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Effect of Patient Characteristics on Performance of an Enzyme Immunoassay for Detecting Cervical *Chlamydia trachomatis* Infection

LAURENCE S. MAGDER,[1]† KARL C. KLONTZ,[2]‡ LINDA H. BUSH,[3] AND ROBERT C. BARNES[1,4]§*

Division of Sexually Transmitted Diseases, Center for Prevention Services,[1] Division of Field Services, Epidemiology Program Office,[2] and Sexually Transmitted Diseases Laboratory Program, Center for Infectious Diseases,[4] Centers for Disease Control, Atlanta, Georgia 30333, and Department of Nursing, Duval County Public Health Unit, Florida Department of Health and Rehabilitative Services, Jacksonville, Florida 32206[3]

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We compared the performance of a commercial enzyme immunoassay (EIA) (Chlamydiazyme; Abbott Diagnostics, North Chicago, Ill.) with that of cell culture for the detection of *Chlamydia trachomatis* cervical infection in 1,417 women attending public health clinics. Confirmatory chlamydial testing by a direct fluorescent-antibody test (MicroTrak; Syva Co., Palo Alto, Calif.) was performed on specimens from women who had positive EIAs. Overall, only 57% of women who had a positive chlamydial test by cell culture were also positive by EIA. We noted a strong association between the number of chlamydial inclusions in cell culture and a positive EIA outcome. The proportion of culture-positive women who also had a positive EIA declined with age and a history of previous sexually transmitted disease and increased among oral contraceptive users. The results of direct fluorescent-antibody confirmatory testing suggested that cell culture was also insensitive for the detection of *C. trachomatis* infection. Our observations demonstrate that the performance of the chlamydial EIA may vary greatly with individual patient characteristics and that the utility of EIA as a screening test may be limited, especially in older women.

The performance of enzyme immunoassays (EIAs) for detecting *Chlamydia trachomatis* infections of the cervix has been evaluated in many studies (1, 3, 5, 9, 11, 12, 14, 15, 18–21, 23–25, 27, 28; for a review, see reference 26), with various results. The proportion of chlamydial culture-positive patients who were found to be positive by EIA has varied from 67% (25) to 100% (15), while the proportion of chlamydial culture-negative patients who were found to be negative by EIA has ranged from 91% (15) to 98% (1, 5, 9, 14, 19). Differences in the reported performance of EIAs can be attributed to differences in chlamydial culture systems or to clinical and statistical sampling variabilities. However, another possible source of variation might be the characteristics of the patients being tested (2, 24, 26). The performance of EIAs has been shown to be related to the number of inclusions found in positive cultures (1, 25); and the number of inclusions found in positive cultures was found to be significantly associated with age, birth control method, and other patient characteristics in several studies (2, 10). Therefore, it seems plausible that the performance of an EIA may vary according to these factors. To investigate this possibility, we performed a chlamydial culture and an EIA (Chlamydiazyme; Abbott Diagnostics, North Chicago, Ill.) for chlamydia on more than 1,400 women attending public health clinics in Jacksonville, Fla., and compared the results in subgroups defined by various patient characteristics.

* Corresponding author.
† Present address: Department of Biostatistics, Johns Hopkins University, Baltimore, MD 21205.
‡ Present address: Clinical Nutrition Section, Food and Drug Administration, Washington, D.C. 20204.
§ Present address: Consultants in Medicine, 800 East Chestnut Street, Bellingham, WA 98225.

MATERIALS AND METHODS

Enrollment of patients. Women presenting at 11 Duval County, Fla., Public Health Unit clinics (including clinics for family planning, gynecology, sexually transmitted diseases [STDs], and adolescent health) from January to July 1987 for any service requiring a pelvic examination were asked to participate in the study. Women were excluded if they refused to participate in the study, if they were pregnant at the time of the evaluation, or if they were allergic to erythromycin. Women who volunteered were interviewed by using a standardized questionnaire which included questions regarding the reason for their clinic visit; their method of birth control; the date of their last menstrual period; their number of sexual partners in the preceding 6 months; the location and nature of genitourinary or abdominal symptoms; and a prior history of gonorrhea, syphilis, chlamydia, trichomoniasis, genital herpes, or pelvic inflammatory disease.

Sample collection and laboratory methods. During the pelvic examination, an unlubricated speculum was inserted to visualize the uterine cervix. A visual examination of the cervix was made which included the amount and quality of cervical discharge, the presence of menstrual blood, and the presence of cervical ectopy. A cotton swab was used to remove excess ectocervical mucus. If bleeding of the ectocervix occurred during cervical manipulation, cervical friability was recorded. Women who were determined to be at high risk for chlamydial infection by recommendations of the Centers for Disease Control (4) were treated empirically with erythromycin.

Following ectocervical cleaning, a Papanicolaou smear specimen was obtained, if indicated, followed by a specimen from the endocervix for culture of *Neisseria gonorrhoeae*. Specimens were next collected for chlamydial direct fluorescent-antibody (DFA) testing and EIA. Collection of speci-

mens for chlamydial antigen detection by the DFA test or EIA was performed according to the instructions of the manufacturers. The order of samples for chlamydial antigen detection was randomized by a consecutive numbering scheme. Specimens for EIA were transported daily to a local laboratory, and tests were conducted according to the package insert provided by the manufacturer. DFA specimen slides were stored at −70°C until they were tested. Slides from women with positive Chlamydiazyme test results were thawed and stained for DFA testing with the MicroTrak reagent (Syva Co., Palo Alto, Calif.) by the method described in the package insert provided by the manufacturer.

Following collection of specimens for chlamydial antigen detection, a specimen was obtained for chlamydial culture. A Dacron swab was rotated in the endocervical canal for 20 to 30 s and was then placed in 2-SP (8) transport medium at 4°C. These specimens were transported on ice to a local laboratory daily and stored at −70°C pending a weekly shipment to the Chlamydia Laboratory at the Centers for Disease Control, where cell culture was performed. Chlamydia isolation was performed in cycloheximide-treated McCoy cells in glass vials as described by Ripa and Mårdh (22). Chlamydial inclusions were detected by using fluoresceinated monoclonal antibody preparations.

Statistical methods. To assess the statistical significance of the difference between two proportions, we used a Pearson chi-square statistic. To test the significance of a trend in proportions by age group and to test the effect of one factor controlling for others by stratification, we used an extension of the H statistic of Mantel (17).

RESULTS

More than 90% of the eligible women who were asked volunteered to participate in the study. Of the 1,481 women originally enrolled, no culture or EIA results were available for 64 women, leaving results for a total of 1,417 women to be included in the data analysis. The mean age of the women was 24.8 years, with 41% of the women being 25 years of age or older. A total of 55% of the women were black, and 44% were white. The primary reason for the clinic visit for 53% of those enrolled was related to birth control, while 15% came to the clinic because of symptoms, 5% came to the clinic because of contact with STD, 8% came for a pregnancy check, and 18% came for other reasons.

Overall, 12% of the women were positive for *C. trachomatis* by cell culture. The proportion of women who were positive ranged from 8 to 16% in the 11 clinics, with the exception of the adolescent clinic, where, of only 19 patients enrolled, 6 (32%) were culture positive. The proportion of women with positive cultures was somewhat higher in blacks (13%) than in whites (9%) ($P = 0.022$); much higher in women under age 25 (15%) than in older women (6%) ($P < 0.001$); and higher in those with purulent discharge (20%) than in those with mucoid (9%), clear (10%), or no discharge (11%) observed on examination ($P < 0.014$). Among the women who used oral contraceptives, 13% were culture positive compared with 9% of those who reported using other birth control methods and 12% of those who used no birth control.

Tables 1 and 2 show the degree of agreement between culture and EIA results by various patient characteristics. Overall, 57% of the women who were culture positive were also found to be positive by EIA. This proportion declined significantly by age group ($P < 0.001$), was lower in those with a history of STD ($P = 0.054$), and was somewhat higher among those who used oral contraceptives ($P = 0.061$) (Table 1). The decline by age was still significant ($P < 0.001$) after controlling, by stratification, for whether the patients had a history of a previous STD. Similarly, the association with a history of STD was still significant ($P = 0.013$) after controlling for age group. There were no significant associations between genital symptoms or signs and the proportion of culture-positive women who were found to be positive by EIA (Table 2); however, the sample size was not large enough to rule out important differences. A strong association was observed between the number of inclusions found in positive chlamydial cultures and the results of the EIA ($P < 0.001$; Table 2).

TABLE 1. Agreement between chlamydial culture and EIA results by patient characteristics[a]

Patient characteristic	No. positive/total no. (%) of culture-positive subjects who were also EIA positive	No. positive/total no. (%) of culture-negative subjects who were also EIA negative
Overall	93/163 (57)	1,118/1,254 (89)
Age (yr)		
≤19	44/55 (80)	232/273 (85)
20–24	35/71 (49)[b]	387/433 (89)[c]
≥25	12/35 (34)	487/535 (91)
Race		
White	30/59 (51)	517/571 (91)
Black	62/103 (60)	591/672 (88)
Other	1/1 (100)	10/11 (91)
History of STD		
Yes	12/31 (42)	272/300 (94)
No	74/121 (61)[d]	762/859 (94)
No. of inclusions found per cover slip		
1–10	25/66 (38)	
11–100	40/62 (65)[b]	
≥101	25/30 (83)	

[a] Because of missing data items, the sum of variable denominators may not equal the overall denominator. *P* values for the association between each factor and EIA results (based on the Pearson chi-square statistic) are provided when they are less than 0.1.
[b] $P < 0.001$.
[c] $P = 0.032$.
[d] $P = 0.054$.

Of 1,254 women who were found to be negative by cell culture, 89% were also found to be negative by EIA (Table 1). This proportion increased somewhat with age ($P = 0.032$; Table 1) and was significantly higher in women without ectopy compared with that in women with ectopy ($P = 0.012$; Table 2).

There were 123 patients who tested positive by EIA but negative by cell culture. Of the 104 of these patients for whom there were unequivocal DFA test results, 65 (63%) were also positive by the DFA test. If the DFA-positive patients were removed from the analysis, there was no longer a statistically significant association between the proportion of culture-negative patients identified as negative by EIA and either age or cervical ectopy.

DISCUSSION

As hypothesized, a significant association was observed between the proportion of chlamydial culture-positive

TABLE 2. Agreement between chlamydial culture and EIA results by patient symptoms and clinical findings[a]

Patient symptom or clinical finding	No. positive/total no. (%) of culture-positive subjects who were also EIA positive	No. positive/total no. (%) of culture-negative subjects who were also EIA negative
Reported cervical or vaginal discharge		
Yes	20/39 (51)	299/334 (90)
No	66/108 (61)	721/810 (89)
Cervical discharge on examination		
None	43/78 (55)	566/622 (91)
Clear	5/10 (50)	82/92 (94)
Mucoid	15/22 (68)	183/214 (86)
Purulent	19/27 (68)	99/109 (93)
Cervical friability		
Yes	25/35 (71)	203/235 (95)[b]
No	61/106 (58)	756/837 (90)
Cervical ectopy		
Yes	23/37 (62)	230/269 (86)[c]
No	61/102 (60)	731/804 (91)
Contraceptive method		
None	28/58 (48)	390/442 (88)
Oral	46/70 (66)[d]	436/487 (90)
Other	7/17 (41)	160/175 (91)

[a] Because of missing data items, the sum of variable denominators may not equal the overall denominator. *P* values for the association between each factor and EIA results (based on the Pearson chi-square statistic) are provided when they are less than 0.1.
[b] $P = 0.082$.
[c] $P = 0.012$.
[d] $P = 0.061$.

women who were found to be positive by an EIA and various patient characteristics. Most notably, the proportion declined with age, was lower among those who had a history of STDs, was higher among those who used oral contraception, and was positively associated with the number of inclusions observed in the cultures.

We have deliberately avoided the use of the words sensitivity and specificity in comparing the EIA results with the cell culture results because of our concern that cell culture is inadequate as a "gold standard" for detection of *C. trachomatis* (20). Although the specificity of the cell culture method of *C. trachomatis* detection is reasonably presumed to be 100%, recent studies have cast doubt on the sensitivity of culture diagnosis. In several studies, repeated sampling and culturing of the cervix yielded approximately 40% more chlamydial infections than did a single culture swab (6, 7). In another laboratory, culture in microdilution plates with one blind passage detected only 64% of infections that were eventually detected after exhaustive passage (13).

In many studies that compare methods of *C. trachomatis* detection, a high sensitivity for cell culture is presumed; however, it seems possible that there are some chlamydial infections which are difficult to detect by culture by virtue of low-level excretion of organisms or effects of host immunity. If these infections constitute a substantial portion of all chlamydial infections, the sensitivity of culture might be much less than that estimated previously. One way of quantifying the level of chlamydial shedding is to record the number of inclusions found per cover slip in positive cultures. Unpublished data (L. S. Magder, unpublished data) from a previous study (16) found a direct relationship between the number of inclusions found in one chlamydial culture and the positivity of a second culture obtained immediately after the first culture. This suggests that low-level infections are indeed more difficult to culture.

It also seems that low-level chlamydial infections are more difficult to identify by EIA, since we and others (1, 25) have shown that the performance of EIAs is directly related to the number of inclusions found in culture. Thus, the sensitivity of an EIA for chlamydial infections that were not detected by culture is probably lower than the sensitivity of EIA among culture-positive individuals. This suggests that the overall proportion of culture-positive individuals found to be EIA positive in our study, 57%, is an overestimate of the true sensitivity of EIA in our laboratories. This and the remainder of our study data suggest that EIA sensitivity is lower for older women and for women with previous STDs and is somewhat higher for women who use oral contraceptives.

The specificity of EIA is difficult to estimate from our data because many of the culture-negative, EIA-positive women were found to be positive by the DFA test and, therefore, may have actually been infected (true positive). If those women with positive DFA confirmatory test results were assumed to be infected (true positive), then the EIA had a determined specificity of 94% in our study. Under the same assumption, there was no statistically significant association between the specificity of EIA and any of the patient characteristics we considered.

Although a diagnostic test that misses almost half of the infections would seem inadequate in most contexts, the resource-limited world of public health might be an exception. It is likely that the currently available alternatives to EIA commonly used for chlamydial detection, cell culture and the DFA test, are not dramatically more sensitive. Despite the low sensitivity of the EIA, a negative EIA may have a high predictive value. In our study, using culture as the standard, the predictive value of a negative EIA was 94%. However, it must be recognized that a high predictive value of a negative test result is a natural consequence of the rarity of infection in a low-prevalence population. Thus, a high predictive value of a negative test alone does not justify the use of a diagnostic test as a screening test in populations with low pretest probabilities of infection. It is clear that no current clinical or laboratory diagnostic test has proven satisfactory for screening low-prevalence populations for chlamydial cervical infection. The current methods are, however, suitable for finding cases of infection in populations at moderate or high risk for infection. In laboratory settings with less limited resources for testing, sequential diagnostic testing to enhance the overall detection rate would seem appropriate. However, reports of highly discordant detection rates in chlamydial testing by several methods (20) may indicate that only improved laboratory diagnostic methods will satisfactorily address the difficulty of diagnosing chlamydial infections.

In conclusion, our results suggest that the performance of EIA for diagnosing *C. trachomatis* cervical infections in public health clinics may not be as good as was previously estimated. In particular, our observation that the sensitivity of EIA varies with the age and other characteristics of the patient demands further study. As a relatively easy way to explore this issue, we suggest that investigators with access

to data from previous evaluations of EIA for *C. trachomatis* detection report EIA performance by age and other patient characteristics.

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