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Comparison of a Chemiluminometric Immunoassay with Culture for Diagnosis of Chlamydial Infections in Infants

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The performance of Magic Lite (CIBA-Corning), a chemiluminescent immunoassay (CIA), was compared with that of culture for the diagnosis of neonatal chlamydial conjunctivitis and respiratory infection. We performed a retrospective evaluation of 51 ocular and 96 nasopharyngeal specimens previously collected for culture testing with the CIA. The sensitivities for the ocular and the nasopharyngeal specimens were 91 and 91.7%, respectively. The specificities for both sites were 100%. A subsequent prospective study evaluating 71 ocular and 38 nasopharyngeal specimens revealed sensitivities of 83.3 and 20%, respectively. The specificities for both sites were 100%. The CIA performed favorably, compared with culture, for the diagnosis of chlamydial conjunctivitis; however, the CIA appeared less sensitive for the diagnosis of respiratory infection, including pneumonia.

Chlamydia trachomatis is the major infectious cause of neonatal conjunctivitis. Approximately 20 to 40% of the cases of neonatal conjunctivitis seen at our institution are caused by *C. trachomatis* (4, 6–8). The organism also plays a major role in pneumonia in infants, which can have significant morbidity (4, 5). Previous studies have demonstrated that enzyme immunoassays and the direct fluorescent-antibody test are specific and sensitive for the diagnosis of chlamydial conjunctivitis and pneumonia in infants (10, 11). Magic Lite Chlamydia Collection System (CIBA-Corning Diagnostics Corp., East Walpole, Mass.) represents a new method of chlamydial antigen detection. It employs a chemiluminescent reaction and takes 2½ to 3 h to run. The chemiluminescent immunoassay (CIA) has recently been approved for cervical and urethral specimens from adult men and women and for urine specimens from men. Although the CIA has been approved for clinical use, very few data from studies using this assay for diagnosing chlamydial infection have been published. Danielsson et al. reported a sensitivity of 97.1% and a specificity of 97.3% in a study of 291 cervical specimens and a sensitivity of 88.8% and a specificity of 97.4% for urine specimens from men (3).

In this study, we evaluated the performance of Magic Lite compared with that of culture for the detection of *C. trachomatis* in ocular and nasopharyngeal specimens from infants with suspected chlamydial conjunctivitis and respiratory infection, including pneumonia. Infants (age, <6 weeks) with conjunctivitis and infants (age, <6 months) with suspected chlamydial pneumonia were enrolled in the study. These infants were seen in the emergency room or the neonatal service facility at Kings County Hospital Center, Brooklyn, N.Y.

Specimens for culture of *C. trachomatis* were collected with wire-shafted Dacron tip swabs (Spectrum, Houston, Tex.) from the conjunctivae and nasopharynges of infants with conjunctivitis and from the nasopharynges of infants with pneumonia. The swabs were immersed in 2 ml of transport medium containing a sucrose-phosphate buffer with 10% fetal bovine serum, 10 mg of vancomycin per ml, 10 mg of gentamicin per ml, and 1 mg of amphotericin B per ml. They then were refrigerated for up to 24 h or frozen at −80°C if not cultured within that period. Specimens for Magic Lite were collected with swabs (Magic Lite Chlamydia Collection System; CIBA-Corning) which were then immersed in 100 μl of specimen collection storage solution, stored at 2 to 8°C, and tested within 5 days of collection.

Isolation was performed with cycloheximide-treated McCoy cells grown on 96-well microtiter plates. Following 48 to 72 h of incubation, the wells were fixed and stained with an anti-lipopolysaccharide fluorescein-conjugated monoclonal antibody (Pathfinder Culture Confirmation System; Kallestad Diagnostics, Chaska, Minn.) (12).

The Magic Lite assays were performed according to the instructions of the manufacturer; reagents and equipment were provided by CIBA-Corning. Results were expressed as relative light units. Specimens with relative light unit values greater than the calculated cutoff value were considered positive for *C. trachomatis*. A negative result occurred when relative light unit values were less than the cutoff. When indeterminate results were obtained, specimens were retested and/or new specimens were obtained and tested. This was also done for negative CIA results which conflicted with culture results.

Retrospective and prospective studies were done. For the retrospective study, we used the Magic Lite CIA to evaluate specimens that had been previously collected for chlamydial culture and frozen at −80°C since 1990. A 200-μl aliquot of each of these specimens was tested as described above. For the prospective study, duplicate specimens for culture and CIA were obtained from each patient. The order of swab collection was alternated.

Sensitivity, specificity, and predictive values of positive and negative tests were evaluated by standard definitions. Statistical evaluation of data was performed by using a general form of the Pearson chi-square test for a three-dimensional table. A significance level of $P < 0.05$ (2, 5) was used.

Fifty-one conjunctival specimens were evaluated in the retrospective study; 11 (21.6%) were positive by culture.

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TABLE 1. Comparison of Magic Lite CIA with culture for the detection of *C. trachomatis* in conjunctival and nasopharyngeal specimens

CIA result	No. of culture results		
	Positive	Negative	Total
Retrospective			
Ocular			
Positive	10	0	10
Negative	1	40	41
Nasopharynx			
Positive	11	0	11
Negative	1	84	85
Prospective			
Ocular			
Positive	20	0	20
Negative	4	47	51
Nasopharynx			
Positive	1	0	1
Negative	4	33	37

The results are summarized in Table 1. Ten specimens were both CIA and culture positive; one specimen was CIA negative and culture positive. The sensitivity and specificity were 91 and 100%, respectively. The predictive values of positive and negative tests were 100 and 97%, respectively. An additional 96 nasopharyngeal specimens from infants with suspected chlamydial conjunctivitis and pneumonia were also evaluated. Twelve (12.5%) of the nasopharyngeal specimens were positive by culture. Eleven were both CIA and culture positive. One specimen was CIA negative and culture positive. The sensitivity and specificity were 91.7 and 100%, respectively. The predictive values of positive and negative tests were 100 and 98.8%, respectively.

For the prospective study, duplicate ocular specimens for chlamydial culture and CIA were obtained from 71 infants with conjunctivitis. Twenty-four (33.8%) were positive by culture; 20 were both CIA and culture positive, and 4 were CIA negative and culture positive. The sensitivity and specificity were 83.3 and 100%, respectively. The predictive values of positive and negative tests were 100 and 92%, respectively. Duplicate nasopharyngeal specimens were collected from 38 infants with suspected chlamydial pneumonia for chlamydial culture and CIA. Five infants (13%) were positive by culture. One specimen was CIA and culture positive; four were CIA negative and culture positive. The sensitivity and specificity were 20 and 100%, respectively. The predictive values of positive and negative tests were 100 and 89%, respectively.

The CIA compared favorably with culture for the diagnosis of chlamydial conjunctivitis in both the retrospective and the prospective studies. The CIA performed better in the retrospective study than in the prospective study. The poorer performance in the prospective study was probably a result of variations in specimen collection. The CIA compared favorably with culture for the detection of *C. trachomatis* in nasopharyngeal specimens in the retrospective study, but it was significantly less sensitive in the prospective study ($P = 0.013$). Other immunoassays have shown similarly disparate results between ocular and nasopharyngeal specimens (8, 9, 11). Although some comparative studies have found that the order of swab collection of the specimen may have an influence on performance, this was not our experience. Moreover, there was probably less variation in the collection of conjunctival specimens than in the collection of nasopharyngeal specimens because of the ease of collection and the greater number of organisms present. We also found that nasopharyngeal specimens that were excessively mucoid resulted in indeterminate readings, which required reevaluation, suggesting that the assay may need a more effective mucolytic agent or that these specimens may require longer vortexing. It was found that immunoassay detection failure was attributable to the low number of organisms present. Specimens that were negative by CIA had ≤5 inclusions per well in cell culture. Magic Lite was unable to detect *C. trachomatis* in specimens with $\leq 10^3$ inclusion-forming units/ml without falling into the "grey zone" or indeterminate area. Although 10 specimens were negative, the test was highly specific, exhibiting no false-positive results.

A major concern of this study was the small number of positive specimens in the prospective nasopharyngeal analysis. Since only five specimens were positive by culture, it appeared too small a number on which to base significant claims regarding sensitivity and specificity. The data for the nasopharyngeal specimens from both the retrospective and the prospective studies were combined and evaluated. The combined sensitivity value for the nasopharyngeal data was 70.6%, with a specificity of 100%, making the overall performance of Magic Lite comparable to the performance of the already commercially available enzyme immunoassays and direct fluorescent-antibody assays. Comparative studies of culture versus enzyme immunoassays and direct fluorescent-antibody assays have shown sensitivities ranging from 60 to 90%, with specificities ranging from 70 to 100% for similar anatomical sites (4, 7–9).

While the performance of Magic Lite was comparable to that of other rapid diagnostic tests available, the CIA offered no major advantages. There is however, an urgent need for quality assurance for these variant assays. This is especially important for the performance of automated tests, largely because of the complexity and sensitivity of the procedures. Routine monitoring of test processes and pretest inspection of controls and test specimens are necessary for the accuracy of these tests (1). The variations in the designs of tests for clinical use point to the need for continued research and development in these areas.

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