Evaluation of Syva Enzyme Immunoassay for Detection of Chlamydia trachomatis in Genital Specimens

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Detection of Chlamydia trachomatis infection was evaluated by culture and a new Syva enzyme immunoassay (EIA) in 1,012 patients at two Baltimore, Md., sexually transmitted disease clinics. The overall chlamydia prevalence determined by culture was 12%. For 506 fresh cervical and urethral specimens, the sensitivity of Syva EIA was 90% and its specificity was 94% compared with culture. Discordant Syva EIA results were further evaluated by staining the sediment in centrifuged culture transport media and Syva EIA transport tubes with a fluorescent monoclonal antibody to C. trachomatis to detect elementary bodies. Reanalysis of the data after use of this technique to resolve discordant results increased sensitivity and specificity to 92% and 96%, respectively. A subsample of 307 fresh cervical specimens was also tested in a three-way comparison using Abbott Chlamydiazyme, Syva EIA, and culture. In this sample, compared with culture, the sensitivity and specificity of Syva EIA were 87% and 95%, respectively, and for Chlamydiazyme they were 77% and 98%, respectively. Syva EIA is a 4-h, easy-to-perform enzyme-linked immunosorbent assay which has a high sensitivity with fresh genital specimens and offers an excellent alternative to culture.

Chlamydia trachomatis infections are recognized as the most prevalent sexually transmitted bacterial infections in the United States, with an estimated 4 million cases annually (2, 4, 9, 11). Men, women, and infants may be affected, but women are especially at risk for serious complications, such as pelvic inflammatory disease, infertility, and ectopic pregnancy (14). Infants born to infected mothers may develop neonatal conjunctivitis or pneumonia (1). C. trachomatis causes 40 to 50% of cases of nongonococcal urethritis (2), and acute epididymitis may develop as a complication. In homosexual men, proctitis can be caused by either lymphogranuloma venereum or non-lymphogranuloma venereum strains of C. trachomatis (8).

While the presumptive diagnosis of chlamydial infection is more difficult to make in women, who may be asymptomatic for months (6), early diagnosis and treatment is important to prevent complications and to limit the spread of infection. Currently, cell culture is the accepted standard for chlamydia diagnosis, but its utility is limited because of its inherent technical complexity, time requirement (48 to 72 h), specimen-handling requirements, and expense. Thus, there is a need for alternative rapid, accurate, and easy-to-perform diagnostic screening tests. We therefore evaluated a new enzyme immunoassay (EIA) from Syva (Palo Alto, Calif.) for detection of C. trachomatis in genital specimens from patients at two large-city sexually transmitted disease clinics. A subset of female patients was also evaluated with the Chlamydiazyme EIA (Abbott Laboratories, North Chicago, Ill.), and both test results were compared with those of culture.

MATERIALS AND METHODS

Study population. Genital specimens were collected from 792 females and 240 males at two inner-city sexually transmitted disease clinics for reasons other than test of cure.

Specimen collection. Swab specimens were sequentially obtained from consenting patients for testing. Endocervical swab 1 was used to prepare a specimen for Gram stain and to inoculate modified Thayer-Martin medium for Neisseria gonorrhoeae culture. The Gram stain was examined for signs of inflammation, which was defined as ≥30 polymorphonuclear leukocytes per high-power field for females and ≥5 polymorphonuclear leukocytes per high-power field for males. Specimen 2 for chlamydia culture was taken with a Dacron swab which was placed into transport medium containing sucrose-phosphate buffer with 2% fetal bovine serum and antibiotics (mystatin, gentamicin, and vancomycin). Swabs 3 and 4 were alternately used to obtain specimens for Syva EIA and Abbott Chlamydiazyme from patients on whom both tests were performed. In each case, manufacturer-supplied swabs and transport tubes were used. From 1,012 evaluable patients, 506 fresh specimens (stored at 4°C for up to 7 days) and 506 frozen specimens (−70°C) were tested by Syva EIA and the results were compared with those of culture. From these patients, 694 specimens from females were tested by Chlamydiazyme. These were stored at 4°C for up to 5 days. A subset of this population contained 307 females from whom swabs were also collected for both EIA (fresh) and culture.

Chlamydia cultures. Cultures were performed in duplicate by using McCoy cell monolayers in 96-well microtiter plates pretreated with 30 μg of DEAE-dextran per ml for 30 min at 35°C. Inoculated cultures (100 μg per well) were centrifuged for 60 min at 800 × g at 35°C and incubated for 30 min at 35°C. Following aspiration of the supernatant, 0.2 ml of inoculation medium containing 1.0 μg of cycloheximide per ml was added to each well. After 48 h of incubation at 35°C, cultures were fixed and stained with a fluorescein-conjugated monoclonal antibody (Syva Microtrak) and read for the presence of inclusion bodies with a Zeiss epifluorescence microscope. A second passage was performed on all negative and toxic cultures, as well as on positive cultures.
TABLE 1. Sensitivity and specificity of Syva EIA compared with those of culture of fresh and frozen genital specimens

<table>
<thead>
<tr>
<th>Syva EIA result&lt;sup&gt;a&lt;/sup&gt;</th>
<th>No. of culture results</th>
<th>% Sensitivity</th>
<th>% Specificity</th>
<th>PPV&lt;sup&gt;b&lt;/sup&gt; (%)</th>
<th>NPV&lt;sup&gt;c&lt;/sup&gt; (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
<td>Original</td>
<td>Revised&lt;sup&gt;d&lt;/sup&gt;</td>
<td>Original</td>
</tr>
<tr>
<td>Fresh specimens</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>64</td>
<td>28&lt;sup&gt;e&lt;/sup&gt;</td>
<td>90</td>
<td>92</td>
<td>94</td>
</tr>
<tr>
<td>Negative</td>
<td>7</td>
<td>407</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frozen specimens</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>51</td>
<td>15&lt;sup&gt;f&lt;/sup&gt;</td>
<td>85</td>
<td>86</td>
<td>97</td>
</tr>
<tr>
<td>Negative</td>
<td>9</td>
<td>431</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> There were 506 frozen specimens and 506 fresh specimens.
<sup>b</sup> PPV, Positive predictive value.
<sup>c</sup> NPV, Negative predictive value.
<sup>d</sup> Calculated after discrepant analysis to yield truly positive results.
<sup>e</sup> The EIA tube contained elementary bodies in 13 of these specimens.
<sup>f</sup> The EIA tube contained elementary bodies in four of these specimens.

containing fewer than three inclusions. Presence of one inclusion body was considered positive.

**Syva EIA procedure.** In the Syva EIA, treatment solution (1.0 ml) was added to each specimen and control tube. All tubes were placed in a heat block at 95 to 100°C for 15 min, cooled, and vortexed, and 100 μl was added to the well of a microtiter plate containing 100 μl of polyclonal anti-chlamydia antibody. Following incubation for 90 min at 37°C and washing, 100 μl of goat anti-rabbit substrate-conjugated antibody was added and the plate was incubated for 30 min at 37°C. After washing, 100 μl of substrate was added, followed by incubation for 30 min at room temperature. Stop solution was added, and the A<sub>492</sub> was read.

**Abbott Chlamydiazyme procedure.** The Chlamydiazyme procedure was as follows. After addition of buffer, room temperature incubation for 10 min, and vortexing, 200 μl of a specimen or a control was transferred into a well with a treated bead. After 1 h of incubation at 37°C and washing, chlamydial antiserum (200 μl) was added to each well followed by 1 h of incubation at 37°C. Another wash was performed, and 200 μl of enzyme conjugate was added. Following a third, identical incubation, the beads were washed and transferred to assay tubes containing 300 μl of O-phenylenediamine dihydrochloride substrate and the A<sub>650</sub> was read.

**Resolution and analysis of discrepant results.** Specimens with discrepant Syva results were further evaluated by both repeat culture and EIA. Additionally, the remaining transport media in both the culture vial and the EIA tube were centrifuged at 2,000 × g for 15 min and the resultant sediment was stained for the presence of elementary bodies. When elementary bodies were noted in culture-negative specimens, the sample sensitivity and specificity were calculated by placing the specimens into the Syva EIA-positive—culture-positive category.

**RESULTS**

The median age of the patient population was 23 (range, 12 to 61) years. Twenty-three (10%) of 240 males were culture positive for *C. trachomatis*, as were 106 (13%) of 792 females tested. Forty-six (19%) of 240 males were culture positive for *N. gonorrhoeae*, as were 130 (16%) of 792 women. Fourteen percent of females had yeast cells present in the wet mount. Patients positive for both *C. trachomatis* and *N. gonorrhoeae* comprised 3% of the men and 5% of the women.

Sixty percent of the men were symptomatic, and 43% had signs of inflammation noted on urethral Gram stain (≥5 polymorphonuclear leukocytes per high-power field). Sixty-eight percent of the women had symptoms, and 48% had signs of inflammation on Gram stain of cervical mucus (≥30 polymorphonuclear leukocytes per high-power field).

When fresh specimens (n = 506) and frozen specimens (n = 506) were compared with culture, the sensitivities of Syva EIA were 90 and 85%, respectively (no significant difference). After discrepant samples were analyzed for the presence of elementary bodies, the sensitivity for fresh specimens increased to 92% and that for frozen specimens increased to 86%. Specificity for fresh specimens increased from 94 to 96%, and that for frozen specimens increased from 97 to 98% (Table 1).

When fresh specimens for Syva EIA versus culture were analyzed for males and females, the sensitivity was higher for males (95%) than for females (88%). After elementary body analysis of discrepant results, the sensitivities increased to 97 and 89% for men and women, respectively. Specificity for males increased from 91 to 98%, and that for females increased from 95 to 96% (Table 2). The positive predictive value for males increased from 61 to 91%, and that for females increased from 75 to 80%. The negative predictive values remained unchanged at 99% for males and 98% for females.

Analysis of fresh discrepant specimens which were culture positive and Syva EIA negative demonstrated that six of seven specimens did have elementary bodies present in the EIA transport tube (falsely negative by EIA). When 18 culture-negative, EIA-positive specimens were evaluated, 15 had no elementary bodies in the EIA tube (falsely positive by EIA). In addition, six specimens contained elementary bodies in the culture vial (truly false negative by culture).

For a subset of female specimens (n = 694) that were tested by Abbott Chlamydiazyme, the sensitivity was 72% and the specificity was 96% compared only with culture. The positive predictive value was 77%, and the negative predictive value was 95%. Of 307 females who had specimens collected for culture, Syva EIA, and Chlamydiazyme, 35 were positive by all three tests and 244 were negative by all three tests. Compared with culture, Syva EIA had a sensitivity and a specificity of 87 and 95%, respectively, whereas Chlamydiazyme had a sensitivity of 77% and a specificity of 98% (Table 3). Compared with culture, Syva sensitivity was
not significantly different from Chlamydiazyme sensitivity ($P = 0.21$).

**DISCUSSION**

This study compared a new EIA by Syva with culture and Chlamydiazyme for the detection of *C. trachomatis* in genital specimens. Rapid screening assays could potentially fulfill the need to survey populations at risk for *C. trachomatis* infections accurately and inexpensively. In a recent review of alternative tests to culture for detection of chlamydia, Stamm reported that when 12 previous studies of the Chlamydiazyme test were combined, the sensitivities were 89% for high-prevalence female populations, 85% for intermediate-prevalence female populations, 79% for symptomatic male populations, and 49% for asymptomatic male populations (10). Specificities were uniformly high, in the range of 95 to 97% (10). Other rapid diagnostic tests for chlamydia include direct immunofluorescence, IDEIA (Boots-Celltech, Berkshire, United Kingdom), a nonisotopic nucleic acid probe (Gen-Probe, San Diego, Calif.), Kodak Surecell (Eastman Kodak Co., Rochester, N.Y.), and the Abbott TestPack (Abbott Laboratories, North Chicago, Ill.). The reported specificities of these tests range from 60 to 97% (3, 5, 7, 10, 12, 13; R. Chauncey, J. Mauch, and J. Gilbert, Abstr. Annu. Meet. Am. Soc. Microbiol. 1989, abstr. no. C-112, p. 412).

In our study, in which the prevalence of chlamydia by culture was 12% and 66% of the population had symptoms, the Syva EIA had a sensitivity of 90% and a specificity of 94% in screening 506 fresh specimens. Since the sensitivity was lower with frozen specimens (85%; no significant difference), the manufacturer does not recommend using frozen specimens. The reasons for this decreased sensitivity remain obscure. Additionally, since this study was performed in a high-risk, high-prevalence population, caution should be taken in application of these results when screening a low-prevalence population, since the positive predictive value may decrease with decreasing prevalence. False-positive results in such a low-prevalence population may be a cause for concern in informing patients of positive results of a sexually transmitted disease.

Because the sensitivity of cell culture is accepted as less than 100%, we analyzed discrepant specimens for the presence of elementary bodies in the transport tubes to evaluate the possibility of false-negative culture results or false-positive Syva EIA results. Following this analysis, the sensitivity of the Syva EIA improved from 90 to 92% and the specificity improved from 94 to 96%. More importantly, the positive predictive value improved substantially from 70 to 84% while the negative predictive value remained at 98%. It is interesting that when fresh specimens from males and females were tested separately, both the initial and revised sensitivities of the Syva EIA were higher for males than females. This is a reflection of the fact that EIA missed only 1 of 21 culture-positive males but missed 6 of 50 culture-positive females. The reason for the lower sensitivity remains unclear. Perhaps constituents of cervical mucus or cervical flora interfered with chlamydia detection. Lower test sensitivity in women has a number of potential problems, such as misdiagnosis of the severe consequences of chlamydia infection, including pelvic inflammatory disease, infertility, and ectopic pregnancy.

This study demonstrated that analysis of elementary bodies in discrepant specimens offers an alternative method for evaluation of truly positive and truly negative specimens. For example, it initially appeared that culture missed 10 of 13 EIA-positive samples from males. However, when these culture tubes were analyzed, only four had elementary bodies. This also illustrates the problem of sampling technique and the amount of specimen usually obtained from males. This will always be a problem when comparing two tests using two different swabs. Among the 15 discordant specimens among females (culture negative and EIA positive), only 3 had elementary bodies in the EIA tube, indicating that 12 specimens were truly false positive by EIA.

**TABLE 2. Sensitivity and specificity of Syva EIA compared with those of culture for fresh genital specimens with regard to sex**

<table>
<thead>
<tr>
<th>Syva EIA result</th>
<th>No. of culture results</th>
<th>% Sensitivity</th>
<th>% Specificity</th>
<th>PPV* (%)</th>
<th>NPV* (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive Negative</td>
<td>Original Revised</td>
<td>Original Revised</td>
<td>Original Revised</td>
<td>Original Revised</td>
</tr>
<tr>
<td>Females</td>
<td>Positive</td>
<td>44 15</td>
<td>88 89</td>
<td>95 96</td>
<td>75 80</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>6</td>
<td>280</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>Positive</td>
<td>20 13</td>
<td>95 97</td>
<td>91 98</td>
<td>61 91</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>1</td>
<td>127</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* There were 345 specimens from females and 161 from males.
* PPV, Positive predictive value.
* NPV, Negative predictive value.
* Calculated after discrepant analysis to yield truly positive results.
* The EIA tube contained elementary bodies in three of these specimens.
* The EIA tube contained elementary bodies in 10 of these specimens.

**TABLE 3. Sensitivity and specificity of Syva EIA and Abbott Chlamydiazyme compared with those of culture for 307 fresh genital specimens**

<table>
<thead>
<tr>
<th>No. of specimens</th>
<th>Result by:</th>
<th>Culture</th>
<th>Syva EIA*</th>
<th>Chlamydiazyme*</th>
</tr>
</thead>
<tbody>
<tr>
<td>35</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>+</td>
<td></td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>-</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>244</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

* For the Syva EIA and Chlamydiazyme, respectively, the sensitivities were 87 and 77%, the specificities were 95 and 98%, the positive predictive values were 75 and 90%, and the negative predictive values were 98 and 96%.
Additionally, when the culture-positive, EIA-negative female category was analyzed, EIA was shown to have missed 6 of 71 culture-positive samples or 8.5% of all truly positive samples.

Technological advances in the rapid detection of *C. trachomatis* in clinical specimens have the potential to greatly enhance chlamydia diagnosis. While culture remains the “gold standard,” EIA offers many advantages over culture because of its speed, lower cost, and lack of need for cell culture capability and technical expertise. The Syva EIA has the additional advantage of allowing batching of large numbers of tests. It is a highly sensitive and specific test and should provide a useful method for laboratories without culture capability to improve their abilities to diagnose this important cause of worldwide morbidity.

**ACKNOWLEDGMENT**

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**LITERATURE CITED**


