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Limited Value of Two Widely Used Enzyme Immunoassays for Detection of *Chlamydia trachomatis* in Women

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Enzyme immunoassays (EIAs) are widely used to diagnose chlamydial infections in patients attending genitourinary medicine clinics. They are relatively easy to perform and are suitable for testing large numbers of samples. The objective of this study was to determine what proportion of women with chlamydial infection, defined as the presence of *Chlamydia trachomatis* in a cervical smear or deposit and/or in the urinary tract, detected by means of a sensitive direct fluorescent antibody test could also be identified by using two commercially available EIAs to test cervical samples. One hundred fifty-one women attending the genitourinary medicine clinic at St. Mary's Hospital, London, were enrolled. The use of the Chlamydiazyme (Abbott Diagnostics, UK) and MicroTrak (Syva, UK) EIAs resulted in the identification of only 56 % and 63 %, respectively, of women with chlamydial infection detected by direct fluorescent antibody staining. Thus, the EIAs available for detection of chlamydiae in cervical samples are inadequate for identifying all infected women. Improvement might be achieved by testing multiple samples or by resorting to tests of greater sensitivity.

In many clinical laboratories the enzyme immunoassay (EIA) has become the method of choice for the detection of *Chlamydia trachomatis*. It is less labour-intensive than culture and more suitable than a direct fluorescent antibody (DFA) test for screening large numbers of samples. We have reviewed the reported sensitivity values for many commercially available EIAs (1). They vary widely and, indeed, values for a single assay reputedly vary depending upon the prevalence of chlamydiae in the population being studied and whether patients are symptomatic or asymptomatic (2). The most relevant factor, however, must be the detection method with which an assay is compared, since an assay may be made to appear more sensitive than it actually is by using an insensitive comparator. Only by comparing a new assay with the most sensitive detection method available will the real value of the assay be ascertained.

The results of recent studies have shown that centrifuging samples and staining the deposit in a DFA test is a very sensitive method for detecting chlamydial infection in women (3). In addition, some chlamydia-positive women can be identified only by examining samples from the urinary tract, that is urethral swabs and/or urine (4). If all these factors are considered, the sensitivity of detecting *Chlamydia trachomatis* in women has been improved by 18 % (4).

We tested cervical samples by two EIAs (MicroTrak EIA and Chlamydiazyme) and compared the results with those obtained by examining cervical smears, centrifuged cervical deposits and urinary tract samples by the DFA test in order to assess what proportion of women with chlamydial infection is identified by these two EIAs.

Materials and Methods

Subjects. Samples were taken from women who were recruited during a study of the aetiology of mucopurulent cervicitis (4). One hundred fifty-one women attending the Jefferiss Wing (genitourinary medicine clinic) of St. Mary's Hospital, London, were included. Subjects were excluded if they had received antibiotics with activity against *Chlamydia trachomatis* in the last three months.

Procedure. Samples were collected as follows. The urethral meatus was cleaned with saline-soaked gauze. A smear for the DFA test (MicroTrak, Syva, UK) to detect *Chlamydia trachomatis* was prepared from a fine cotton-tipped swab introduced 2–3 cm into the urethra. A non-lubricated specu-

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lum was passed and the cervix cleaned with a cotton-tipped swab. Endocervical material was collected by swabbing the endocervix and areas of ectopy on the ectocervix; one swab was expressed in transport medium for the Chlamydiazyme EIA (Abbott Diagnostics, UK). A second swab was rolled on a slide to produce a smear for DFA staining and then placed in transport medium for the MicroTrak EIA (Syva, UK). The order of collecting the two swabs was alternated between clinic sessions. The patient then collected the first 20 ml of voided urine.

Handling of Samples. Urine samples were stored at 4 °C for a maximum of three days. They were warmed to 37 °C to dissolve any deposit which had formed on cooling, vortexed to break up threads and distribute the cell content evenly, and centrifuged at 3,000 rpm for 30 min in an MSE Mistral 2000 centrifuge. Deposits were resuspended in 1 ml volumes of phosphate-buffered saline and stored at -70 °C. Prior to testing by the DFA method, these deposits were thawed and centrifuged at 13,000 rpm in a microcentrifuge (MSE MicroCentaur). The resulting deposit was suspended in 20 µl of distilled water and dried on a MicroTrak slide. One ml of "specimen treatment solution" was added to each cervical swab to be tested by the MicroTrak EIA, which was performed according to the manufacturer's instructions. The sample remaining after 200 µl had been removed for the EIA was centrifuged at 13,000 rpm in the MicroCentaur for 10 min. The deposit was resuspended in 20 µl of distilled water and dried on a MicroTrak slide for DFA testing.

Techniques for Detecting Chlamydia trachomatis. The MicroTrak DFA test was used as described previously (5). Urethral smears and urine deposits were fixed in acetone and stained with 15 µl of MicroTrak direct specimen test reagent. Smears were considered inadequate if they contained few epithelial cells in the absence of chlamydial elementary bodies (EBs) or if they were too thick for individual cells to be brought into focus on microscopy. A sample was regarded as positive if one or more EB was seen. The MicroTrak EIA and the Chlamydiazyme EIA procedures were undertaken according to the manufacturers' instructions. Positive Chlamydiazyme results were confirmed by a blocking assay.

Specimens Tested. Specimens from 151 women were tested by the MicroTrak DFA test. For various technical reasons, samples from only 146 of these women were tested by the MicroTrak and Chlamydiazyme EIAs. The exclusion of inadequate specimens accounts for the analysis of specimens from only 139 women.

Results

Detection of Chlamydia trachomatis in Cervical Smears, Deposits and Urinary Tract Samples by DFA Staining. Cervical smears, cervical deposits and urinary tract samples (urethral smear, urine deposit) were available from 151 women (Table 1). *Chlamydia trachomatis* was detected by DFA staining in the cervix of 37 (24.5 %) of them. More cervical deposits (n = 37) than smears (n = 26) were positive, and many more cervical smears (n = 9) than deposits (n = 1) were inadequate. Overall, 36 women had *Chlamydia trachomatis* detected by DFA staining of samples from the urinary tract, and five (14 %) of these women were chlamydia-negative in their corresponding cervical deposits and/or smears.

Comparison of Results of MicroTrak and Chlamydiazyme EIAs in Cervical Samples with Those of Staining Cervical Smears by DFA. The results of the three tests in samples from 146 women are shown in Table 2. In comparison with the results of staining cervical smears with DFA, the sensitivities of the MicroTrak and Chlamydiazyme EIAs were 87.5 % and 75 %, respectively, and the specificities 98 % and 99 %, respectively. However, all samples positive by both or either of the EIAs when the cervical smear was negative by DFA were from patients whose cervical deposit and/or urinary tract samples were positive by DFA staining. If these EIA results are considered to be truly positive, the specificity of both EIAs becomes 100 %. Similarly, all the women whose cervical samples were positive by either EIA and who had inadequately DFA-stained cervical smears had at

Table 1: Detection of *Chlamydia trachomatis* in cervical smears and deposits and in urinary tract samples by DFA staining.

Results for urinary tract samples	Results for cervical smears and deposits					
	C. trachomatis present		*C. trachomatis* absent		Samples inadequate*	
	Deposit	Smear	Deposit	Smear	Deposit	Smear
C. trachomatis present	31	26	4	5	1	5
C. trachomatis absent	6	0	109	111	0	4
Total	37	26	113	116	1	9

*See text.

Table 2: Comparison of testing cervical samples for *Chlamydia trachomatis* by MicroTrak and Chlamydiazyme EIAs with testing cervical smears by the DFA test.

DFA results for cervical smears	Results for cervical samples			
	MicroTrak EIA		Chlamydiazyme EIA	
	Positive	Negative	Positive	Negative
Positive	21	3	18	6
Negative	2[a]	110	3[a]	109
Samples inadequate	4[a]	6[b]	2[a]	8[b]
Total	27	119	23	123

[a] All positive by DFA test in another sample.
[b] Two of 6 and 4 of 8 positive by DFA test in another sample.

Table 3: Comparison of testing cervical samples for *Chlamydia trachomatis* by MicroTrak and Chlamydiazyme EIAs with testing cervical deposits by DFA.

DFA results for cervical deposits	Results for cervical samples			
	MicroTrak EIA		Chlamydiazyme EIA	
	Positive	Negative	Positive	Negative
Positive	26	9	22	13
Negative	0	108	1[a]	107
Inadequate	0	3[b]	0	3[b]
Total	26	120	23	123

[a] Positive by DFA test in the cervical swab and urinary tract.
[b] One of 3 positive by DFA test in the urinary tract.

Table 4: Comparison of MicroTrak and Chlamydiazyme EIA results with a positive *Chlamydia trachomatis* result in any sample.

DFA result for any sample	Results for cervical samples			
	MicroTrak EIA		Chlamydiazyme EIA	
	Positive	Negative	Positive	Negative
Positive	26	15	23	18
Negative	0	98	0	98
Total	26	113	23	116

least one other sample positive by DFA, as did four women whose samples were negative by one or both EIAs.

Comparison of Results of MicroTrak and Chlamydiazyme EIAs in Cervical Samples with Those of Staining Cervical Deposits by DFA. The results of the three tests in samples from 146 women are shown in Table 3. In comparison with the results of staining cervical deposits with DFA, the sensitivities of the MicroTrak and Chlamydiazyme EIAs were 74 % and 65 %, respectively. This represents a reduction in the sensitivity of these tests compared to that seen when DFA staining of cervical smears was used as the comparator.

Comparison of Results of MicroTrak and Chlamydiazyme EIAs in Cervical Samples with Those of

Staining Cervical Smears, Deposits and Urinary Tract Samples by DFA. The results of the three tests in samples from 139 women are shown in Table 4. In comparison with the results of staining cervical smears, deposits and urinary tract samples with DFA, and regarding a positive result for any sample as indicative of an infected woman, the sensitivities of the MicroTrak and Chlamydiazyme EIAs were 63 % and 56 %, respectively. These sensitivities were, again, less than those noted previously in this study.

EIA Results. Of 41 women who were chlamydia-positive in at least one sample by the DFA test, 20 were positive and 12 were negative by both EIAs. Five of the 12 negative women also had negative or inadequate cervical tests by DFA staining, and chlamydiae were detected in their urinary tract alone; a further four of them had chlamydiae only in their DFA-stained cervical deposits but, in the remaining three, most cervical and urinary tract samples were DFA-positive. All six women whose cervical samples were negative in the Chlamydiazyme test but positive by MicroTrak EIA had chlamydiae detected in most samples by the DFA test; three women whose samples were positive by Chlamydiazyme but negative by MicroTrak EIA had at least one other sample positive by the DFA test.

Discussion

The sensitivity and specificity values of any detection method are not absolute but vary relative to the quality of the method with which it is compared. Thus, the values for the sensitivity of the MicroTrak and Chlamydiazyme EIAs, examined in this study, varied between 88 % and 65 % and 75 % and 56 %, respectively, in relation to the least and most sensitive comparators. However, as reported elsewhere (6), both EIAs were found to be extremely specific, apparently false-positive EIA results being confirmed as truly positive by using a sensitive procedure to test samples from the same or another site in the same patient.

The MicroTrak EIA was found consistently to be more sensitive than the Chlamydiazyme EIA, despite the samples for the former being used initially to make smears for DFA staining. When a false-negative result occurred with the MicroTrak EIA, at least one of the DFA tests in cervical samples was also negative, indicating that the swab contained only a small number of chlamydial EBs. However, when a false-negative result occurred with the Chlamydiazyme EIA, all the other cervical samples stained by the DFA test were either inadequate or chlamydia-positive, suggesting poor sampling or a relatively insensitive test.

For the routine diagnosis of *Chlamydia trachomatis* infection in patients attending genitourinary medicine clinics, EIAs are labour-saving and objective alternatives to DFA staining. However, the results of this study indicate that in only about 60 % of women who are infected with *Chlamydia trachomatis* at some site will an accurate diagnosis be made by two of the assays used most widely. The detection rate might be improved by combining cervical and urethral samples into the same transport medium, thus increasing the antigen content of the sample, in the same way that we increased sensitivity previously by combining multiple samples from the cervix (7). However, women who are chlamydia-positive in the urethra alone or in the urine alone have only small numbers of EBs (4), and it is unlikely that these would be detected by the current EIAs.

Routine detection of *Chlamydia trachomatis* by DFA staining is impractical if large numbers of samples are involved. Moreover, if not performed by a skilled observer and if centrifugation of specimens is not undertaken, DFA staining is probably no more sensitive than the best EIA (B.J. Thomas, unpublished data). It is important that clinicians and manufacturers of the EIAs are aware of the large number of women whose chlamydial infections remain undetected and, therefore, untreated when samples are subjected to examination by these currently available and widely used diagnostic methods. Use of the polymerase chain reaction and/or the ligase chain reaction may resolve the problem in the future.

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