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Evaluation of a Nonisotopic Probe for Detection of *Chlamydia trachomatis* in Endocervical Specimens

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A nonisotopic probe (Gen-Probe PACE; Gen-Probe, Inc., San Diego, Calif.) for detection of *Chlamydia trachomatis* in endocervical specimens was evaluated in 344 women attending a dysplasia clinic or an obstetrics clinic and 158 women who visited an emergency room. For each patient, the probe, a tissue cell culture, and a direct immunofluorescent-antibody test (DFA; MicroTrak; Syva Co., Palo Alto, Calif.) were used. *C. trachomatis* was detected in 54 specimens by at least one method. Forty-four, 44, and 37 specimens were positive by culture, probe, and DFA, respectively, and 31 were positive by all three methods. Considering culture-positive plus both probe- and DFA-positive results as the "gold standard," we determined the overall sensitivity, specificity, and positive and negative predictive values of the probe to be 80, 98, 82, and 98%, respectively. These values were 94, 98, 84, and 99%, respectively, in emergency room patients and 71, 98, 80, and 97%, respectively, in clinic patients. The sensitivities, specificities, and negative predictive values of the DFA and probe were comparable. The positive predictive values of the DFA in all patients and in emergency room and clinic patients were 97, 100, and 95%, respectively. Given the number of probe-positive results that were not confirmed by culture, we do not recommend using the Gen-Probe PACE to screen for *C. trachomatis* in women with a low to moderate risk for infection.

Chlamydia trachomatis is the most common cause of sexually transmitted disease in the United States today (3). Tissue cell culture is considered the reference method for detection of *C. trachomatis* in clinical specimens. However, tissue culture requires a minimum incubation of 48 h and is costly. Antigen detection methods have made rapid and cost-effective screening for *C. trachomatis* possible. A direct immunofluorescent-antibody test (DFA) that uses monoclonal antibodies to the species-specific outer membrane protein was the first such test introduced. The sensitivity of the DFA in females has ranged from 70 to 100% and is dependent upon the type of patient population studied and the cutoff used for a positive result (2, 4, 6–9). An enzyme immunoassay was developed shortly after the DFA. The sensitivity of the enzyme immunoassay in females has ranged from about 70 to 90%, depending on the patient population (1, 5, 10). In general, the specificity and positive predictive value of the enzyme immunoassay have been lower than those of the DFA, especially in the low-risk group of women.

Recently, a nonisotopic DNA probe for detection of *C. trachomatis* in genital specimens became commercially available (Gen-Probe PACE; Gen-Probe Inc., San Diego, Calif.). Between July 1988 and February 1989, we evaluated this probe assay in 158 women visiting the emergency room and in 344 women attending the dysplasia clinic or the obstetrics clinic at the University of Nebraska Medical Center. In addition to the probe, single-passage tissue cell culture (13) and the DFA, which has been the method routinely used to screen for *C. trachomatis* at this institution for the past 3 years, were also used.

Specimen collection. Three endocervical samples were collected after the exocervix was cleaned. All house staff in the two departments participated in the study. The order of specimen collection was rotated as follows: DFA, probe, and culture for the first 174; probe, culture, and DFA for the second 172; and culture, DFA, and probe for the remainder. For collection and preparation of the DFA specimens, we used the MicroTrak collection kit (Syva Co., Palo Alto, Calif.). The specimens were air dried, fixed with methanol, and refrigerated until stained (within 24 h of receipt). For the probe assay, the Gen-Probe PACE collection kit was used. Samples were stored at room temperature for up to 1 week prior to being tested. For culture, samples were collected with a calcium alginate swab and placed in 1.5 ml of sucrose phosphate buffer transport medium supplemented with 3% fetal bovine serum and containing gentamicin and amphotericin B. Samples were stored at 4°C (if inoculated within 24 h) or at −70°C; all samples were cultured within 1 week.

DFA. Methanol-fixed smears were stained with the MicroTrak fluorescein-conjugated monoclonal antibodies in accordance with manufacturer directions. Positive and negative control slides (Syva Co.) were included with each specimen run. Using a Nikon epifluorescence microscope, we scanned slides at a magnification of ×400 and examined them at a magnification of ×1,000 for confirmation of morphology. The presence of three or more characteristic elementary bodies was considered positive (M. J. Jaqua-Stewart, J. Tichota-Lee, L. H. Amundson, J. L. Simmons, and R. A. Jaqua, Abstr. Annu. Meet. Am. Soc. Microbiol. 1988, C223, p. 369). Samples containing less than 10 columnar epithelial cells or one to two elementary bodies and those with excessive amounts of mucus were considered inconclusive, and collection of a new specimen was recommended.

DNA probe. Specimens were processed in accordance with manufacturer directions. One positive control and three negative controls were included with each specimen run. Briefly, a mixture of probe (single-stranded DNA, labeled with acridinium ester, complementary to the rRNA of *C. trachomatis*) and specimen or control was incubated in a 60°C water bath for 1 h. Separation reagent was added, and tubes were incubated for 5 min. The tube rack was placed on

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the separation base for 2 to 3 min, and specimens were decanted. Three washes with warm wash solution were performed. Elution reagent was added, and tubes were incubated for 5 min. Magnetic separation was performed, and samples were read with a luminometer (LEADER I). A result, measured in relative light units, was considered positive if its value exceeded the mean of the negative reference values plus 1,200.

Culture. Specimens were vortexed and sonicated for 3 min in a Mettler ultrasonic cleaner. To each specimen, 1 ml of Eagle minimum essential medium (GIBCO Laboratories, Grand Island, N.Y.) containing 3% chlamydia-free fetal bovine serum (Whittaker M.A. Bioproducts, Walkersville, Md.), gentamicin, and amphotericin B (Fungizone) was added. Each of two wells of a 24-well plate containing McCoy cell monolayers on 12-mm glass cover slips was inoculated with 0.5 ml of that mixture. Positive and negative controls were included with each specimen run. Plates were centrifuged at 2,500 × *g* for 1 h at 33°C. After incubation at 35°C in 5% CO_2 for 2 h, 1 ml of chlamydial culture medium with cycloheximide was added and plates were incubated for 48 h. Medium was removed, and cover slips were fixed in 95% ethanol for 10 min, washed twice, and stained with monoclonal antibodies (MicroTrak). Cover slips were mounted on glass slides and examined at magnifications of ×100 and ×400 with a Nikon epifluorescence microscope. The presence of one or more positively fluorescing inclusions was considered positive. If the culture was negative and either the DFA or the probe was positive, cell culture was repeated.

Chart review. Charts of patients with a positive probe and/or MicroTrak result(s) that was not confirmed by culture were reviewed.

C. trachomatis was detected in a total of 54 specimens (11%), 20 (13%) from emergency room and 34 (10%) from clinic patients, by at least one method. Forty-four specimens (9%) from 16 (10%) emergency room and 28 (8%) clinic patients were positive by cell culture, and 1 of these (initially positive by the probe and DFA but negative by culture) was detected only on the repeat culture. The probe was positive in 44 cases (20 emergency room and 24 clinic patients), 35 of which were also culture positive. Thirty-seven specimens from 16 emergency room and 21 clinic patients were positive by the DFA, and 35 of these were confirmed by culture. One of the two DFA-positive, culture-negative specimens was positive by the probe. The DFA yielded inconclusive results for 20 (4%) specimens (14 had excessive mucus and 6 had less than 10 columnar cells). Although repeat testing of patients whose specimens yielded inconclusive results was recommended, no additional specimens were collected from any of these 20 patients. One specimen with excessive mucus was positive by the probe. *C. trachomatis* was detected by all three methods in 31 specimens from 15 emergency room and 16 clinic patients.

There were 18 discrepancies between the culture and probe results. Nine were culture positive and probe negative. Four of these were also positive by the DFA. Culture yielded less than or equal to five inclusions on one or both cover slips for three of the nine; however, culture yielded a similar number of inclusions for three specimens positive by all methods. The probe result for two of these nine specimens was within 200 relative light units of the positive cutoff value; two culture-negative specimens also yielded probe results in this range. The probe sample was collected first, second, and third from three, four, and two, respectively, of the nine patients. Of the nine probe-positive, culture-negative specimens, one was positive by the DFA and one yielded inconclusive DFA results. The probe sample was collected first, second, and third from five, zero, and four, respectively, of the nine patients.

TABLE 1. Reliability of the probe and DFA for detection of *C. trachomatis* in clinical specimens

Type of patient	Test	Sensitivity (%)	Specificity (%)	Predictive value (%)	
				Positive	Negative
Emergency room	Probe	94	98	84	99
	DFA	94	100	100	99
Clinic	Probe	71	98	80	97
	DFA	71	99.7	95	97.5
All	Probe	80	98	82	98
	DFA	80	99.8	97	98

Eleven patients had discrepant culture and DFA results. Nine were culture positive and DFA negative. Culture yielded less than or equal to five inclusions on both cover slips for two of these. The DFA sample was collected first, second, and third from three, three, and three, respectively, of these nine patients. Both of the two DFA-positive, culture-negative specimens were collected third.

The sensitivities, specificities, and positive and negative predictive values of the probe and DFA were calculated by using as the "gold standard" the number of specimens positive by culture and by both the probe and the DFA. The reliability of each method in clinic patients, emergency room patients, and all patients is shown in Table 1. The sensitivities of the probe and DFA were identical in all patient groups (71% in clinic patients, 94% in emergency room patients, and 80% overall). The negative predictive values of both tests were comparable. The probe had a specificity of 98% in all patients groups, and the specificity of the DFA approached 100%. The positive predictive value of the probe (80 to 84%) was lower than that of the DFA (95 to 97%) in all patient groups. Culture had a sensitivity of 98%.

Six of the 10 patients with negative culture and positive probe and/or DFA results were symptomatic, as determined by chart review. Two had physical findings (i.e., mucopurulent cervical discharge, pain on motion of the cervix) highly suggestive of a *C. trachomatis* infection, and both were treated as outpatients with doxycycline. The remaining four presented with right-lower-quadrant pain. One had candida vaginitis, one was diagnosed with periappendiceal fibrosis by exploratory laparotomy, one had a urinary tract infection, and one was released from the emergency room without a specific gynecologic diagnosis. Of the four asymptomatic patients, three were attending the dysplasia clinic for colposcopy and biopsy following Pap smear-diagnosed dysplasia, and one was attending the obstetrics clinic for a routine prenatal examination.

Problems with the probe assay of low sensitivity and false-positive results identified in this evaluation have been observed by other investigators. In an evaluation of 298 obstetric-gynecologic clinic patients, PACE had a sensitivity of 80%, a specificity of 91.5%, and positive and negative predictive values of 59.3 and 96.7%, respectively (M. N. Steinman, S. B. Overman, and N. L. Goodman, Abstr. Annu. Meet. Am. Soc. Microbiol. 1989, C115, p. 412). Peterson et al. tested 196 women, and the probe assay had a sensitivity of 60%, a specificity of 95%, and positive and

negative predictive values of 60 and 95%, respectively (E. M. Peterson, R. Oda, R. Alexander, J. R. Greenwood, and L. M. de la Maza, Abstr. Annu. Meet. Am. Soc. Microbiol. 1989, C118, p. 413). DeBates et al., who evaluated the probe assay in 113 women attending a sexually transmitted disease clinic, reported a sensitivity of 76.5% (M. DeBates, K. Thompson, M. Case, K. Wieczorek, P. O'Keefe, and C. Libertin, Abstr. Annu. Meet. Am. Soc. Microbiol. 1989, C116, p. 412). In a study of patients (117 females, 70 males) attending sexually transmitted disease and infertility clinics, PACE had a sensitivity of 83% and a specificity of 75% in females and a sensitivity of 68% and a specificity of 75% in males (C. A. Gratton, P. C. Kibsey, R. Lim-Fong, E. Prasad, and B. Romanowski, Abstr. Annu. Meet. Am. Soc. Microbiol. 1989, C117, p. 413). Lowering the cutoff for a positive probe result by 200 relative light units would improve the sensitivity slightly, but concomitantly, the positive predictive value would decrease. Therefore, altering the cutoff does not appear to offer any advantage.

As with any evaluation of a test for detection of *C. trachomatis*, there were inherent problems in our study design. First, when multiple samples are collected from one site, the possibility exists that the order of collection will bias the results. Therefore, the order of sample collection was rotated. The order of collection did not appear to have a major influence on results. Specimen randomization was further enhanced by the fact that a large number of clinicians, each collecting the specimen with different vigor, were involved in the evaluation. Second, and most importantly, the sensitivity of culture, which is the gold standard against which new tests are compared, is less than 100% (6, 11, 12). Consequently, interpretation of positive probe results that were not confirmed by culture was difficult. One possible explanation for these results is that we failed to detect all positive specimens by culture since we did not perform blind passage; however, in our experience blind passage has not significantly increased the detection of positive specimens. Likewise, others have failed to demonstrate increased detection by performing blind passage (13). Another possible reason for probe-positive, culture-negative results is that the probe was hybridizing to an unidentified substance in the specimen. Charts were reviewed to discern the likelihood of *C. trachomatis* infection. However, because many patients infected with *C. trachomatis* are asymptomatic, this approach does not adequately address the discrepant test results. A final criticism involves the relatively small sample size. Although we were unable to document a significant difference between tissue cell culture and probe results, the number of tests that were positive by the probe but not confirmed by culture is disconcerting and should be used as preliminary data to initiate a larger study.

In summary, given the number of probe-positive, culture-negative samples, we cannot recommend using the PACE system to screen for *C. trachomatis* in women with a low to moderate risk for infection. Moreover, when the probe assay is used, it is not possible to assess the quality of the specimen, which is one advantage of the DFA. However, a modified Gen-Probe PACE assay, which requires less hands-on time, has been developed. We are presently evaluating this improved system, and we plan to perform a more in-depth evaluation of specimens yielding discrepant results.

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