostics, by the variation in prevalence rates found in rural and urban clinics, and by the need to screen and treat asymptomatic infections. Previous studies have focused on models of the cost-effectiveness of screening in specific populations without a comparative analysis of the performance of diagnostic tests (4, 20, 24, 29, 32). Other studies have addressed the comparative performance of two or three chlamydial assays among specific prevalence groups (2, 10, 19, 23, 30) without a determination of cost.

In this study, we evaluated the performance and quantified the time and cost differences for three chlamydial diagnostic tests in a side-by-side comparison for the first time. In order to avoid intraindividual variation in the samples, only one sample was collected from each patient. This ensured that the same patient sample was used for EIA, DFA, commercial PCR and LCR, and in-house PCR and that the results would be comparable. Using the EIA remnant sample for the commercial PCR and LCR assays may theoretically have affected the performance of these tests. However, in a previous study by Østergaard and Møller (21), the use of EIA remnant samples for commercial PCR resulted in the detection of additional true-positive samples over those found by DFA and a concordance rate of 98.2% with DFA results. We found similar results. An additional 10 (7%) true-positive samples were detected by commercial PCR, and the concordance rate was 89.3% (216/242), with no false positives detected by either test. Although LCR has not previously been performed on EIA remnant samples, we found 6 (5%) additional true-positive samples by LCR compared with 1 (1%) by DFA. The concordance rate was 92.3% (217/235). Although commercial test instructions require that samples be obtained in the respective commercial

TABLE 2. Comparison of DFA with commercial LCR (group 2) as EIA verification tests[a]

DFA result	No. of samples with indicated EIA and LCR results				Total
	EIA reactive n = 113)		EIA NGZ (n = 122)		
	LCR +	LCR −	LCR +	LCR −	
+	96	0	6	1	103
−	7	10	10	105	132
Total	103	10	16	106	235

[a] The NGZ was defined as extending to 50% below the cutoff value. +, positive result; −, negative result.

TABLE 3. Performance of EIA/DFA versus EIA/PCR and EIA/LCR for true-negative and true-positive specimen results

Test[a]	No. of samples that were:				Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)	Agreement with true results (%)
	True positives	False negatives	True negatives	False positives					
EIA/PCR[b]									
DFA	119	18	105	0	87	100	100	93	93
PCR	129	8	105	0	94	100	100	93	97
EIA/LCR[c]									
DFA	103	7	125	0	94	100	100	94	97
LCR	109	1	115	10	99	92	92	99	95

[a] Test to which EIA/DFA was compared.
[b] Number of true-positive specimens, 137 (n = 242).
[c] Number of true-positive specimens, 110 (n = 235).

PCR or LCR transport buffer, the EIA remnant does not appear to alter test performance and thus may be an appropriate alternative sample type for either assay.

EIA/LCR detected 10 false positives, a result which is of concern if LCR is to be used as a broad-based screening test. Lack of verification of these 10 samples by DFA and in-house PCR may be due to inhibitors for PCR, as has been suggested by other investigators (2, 25). However, in-house PCR was performed on the same EIA remnant samples in all cases of discrepancy, and each DFA and commercial PCR discrepancy could be resolved by this test. Thus, it seems unlikely that inhibitors were suddenly present in the EIA remnant samples used for discrepancy resolution of the LCR results. Also, no inhibitors were detected in the spiked samples where the LCR and in-house PCR tests showed equivalent results. Alternatively, the LCR test may be a more sensitive assay, as it detects the plasmid gene which has multiple copies in the EB, whereas in-house PCR detects the single-copy MOMP gene. Thus, since the 10 false positives were in the NGZ, low copy numbers may have limited the detection of the organism by in-house PCR. Other investigators have been able to resolve LCR discrepancies by culture, EIA using a blocking antibody, DFA, and MOMP PCR. It also is possible that amplicons from the LCR assay may produce low-level contamination and contribute to false positives. Further studies are required to address the cause of false positives identified in this and other studies (10, 18).

None of the specimens with absorbances below the 50% NGZ were tested by commercial PCR and LCR. Since it is common practice among clinics, hospitals, and public health laboratories in the United States to use an EIA with verification testing of reactive and NGZ samples to diagnose *C. trachomatis* infections in an effort to contain costs, this study was designed to compare the performance of DFA and commercial PCR and LCR assays as verification tests for EIA-reactive and NGZ samples. We also analyzed the cost-effectiveness of each as a verification test and as the primary test in the case of commercial PCR and LCR. In low- to moderate-prevalence populations, such as those sampled in this study, the number of true-positive samples below the 50% NGZ would be expected to be very small. One study in progress at site 2 has shown that 15 of approximately 55,000 specimens had absorbances in an NGZ of 41 to 50% of the cutoff value and that only 1 was a true positive by DFA verification (unpublished data).

The increased detection of true positives in NGZ samples in this and other studies (7, 15, 16) supports the notion that verification of NGZ samples would be the most cost-effective approach to increasing the sensitivity of EIA. Verification results showed greater sensitivity for both commercial PCR and LCR compared with DFA, but the specificity was 100% for DFA and commercial PCR and only 92% for LCR. The performance analysis, then, would suggest that DFA or commercial PCR or LCR should be used for verification of EIA-reactive and NGZ samples. This is supported by the fact that the time and cost required for DFA verification of one EIA plate with 92 samples were essentially the same as those for commercial PCR or LCR verification of one plate. Further, the commercial amplification tests, if used alone for all samples, would be twice as expensive to perform as any of the EIA verification algorithms despite a significant cost decrease from

TABLE 4. Evaluation of labor and material costs by diagnostic test

Test	Hands-on time		Cost (dollars)/92 specimens				Total cost (dollars)/specimen[g]	
	Min/92 specimens[c]	Min/specimen	Labor[d]	Kit[e]	Reagents and materials[f]	Total	Public health	Non-public health
EIA/DFA	100	1.1	39.58	176.00	79.77	296.15	3.21	
EIA/PCR	160	1.7	40.38	176.00	100.16	316.54	3.44	3.76
PCR[a]	190	2.1	75.20	447.11	91.08	613.39	6.66	11.86
EIA/LCR	160	1.7	40.38	176.00	100.24	316.62	3.44	3.75
LCR[b]	246	2.7	97.38	473.80	58.14	636.67	6.92	12.00

[a] PCR refers to a commercial PCR (Roche Diagnostics) test that would be used as the sole test on cervical specimens and not as a verification test.
[b] LCR refers to a commercial LCR test that would be used as the sole test on cervical specimens and not as a verification test.
[c] Includes time to handle seven DFA, seven PCR, or seven LCR verification test kits per EIA plate.
[d] Calculated at $23.75 per h fully burdened, which includes employee benefits.
[e] Does not include tax as this cost will vary from state to state.
[f] Based on an estimate of reagent costs for a laboratory with a similar testing volume. The values include the costs of the reagents and materials for seven DFA, seven PCR, or seven LCR verification tests per EIA plate.
[g] The difference in cost reflects the difference between the cost of the kit at public health pricing versus the cost if purchased by a moderate- to high-volume laboratory.

public health pricing and would take two to three times longer to perform. Using the non-public health costs, the price was prohibitive, i.e., more than 3.7 times the cost of the EIA verification algorithms.

Ideally, a screening test should not miss any true-positive case, as this would impact the cost of potential complications such as pelvic inflammatory disease and related sequelae and the spread of chlamydia to secondary cases. Neither the EIA verification test algorithms nor the amplification tests used alone are 100% sensitive. Where cost containment is important, our data support the use of EIA verification test algorithms for low-prevalence populations where a larger number of patients could be screened because of the lower cost compared with commercial PCR and LCR used alone and where the false-negative rate would be relatively low. Further, amplification tests should be used for screening in high-prevalence populations. In a recent study by Genc and Mardh (12), the most cost-effective strategy for screening asymptomatic women when the prevalence of infection was greater than 6% was a DNA amplification test. Although there was no comparative evaluation with the EIA verification test algorithms, the most sensitive test regardless of cost and labor time would be the most appropriate in high-prevalence populations, as this would detect most but not all cases and would thereby presumably prevent more ascending infections and costly sequelae. Alternatively, specific high-risk target groups, for example, adolescents, pregnant women, or patients attending STD clinics, could be targeted for screening with amplification tests.

In summary, to balance test and cost-effectiveness for screening cervical samples for chlamydial infection, we recommend the following: the use of DFA or commercial PCR or LCR as verification tests for EIA-reactive and NGZ samples in low- to moderate-prevalence populations and the use of commercial amplification tests for screening high-risk groups or high-prevalence populations. This approach to test and cost-effectiveness may be the compromise needed to provide the best service to the public at large. Additional studies will be required to evaluate both the cost-effectiveness and head-to-head performance comparisons of different diagnostic tests among moderate- and high-prevalence populations and to determine the best approach to screening these populations.

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