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Evaluation of a New Optical Immunoassay for Diagnosis of Neonatal Chlamydial Conjunctivitis

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The BioStar OIA *Chlamydia* test (BioStar, Inc., Boulder, Colo.) is a novel immunoassay system that uses changes in reflection of light to directly detect chlamydial antigen in clinical specimens. We compared the optical immunoassay (OIA) with culture for detecting *Chlamydia trachomatis* in ocular specimens from infants with suspected chlamydial conjunctivitis. We initially performed a retrospective evaluation, testing 152 ocular specimens previously collected for culture with the OIA. The sensitivity and specificity were 94.2 and 97%, respectively. A subsequent prospective study evaluating 37 ocular specimens from infants with suspected *C. trachomatis* conjunctivitis revealed a sensitivity and specificity of 100 and 92.6%, respectively.

Chlamydia trachomatis is the most common identifiable infectious cause of neonatal conjunctivitis (3–5, 7, 8). Approximately 20 to 40% of the cases of neonatal conjunctivitis seen at our institution are caused by *C. trachomatis* (3–5, 8). Previous studies of enzyme immunoassays and direct fluorescent antibody tests (DFA) in infants with chlamydial conjunctivitis have found sensitivities and specificities usually exceeding 92% (3–5, 7, 8).

The BioStar OIA *Chlamydia* test (BioStar, Inc., Boulder, Colo.) is a rapid, self-contained, optical immunoassay (OIA) based on changes in the reflection of light. The assay takes approximately 25 min to run and requires no special equipment. The BioStar OIA *Chlamydia* test was recently approved for identifying *C. trachomatis* from endocervical specimens in women. In this study we compared the performance of the BioStar OIA *Chlamydia* test with that of culture for the detection of *C. trachomatis* in ocular specimens from infants with suspected chlamydial conjunctivitis. Infants less than 6 weeks of age with conjunctivitis were enrolled in the study. These infants were seen in the pediatric emergency room or neonatal services at Kings County Hospital Center and University Hospital of Brooklyn, Brooklyn, N.Y.

Specimens for culture of *C. trachomatis* were collected with wire-shafted Dacron swabs (Dacroswab, Spectrum, Houston, Tex.) from the conjunctivae of infants with conjunctivitis. The swabs were immersed in 2 ml of transport medium containing a sucrose-phosphate buffer with 10% fetal bovine serum and 10 μl of gentamicin, 10 μl of vancomycin, and 1 μl of amphotericin B per ml. They then were refrigerated for up to 24 h or frozen at −70°C if they were not cultured within that period. For the prospective study specimens for OIA were also collected with wire-shafted Dacron swabs, which were placed in plastic tubes and refrigerated until the assay was run, usually within 1 to 2 h of collection.

Isolation of *C. trachomatis* was performed with cycloheximide-treated McCoy cells grown in duplicate 96-well microtiter plates as previously described (5). After 48 to 72 h of incubation, the wells were fixed and stained with two different monoclonal antibodies, one directed against chlamydia lipopolysaccharide (Kallestad, Chaska, Minn.) and the other one directed against the major outer membrane protein (Syva MicroTrak, Palo Alto, Calif.). The duplicate plate was passed and then fixed and stained 48 h later.

The OIA was run in accordance with the instructions of the manufacturer. A solid reflective support is coated with a thin film selected to make it sensitive to changes in the reflection of specific wavelengths. Once chlamydial lipopolysaccharide is extracted from the specimen, it is added to the membrane and bound. Subsequently, an anti-LPS antibody–horseradish peroxidase conjugate and substrate are added, which bind to the OIA surface. The increased thickness of the thin film due to the antigen-antibody reaction changes the optical path of light causing a color change on the surface from gold to purple.

Retrospective and prospective studies were done. For the retrospective study we tested ocular specimens that had been previously collected for chlamydial culture with the OIA. These specimens were originally collected and cultured in 1992 and had been frozen at −70°C. A 0.1-ml aliquot of each of these specimens was tested as described above. For the prospective study duplicate specimens for culture and OIA were obtained from each patient. The order of swab collection was alternated.

Discrepancies between clinical samples which were OIA positive but culture negative were resolved by examining the original culture specimen. A 1-ml fraction of the discordant specimen was added to 1 ml of phosphate-buffered saline and centrifuged at 3,000 × *g* for 1 h. The supernatant was discarded, and the pellet was suspended in a volume of 100 to 200 μl with phosphate-buffered saline. One drop of this suspension was spotted onto a glass slide, fixed, stained with fluorescein-conjugated antichlamydia antibody (Syva MicroTrak), and examined for the presence of elementary bodies (EBs). Positive and negative controls were examined at the same time.

A total of 152 previously cultured ocular specimens were evaluated in the retrospective study. Forty-nine specimens (32.2%) were culture and OIA positive, three were culture negative and OIA positive, and three were culture positive and OIA negative. The sensitivity and specificity compared to culture were 94.2 and 97%, respectively, and the predictive values of positive and negative tests were 94.2 and 97%, respectively. DFA staining of the culture specimens of two of the three

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culture-negative, OIA-positive specimens revealed EBs, so the specimens were probably true positives. The resolved sensitivity and specificity and positive and negative predictive values were 94.4, 99, 98, and 97%, respectively.

For the prospective study, duplicate specimens for culture and OIA were obtained from 37 infants with conjunctivitis. Ten (27%) infants were culture and OIA positive and two infants were culture negative and OIA positive. Neither of these discrepant specimens was resolved by DFA staining of the culture specimens, so the specimens were probably false positives. The sensitivity and specificity compared to culture were 100 and 92.6%, respectively, and the predictive values of positive and negative tests were 83.3 and 100%, respectively.

The BioStar OIA *Chlamydia* test compared favorably with culture for the diagnosis of neonatal chlamydial conjunctivitis in both the retrospective and prospective studies. The sensitivity and specificity are comparable to those previously reported for enzyme immunoassays and DFAs for use with conjunctival specimens from infants (3–5, 7, 8). The only other rapid test approved for use with conjunctival specimens, Kodak Surecell, was recently withdrawn from the market, ostensibly for economic, not performance, reasons. The BioStar OIA *Chlamydia* test performed equivalently to Kodak Surecell, which had reported sensitivities of 93 and 96.7% and specificities of 93.3 and 96% compared to culture for detecting *C. trachomatis* in the conjunctivae of infants with conjunctivitis (3, 5).

The OIA technology has been approved as a rapid test for detection of group A streptococci in throat swabs (Strep A OIA; BioStar, Inc.) and group B streptococci in vaginal swabs (Strep B OIA; BioStar, Inc.) (1, 2, 6). Data on the use of this test for *C. trachomatis* infections are limited. Recently, Wells reported an evaluation of the BioStar OIA *Chlamydia* test for detection of *C. trachomatis* in endocervical specimens (9). The OIA was compared to culture and PCR (Amplicor; Roche Molecular Systems, Branchburg, N.J.). When a positive result with any assay was considered to be diagnostic, the OIA was found to have a sensitivity of 61% compared to only 51% for culture. Patients that were only PCR positive were considered true positives, and the OIA detected 67% of PCR-positive specimens, whereas culture detected only 46%; but four specimens were only culture positive. The author concluded that OIA was comparable to a single-pass culture. In the present study, OIA was comparable to culture in ocular specimens; however, the eye is an easier site to sample. Two of three OIA-positive, culture-negative cultures in the retrospective study contained EBs on DFA staining of the original specimen, and so they probably contained organisms that were not viable. The two discrepant specimens in the prospective evaluation may have been due to variation in specimen collection technique.

The BioStar OIA *Chlamydia* test may fill a niche for use in physicians' offices or small hospital laboratories which see small numbers of infants with suspected chlamydial conjunctivitis.

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