Evaluation of a Rapid Assay for Detection of *Chlamydia trachomatis* Infections in Outpatient Clinics in South Kalimantan, Indonesia

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Received 15 April 1999/Returned for modification 8 June 1999/Accepted 20 September 1999

A multicenter cross-sectional survey was conducted comparing a commercially available chlamydial optical immunoassay (OIA) to the chlamydial ligase chain reaction (LCR). Endocervical samples from 415 outpatients visiting clinics from three hospitals in South Kalimantan, Indonesia, were evaluated. Relative to the LCR, the overall sensitivity and specificity of the OIA were 31.6 and 98.9%, respectively. The sensitivity of the OIA varied among the three hospital laboratories, ranging from 20 to 50%. The OIA performance was slightly lower on samples from patients attending dermatovenerology clinics than on samples from nondermatovenerology clinic patients. The results indicate that the OIA did not perform well compared to LCR.

*Chlamydia trachomatis* has been reported as one of the most common sexually transmitted pathogens in the developed world (1, 9, 10). To reduce the reservoir of infected asymptomatic individuals, who make up the bulk of prevalent infections and are responsible for maintaining transmission of the infection within a community, active chlamydia control programs are needed. For such a program to be successful and accurate, a simple and rapid screening diagnostic test is required so that prompt and effective therapy can be initiated, particularly in asymptomatic cases. Although treatment is relatively simple and straightforward (4, 8), bringing patients to treatment still remains a challenge (3).

Traditionally, the diagnosis of *C. trachomatis* infections has relied on the isolation of the organism in tissue culture. This method is time-consuming and requires considerable technical expertise and a suitable cell culture facility. Molecular biology-based tests, such as PCR and ligase chain reaction (LCR), are now firmly established as sensitive and specific techniques for detecting chlamydia in clinical specimens (2). However, the advent of direct antigen detection methods has provided more rapid and less expensive alternatives to these molecular biology approaches. An important advantage of the antigen detection assay is that it can greatly increase the availability of chlamydia diagnostic services. One such immunoassay, the CHLAMYDIA OIA (BioStar, Boulder, Colo.), has recently become commercially available for office- or clinic-based detection of *C. trachomatis*. To evaluate the sensitivity and specificity of the optical immunoassay (OIA), we conducted a multicenter cross-sectional survey comparing this assay to the chlamydia LCR.

Three hospital outpatient clinics located in South Kalimantan, Indonesia, were selected for study: Ulin Provincial Hospital, Banjar Baru District Hospital, and Kandangan District Hospital clinics. Between May 1996 and October 1996, a total of 415 outpatients were enrolled into the study for screening. The patients were part of a larger study seeking to assess issues related to integrating sexually transmitted disease screening and management services as part of a maternal health care package. Patients attended either dermatovenerology (DV) clinics or nondermatovenerology (NDV) clinics.

Following routine gynecological examination, endocervical swabs were collected for CHLAMYDIA OIA and LCR testing, according to the manufacturers’ instructions. Swabs for CHLAMYDIA OIA were placed into collection device tubes, and those for LCR assay were placed into transport vials containing 1 ml of sterile double-distilled water. All specimens were held at 4°C until arrival at the laboratory of each hospital. The chlamydia OIA was performed within 3 days of collection, which was within the manufacturer’s parameters. The LCR specimens were stored in liquid nitrogen and transported to the U.S. Naval Medical Research Unit No. 2, Jakarta, for testing by LCR.

For performance of the OIA, chlamydial lipopolysaccharide was first extracted from the sample, and then the extracted sample was applied to the CHLAMYDIA OIA device. This device consists of a solid reflective support coated with a thin film selected to make it sensitive to changes in the reflection of specific wavelengths of light. Subsequently, an anti-lipopolysaccharide antibody-horseradish peroxidase conjugate and substrate is added, which binds to the thin film via the antigen-antibody reaction. Binding of antigen to antibody results in alterations in the thickness of the film. The increased film thickness causes optical changes to the path of light, with a subsequent color change on the film’s surface from gold to purple. The CHLAMYDIA OIA was performed in the clinical laboratory of each hospital. Prior to evaluating clinical samples, laboratory personnel at each hospital were thoroughly trained in the use of OIA by experienced technicians.

For *C. trachomatis* diagnosis by LCR, a commercially available assay was used (LCx *Chlamydia trachomatis* assay; Abbott Laboratories, Chicago, III.). Specimens were thawed completely and vortexed, and the suspension was transferred to a microcentrifuge tube and centrifuged at 10,000 rpm (Beckman 5415C) for 10 min. After removal of the supernatant fluid, the pellet was resuspended in 0.5 ml of resuspension buffer provided in the LCx kit and heated to 95°C for 15 min. After cooling at room temperature, processed specimens were either tested immediately by LCR or stored at −70°C until used. Each step was performed according to the instructions of the manufacturer and was described earlier (5).
The BioStar CHLAMYDIA OIA is a new OIA that has previously been evaluated in the detection of C. trachomatis in ocular specimens from infants with suspected chlamydial conjunctivitis. In that study, the OIA was compared to chlamydial culture and demonstrated a sensitivity of 100% and a specificity of 92.6% when prospectively evaluating 37 ocular specimens from infants (6). Our study was designed to further evaluate the BioStar OIA relative to LCR in diagnosing chlamydial infections in women attending outpatient reproductive health clinics in a resource-limited primary-care setting. The results showed that the overall sensitivity of the assay was poor at 32%, while the specificity was acceptable at 99%. The best sensitivity was obtained at Kandangan (50%) but was still considered relatively low.

The low sensitivity observed in our study is not unlike that seen in an earlier study describing the evaluation of another solid-phase rapid antigen detection test for chlamydia (3). In that study, with chlamydial cultures used as the predicate test, a higher sensitivity (47%) and specificity (99.7%) were obtained. Although the overall sensitivity of the OIA was lower than that reported for this other rapid detection assay, the difference could be explained in part by the fact that a more sensitive comparative test (LCR) was used in our study. The superiority of the LCR over the conventional chlamydia culture has been demonstrated in a prior study (7). Another plausible explanation is that the format of the other assay renders it slightly more sensitive than the OIA.

When considering that the OIA previously performed well against chlamydial culture (6), we speculate that the sensitivity and specificity of the OIA in our study may have been comparable to that in the other OIA study if culture instead of LCR was used as the comparative test. A more likely reason why the OIA performed better in the study by Roblin et al. (6) is that conjunctival samples instead of cervical samples were used. The mucous and red blood cells present in cervical specimens may have had an inhibitory effect on the CHLAMYDIA OIA in our study. Because we present no data directly evaluating the influence of mucous and red blood cells on CHLAMYDIA OIA performance, definitive reasons for the differences remain unknown.

The sensitivity of the CHLAMYDIA OIA in this study varied greatly, depending on the location of the clinical laboratory where the tests were performed. The precise reason(s) for this is not clear. One possible explanation is that although the test is rather simple to perform, a certain level of technical expertise is needed to process the cervical samples and apply the material to the chlamydia OIA device. Varying technical capabilities of the staff at each clinical laboratory may have contributed to the varying sensitivities. Another possible reason is that the interpretation of the assay is very subjective and is based on optical refraction of light creating a halo of purple color surrounding the specimen dot. Under different lighting conditions, weakly positive samples may be read as negative.

To explore the effect of the variables mentioned above on the performance of the CHLAMYDIA OIA, more information about the hospitals, hospital laboratories, and laboratory personnel was obtained. Ulin Hospital is a type B hospital with a capacity of 405 beds. Both Banjar Baru and Kandangan are type C hospitals with 89 and 75 beds, respectively. The clinical laboratory in Ulin Hospital is equipped with more sophisticated instrumentation, and as a result, laboratory personnel possess more in-depth experience with various laboratory procedures. The educational background of the Ulin laboratory technicians ranges from the associate degree level to the high

### Table 1. Comparison of CHLAMYDIA OIA and LCR for the detection of C. trachomatis in NDV and DV patients

<table>
<thead>
<tr>
<th>Patients (n)</th>
<th>Prevalence (%)</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>+PV (%)</th>
<th>−PV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NDV (342)</td>
<td>7.9</td>
<td>33.3</td>
<td>99</td>
<td>75</td>
<td>94.5</td>
</tr>
<tr>
<td>DV (73)</td>
<td>15.1</td>
<td>27.3</td>
<td>98.4</td>
<td>75</td>
<td>88.4</td>
</tr>
<tr>
<td>Total (415)</td>
<td>9.2</td>
<td>31.6</td>
<td>98.9</td>
<td>75</td>
<td>93.5</td>
</tr>
</tbody>
</table>

* +PV, predictive value of positive test.
* −PV, predictive value of negative test.

Endocervical swab specimens were collected from 415 outpatients for screening and tested in three different clinical laboratories for C. trachomatis by CHLAMYDIA OIA. Of the 415 outpatients participating in this study, 342 were from NDV clinics (antenatal clinics and obstetric and gynecology clinics) and 73 were from DV clinics. No information was available to us regarding the proportion of participants in each group that were symptomatic or asymptomatic. The mean age for persons in the NDV group was 32 years, and the mean age for the DV group was 31 years.

The prevalence of C. trachomatis in NDV patients by LCR was 7.9% (27 of 342), and the prevalence in DV patients was 15.1% (11 of 73), with an overall prevalence rate of 9.2% (38 of 415) (Table 1). The OIA was positive in 9 of the 27 positive NVP patients (33%) and in 3 of the 11 positive DV patients (27%). Samples from four patients tested positive by CHLAMYDIA OIA and negative by LCR. To rule the possibility of false negative LCR results due to the presence of inhibitors, these samples were diluted 1:10 and 1:100 and then tested again by LCR. The results showed that these samples remained negative at both dilutions. When using LCR as the gold standard, the sensitivity and specificity of the OIA were 31.2 and 98.9%, respectively. The test performance values of the CHLAMYDIA OIA in DV patients were lower than those in NDV patients. The sensitivity of the assay in NDV patients was 33.3%, compared to 27.3% for DV patients. The predictive value of a positive test was the same in both groups (75%), but the negative predictive value was lower in the DV group (88.4%).

The sensitivity and specificity of the OIA varied between the different laboratories and ranged from 20 to 50% and 98.1 to 100%, respectively (Table 2). Samples from 166 patients were tested at Ulin, samples from 174 patients were tested at Banjar Baru, and samples from 75 patients were tested at Kandangan. The lowest sensitivity and specificity were seen at Banjar Baru, and the highest were seen at Kandangan. The negative predictive value of the CHLAMYDIA OIA at all three sites was greater than 90%, with the highest value being 98% at Kandangan. The positive predictive values varied greatly among the three sites. The poorest was seen at Banjar Baru at 50%, and the best was seen at Kandangan at 100%.

The BioStar CHLAMYDIA OIA is a new OIA that has previously been evaluated in the detection of C. trachomatis in ocular specimens from infants with suspected chlamydial conjunctivitis. In that study, the OIA was compared to chlamydial culture and demonstrated a sensitivity of 100% and a specificity of 92.6% when prospectively evaluating 37 ocular specimens from infants (6). Our study was designed to further evaluate the BioStar OIA relative to LCR in diagnosing chlamydial infections in women attending outpatient reproductive health clinics in a resource-limited primary-care setting. The results showed that the overall sensitivity of the assay was poor at 32%, while the specificity was acceptable at 99%. The best

### Table 2. Comparison of CHLAMYDIA OIA and LCR for detection of C. trachomatis in different laboratories

<table>
<thead>
<tr>
<th>Hospital (no. of patients)</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>+PV (%)</th>
<th>−PV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ulin (166)</td>
<td>38.1</td>
<td>99.3</td>
<td>88.9</td>
<td>91.7</td>
</tr>
<tr>
<td>Banjar Baru (174)</td>
<td>20</td>
<td>98.1</td>
<td>50</td>
<td>92.9</td>
</tr>
<tr>
<td>Kandangan (75)</td>
<td>50</td>
<td>100</td>
<td>100</td>
<td>98.6</td>
</tr>
<tr>
<td>All sites (415)</td>
<td>31.6</td>
<td>98.9</td>
<td>75</td>
<td>93.5</td>
</tr>
</tbody>
</table>

* +PV, predictive value of positive test.
* −PV, predictive value of negative test.

The mucous and red blood cells present in cervical specimens may have had an inhibitory effect on the CHLAMYDIA OIA in our study. Because we present no data directly evaluating the influence of mucous and red blood cells on CHLAMYDIA OIA performance, definitive reasons for the differences remain unknown.
school graduate level. The educational background of the technicians at the other hospital clinical laboratories is at the high school graduate level. Since the highest sensitivity of the CHLAMYDIA OIA was not seen at the Ulin laboratory, we concluded that educational level and experience were not major factors in determining how well the assay was performed.

Compared to other clinical laboratories, the Kandangan laboratory possessed the best source of light. The Kandangan light source consisted of a combination of natural sunlight and fluorescent lighting, whereas at the other clinical laboratories, natural sunlight was limited and fluorescent fixtures provided most of the lighting. Since the best sensitivity was seen at the Kandangan clinical laboratory, light source may be crucial to maximizing the performance of the CHLAMYDIA OIA. Because our study was not designed to analyze the effect of light source on test performance, a more systematic study is needed to assess the true influence of this variable on the OIA outcome.

The sensitivity of CHLAMYDIA OIA was slightly higher in the NDV group than in the DV group. This result was somewhat unexpected, and the exact reason for this remains unclear. The data generated by this study are not sufficient to provide a definitive explanation for this observed difference. The similar positive predictive value obtained for both groups indicates that the assay was performed equally well in the two groups.

The BioStar OIA is a rapid antigen detection assay that has a sensitivity and specificity comparable to the sensitivity and specificity of chlamydia culture. When compared to the most sensitive chlamydia detection methods, such as the LCR, the OIA performs poorly and cannot be considered a viable alternative to these tests.

We thank the Ministry of Health of Indonesia at the national level and in South Kalimantan for allowing us to conduct this study. We are very grateful to the hospital physicians, midwives, and laboratory technicians for their efforts in collecting the data. PATH and J. Lewis of the Center for Disease Control are gratefully acknowledged. We also thank Margaret Wirth and the MotherCare staff in South Kalimantan for their support. Endang Achadi, Marjorie Koblinsky, Tom Marshall, Jeanne McDermott, John Moran, and Carine Ronsmans provided valuable input to the analysis and writing of this paper.

This work was supported by grants from the MotherCare Project, John Snow, Inc., and the Office of Health, Bureau of Global Programs, Field Support and Research, and the United States Agency for International Development.

REFERENCES