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Comparison of Two Enzyme Immunoassays to Culture for the Diagnosis of Chlamydial Conjunctivitis and Respiratory Infections in Infants

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Data are limited for the performance of enzyme immunoassays for the detection of *Chlamydia trachomatis* in conjunctival and nasopharyngeal specimens from infants. The only available data are for one assay, Chlamydiazyme (Abbott Diagnostics). The purpose of this study was to compare a new enzyme immunoassay, Pathfinder (Kallestad Diagnostics), with Chlamydiazyme and culture for the diagnosis of chlamydial conjunctivitis and pneumonia in infants. Pathfinder differs from Chlamydiazyme in that it uses a monoclonal antibody directed against the chlamydial lipopolysaccharide in addition to a polyclonal antichlamydial antibody. Triplicate conjunctival and nasopharyngeal specimens were obtained from 97 infants with conjunctivitis, and additional nasopharyngeal specimens were obtained from 14 infants with suspected chlamydial pneumonia (total, 111 nasopharyngeal specimens). Twenty-nine (30%) of the conjunctival specimens from infants with conjunctivitis and four (28.6%) of the nasopharyngeal specimens from the infants with pneumonia were positive for *C. trachomatis* by cell culture. The sensitivities, specificities, and positive and negative predictive values for Pathfinder for conjunctival specimens were 96.6, 98.5, 96.6, and 98.5%, respectively. The results for Chlamydiazyme were 96.6, 100, 100, and 98.6%, respectively. For nasopharyngeal specimens, the results for Pathfinder were 77.8, 94.6, 73.7, and 95.7%, respectively. The results for Chlamydiazyme were 66.7, 95.7, 75, and 93%, respectively. Pathfinder and Chlamydiazyme appeared to perform equivalently for the detection of *C. trachomatis* in both eye and nasopharyngeal specimens from infants with chlamydial conjunctivitis and pneumonia.

Enzyme immunoassays (EIAs) for the detection of *Chlamydia trachomatis* antigens have been adopted into routine use in many laboratories. The performance of any EIA may be influenced by several factors, including specimen site, gender, number of specimens collected, swab type, transportation and storage conditions, laboratory skills, and indicator reagents in the assays. Most studies have compared culture with EIA for diagnosis of genital infections in adults. Data are more limited for the evaluation of the performance of these assays compared with culture for the diagnosis of ocular and respiratory infection in infants. Chlamydiazyme (Abbott Diagnostics, North Chicago, Ill.) is the only EIA that has been extensively evaluated for the diagnosis of neonatal eye and respiratory infection (2, 3). Chlamydiazyme employs a polyclonal detector antibody and measures the presence of several antigens, including the major outer membrane protein and lipopolysaccharide. Pathfinder (Kallestad Diagnostics, Chaska, Minn.), a recently introduced EIA, differs from Chlamydiazyme in that it employs a murine monoclonal antibody directed against the lipopolysaccharide in addition to a polyclonal antibody. This study compares the ability of these two EIAs to detect *C. trachomatis* antigen in conjunctival and nasopharyngeal specimens from infants with suspected chlamydial conjunctivitis or pneumonia with cultural isolation of the organism from the same sites.

MATERIALS AND METHODS

Patients. 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 neonatal service at Kings County Hospital Center, Brooklyn, N.Y. Clinical criteria for suspected chlamydial pneumonia included the following: (i) no fever, (ii) hyperinflation and variable infiltrates on chest X ray, (iii) the presence of rales on auscultation, and (iv) peripheral eosinophilia (>300 eosinophils per mm^3).

Specimen collection. Specimens for culture of *C. trachomatis* were collected with wire-shafted cotton-tipped swabs (Cottonswab type 1; Spectrum, Houston, Tex.) from the conjunctivae and nasopharynx of infants with conjunctivitis and from the nasopharynx 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 μg of gentamicin per ml, 10 μg of vancomycin per ml, and 1 μg of amphotericin B per ml and refrigerated for up to 24 h or frozen at −80°C if not cultured within that period.

Specimens for Pathfinder and Chlamydiazyme were collected with swabs (Pathfinder Chlamydia EIA Swab Collection System [Kallestad Diagnostics] and STD-PEN [Abbott Laboratories, North Chicago, Ill.]), which were immersed in 100 μl of specimen storage solution, stored at 2 to 8°C, and tested within 5 days of collection.

Culture of *C. trachomatis*. Isolation was performed with cycloheximide-treated McCoy cells grown in 96-well microtiter plates (3). After 48 to 72 h of incubation, the wells were fixed and stained with fluorescein-conjugated monoclonal antibody (Pathfinder).

EIAs. The Pathfinder and Chlamydiazyme assays were performed according to the instructions of the manufacturer; reagents and equipment were supplied by Kallestad Diagnostics. The two assays are very similar. Pathfinder utilizes tubes coated with monoclonal antibody. Chlamydiazyme utilizes treated polystyrene beads. Each assay takes approx-

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TABLE 1. Comparison of Kallestad Pathfinder and Abbott Chlamydiazyme with culture for the detection of *C. trachomatis* in the eyes of 97 infants with conjunctivitis

Chlamydia culture result	No. of EIA results			
	Positive		Negative	
	Pathfinder	Chlamydiazyme	Pathfinder	Chlamydiazyme
Positive	28	28	1	1
Negative	1	0	67	68

imately 5 h to run. The absorbance was determined in a spectrophotometer (miniSTATUS [Kallestad Diagnostics] or Quantum II [Abbott Laboratories]).

Kallestad blocking assay. Clinical samples which were Pathfinder positive but culture negative were further evaluated by a blocking assay (Kallestad Diagnostics). Briefly, 200 μl of the treated discordant EIA specimen was placed into two *Chlamydia* EIA Monoclonal Antibody Tubes. One tube was designated "blocking," and the other was designated "blocking control." A treated positive and negative control were also included. After 1 h of incubation at room temperature, 100 μl of blocking antibody was added to each tube designated "blocking control." After another 1 h of incubation at 20°C, the assay was performed as for the original specimens.

A specimen with a true-positive result should show a decrease in absorbance of 50% or more in the blocking tube compared with the blocking control tube. Those specimens with less than a 50% decrease or that failed to repeat the positive signal in the blocking control tube were considered false-positives.

Specimen confirmation test with fluorescein-conjugated monoclonal antibody. Clinical samples which were EIA positive but culture negative were also reevaluated 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, air dried, and fixed with acetone. The specimen was stained with a fluorescein-conjugated antichlamydia monoclonal antibody (MicroTrak; Syva Co.) and examined for the presence of apple-green-fluorescing elementary bodies (EBs). Positive and negative controls were examined at the same time.

RESULTS

Triplicate eye and nasopharyngeal specimens were obtained from 97 infants with conjunctivitis. Additional nasopharyngeal specimens were also obtained from 14 infants with suspected chlamydial pneumonia (total, 111 nasopharyngeal specimens). Twenty-nine (30%) infants with conjunctivitis had positive conjunctival cultures for *C. trachomatis*. Fourteen (48.3%) of the infants with culture-positive chlamydial conjunctivitis also had positive nasopharyngeal cultures. Four (28.6%) of the 14 infants with suspected chlamydial pneumonia had positive nasopharyngeal cultures. A total of 18 (16.2%) infants had positive nasopharyngeal cultures.

TABLE 2. Comparison of Pathfinder and Chlamydiazyme with culture for the detection of *C. trachomatis* from nasopharyngeal specimens of 111 infants with conjunctivitis and pneumonia

Chlamydia culture result	No. of EIA results			
	Positive		Negative	
	Pathfinder	Chlamydiazyme	Pathfinder	Chlamydiazyme
Positive	14	12	4	6
Negative	5	4	88	89

TABLE 3. Sensitivity, specificity, and predictive values of Pathfinder and Chlamydiazyme compared with culture for the detection of *C. trachomatis* in conjunctival and nasopharyngeal specimens from infants

Assay and specimen source	%			
	Sensitivity	Specificity	Predictive value	
			Positive	Negative
Pathfinder				
Eye	96.6	98.5	96.6	98.5
Nasopharynx	77.8	94.6	73.7	95.7
Chlamydiazyme				
Eye	96.6	100	100	98.6
Nasopharynx	66.7	95.7	75	93

The performance of Pathfinder and Chlamydiazyme compared with culture for conjunctival specimens is shown in Table 1. One conjunctival specimen was Pathfinder positive and culture negative. This specimen did not repeat in the blocking assay and was considered a false-positive. There was one eye specimen that was culture positive but negative by both EIAs.

The performance of Pathfinder and Chlamydiazyme compared with culture for nasopharyngeal specimens is summarized in Table 2. There were five nasopharyngeal specimens that were Pathfinder positive and culture negative. Four of them were resolved as follows. Two samples did not repeat in the blocking assay and thus were probably false-positives. One sample was blockable and thus was probably a true-positive, and one was unrepeatable in the blocking assay, but fluorescent monoclonal antibody (FA) staining of the pellet of the original culture specimen resulted in typical fluorescing EBs. These two discrepant nasopharyngeal specimens were from infants with culture-positive chlamydial conjunctivitis. Four nasopharyngeal specimens were Chlamydiazyme positive and culture negative. Three of these were resolved as follows. Two of these specimens were blockable and also contained EBs on FA staining of the pellet of the original culture specimen; these specimens were also Pathfinder positive as described above. One specimen did not block and did not contain EBs on FA staining. This specimen was from an infant with suspected chlamydial pneumonia. Of the 14 infants with suspected chlamydial pneumonia, three were culture and EIA positive with both assays, one was only culture positive, and one had only a positive Chlamydiazyme test, which was probably a false-positive as described above. The sensitivities, specificities, and predictive values for both assays at each anatomic site are summarized in Table 3.

DISCUSSION

The results of this study demonstrated that Pathfinder was equivalent to Chlamydiazyme for the detection of *C. trachomatis* in conjunctival specimens from infants with suspected

chlamydial conjunctivitis. The prevalence of chlamydial conjunctivitis in our population was relatively high (30%), and 48% of these infants also had positive nasopharyngeal cultures. The performance of both EIAs for diagnosis of conjunctivitis was comparable to that in two previous studies of Chlamydiazyme, which found sensitivities and specificities compared with culture of 93 and 97%, respectively (2), and 98 and 94%, respectively (3).

Both assays did not perform as well for detecting *C. trachomatis* in nasopharyngeal specimens; the sensitivities compared with culture were 77.8 and 66.7% for Pathfinder and Chlamydiazyme, respectively. The sensitivity of Chlamydiazyme in a previous study was 87% (3).

There were more discordant nasopharyngeal samples than eye samples for both assays. At least two EIA-positive, culture-negative samples were probably true-positives on the basis of the results of two confirmatory tests (blocking assay and FA staining). Since both infants had chlamydial conjunctivitis, the chlamydiae may have been nonviable or too scarce to be detected by our culture methods. Alternatively, these discrepant specimens may also have been due to variation in specimen collection. There is probably less variation with collection of conjunctival specimens than with collection of nasopharyngeal samples because of the ease of collection and the large number of organisms present. The order of specimen collection did not appear to affect the probability of a positive test. We have had similar experiences of finding EIA-positive, culture-negative nasopharyngeal specimens from infants with chlamydial conjunctivitis in our previous evaluation of Chlamydiazyme and of two direct FA stains (MicroTrak and Pathfinder [5]). In the previous study, all the discrepant specimens were blockable and contained EBs when evaluated with FA staining.

A major difference between Pathfinder and Chlamydiazyme is that Pathfinder utilizes an antilipopolysaccharide monoclonal antibody in addition to a polyclonal antibody. The addition of the monoclonal antibody did not appear to make a difference in the sensitivity or specificity of Pathfinder compared with Chlamydiazyme for either eye or nasopharyngeal specimens. Possible differences in sensitivity have been observed for another EIA, IDEIA (Cell Tech Diagnostics), which contains only an antilipopolysaccharide monoclonal antibody. Mahony et al. (4) found IDEIA to be more sensitive for detecting *C. trachomatis* in cervical specimens from women (96.3%) than in urethral specimens from men (67.5%), whereas the sensitivities of Chlamydiazyme were roughly the same for both sites (81.8% and 85.2%, respectively). Immunoblotting of the detector reagents from both EIAs indicated that the differences in performance may have been due to measurement of different chlamydial antigens. Results of studies from other investigators have not been consistent. Some have found similar sensitivities for both EIAs (6).

The type of antigen to which the detector antibody is directed may be important for direct FA reagents. A recent in vitro study (1) compared the staining characteristics of six commercially available monoclonal reagents used for direct FAs and found wide variations in degree of brightness, consistency, and specificity of staining when these reagents were used to stain purified EBs from 14 *C. trachomatis* serovars. The reagents directed towards the major outer membrane protein appeared to produce brighter fluorescence, more consistent elementary antibody morphology, and less nonspecific staining than the monoclonal antibody reagents directed towards the lipopolysaccharide. This may be less of a problem for EIAs, since the determination of a positive test is not dependent on the morphological identification of EBs, which has a strong subjective component. However, these data suggest that one may not be able to extrapolate from the performance of one assay to that of another for a given anatomical site.

LITERATURE CITED

1. **Cles, L. D., K. Bruch, and W. E. Stamm.** 1988. Staining characteristics of six commercially available monoclonal immunofluorescence reagents for direct diagnosis of *Chlamydia trachomatis* infections. J. Clin. Microbiol. **26**:1735–1737.
2. **Hammerschlag, M. R., J. E. Herrmann, P. Cox, M. Worku, and L. V. Howard.** 1985. Enzyme immunoassay for the diagnosis of neonatal chlamydial conjunctivitis. J. Pediatr. **105**:741–743.
3. **Hammerschlag, M. R., P. M. Roblin, C. Cummings, T. H. Williams, M. Worku, and L. V. Howard.** 1987. Comparison of enzyme immunoassay and culture for diagnosis of chlamydial conjunctivitis and respiratory infections in infants. J. Clin. Microbiol. **25**:2306–2308.
4. **Mahony, J., S. Castriciano, J. Sellors, I. Stewart, I. Cunningham, S. Landis, W. Seidelman, L. Grant, C. Devlin, and M. Chernesky.** 1989. Diagnosis of *Chlamydia trachomatis* genital infections by cell culture and two enzyme immunoassays detecting different chlamydial antigens. J. Clin. Microbiol. **27**:1934–1938.
5. **Roblin, P. M., M. R. Hammerschlag, C. Cummings, T. H. Williams, and M. Worku.** 1989. Comparison of two rapid microscopic methods and culture for detection of *Chlamydia trachomatis* in ocular and nasopharyngeal specimens from infants. J. Clin. Microbiol. **27**:968–970.
6. **Wissmeier, E., D. A. Bruchner, C. K. Malotte, and L. Manduke.** 1988. Enzyme linked immune absorbent assays in the detection of *Chlamydia trachomatis*: how valid are they? Diagn. Microbiol. Infect. Dis. **9**:219–223.