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Comparison of the Syva MicroTrak Enzyme Immunoassay and Gen-Probe PACE 2 with Cell Culture for Diagnosis of Cervical *Chlamydia trachomatis* Infection in a High-Prevalence Female Population

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Culture is currently considered the "gold standard" for detecting *Chlamydia trachomatis* infections. We evaluated the Syva MicroTrak enzyme immunoassay (EIA) and Gen-Probe PACE 2 tests, which detect chlamydial antigens and rRNA, respectively. These assays were compared with each other and with culture for the detection of *C. trachomatis* in cervical specimens obtained from 217 women attending a clinic for sexually transmitted diseases. The prevalence of infection was 22.1% by culture. The sensitivity, specificity, and positive and negative predictive values were 79.2, 98.2, 92.6, and 94.3%, respectively, for EIA. For PACE 2, the respective values were 77.1, 97.6, 90.1, and 93.7%. After corrections for two false-negative cultures, the sensitivities and specificities were 80 and 99.4%, respectively, for the EIA and 78 and 98.8%, respectively, for the probe assay. Quantitative evaluation of the results showed that false-negative results with either assay were associated with cultures that had low inclusion counts or were negative without subpassage. Analysis of nonculture results revealed that 2.3% of the EIA results and 4.6% of the probe assay results were within ±30% of the respective assay cutoff values. These included four false-negative (one EIA and three probe) and two false-positive (one EIA and one probe) results. The Syva MicroTrak EIA and the Gen-Probe PACE 2 assay are comparable to but significantly less sensitive than culture. Use of a grey zone may help identify the need for repeat or confirmatory testing.

Chlamydia trachomatis is a major cause of sexually transmitted disease. In women, infection is frequently asymptomatic or may be associated with urethritis, mucopurulent cervicitis, and proctitis. The detection of these infections is important not only so that transmission to sexual partners and neonates can be reduced but also because untreated infections may progress to complications, including endometritis, salpingitis, and infertility (4, 12, 24, 27). Laboratory tests are necessary to detect asymptomatic infections and to differentiate chlamydial infections from those caused by other microorganisms. Isolation in cell culture is considered the most sensitive and specific laboratory method for the diagnosis of chlamydial infections (1, 13, 24, 27). However, culture is labor intensive, requires cold specimen transport, and takes up to 7 days to produce results. Thus, there is a need for sensitive and specific nonculture assays that make rapid and cost-effective screening possible. Several assays for the detection of chlamydial antigens by immunofluorescence (IF) and enzyme immunoassay (EIA) are currently available (1, 13, 27). More recently, a nucleic acid hybridization assay that detects chlamydial rRNA has become available for diagnostic use (1). EIA and the hybridization assay are suitable for automated high-volume testing and have comparable turnaround times. There is a need to compare nonculture assays that have different chlamydial targets with each other and with cell culture.

The objective of this study was to evaluate the MicroTrak EIA (Syva Corp., Palo Alto, Calif.) and the PACE 2 (Gen-Probe, San Diego, Calif.), which detect chlamydial antigens and rRNA, respectively. These assays were compared with each other and with quantitative culture for the detection of cervical infection by *C. trachomatis* in a high-prevalence female population.

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MATERIALS AND METHODS

Specimen collection and processing. Endocervical specimens for each assay were collected from 217 nonpregnant female patients (ages 18 to 35 years) attending a sexually transmitted disease clinic in Brooklyn, N.Y. All subjects denied antibiotic use in the previous 7 days. After the exocervix had been cleansed with a Dacron swab, three separate endocervical samples were collected for this study. Swab specimens for the MicroTrak EIA and the PACE 2 assay were collected by using each manufacturer's collection kit; culture specimens were collected with a cytobrush and placed into 0.8 ml of 2-SP (0.2 M sucrose, 0.02 M sodium phosphate, 20 mM HEPES [*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid], vancomycin, nystatin) with 2% heat-inactivated fetal bovine serum. All specimens throughout the study were collected in rotating order by the same individual and were delivered to the laboratory within 4 h of collection. Specimens for EIA were stored at 4°C and tested within 7 days of collection. The specimens for hybridization were vortexed prior to discarding the swabs and then stored at −70°C. After testing, EIA samples were stored at 4°C and probe samples were stored at −70°C. Culture specimens either were stored at 4°C (overnight) or were snap frozen and

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placed at −70°C after the swab had been vortexed and discarded.

Chlamydial cultures. Culture was performed by using a modification of the procedure of Ripa and Mardh (21) with 24-h confluent McCoy cell monolayers in shell vials. Following aspiration of the cell culture medium, duplicate vials were each inoculated with 0.2 ml of vortexed specimen or control preparation and then centrifuged for 1 h at 35°C at 2,500 × *g*. After centrifugation, 1.0 ml of Eagle's minimum essential medium containing 10% heat-inactivated fetal bovine serum, 30 mM glucose, antibiotics, and cycloheximide (1 μg/ml) was added to each vial, and the cultures were incubated at 35°C with 5% CO_2. After 48 to 72 h, monolayers were fixed for 10 min with 95% ethanol, stained by using a fluorescein-conjugated genus-specific monoclonal antibody (Kallestad, Austin, Tex.), and examined at magnifications of ×100 and ×400 with a Nikon epifluorescence microscope (Nikon Inc., Melville, N.Y.). Cultures with one or more fluorescent inclusions were considered positive. The number of inclusions per coverslip was determined; specimens yielding low inclusion counts, i.e., 1 to 10, were examined by a second observer. Subpassage was performed by using the unstained duplicate vials of negative cultures and positive cultures with fewer than five inclusions.

Syva MicroTrak EIA. The Syva MicroTrak EIA was performed in accordance with the manufacturer's instructions. Briefly, specimen treatment solution (1.0 ml) was added to each specimen and control tube, and the tubes were placed in a heat block at 95 to 100°C for 15 min, cooled, and vortexed (15 s). Treated sample (100 μl) or control was added to a well of a microtiter plate containing 100 μl of rabbit anti-chlamydial lipopolysaccharide antibody. Following incubation for 90 min at 37°C and washing, 100 μl of peroxidase-labeled goat anti-rabbit immunoglobulin was added, and the mixture was incubated for 30 min at 37°C. After washing, 100 μl of substrate mixture (peroxide and TMB) was added to each well. Stop solution was added after incubation for 30 min at room temperature, and the A_{450}s were read. Samples with absorbances equal to or greater than the cutoff value were considered positive.

Gen-Probe PACE 2. The chemiluminescent-probe assay utilizes an acridinium ester-labeled single-stranded DNA probe complementary to the rRNA of *C. trachomatis* and was performed in accordance with the manufacturer's instructions. Briefly, 100 μl of the probe reagent was added to a tube containing 100 μl of vortexed (5 s) specimen or control and incubated for 1 h in a 60°C water bath. One milliliter of separation solution (containing metallic microbeads which bind hybridized probe) was added to each tube, and after incubation for 10 min at 60°C, the rack of tubes was placed on the magnetic separation base for 5 min at room temperature and the supernatants were decanted. Wash solution was added, and after 20 min at room temperature on the magnetic separation base, the supernatants were decanted and the relative light units (RLU) emitted by the chemiluminescent label were measured with a luminometer. Samples with an RLU valve equal to or greater than 300 plus the mean value obtained with the negative controls were considered positive.

Syva MicroTrak direct fluorescent-antibody assay. The residual EIA samples of culture-negative discordant specimens were tested by using the MicroTrak direct fluorescent-antibody assay, which employs fluorescein-conjugated monoclonal antibodies to the species-specific major outer membrane protein. The sample residue was centrifuged for 15 min at 2,000 × *g*, the supernatant was removed, and 10 μl of the resuspended cell pellet was spotted onto an 8-mm slide well. The slide was air dried and fixed with methanol, and the direct fluorescent-antibody assay was performed in accordance with the manufacturer's instructions, with a single incubation for 15 min at room temperature. Slides were screened at a magnification of ×400 and confirmed at ×1,000 with a Nikon epifluorescence microscope. Samples with one or more fluorescent elementary bodies were considered positive.

TABLE 1. Comparison of MicroTrak EIA and PACE 2 with cell culture for detection of *C. trachomatis* in cervical specimens

Culture results	No. of specimens			
	Positive		Negative	
	EIA	PACE 2	EIA	PACE 2
Positive (n = 48)[a]	38	37	10	11
Negative (n = 169)[b]	3	4	166	165

[a] Forty-three specimens were positive without subpassage.
[b] Includes two specimens that were positive by PACE 2 and EIA with IF confirmation and were considered culture false negatives.

Statistical analyses. Calculations for test performance were made with standard formulas for sensitivity, specificity, and predictive values (11). The chi-square test was used to test the association between test results and the effect of the order of swab collection on test performance.

RESULTS

Comparison of culture, EIA, and probe assay. *C. trachomatis* was detected by culture in 48 (22.1%) of 217 endocervical samples. Of these, 38 corresponding samples were positive by MicroTrak EIA, and 37 were positive by PACE 2 (Table 1). There was a significant difference ($P < 0.001$) between culture and both nonculture assays. Three positive EIA and four positive probe results were obtained with specimens from patients with negative cultures. IF testing revealed elementary bodies in two of the three EIA-positive sample residues; of the four samples discrepant by probe, two were negative when the probe assay was repeated. The sensitivity and specificity compared with culture were 79.2 and 98.2%, respectively, for the MicroTrak EIA (Table 2). When the two false-negative culture results are taken into account, the sensitivity increases to 80% and the specificity increases to 99.4%. With PACE 2, the sensitivity and specificity compared with culture were 77.1 and 97.6%, respectively, and the respective values when adjusted results are used increase to 78 and 98.8%.

Quantitative evaluation of results. Thirty-six specimen sets were positive and 164 sets were negative by all three methods. Inclusion counts were compared with absorbance (MicroTrak EIA) and RLU (PACE 2) values for the 36

TABLE 2. Sensitivities, specificities, and predictive values of MicroTrak EIA and PACE 2 compared with culture and with adjusted results[a]

Assay	Sensitivity (%)	Specificity (%)	Predictive value (%)	
			Positive	Negative
MicroTrak EIA	79.2 (80.0)	98.2 (99.4)	92.6 (97.4)	94.3 (94.6)
PACE 2	77.1 (78.0)	97.6 (98.8)	90.1 (94.9)	93.7 (94.1)

[a] Values in parentheses were calculated by using adjusted results.

TABLE 3. Analysis of MicroTrak EIA, PACE 2, and quantitative culture results for 36 concordant positive samples

Inclusion count	Mean result ratio[a] (range)	
	MicroTrak EIA	PACE 2
>1,000 (n = 26)	6.1 (1.3–13.6)	428.3 (1.3–3566.1)
101–999 (n = 5)	4.9 (1.6–9.1)	12.0 (1.9–23.9)
11–100 (n = 3)	4.3 (2.1–6.4)	3.9 (1.6–7.6)
1–10 (n = 0)		
0[b] (n = 2)	2.9 (2.1–3.7)	39.0 (1.0–78.0)

[a] Calculated by dividing sample absorbance (EIA) or RLU (PACE 2) by the cutoff value for the run. Ratios of ≥1 are positive.
[b] Specimens were negative without subpassage.

concordant positive specimen sets (Table 3). Twenty-six specimens (72%) yielded inclusion counts of greater than 1,000. Overall, specimens with higher inclusion counts had greater absorbance and RLU values in the corresponding samples. Five (2.3%) EIA and 10 (4.6%) PACE 2 results fell within ±30% of the respective assay cutoff value.

Analysis of discordant specimens. Seventeen specimen sets yielded discordant results (Table 4). Of the five culture-negative discordant specimens, two were culture false negatives; corresponding samples were positive in both nonculture assays. Of the 12 culture-positive sets, false-negative results by either nonculture assay occurred primarily when corresponding specimens had low inclusion counts. One (10%) EIA and three (27%) PACE 2 false-negative results were within 30% of the respective assay cutoff value; 50% of the false negatives for each assay were within 60% of the respective cutoff value.

DISCUSSION

We have compared the Syva MicroTrak EIA and the Gen-Probe PACE 2 assay with culture for the diagnosis of cervical *C. trachomatis* infection in a high-prevalence female population. A previous study, which used isolation in McCoy cells in 96-well microtiter plates with subpassage as the reference method, showed a sensitivity of 89% and a specificity of 96% for the MicroTrak EIA with endocervical specimens obtained from a high-prevalence (14%) female population (9). In five studies comparing PACE 2 with culture, the sensitivities for the probe assay ranged from 86.1 to 93% and the specificities ranged from 97.7 to 100% (12, 14–16, 28). Several factors, including patient characteristics and infection prevalence (2, 8, 13, 17, 27) as well as differences in specimen collection (19) and culture techniques (1, 13, 25, 27, 29), may affect test performance. Isolation in shell vials has been shown to be more sensitive than culture in 96-well microtiter plates when McCoy cells are used (24, 29), and the subpassage of negative specimens has been shown to increase the number of chlamydial isolates by approximately 10% (25). In this study, subpassage yielded five additional isolates. While it has been suggested that the order of sample collection may affect test results (7, 10), our findings are consistent with those of other studies that have shown the swab order not to be a significant factor (3, 6, 20, 22). Chi-square analysis showed that the swab order did not adversely affect the outcome of either nonculture assay (data not shown).

Our results included two specimens that were determined to be culture false negatives. While specific reasons could not be identified for these results, culture false negatives can occur as a result of technical failures, the presence of nonviable organisms, or the presence of inhibitory materials at the sampled site (13). Culture false positives have been reported when immunoassays other than IF were used for culture confirmation (5) and when polymerase chain reaction was the reference method (14). In this study, specimens with one or more stained intracytoplasmic inclusions were considered positive. Cultures with low inclusion counts were examined by a second observer; one specimen had fewer than five inclusions and was positive after subpassage.

We analyzed the results to determine whether the use of a grey zone would improve the diagnostic utility of the assays. Eight negative results (two by EIA and six by probe) fell within 30% of the cutoff value. While all corresponding samples were negative by the alternate direct assay, four (50%) were positive by culture. Seven positive results (three by EIA and four by probe) fell within the 30% grey zone. Of these, two EIA and three probe results were true positives. With EIA, 3 (27%) of the 11 false-negative results and the 1 false-positive result fell within the ±30% grey zone. Confirmatory IF testing of the EIA sample residue of the latter specimen was negative. With the probe assay, 4 (33%) of the 12 false-negative results and 1 of the 2 false-positive results fell within the ±30% grey zone. Consistent with the suggestion to retest samples with low cutoff ratios in the probe assay (28), both samples with false-positive results were negative when repeated. Others have shown that confirmatory (19) or supplemental (26) testing may improve the utility of nonculture chlamydial assays. Our results suggest that a grey zone may be useful for identifying specimens warranting repeat or confirmatory testing. Further evaluation is necessary to determine whether a different pattern will emerge with a lower-prevalence patient population.

TABLE 4. Analysis of discordant results

Culture result	No. of specimens[a]					
	Positive			Negative		
	EIA	PACE 2	EIA and PACE 2	EIA	PACE 2	EIA and PACE 2
Negative (n = 5)	3 (1.18–1.35)	4 (1.10–1.65)	2	2 (0.26–0.37)	1 (0.32)	0
Positive						
With subpassage (n = 3)	1 (1.09)	0	0	2 (0.29–0.40)	3 (0.21–0.31)	2
Without subpassage						
1–10 inclusions (n = 6)	1 (1.28)	1 (1.10)	0	5 (0.25–0.92)	5 (0.22–0.52)	4
11–100 inclusions (n = 3)	0	0	0	3 (0.20–0.47)	3 (0.76–0.98)	3

[a] Numbers in parentheses represent ranges of result ratios obtained by dividing sample absorbance (EIA) or RLU (PACE 2) by the cutoff value for the run. Ratios of ≥1 are positive.

The Syva MicroTrak EIA and the Gen-Probe PACE 2 assay are comparable for the detection of cervical infections in a high-prevalence female patient population, with isolation in cell culture being significantly more sensitive than either nonculture assay. The EIA and the probe assay are useful for high-volume screening and can provide results with a shorter turnaround time than is possible for culture. With either assay, implementation of a grey zone appears useful for identifying the need for additional testing.

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