

Evaluation of the Biostar Chlamydia OIA Assay with Specimens from Women Attending a Sexually Transmitted Disease Clinic

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Chlamydia trachomatis infections are the most prevalent sexually transmitted diseases (STDs) in the United States. In acute-care settings such as clinics and emergency rooms, a desirable chlamydia screening assay should exhibit good sensitivity and good specificity and should provide test results while the patient is still present. The Biostar Chlamydia OIA (Biostar, Inc., Boulder, Colo.) is an optical immunoassay (OIA) that provides test results in less than 30 min and that uses a test format that allows office-based testing. This assay is performed entirely at room temperature without the need for rotators or other specialized equipment. The goal of this study was to compare the performance of the Biostar Chlamydia OIA for the detection of *C. trachomatis* with the performance of cell culture, direct fluorescent-antibody (DFA) assay (Syva MicroTrak; Syva Co., Palo Alto, Calif.), and PCR (Roche Amplicor *Chlamydia trachomatis*; Roche, Branchburg, N.J.) for the detection of *C. trachomatis* infections in women attending an urban STD clinic. For calculations of relative test performance (sensitivity, specificity, and positive and negative predictive values), patient specimens that yielded positive results by two or more of the four assays (cell culture, DFA assay, PCR, and OIA) were classified as “true infections.” By these criteria, 42 of 306 total specimens were classified as positive for *C. trachomatis* (positive prevalence, 13.7%), 11 (3.6%; 10 by PCR and 1 by DFA assay) were positive by a single assay, and 253 (82.7%) were negative by all four tests. All culture-positive specimens were also positive by at least one other assay. Among the culture-negative specimens, 14 (5%) specimens were positive by two of the three non-culture-based assays used. By using the criterion that positivity by at least two of the tests indicated a true infection, the relative sensitivities were as follows: culture and PCR, 92.9% each; Biostar Chlamydia OIA, 73.8%; and DFA assay, 59.5%.

Chlamydia trachomatis infections are the most prevalent sexually transmitted diseases (STDs) in the United States and are a major preventable cause of infertility, ectopic pregnancy, and chronic pelvic pain in women (3). In addition, because the signs and symptoms of infection are often mild or even absent, laboratory testing plays a central role in efforts to control chlamydia.

Optimally, tests for the detection of chlamydia should be sensitive and specific and should provide results quickly to guide patient management. Until recently, the standard for the diagnosis of *C. trachomatis* infection has been cell culture (8, 9). However, in most clinical settings, antigen or nonamplified nucleic acid detection tests have been preferentially used for testing for chlamydia because of their logistical advantages and lower costs, despite the observation that these tests are generally less sensitive than cell culture (8). More recently, evaluations of newer nucleic acid amplification assays for *C. trachomatis* diagnosis such as PCR or ligase chain reaction have shown the true sensitivity of cell culture for chlamydia detection to be 65 to 85% (15, 18). Despite their greater sensitivities, amplified nucleic acid detection tests are not used in many clinical settings, in part because of their substantially higher costs compared to those of nonamplification assays. In addition, like most currently available tests for the diagnosis of *C. trachomatis* infection, the nucleic acid amplification tests usually do not provide test results at the time of screening but typically require turnaround times of a day or more, a charac-

teristic that may introduce delays in translating test results into treatment (6, 16). In some settings there are demonstrable advantages to tests that can provide results while patients are still present in clinical settings, particularly if the performance of such tests is comparable to the performance of alternate tests (6, 16).

The Biostar Chlamydia OIA (Biostar, Inc., Boulder, Colo.) is an optical immunoassay (OIA) designed to rapidly detect chlamydia infection in women, providing test results in less than 30 min in a test format that allows office-based testing. To evaluate the performance of the Biostar Chlamydia OIA for the detection of *C. trachomatis*, we compared the performances of four assays, i.e., cell culture, an immunofluorescence antigen detection assay (Syva MicroTrak; Syva Co., Palo Alto, Calif.), PCR (Roche Amplicor *Chlamydia trachomatis*; Roche, Branchburg, N.J.), and the Biostar Chlamydia OIA, for the detection of *C. trachomatis* infections in women attending an urban STD clinic.

MATERIALS AND METHODS

Patient population and specimen collection. Between 2 November 1994 and 30 December 1994, women attending the Jefferson County Department of Public Health STD Clinic in Birmingham, Ala., were asked to participate in the present investigation. After swab specimens for *Neisseria gonorrhoeae* cultures were taken, three endocervical swab specimens were collected from consenting participants for *C. trachomatis* testing. The first specimen collected from each patient for chlamydia testing was always obtained with a Dacron-tipped plastic shaft swab placed in 1.5 ml of 0.2 M sucrose phosphate chlamydia cell culture transport medium containing 2% fetal bovine serum and antibiotics (gentamicin, mycostatin, and vancomycin). Next, a specimen was collected with a Dacron polyester swab that was placed in the OIA transport container. Finally, a swab specimen was collected for direct-fluorescent antibody (DFA) evaluation and was rolled over a slide, which, upon drying, was immediately fixed with methanol and placed in the transport unit. PCR testing was performed with an aliquot of the Biostar Chlamydia OIA antigen extract solution. All specimens were main-

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tained at 3 to 5°C and were transported to the laboratory within 18 h of collection, where they were stored before evaluation. Cell culture vials were stored for up to 72 h at -70°C before cultivation (2). The swab for OIA and the slide for the DFA evaluation were stored at 3 to 5°C for no more than 24 h, batched, and processed for determination of the test results. The technologists who were processing the specimens were unaware of the results of the other chlamydial assays which had been performed with specimens from the same patient.

Cell culture method. Cell culture for *C. trachomatis* was performed as described previously (11). Briefly, chlamydia culture medium was inoculated, in triplicate, into a 96-well microtiter format culture system. The cultures were incubated at 37°C, and if no chlamydia inclusions were noted at 48 to 72 h with two different fluorescein-conjugated antibody reagents (Syva MicroTrak [Syva Co.] and Kallestad Pathfinder [Sanofi Diagnostics Pasteur, Chaska, Minn.]), a blind passage was performed.

PCR assay. The Roche Amplicor *Chlamydia trachomatis* PCR assay was performed twice weekly with previously collected and assayed samples in the OIA transport medium. A 100- μ l aliquot of the sample processed for OIA (see below) was added to 900 μ l of the Roche Amplicor swab transport medium. From this point forward, the diluted sample was processed by the PCR assay performed according to the manufacturer's package insert.

DFA assay. For DFA testing, methanol-fixed slides were allowed to come to room temperature, placed in a humidified chamber, and stained according to the manufacturer's directions with the Syva MicroTrak *Chlamydia trachomatis* Direct Specimen Test stain (Syva Co.). The slides were then examined with a \times 100 oil immersion lens with a fluorescence microscope (Zeiss, Flushing, N.Y.). Each slide was graded and interpreted as adequate according to the manufacturer's directions. Adequate specimens had at least 10 columnar and/or cuboidal epithelial cells. By using the manufacturer's criteria for positivity, slides were considered positive for *C. trachomatis* if 10 elementary bodies (EBs) were present and negative if fewer than 10 EBs were observed.

OIA assay. The Biostar Chlamydia OIA assay was performed daily with batched samples in the laboratory. This assay is performed entirely at room temperature without the need for rotators or other specialized equipment. Reagents, detection devices, and swab specimens were allowed to warm to room temperature before testing began. The test was performed as follows. The swabs on which the specimens had been collected were initially placed in the manufacturer's polypropylene extraction tube, and 2 drops of an extraction reagent (reagent 1A) was added, followed by brief agitation, and the tubes were allowed to stand for 2 min. At this point, 6 drops of a second extraction reagent (reagent 1B) were added and another 2-min incubation was carried out. Following this step, six drops of a neutralizing reagent (reagent 2) were added and the swab used for specimen collection was used to vigorously mix the reagent solution. Next, the swab was expressed against the walls of the tube to remove excess liquid and was discarded. Then, with a disposable pipet, 1 drop of the final extraction solution was placed directly onto the center of the reflective optical surface of the test device and the remaining extraction solution was refrigerated for subsequent PCR testing. The liquid specimen droplet was allowed to remain on the surface of the test device for 5 min. Then, 1 drop of Biostar murine monoclonal antibody reagent (reagent 3) was added directly to the sample specimen on the OIA device and the mixture was allowed to incubate for an additional 5 min. The test surface was then washed vigorously by squirting a wash solution (reagent 4) across the reflective optical surface, and the remaining wash solution was blotted from the optical surface with the blotter located in the test device lid. One drop of the substrate (reagent 5) was placed onto the center of the device and was allowed to remain in place for 10 mins. The surface was again washed with reagent 4 and the remaining wash solution was absorbed. The lid was then opened and the result was observed in reflected light. An internal procedural control (a 1- to 2-mm-diameter blue-purple dot) contained in each test unit became visible following proper processing, allowing the technician to confirm that all steps of the detection process had been followed for all samples. Negative samples were identified by observation of the internal control dot only. Positive assays were apparent as a large blue to purple filled circle on the yellow reflective surface. If the result was positive, the more intensely colored internal control dot was visible within the larger test specimen area. Positive and negative control samples provided by the manufacturer were included with each run. The results were recorded, and all test devices were stored for confirmation of the results by a third party if necessary. The result obtained with the device is stable for an unlimited period at room temperature.

DFA discrepant analysis. For further evaluation of the results with specimens from patients which yielded only a single positive test result, the questionable specimens were analyzed by the DFA assay. Briefly, the 0.2 M sucrose phosphate culture transport medium was centrifuged, the pellet was resuspended, and the suspension was placed on a slide, stained with a DFA stain (Syva MicroTrak), and evaluated for the presence of chlamydial EBs as described previously (11).

Analyses. Recent studies have suggested that cell culture fails to detect a substantial proportion of *C. trachomatis* infections, and that, other than cell culture, all non-culture-based *C. trachomatis* detection assays have the potential to give false-positive results (8). At the same time, efforts to resolve differences in test results through further testing of specimens yielding discrepant results (i.e., specimens from the same patient yielding different results when tested by different assays) have been criticized because of concerns that this approach introduces biases which favor the new test under consideration (4, 5). For this

TABLE 1. Results of all diagnostic tests evaluated for detection of *C. trachomatis* by using positive results by at least two tests as the criterion for inclusion^a

No. of specimens	Culture result	DFA assay result	PCR result	OIA result	No. of specimens positive by two-test minimum criterion
20	+	+	+	+	20
2	+	+	+	-	2
3	+	+	-	+	3
5	+	-	+	+	5
9	+	-	+	+	9
3	-	-	+	-	3
10 ^b	-	-	+	-	0
1	-	+	-	-	0
253	-	-	-	-	0

^a Of 306 specimens tested, 39 were positive by culture, 26 were positive by DFA assay, 49 were positive by PCR, and 31 were positive by OIA. Forty-two specimens met the criterion of positivity by a minimum of two tests.

^b The culture transport media for 7 of 10 specimens which were only PCR positive were subsequently shown to contain *C. trachomatis* EBs by a modified DFA technique.

study, for assessment of test performance, patient specimens that yielded positive results by any two assays (cell culture, DFA assay, PCR, and OIA) were considered "true infections" for calculation of sensitivity, specificity, and positive and negative predictive values. Thus, for our analyses any specimen that yielded only a single positive result when all four assays were used to test the specimen was considered "false positive." While these definitions may underestimate the true proportion of infected study participants (see Discussion), this approach permits internally consistent analyses and comparison of the relative performance of the four assays.

RESULTS

The age, race or ethnicity, and reasons for clinic attendance of the study population ($n = 306$) were typical of those of female patients attending the Birmingham STD clinic. The age range of the participants was 15 to 52 years (median, 27 years); 87% were African-American, 13% were white, and one subject was of Hispanic ethnicity; and 56% acknowledged symptoms including genital discharge, dysuria, or abdominal pain.

Individuals with positive test results by two or more of the four assays were classified as "infected" for calculation of the relative performance of the assays. By using these criteria, specimens from 42 of 306 total participants were classified as positive for *C. trachomatis* (positive prevalence, 13.7%), 11 (3.6%; 10 by PCR and 1 by DFA assay) were positive by a single assay, and 253 (82.7%) were negative by all four tests (Table 1). All culture-positive specimens were also positive by at least one other assay. Among the culture-negative specimens, specimens from 14 (5%) participants were positive by two of the three non-culture-based assays used in this study (most often both the PCR and the OIA were positive). By using the criterion of positivity by at least two of the tests, the sensitivities of culture and PCR were each 92.9% (Table 2). The sensitivity of the Biostar Chlamydia OIA was 73.8%, and the sensitivity of the DFA assay was 59.5%.

The culture transport medium from the 11 specimens obtained from participants with a single positive non-culture-based assay result was tested for the presence of chlamydial EBs by the DFA assay. Chlamydial EBs were seen in specimens from 7 of 10 PCR-positive patients, while EBs were not seen on testing of the single specimen which was positive only by the DFA assay. If the seven PCR-positive specimens for which EBs were seen in culture transport medium were recorded as true positives, the revised sensitivities of culture, OIA, and the DFA assay decreased to 79.6, 63.2, and 50%,

TABLE 2. Relative performance of each assay compared to that when the criterion was positivity by at least two of the tests^a

Test	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Culture	92.9	100	100	98.9
DFA assay	59.5	99.6	96.2	93.9
PCR	92.9	96.2	79.6	98.8
OIA	73.8	100	100	96

^a These calculations do not consider data for 7 of 10 patients with positive PCR results, in whom chlamydial EBs were seen. Had data for these patients been included, the sensitivity of PCR would be 93.8%, while the sensitivities of culture, the DFA assay, and OIA would have decreased to 79.6, 50, and 63.2%, respectively (see Discussion). PPV, positive predictive value; NPV, negative predictive value.

respectively, while the sensitivity of PCR would increase to 93.8%.

DISCUSSION

In the present investigation the Biostar Chlamydia OIA, an antigen detection assay suitable for on-site use in acute-care settings such as clinics and emergency rooms, accurately detected 74 to 63% of *C. trachomatis* infections, depending on how sensitivity was calculated. Although many published studies have evaluated non-culture-based tests for the diagnosis of chlamydial infections, few of these studies have compared the performance of non-culture-based tests to each other (17). When compared to cell culture and the Roche Amplicor PCR, the Biostar Chlamydia OIA was less sensitive for the detection of chlamydia infection, while when compared to direct immunofluorescence microscopy, the OIA appeared to be somewhat more sensitive. The sensitivities reported in Table 2, however, reflect the relative performance of the four assay methods used in this study. For 10 patients, only the PCR-based tests were positive, and 7 of these patients were subsequently judged to most likely have a true infection on the basis of the demonstration of chlamydial EBs by immunofluorescence microscopic examination of the culture transport medium. Thus, the performance reported in Table 2 likely underestimates the true sensitivity of the PCR-based assay and somewhat inflates the relative sensitivities of cell culture, the MicroTrak DFA assay, and the OIA for the detection of chlamydial infection.

The sensitivity of the Biostar Chlamydia OIA in this study is less than the 83.8% sensitivity reported in the manufacturer's package insert, a finding that most likely reflects differences in analytic approach rather than variations in test sensitivity. The studies used to calculate the test performance reported in the manufacturer's package insert were performed, in part, by our laboratory; however, the U.S. Food and Drug Administration's practice for considering the performance of new tests is to consider only the results of initial cell culture, without blind passage. This practice helps to explain the differences in sensitivities between our findings and those in the package insert, as well as to illustrate the complexities of evaluating the performance of non-culture-based tests for the detection of chlamydia.

To date, few published studies have compared the performance of multiple diagnostic tests to one another (9). Instead, there have been comparisons of the performance of a non-culture-based test to that of culture, sometimes supplemented by immunofluorescence microscopy of specimens positive by non-culture-based tests in order to compensate for possible false-negative culture results. This approach, however, fails to address the theoretical possibility that for substantial numbers of specimens results for both culture and the non-culture-

based assay are false negatives (4, 5). For this study, which included four different tests, we chose a comparative method that evaluated the performance of the four tests relative to each other. However, given what appears to be the greater sensitivity of the PCR test, the positive results for 42 specimens classified as *C. trachomatis* positive by using for these analyses the criterion of positivity by two or more of the four tests may slightly overestimate the true performance of OIA, DFA assay, or culture.

Several other limitations of this study should be acknowledged. In particular, the performance of the DFA test for the diagnosis of chlamydial infection may have been diminished by two potentially confounding factors. The performance of the DFA assay in this study may have been suboptimal since the specimen collection order was not randomized and the specimens for DFA testing were collected fourth (last). Good patient specimen collection with appropriate numbers of cells and proper slide preparation techniques are essential for the maximum performance of the DFA assay. Seventy-two percent of all specimens tested by the DFA assay in the course of this study were deemed inadequate on the basis of having fewer than 10 columnar and/or cuboidal epithelial cells, but in the performance calculations the results for those specimens were considered negative for *C. trachomatis*. Typical proportions of inadequate specimens associated with routine cervical swab specimen collection for the DFA assay range between a low of 1% and a high of 79% (7, 14). Thus, while the number of inadequate specimens obtained during the course of this study was high, they are within the range reported in the literature in the testing of endocervical swab specimens. In addition, the DFA assays in this study were evaluated with a requirement that the specimen contain 10 EBs to be classified as positive, according to the manufacturer's instructions. In prior studies (1, 7, 10, 13), other investigators have demonstrated that the sensitivity of this assay may be increased by using a cutoff of one EB as the criterion for a positive assay result. In our study, 12 specimens were found to contain fewer than 10 EBs (range, 2 to 7 EBs), and if these were considered positive, the result would have been a relative sensitivity for the DFA test of 88%.

It should be acknowledged that for PCR testing, although a commercially available assay was used, the material used for testing was the specimen processed for OIA rather than a swab specimen. Studies in our laboratory suggest that the use of this material for testing did not detract from the performance of the PCR (data not shown).

Finally, another issue which should be acknowledged is that this study was performed in a research laboratory by experienced laboratory staff. Thus, the results may not reflect the performance characteristics of these tests in the office- or clinic-based settings for which they are designed and where non-laboratory staff are performing the assay. Real-world use of the OIA in busy clinical settings may not yield the same sensitivity reported here. Nonetheless, the performance of the Biostar Chlamydia OIA in this study is comparable to that in laboratory-based enzyme immunoassays previously performed in our laboratory (12). While the sensitivity of the OIA for the detection of chlamydial infection was not as great as that of cell culture or PCR, the performance of the OIA in selected settings, with patients present, could result in the expeditious treatment of chlamydia-infected individuals. The Biostar Chlamydia OIA is classified as a moderately complex assay. It is also considered a quick method for the detection of *C. trachomatis* in endocervical samples.

In summary, in this prospective study, the relative comparisons of the four assays (cell culture, PCR, DFA assay, and OIA) showed that there was a good correlation between the

results of PCR and cell culture, that the relative sensitivity of the OIA was 73.8% and that the relative sensitivity of the DFA assay was 59.5%. In settings in which the prevalence of *C. trachomatis* is high, the Biostar Chlamydia OIA should be considered a patient point-of-care screening device and should be a useful addition to the bench-top assays currently available for the detection of chlamydia.

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