Comparison of Monoclonal Antibody Staining and Culture in Diagnosing Cervical Chlamydial Infection

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We compared a fluorescein-conjugated monoclonal antibody (FA) direct specimen test (MicroTrak; Syva Co., Palo Alto, Calif.) with culture (TC) in McCoy cells (vials, with blind passage and iodine staining of inclusions for diagnosis of Chlamydia trachomatis infection in the cervix. Duplicate specimens were collected from 1,230 women, but for 262 of these subjects, both results were unavailable (150 FA smears were inadequate, indicating a need for clinician training in specimen collection), leaving 968 comparisons. Prevalence of chlamydiae by culture was 13% (126/968). Compared with TC results, the sensitivity of FA was 70% (88/126) and the specificity was 94% (795/842). There was a 91% agreement (883/968). The predictive value of a positive FA test was 65% (88/135), and that of a negative FA was 95% (795/833). We reexamined 38 smears for which paired results were discrepant, and the reread would have changed the result in only 5 of these. TC is <100% sensitive and some FA-positive, TC-negative specimens represent positive specimens not detected by TC. Unfortunately, it is not possible to identify which results in this group are truly false-positive. Clearly, the FA procedure has a performance profile which would make it a useful tool in screening high-risk populations (particularly when TC is not available) but it is less suited to screening low-risk populations, for which false-positive results are more important. The greater utility of the FA procedure in a venereal disease clinic was confirmed by testing 172 evaluable specimen pairs, of which 34 (20%) were Chlamydia isolate positive. The FA sensitivity was 76% (26/34) and specificity was 96% (133/138), giving a predictive value of 84% (26/31) for a positive test.

In the last decade, Chlamydia trachomatis has been recognized as one of the most common sexually transmitted pathogens (2, 5). Approximately 3 million new chlamydial infections occur annually in the United States (3). In men, it causes nongonococcal urethritis and epididymitis. In women, it may cause cervicitis, the urethral syndrome, endometritis and salpingitis, or perihepatitis. Many genital infections are asymptomatic. Infants passing through an infected birth canal are at risk of developing inclusion conjunctivitis or pneumonia or both (6).

Chlamydiae are obligate intracellular parasites which infect and subsequently multiply in columnar epithelial cells. Tissue culture of chlamydiae requires facilities that are not generally available, are costly, and require 3 to 7 days for completion. Thus, the management of chlamydial infections is often presumptive, based on clinical indicators (8). Presumptive therapy for symptomatic patients, however, does not control the increasing reservoir of asymptomatic women, who are subject to serious complications and who may unknowingly infect sexual partners and neonates. Development of a rapid, inexpensive, sensitive, and specific diagnostic test for C. trachomatis would aid in efforts to control chlamydial infections by detecting the large number of asymptomatic infections in the population.

The MicroTrak Chlamydia trachomatis direct specimen test (Syva Co., Palo Alto, Calif.) has been presented as a rapid alternative method of detecting C. trachomatis infection. The test uses fluorescein-conjugated monoclonal antibodies, reactive with all 15 known human serovars of C. trachomatis, to detect elementary bodies in clinical smears (9, 10). We compared the fluorescein-conjugated monoclonal antibody (FA) system with TC in diagnosing cervical chlamydial infection in three populations of women.

MATERIALS AND METHODS

Patient population. In this study, clinical specimens were received from three populations of females: (i) asymptomatic females in an adolescent clinic, (ii) asymptomatic females attending a planned parenthood clinic, and (iii) patients with acute salpingitis.

Specimen collection. Duplicate endocervical specimens were collected from women by using a dacron swab for the MicroTrak direct specimen test and a calcium alginate swab for TC. Sampling order was randomized. The exocervix was first cleaned with a cotton or dacron swab to remove mucus and exude. Another dacron swab was inserted into the endocervical canal, rotated against the wall and removed, avoiding contact with any vaginal surface. The swab was rolled onto the MicroTrak slide, covering the 8-mm well evenly with specimen. The slide was air dried, fixed by flooding with 100% acetone, and air dried again. Fixed slides were stored at 4°C until stained. A calcium alginate swab was collected the same way and then immersed in transport medium (2.0 ml of Eagle minimal essential medium in Earle salts containing 10% fetal calf serum, 1% L-glutamine [200 nM solution], 10 μg of gentamycin per ml, 100 μg of vancomycin per ml, 10 U of mycostatin per ml, and 3 μM glucose per ml) for culture. Specimens were stored at 4°C for up to 72 h before inoculation of cells.

MicroTrak procedure. The MicroTrak reagent contains fluorescein-conjugated monoclonal antibodies against the chlamydial major outer membrane protein and a counterstain (Evan blue) in a protein-stabilized buffer solu-

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tion. The acetone-fixed slides were incubated with 30 μl of the reconstituted FA reagent for 15 min at room temperature in a moist chamber. Slides were then rinsed in distilled water and allowed to air dry, and cover slips were applied with the MicroTrak mounting fluid (phosphate-buffered saline-glycerol with antiphotobleach). We used a Zeiss fluorescent microscope equipped with epi-illumination, a quartz halogen light source (12 V, 100 W), and a filter system for fluorescein isothiocyanate examination. Patient and control slides were screened at ×400 magnification, and all positive slides were confirmed at ×1,000 magnification. Every field within the well was scanned. Slides were scored as positive only when 10 or more smooth, evenly fluorescing, bright apple-green disks consistent with chlamydial elementary bodies were seen. Negative slides contained fewer than 10 fluorescing chlamydial particles per well. All slides were graded as follows: 0, no particles seen; 1, <10 particles; 2, 10 to 25 particles; 3, >25 particles seen per slide. Thin acellular smears containing only squamous cells or polymorphonuclear leukocytes indicated that an inadequate specimen had been collected, and specimen pairs producing such smears were excluded from analysis.

TC. Chlamydia TC tests were carried out in cycloheximide-treated McCoy cells in 1-dram (ca. 4-g) shell vials by using a modification of the procedure of Ripa and Märdh (4). Inocula were centrifuged onto the monolayers (2,700 × g for 1 h). After incubation at 35°C under 5% CO2 for 3 days, one vial of each specimen was fixed with methanol and stained with iodine. Cover slips were removed from the vials and examined microscopically for intracytoplasmic inclusions of C. trachomatis. Four days after first passage, the two remaining vials were pooled for a second (blind) passage, which was done in the same manner. Cultures were graded as follows: 0, negative culture; 1, <25 inclusions; 2, 25 to 100 inclusions; 3, >100 inclusions per cover slip.

**Statistical comparisons.** Sensitivity, specificity, and predictive values were calculated by using standard techniques (1).

**RESULTS**

Of 1,230 specimens collected for this study, 262 matched results were unavailable for comparison. Of these 262 specimens, 150 were inadequate FA smears, 64 were inadequate for culture (too old to process or producing an inconclusive result due to cytopathic effect), and the rest were unacceptable due to mislabeling or missing paired specimens. The results of the remaining 968 paired specimens (FA and TC) are shown in Table 1. The prevalence of chlamydiae in TC was 13% (126/968). Compared with TC results, the sensitivity of FA was 70% (88/126) and the specificity was 94% (795/842). There was 91% agreement (883/968) between the tests. The predictive value of a positive FA test was 65% (88/135), and that of a negative FA test was 95% (795/833). We reexamined 38 smears in which paired results were discrepant, and the result changed in only 5 on rereading. The comparison of FA with TC by clinic is shown in Table 2. In different clinics, the sensitivity of FA ranged from 63 to 76%, the specificity of FA ranged from 92 to 97%, and the predictive value of a positive FA test ranged from 55 to 77%. While the differences are not statistically significant, the variation suggests that differences in specimen handling did exist.

A comparison of the degree of positivity between FA and TC results is shown in Table 3. A total of 45 MicroTrak specimens were given a score of 1 (<10 elementary bodies were seen per slide). Forty-one of the 45 paired specimens were negative in TC and 4 were positive in TC. If we had used a cutoff value of 1 elementary body per slide for a positive MicroTrak specimen, an additional 41 false-positive FA slides would have resulted, and the overall sensitivity of the test would have changed from 70 to 71%, but specificity would have dropped from 94 to 89%, and the predictive value of a positive test would have decreased from 64 to 51%. Thus, the recommended cutoff value of 10 elementary bodies or more as the criterion for a positive MicroTrak test was supported by our data.

Smears averaged 3 to 5 min to read, depending on the quality of the smear. The presence of artifacts on the slide increased the time required for interpretation. Positive slides were usually evaluated within 2 min. Only extracellular elementary bodies, visualized microscopically as discrete, evenly fluorescent, bright apple-green disks of about 300 nm in diameter or occasionally larger reticulate bodies were observed. No intact chlamydial inclusions were seen in smears.

**TABLE 1. Comparison of results and projections with FA and TC**

<table>
<thead>
<tr>
<th>FA result</th>
<th>Actual (13% prevalence)</th>
<th>Projectedb</th>
<th>5% Prevalence</th>
<th>25% Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Positive</td>
<td>88</td>
<td>47</td>
<td>35</td>
<td>57</td>
</tr>
<tr>
<td>Negative</td>
<td>38</td>
<td>795</td>
<td>15</td>
<td>893</td>
</tr>
</tbody>
</table>

* Positive predictive value, 65%; negative predictive value, 95%.
* Projected onto a population of 3,000 women.
* Positive predictive value, 38%; negative predictive value, 98%.
* Positive predictive value, 79%; negative predictive value, 90%.

**TABLE 2. Comparison of FA with TC (second passage) for diagnosis of cervical chlamydial infection by clinic**

<table>
<thead>
<tr>
<th>Specimen source</th>
<th>FA result</th>
<th>No. of specimens giving the following TC result:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Adolescent clinic (n = 438)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>37</td>
<td>11</td>
</tr>
<tr>
<td>Negative</td>
<td>22</td>
<td>368</td>
</tr>
<tr>
<td>Planned parenthood (n = 426)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>35</td>
<td>29</td>
</tr>
<tr>
<td>Negative</td>
<td>11</td>
<td>351</td>
</tr>
<tr>
<td>Acute salpingitis patients (n = 104)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>16</td>
<td>7</td>
</tr>
<tr>
<td>Negative</td>
<td>5</td>
<td>76</td>
</tr>
</tbody>
</table>

**TABLE 3. Comparison of degrees of positivity of FA and TC**

<table>
<thead>
<tr>
<th>EB score</th>
<th>% of specimens positive by TC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>First passage</td>
</tr>
<tr>
<td>0 (788)</td>
<td>2</td>
</tr>
<tr>
<td>1-9 (45)</td>
<td>4</td>
</tr>
<tr>
<td>10-25 (54)</td>
<td>22</td>
</tr>
<tr>
<td>&gt;25 (81)</td>
<td>53</td>
</tr>
</tbody>
</table>

* EB score represents the number of elementary bodies observed by fluorescence microscopy as described in the text.
DISCUSSION

The FA system was less sensitive than TC (Table 2). The MicroTrak direct specimen test detected 70% of culture-positive endocervical chlamydial infections in a largely asymptomatic population of women. The large number (12%) of inadequate MicroTrak specimens indicates a need for clinician training in specimen collection. False-negative results occurred mainly in patients whose infections were characterized by a low number of organisms, often requiring a second TC passage for detection. Lowering the cutoff value to one elementary body to try to identify these low-grade positive specimens did not improve sensitivity but did compromise the specificity of the test. Our results support a cutoff value of 10 elementary bodies or more as the criterion for a positive MicroTrak test.

Some false-positive results occurred because TC is less than 100% sensitive. Other false-positive results may in fact have been obtained for women with true chlamydial infections, since dead or otherwise noninfectious organisms can be stained by antibody in the FA test. Unfortunately, it is not possible to identify which specimens of this group are truly false-positive. There are other reasons for false-positive results. Since different swabs were used to obtain specimens for each TC and smear, variable sampling of the cervix may have accounted for some of the discrepant results. Twelve percent of our pairs were excluded from analysis because the smears had inadequate cells. We knew that because of microscope evaluation, but we had no way of knowing whether the TC specimens were inadequate. All we knew was that a smear had been collected for each specimen. If the inadequate specimens were randomly distributed between the two tests, we would have missed 20 TC-positive results that would have been detected had an adequate specimen been taken. If that conjecture is valid, there would be a slight improvement in performance of the test (sensitivity, 74%; specificity, 97%; predictive value of a positive test, 80%). It is likely that many highly FA-positive, TC-negative specimens (elementary body score, >25) (Table 3) represent inadequate or improperly handled TC specimens.

Another potential cause of false-positive FA results is the cross-reaction of antigen in the specimen. The assumption that the use of reagent is a monoclonal antibody will only stain C. trachomatis is wrong. We have seen three different bacteria fluoresce in this test. The morphology is more likely to be confused with reticulate bodies than with elementary bodies, but that statement is based on slides for which we could make the differentiation, not on slides that we misidentified and thus reported as false-positive results. There is a subjective element associated with reading the direct immunofluorescence test, and a careful program of training and standardization is required for proper interpretation of this test.

Our results suggest that the MicroTrak test would be useful for diagnosing chlamydial infection in high-prevalence populations. For low-risk groups, however, our results suggest that the predictive value of a positive test would be low. For example, projection of our results (sensitivity, 70%; specificity, 94%) onto a population of 1,000 women with a 5% prevalence of chlamydial infection would result in correct identification of 35 of the 50 infected women, false-negative results for 15 women (Table 1). Of greater concern is the fact that 57 false-positive results would be obtained from this test; thus, the predictive value of a positive MicroTrak test would only be 35 of 92, or 38% (predictive value of a negative result, 98%). A projection of our results into a high-risk population with a 25% prevalence of chlamydial infection would result in a predictive value of a positive MicroTrak test of 79% and a predictive value of a negative MicroTrak test of only 90% (Table 1). Thus, there is no certainty that chlamydial infection could be ruled out by a negative MicroTrak test in such a high-risk population.

Previous studies have reported a much better performance of the MicroTrak direct specimen test when compared with TC (7, 10–12). Tam et al. (10) have reported a sensitivity of 93%, a specificity of 96%, and a predictive value for a positive test of 87%, when FA was compared with TC in McCoy cells (96-well microtiter plates, blind passage, and iodine staining). This study was performed in a population with a high prevalence of chlamydiae (~22%), men and women who presented with clinical syndromes known to be associated with chlamydial infection (overt urethritis and mucopurulent cervicitis, respectively). Such patients typically have higher inclusion counts than do unselected patients. The improved performance of the MicroTrak test probably resulted in part from the patient selection and in part from the TC technique used in this study: 96-well microtiter plates are less sensitive than vials for detecting inclusions in TC (J. Schachter, Yale J. Biol. Med., in press).

Thomas et al. (11) also reported excellent agreement (94 to 99%) and sensitivity (91 to 100%) in their comparison study of FA with single-passage TC in a high-risk population. This patient population consisted of men with nongonococcal urethritis, men with gonorrhea, female contacts of men with nongonococcal urethritis, and babies with conjunctivitis. Stamm et al. (7) have reported an FA sensitivity of 92% for a test group of 576 men, most of whom had symptoms and signs of urethritis, and a sensitivity of 89% for a test group of 595 high-risk women attending the same sexually transmitted disease clinic.

The studies of Thomas et al. and Stamm et al. were performed in highly expert research settings. The investigators were highly motivated with extremely well-trained clinicians who were experienced at specimen collecting and microscopists with considerable experience in microscopy for chlamydial elementary bodies. The results may not be readily transferable into a more routine clinical setting, which our study may represent.

The MicroTrak test has a sensitivity of 96%, a specificity of 99%, and a predictive value for a positive result of 92% (12) for the MicroTrak test. Their study was of 401 asymptomatic females who were either pre- or postpartum. The prevalence of chlamydial infection in this population was ~7%. The improved performance of the MicroTrak direct specimen test in this case may have been due to the decreased sensitivity of the TC technique used. McCoy cells used were not prepared fresh, whereas they were in our study. Their comparison of the FA was with first-passage TC (an additional 15 to 30% positive results might have been picked up if a second passage had been done).

The FA test has two other features that could enter into a decision to use it routinely. It is less expensive than TC. In our circumstances, excluding capital costs (equipment, etc.) and simply focusing on the costs of reagents and hands-on technician time, the FA test cost approximately $8 per specimen, while TC cost $15 per specimen. In processing large numbers of specimens, that difference in costs could be significant. Conversely, processing large numbers of FA specimens would put extreme demands on microscopists. We often process 100 specimens a day and the FA technique would require a full day of microscopy for evaluating 100
smears. The fatigue factor in the reliability of microscopic evaluation, as well as the reluctance of technicians to spend that much time at that task, would also have to be considered.

In summary, the FA procedure has a performance profile which could make it a useful tool in screening high-risk populations (particularly when tissue culture is not available), but it is less suited to screening low-risk populations, for which false-positive results are more important.

ACKNOWLEDGMENTS

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ADDENDUM

After this manuscript was submitted, we initiated an evaluation of the FA test in a clinic for sexually transmitted disease. One hundred ninety-two paired specimens were collected. Of these, 7 were not evaluable due to cytopathic effect in tissue culture, and 13 slides were inadequate. The 7% rate of inadequate smears was lower than that observed in the previous screening studies and probably reflects the greater specimen-collecting expertise of the sexually transmitted disease clinic personnel. The results are shown in Table 4. With a prevalence of 20% (34/172), the FA test was still less sensitive than TC, being positive in only 76% (26/34) of the specimens in which TC was positive. Specificity of the test was 96% (133/138). Thus, the overall performance profile was similar to that which we had observed before, but because of higher prevalence, the predictive value of a positive FA test was 84% (26/31), and that result, combined with the greater efficiency of specimen collection, supports the concept that the FA test can be a useful procedure in a high-prevalence setting.

LITERATURE CITED