Evaluation of the Vidas Chlamydia Test To Detect and Verify *Chlamydia trachomatis* in Urogenital Specimens

JULIUS SCHACHTER,1* ROBERT B. JONES,2 R. CHRISTOPHER BUTLER,3 BERNIE RICE,4 DAVID BROOKS,1 BARBARA VAN DER POL,2 MARY GRAY,4 AND JEANNE MONCADA1

Department of Laboratory Medicine, University of California, San Francisco, California1; Indiana University School of Medicine, Indianapolis, Indiana2; The Arlington Hospital, Arlington, Virginia3; and bioMérieux Vitek, Rockland, Massachusetts4

Received 14 January 1997/Returned for modification 26 February 1997/Accepted 29 April 1997

Chlamydia trachomatis is a leading cause of sexually transmitted diseases (STD), with approximately four million new cases occurring annually in the United States (3). It is a major cause of upper genital tract infections in women and urethritis and epididymitis in men. Furthermore, newborn infants exposed to *C. trachomatis* during passage through an infected birth canal can develop pneumonia. Currently, the Centers for Disease Control and Prevention recommends that sexually active teens and adults ≤24 years of age be routinely screened for *C. trachomatis*. Thus, accurate laboratory tests for the diagnosis of chlamydia are needed.

Although tissue culture (TC) isolation of *C. trachomatis* was long considered the gold standard (14), it is labor-intensive, time-consuming, and costly. TC is 100% specific but less than 100% sensitive. Direct antigen detection methods have provided more rapid and less expensive alternatives. GenProbe, a DNA hybridization assay, has a sensitivity similar to those of antigen detection methods (8). For high-volume laboratories, enzyme immunoassays (EIA) may be preferable because of the capacity to screen large numbers of specimens. Various systems, such as the Commander (Abbott Laboratories, Abbott Park, Ill.) and Syva XL (Behring Diagnostics Inc., San Jose, Calif.), are totally automated. In addition, EIA has been used successfully with first catch urine (FCU) specimens from symptomatic males as a noninvasive test for *C. trachomatis* (5, 11).

The Vidas Chlamydia test (CHL) is an automated enzyme-linked immunofluorescence assay for the detection of *Chlamydia trachomatis*. Positive and equivocal results are confirmed with a blocking assay. A mouse monoclonal antibody directed against the chlamydial lipopolysaccharides was used for the test. The CHL assay is widely used in Europe, but U.S. experience with it is limited. Three clinical test sites (The Arlington Hospital, Arlington, Va., Indiana University, Indianapolis, and the University of California, San Francisco) compared CHL with tissue culture (TC) for the identification of chlamydia in urogenital specimens (2,453 females and 850 males). True positives (TP) were defined as either TC positive or TC negative and CHL positive by a positive direct fluorescent-antibody assay or PCR test. Overall prevalence was 5.5% for females, 10.3% for male urethral swabs, and 10.7% for combined male TC urethral swabs and CHL with first catch urine (FCU) specimens. Compared to TP, CHL and TC had sensitivities of 89.6 and 94.1% with female cervical swabs and 99.9 and 86.4% with male urethral swabs, respectively. CHL sensitivity was 81.2 for male FCU specimens and 77.7% for matching male TC swabs. There were relatively few false-negative results, with all specificities being >99.4%. With the blocking assay, Vidas CHL specificity was >99.7%. However, male FCU specimen sensitivity was compromised because 9.2% (7 of 76) of the TP were initially positive but were not confirmed. An improvement in the Vidas blocking assay is needed before we can recommend its use with male urine. Alternatively, one could argue that the specificity of the test is so high that a confirmatory assay is not needed. For male and female swabs, the Vidas CHL assay has a performance that is similar to that of TC.

However, EIA still lacks the sensitivity and specificity of TC. False-positive results were a serious problem with EIA until the introduction of confirmatory assays that improved specificity (9). Furthermore, EIA cannot be used with female FCU specimens because of poor sensitivity and false-positive results (5). Recently, amplified DNA methods have been introduced into the diagnostic laboratory. PCR and ligase chain reaction (LCR) are both approved by the Food and Drug Administration; each targets the cryptic plasmid of *C. trachomatis*. Evaluations have shown that these tests are highly sensitive and specific. While the performance of PCR (Amplicor; Roche Molecular Systems, Branchburg, N.J.) with specimens from men is outstanding (7), PCR inhibitors reduce sensitivity with cervical specimens (1). The LCR test (LCx; Abbott Laboratories) is highly sensitive and specific for both men and women (6, 15); however, the low throughput restricts its use in high-volume laboratories. Clearly, there is still a need for a sensitive and specific automated system to test large numbers of urogenital specimens for *C. trachomatis*.

The Vidas Chlamydia test (CHL) (bioMérieux Vitek, Inc., Hazelwood, Mo.) is an automated enzyme-linked immunofluorescence assay for the detection of *Chlamydia trachomatis*. A mouse monoclonal antibody directed against the chlamydial lipopolysaccharides (LPS) is used. Positive and equivocal Vidas CHL specimens are confirmed with a blocking assay. The CHL assay is widely used in Europe, but its use in the United States is limited. Because it allows high-volume testing for chlamydiae, the Vidas assay could be a useful test. Three clinical test sites (The Arlington Hospital, Arlington, Va., Indiana University, Indianapolis, and the University of California, San Francisco)
compared the CHL with TC for the isolation and identification of chlamydiae in urogenital specimens.

**MATERIALS AND METHODS**

**Patient population.** The evaluations were performed at three different test sites. Site 1 (The Arlington Hospital), site 2 (Indiana University), and site 3 (University of California) collected specimens from an STD and teen family planning clinic. Patients were categorized as asymptomatic or symptomatic. Specimens were collected. At each site, two cervical swabs were collected from females and a FCU specimen and two urethral swabs were obtained from males. All the diagnostic test procedures were performed blindly.

The two cervical specimens were collected in random order. The exocervix was cleaned with gauze and exudate. The swab or cytobrush (site 3 only) was rubbed against the endocervical canal and removed; contact with vaginal surfaces was avoided. For the male, 15 to 30 ml of FCU (site 2 collected up to 70 ml) was obtained in a sterile container. Then, the two urethral swabs were collected randomly. Each Dacron swab (type 1) was inserted 2 to 3 cm into the urethra, rotated, and withdrawn. All TC swabs were placed in the appropriate transport medium. CHL swab specimens were collected with the Vidas collection kit. Specimens were held at 4°C until they were transported to the laboratory. TC specimens were processed within 72 h of collection, with the remaining being frozen at −70°C for possible discrepant analysis.

**TC procedure.** Cycloheximide-treated McCoy cells and a direct fluorescent antibody assay (DFA) were used to identify inclusions. Only a primary passage was performed at sites 1 and 2, whereas a blind passage was performed at site 3. At site 1, TC swabs were placed in chlamydia transport media (2.0 ml of RPMI medium with 2% fetal bovine serum, 2.5% HEPEs [1 M], 1% t-glutamine and penicillin-streptomycin-amphotericin solution). Specimens were inoculated into 1-dram (1 dram = 3.7 ml) shell vials. After 48 h of incubation at 35°C with 5% CO₂, vials were cold acetone fixed and stained by an anti-LPS fluorescent antibody (FA) (Bartels Immunodiagnostics).

At site 2, TC swabs were placed into a transport vial containing glass beads with SPG-VGN (sucrose phosphate glutamate with 50 μg of vancomycin per ml, 40 μg of gentamicin per ml, and 125 U of nystatin). Specimens were vortexed, sonicated, and then inoculated in triplicate into microtiter plates by using a Biomek 1000. After 48 h of incubation at 36°C, plates were methanol fixed and stained with an indirect FA by using an in-house monoclonal antibody (anti-MOMP).

At site 3, a modification of the procedure of Ripa and Mårdh (13) was followed. TC swabs or cytobrushes were placed in transport medium (2.0 ml of Eagle’s minimal essential medium in Earle salts containing 10% fetal calf serum, 1% t-glutamine [200 mM solution], 10 μg of gentamicin per ml, 100 μg of vancomycin per ml, and 10 U of nystatin per ml, and 0.003 mM glucose per ml). TC isolation was performed with 1-dram shell vials, an anti-MOMP FA stain (Syva MicroTrak chlamydia culture confirmation reagent) at 48 h, and a blind pass.

**DFA procedure.** All specimens collected were tested with a DFA. An aliquot of the original TC and processed CHL (swab and FCU) specimens was cytospun and stained. Briefly, 250 μl of each sample was microdispensed for 5 min at 12,000 rpm. Supernatant was removed, and the pellet was resuspended in 30 μl of phosphate-buffered saline. Smears were made with 5 μl of the specimen. Slides were air dried; methanol was fixed and stained by the Syva MicroTrak chlamydial LPS binding assay test protocol. The smears were scored. Positive smears contained four or more elementary bodies (EBs).

**Vidas CHL assay.** All samples were kept at 4°C and processed within 7 days of collection. Test procedures were followed according to the manufacturer’s package insert.

Sample treatment reagent was added to the Vidas CHL specimens and incubated at room temperature for 15 min. After tubes were vortexed for 30 s, and the swabs were discarded. Specimens were then placed in a heat block at 100°C for 15 min. These processed samples were tested within 24 h. For FCU specimens, 12 ml was pipetted into round bottom tubes and centrifuged at 3,000 × g for 15 min. Two milliliters of sample treatment reagent was added to the pellets, resuspended, and placed in a heat block at 100°C for 15 min. Processed specimens (swabs and FCU), standards (buffers), and controls (350 μl each) were loaded into the sample wells of CHL reagent strips. These strips contain all the reagents needed for the assay.

The CHL strips were placed into an automated Vidas instrument that processed 30 specimens (including two standards and two controls) each run. Temperature and assay steps are controlled by the instrument, which pipettes the samples to the various wells where the incubation and wash take place. The assay uses a mouse monoclonal antibody directed against the chlamydial LPS, an immunoglobulin G conjugated with alkaline phosphatase, and a fluorescent substrate (4-methylumbelliferyl phosphate). The intensity of fluorescence is measured by the instrument’s optical scanner. After 1.5 h, the test is completed, and results yield a relative fluorescence value (RFV). Positive specimens have an RFV of ≥30, equivocal results are ≥80 to <30, and negative results are <60.

**Vidas CHL blocking assay.** All positive and equivocal Vidas CHL specimens were confirmed with a blocking assay within 24 h of the initial testing. For the blocking assay, processed specimens were added to two CHL strips. One strip contained the mouse α-chlamydial LPS antibody and a normal serum reference reagent (reference strip). The other strip contained the mouse α-chlamydial LPS as well as an additional α-chlamydial LPS antibody (block strip). The two CHL strips were placed in the Vidas instrument, and the assay was initiated. The Vidas CHL blocking reagent binds specifically to the same epitope as does the mouse monoclonal antibody in the CHL strip. The reference reagent does not react to these binding sites. A confirmed chlamydia result had an RFV of ≥30 and a blocked RFV of <50 of the unblocked result.

**Discrepant analysis.** Samples that were TC negative, DFA negative, and Vidas blocking positive (TC+/DFA+/CHL+) were further analyzed. An aliquot of the original TC specimen was tested by Amplicor (Roche Molecular Systems), a PCR test for C. trachomatis. The PCR assay was performed according to the manufacturer’s instructions. For analysis, a positive PCR confirmed the positive CHL assay. A negative PCR resulted in a false-positive CHL assay classification. TC−, DFA+, and CHL+ were accepted as true positive.

## RESULTS

A total of 2,453 cervical swabs and 850 male urethral swabs were evaluated. The latter group had 708 matched FCU specimens. The overall prevalence of chlamydiae by true positives was 5.5% for female cervical swabs, 10.3% for male urethral swabs, and 10.7% for combined male TC on urethral swabs and CHL on FCU specimens. Table 1 shows the performance profile for each test site. The Vidas CHL assay results were similar to those for TC at all three sites but slightly better than...
those for TC at sites 1 and 3. Site 2 had better sensitivities with TC for all specimen types, but none of these differences was statistically significant. CHL sensitivities were lower with male FCU specimens than with male swabs at all sites. For this analysis, the results of TC on urethral swabs and CHL on urethral swabs and on FCU specimens were pooled, and then the three types of specimens were analyzed separately (Table 2). TC sensitivity was marginally lower than that of CHL by swab (74 versus 79%).

Use of the blocking assay identified a total of five (three female and two male FCU specimens) false positives. However, the overall sensitivity of Vidas CHL dropped because the blocking assay failed to confirm some true positives (9). The majority, 77.8% (7 of 9), of the failed confirmations occurred with male FCU specimens, representing 9.2% (7 of 76) of true-positive male FCU specimens. If the blocking assay had not been used, then there would have been a slight loss in specificity with an increase in sensitivity (Table 3). Male FCU sensitivity would have increased to 90.8% (69 of 76), making its specificity better than that of TC, with a slight reduction of sensitivity to 99.8%. The blocking assay did not detect any false positives or miss any true positives with male swabs.

Table 4 represents a breakdown of all DFA positive specimens. Site 1 had outstanding performance with the DFA; only one false positive was detected. DFA sensitivities for males and females were >95%. However, sites 2 and 3 had DFA performances that varied greatly. Sensitivities ranged from 39 to 93%. DFA false positives were identified at site 3 by the use of an additional test (PCR). The majority of false positives detected were with female specimens.

### DISCUSSION

The Vidas CHL assay was evaluated at three different test sites. Although methods for culture and transport were not consistent at the three sites, each site did use its usual and optimal method for chlamydia isolation rather than attempting to standardize methods. Compared to true-positive chlamydia specimens, Vidas CHL and TC had sensitivities of 89.6 and 94.1% for female cervical swabs and 90.9 and 86.4% for male urethral swabs, respectively. CHL sensitivity was 81.2% for male FCU specimens and 77.7% for matching male TC swabs. The evaluations at sites 1 and 3 indicated that Vidas CHL was comparable to TC with all urogenital samples. However, at site 2, CHL had lower sensitivities than TC. The results at site 2 for CHL were similar to those for other nonculture methods for identifying chlamydia. With male FCU specimens, a Vidas CHL sensitivity of only 76.9% (10 of 13) was found at site 2. This variation in results may be due to a blocking problem (two CHL true positives failed to confirm). Also, specimen transport and efficient processing time may increase TC sensitivity. Site 1 had a low prevalence of chlamydia (4.5%), while site 2 had a high prevalence of chlamydia (12 to 16%). The prevalence of chlamydia at site 3 was 4% in females and 16% in males. Most nonculture tests perform better in high-prevalence settings. With female patients, the opposite is seen in this study. Vidas CHL had slightly higher sensitivity in low-prevalence settings.

With male FCU specimens, overall sensitivity was 81.2% with 100% specificity. The lower sensitivity was due to the failure of the blocking assay to confirm true-positive specimens. A total of 9.2% of true-positive male FCU specimens were not confirmed upon retest. If we omit the Vidas CHL blocking assay, the CHL sensitivities increase (Table 3), with the specificity dropping slightly to 99.8%. Thus, the argument could be made that the confirmatory test is not needed.

Without nonculture tests, the Centers for Disease Control and Prevention currently recommends the use of a confirmatory assay for all positive chlamydia results with populations other than those from STD clinics. Clearly, the Vidas CHL blocking assay will identify false-positive results, but there is a minor problem with confirmation of true-positive FCU specimens.

### TABLE 2. Ability of Vidas CHL to detect male patients positive for chlamydia

<table>
<thead>
<tr>
<th>Test result</th>
<th>No. of patients with result</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>PV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Swab</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>63</td>
<td>78.7</td>
<td>99.8</td>
<td>98.4</td>
</tr>
<tr>
<td>Negative</td>
<td>17</td>
<td></td>
<td></td>
<td>627</td>
</tr>
<tr>
<td>FCU specimen</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>62</td>
<td>77.5</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Negative</td>
<td>18</td>
<td>97.2</td>
<td></td>
<td>628</td>
</tr>
</tbody>
</table>

* n = 708.
* Patients positive for chlamydia had either a TC+ or a confirmed CHL (swab or FCU) result.

### TABLE 3. Use of Vidas CHL without blocking assay to detect C. trachomatis

<table>
<thead>
<tr>
<th>Site and group</th>
<th>n</th>
<th>Prev</th>
<th>TC sensitivity (%)</th>
<th>Vidas CHL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site 1 (Arlington, Va.) Females</td>
<td>1,251</td>
<td>18</td>
<td>4.4</td>
<td>96.4</td>
</tr>
<tr>
<td>Males</td>
<td>384</td>
<td>5.2</td>
<td>85.0</td>
<td>95.0</td>
</tr>
<tr>
<td>Site 2 (Indianapolis, Ind.) Females</td>
<td>368</td>
<td>13.9</td>
<td>96.7</td>
<td>93.3</td>
</tr>
<tr>
<td>Males</td>
<td>73</td>
<td>16.4</td>
<td>92.3</td>
<td>84.6</td>
</tr>
<tr>
<td>Site 3 (San Francisco, Calif.) Females</td>
<td>834</td>
<td>4.1</td>
<td>88.2</td>
<td>91.2</td>
</tr>
<tr>
<td>Males</td>
<td>251</td>
<td>15.1</td>
<td>78.9</td>
<td>86.8</td>
</tr>
<tr>
<td>Total</td>
<td>2,453</td>
<td>5.5</td>
<td>94.1</td>
<td>91.1</td>
</tr>
<tr>
<td>Females</td>
<td>380</td>
<td>10.3</td>
<td>86.4</td>
<td>90.9</td>
</tr>
<tr>
<td>Males</td>
<td>708</td>
<td>10.7</td>
<td>77.6</td>
<td>90.8</td>
</tr>
</tbody>
</table>

* CHL on FCU specimens versus TC on swabs.
* Prev, prevalence. Results of TC+, TC−/CHL+/DFA+, or TC−/CHL+/DFA−/PCR− were considered true positive.
* PV, predictive value.
With EIA, confirmatory assays are needed because of cross-reacting bacteria and nonspecific binding of α-LPS antibodies. In this study, only five false positives were detected. However, different populations and the variation in patient sampling may cause a higher false-positive rate in other settings. Thus, a confirmatory assay is still warranted.

The use of cytospin-DFA on the original specimen to confirm true positives is an alternative to the blocking assay (10). The advantages of using DFA are the faster turnaround time (approximately 30 min) and the visualization of chlamydia EBs in the specimen. Østergaard and Möller (12) reported that the cytospin-DFA can be used to confirm positive Syva EIA in a low-prevalence population. Other investigators have used DFA to confirm gray zone EIA specimens (2, 4). In our study, all specimens (TC and CHL) were tested by cytospin-DFA. Site 1 had remarkable results; it confirmed 84% (11 of 14) of the true positives tested and reported a DFA sensitivity of >95%. Sites 2 and 3 had results that are consistent with their previous experience with the MicroTrak DFA with different specimens. DFA sensitivities were in the 39 to 93% range. Only 49.1% (28 of 57) of the true positives were confirmed. Clearly, to be accurate, the DFA cannot be used as the only confirmatory assay; the PCR test confirmed 29 DFA+/CHL+ specimens. False positives (TC−/CHL−/DFA+) were seen (Table 4) with the DFA procedure. The majority (29 of 40) of these false positives had EB counts in the low range (4 to 25).

Site 3 performed an additional PCR test on false-positive specimens; all 24 TC−/CHL−/DFA+ were negative by a PCR test. Thus, 7,374 cytospin-DFAs indicated no positive chlamydia specimens beyond those detected by CHL and TC, but the DFA alone had 0.6% false-positive results (specificity, 99.4%). This study has shown that the Vidas CHL has a performance that is similar to that of TC for male and female swabs and male FCU specimens. The ability of these tests to detect a positive chlamydia patient was 77.5% for CHL with male FCU specimens, 78.7% for CHL on male urethral swabs, and only 73.8% for TC on male urethral swabs. Because the blocking assay failed to confirm 9.2% of true-positive male FCU specimens, we cannot recommend its use as a confirmatory test for urine specimens. The variability between sites suggests the need to fine-tune the blocking test before it can be used with FCU specimens. As noted above, it may not be needed, but that will depend on further experience. However, with swabs, the sensitivity of CHL was similar to that of TC at all sites, and specificity was excellent. With high throughput, reasonable sensitivity, and excellent specificity, this test could compete with the more sensitive DNA amplification procedures if the cost was appropriate.

### REFERENCES


