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Comparison of Two Rapid Microscopic Methods and Culture for Detection of *Chlamydia trachomatis* in Ocular and Nasopharyngeal Specimens from Infants

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The data available for the diagnosis of chlamydial infections in infants which compare direct fluorescent-monoclonal-antibody stains (DFAs) with culture are limited to one reagent, MicroTrak (Syva Inc., Palo Alto, Calif.). We therefore performed a comparison of Pathfinder (Kallestad Diagnostics, Chaska, Minn.) and MicroTrak with chlamydia culture. Paired conjunctival and nasopharyngeal specimens for DFAs and cultures were obtained from 56 infants <1 month of age with conjunctivitis. The sensitivities for detecting *C. trachomatis* in conjunctival specimens with MicroTrak and Pathfinder were 93.8 and 88.2%, respectively, and the specificities were 87.5 and 94.9%, respectively. The DFA tests on nasopharyngeal specimens from infants with conjunctivitis did not perform as well. The sensitivities for Pathfinder and MicroTrak were 33 and 50%, respectively. There were a total of six patients with culture-positive chlamydial conjunctivitis whose nasopharyngeal specimens were DFA positive and culture negative; four of the specimens were positive by both DFAs. These six discordant specimens were further evaluated by preparing pellets and smears of the original culture specimens. All six contained typical fluorescing elementary bodies when stained with the Syva DFA reagent.

Chlamydia trachomatis is the most common identifiable cause of neonatal conjunctivitis and an important cause of pneumonia in young infants. The data available for the diagnosis of chlamydial infections in infants, using direct fluorescent-monoclonal-antibody stains (DFAs), are limited to one reagent, MicroTrak (Syva Inc., Palo Alto, Calif.) (1, 4). We therefore did a comparison of Pathfinder (Kallestad Diagnostics, Chaska, Minn.), another DFA reagent, and MicroTrak with chlamydia culture from infants with suspected chlamydial conjunctivitis or pneumonia.

MATERIALS AND METHODS

Patients. Infants (age <6 weeks) with conjunctivitis and infants (age <6 months) with probable chlamydial pneumonia were enrolled in the study. These infants were seen either as part of a prospective study of neonatal ocular prophylaxis (born to chlamydia-positive mothers) or were presented to 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) presence of rales on auscultation, and (iv) peripheral eosinophilia (>300 cells per mm^3).

Specimen collection. Cultures for *C. trachomatis* were collected with wire-shafted cotton-tipped swabs (Cotton Swab type 1; Spectrum, Houston, Tex.) from the conjunctivae and nasopharynxes of infants with conjunctivitis and from the nasopharynxes 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 were refrigerated for up to 24 h or were frozen at −80°C if not cultured within that period. For the DFAs, specimens were collected on wire-shafted Dacron swabs provided in each DFA kit. Each swab was rolled over the circular enscribed area of the glass slides provided and was appropriately labeled. Pathfinder slides were fixed with methanol, and the Syva slides were fixed with acetone. The control slides provided were similarly processed.

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

DFAs. DFAs were performed according to the instructions of the manufacturer; reagents for both DFA tests were supplied by Kallestad Diagnostics. After slide fixation, approximately 30 μl of either antibody reagent was added to each well, and the slides were incubated for 15 min at room temperature in a moist chamber. The slides were rinsed with distilled deionized water for 10 to 15 s and were air dried. One drop of the provided mounting fluid was added to each well, and then a cover slip was carefully placed over the well. The corners were sealed with clear nail polish. The entire circumscribed area on the slide was visually examined with an epifluorescence microscope at a total magnification of 500× under oil. Slides were scored as positive if ≥5 distinct apple-green fluorescing elementary bodies were observed (both DFAs). Positive slides were confirmed by using a total magnification of 1,000× with an oil immersion objective. Slides with no cellular material present were rejected as representing inadequate specimen collection.

Specimen confirmation test with fluorescein-conjugated monoclonal antibody. Clinical samples which were DFA 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 was centrifuged at 3,000 × *g* for 1 h.

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TABLE 1. Comparison of two DFA tests for *C. trachomatis* with culture in 56 conjunctival specimens from infants with conjunctivitis

Chlamydia culture result	No. of results by DFA[a]			
	Positive		Negative	
	KP	SM	KP	SM
Positive	15	15	2	1
Negative	2	5	37	35

[a] KP, Kallestad Pathfinder; SM, Syva MicroTrak.

The supernatant was discarded, and the pellet was suspended to 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 anti-chlamydia monoclonal antibody (MicroTrak, Syva) and was examined for the presence of apple-green fluorescing elementary bodies. Positive and negative controls were examined at the same time.

RESULTS

Separate ocular and nasopharyngeal specimens were obtained from 56 infants with conjunctivitis. Paired nasopharyngeal specimens were obtained from two infants with possible chlamydial pneumonia. The majority of the specimens were collected by the pediatric house staff. Of the 56 infants with conjunctivitis, 18 (32%) had conjunctival cultures positive for *C. trachomatis*. Of these 18 infants, 2 (11%) also had positive nasopharyngeal cultures. One of the two infants with suspected chlamydial pneumonia had a positive nasopharyngeal culture. One of the infants with conjunctivitis was withdrawn from the analysis because the MicroTrak DFA slide did not have an adequate number of cells.

Of the conjunctival specimens tested, 15 were positive by both Pathfinder DFA and culture, 2 specimens were DFA negative and culture positive, and 2 were DFA positive and culture negative (Table 1). One of these infants was partially treated with erythromycin at the time that the specimens were obtained. The cultures of both discordant specimens contained 1 to 2 inclusions per well. The original culture specimens of the two DFA-positive culture-negative specimens were examined by fluorescent-antibody staining; both specimens had typical fluorescing elementary bodies on smears of the pellets. Of the nasopharyngeal specimens tested, two were both Pathfinder DFA and culture positive, one was DFA negative and culture positive, and four were DFA positive and culture negative (Table 2). All of the DFA-positive culture-negative specimens were from infants who had conjunctival cultures positive for *C. trachomatis*.

TABLE 2. Comparison of two DFA tests for *C. trachomatis* with culture in 56 nasopharyngeal specimens from infants with conjunctivitis

Chlamydia culture result	No. of results by DFA[a]			
	Positive		Negative	
	KP	SM	KP	SM
Positive	2	1	1	2
Negative	4	6	49	47

[a] KP, Kallestad Pathfinder; SM, Syva MicroTrak.

TABLE 3. Summary of performance of two DFA tests for *C. trachomatis* with culture from infants with conjunctivitis

Culture specimen and DFA test[a]	% Sensitivity	% Specficity	Predictive value (%)	
			Positive	Negative
Conjunctival				
KP	88.2	94.9	88.2	94.9
SM	93.8	87.5	75	97
Nasopharyngeal				
KP	50	92.5	33	98
SM	33	88.7	14.3	96

[a] KP, Kallestad Pathfinder; SM, Syva MicroTrak.

All four discordant nasopharyngeal specimens were evaluated with fluorescent-antibody staining; smears of the pellets of all four contained typical fluorescing elementary bodies.

Conjunctival specimens stained with MicroTrak DFA were as follows: 15 were both DFA and culture positive, 1 was DFA negative and culture positive, and 5 were DFA positive and culture negative (Table 1). One of the DFA-positive culture-negative specimens was from the same infant who was partially treated with erythromycin. The four discordant eye specimens were examined by DFA after concentration of the original specimen; three were negative. The remaining specimen had typical fluorescing elementary bodies. Of the nasopharyngeal specimens tested, one was both DFA and culture positive, two specimens were DFA negative and culture positive, and six were DFA positive and culture negative (Table 2). The six DFA-positive culture-negative nasopharyngeal specimens were evaluated with fluorescent-antibody staining; smears of the pellets of all six contained typical fluorescing elementary bodies. As previously mentioned, there were two infants with pneumonia. One was culture and DFA positive by both Pathfinder and MicroTrak. There were a total of 20 DFA-positive conjunctival specimens; 17 were positive by both methods and 3 were positive by MicroTrak alone. Two of these were from one patient on two separate occasions. The numbers for the nasopharyngeal specimens were considerably smaller, giving a total of seven DFA-positive specimens; five were positive by both methods, and two were positive only by MicroTrak. The performances of MicroTrak and Pathfinder DFAs compared with that of tissue culture are summarized in Table 3.

DISCUSSION

Pathfinder DFA appears to be equivalent to MicroTrak DFA in both sensitivity and specificity for the detection of *C. trachomatis* in conjunctival specimens. Although the number of infants tested with relatively small, the prevalence of chlamydial conjunctivitis was high (35.7%) in this population. The prevalence of positive nasopharyngeal specimens was too small to accurately assess the performance of either DFA. However, of special interest was the presence of what appeared to be a large number of false-positive nasopharyngeal specimens from infants with conjunctivitis. It was observed, however, that these DFA-positive culture-negative specimens occurred only in infants who had culture-positive chlamydial conjunctivitis. That these specimens were probably true positives was confirmed by the presence of typical fluorescing elementary bodies in pellets of the original culture specimens when examined with MicroTrak DFA. Therefore, the specificity and predictive value of a

positive nasopharyngeal DFA would be higher than originally calculated. The chlamydiae may have been nonviable or too scarce to be detected by our culture methods. We had a very similar experience when evaluating an enzyme immunoassay, Chlamydiazyme (Abbott Laboratories, North Chicago, Ill.), to culture for the diagnosis of chlamydial respiratory and ocular infections in infants (3). We found a sizable number of enzyme immunoassay-positive culture-negative nasopharyngeal specimens, which also occurred in infants with culture-positive chlamydial conjunctivitis, but there were no discordant specimens among 16 infants with chlamydial pneumonia. Infants with chlamydial pneumonia generally have much higher titers of the organism present in the nasopharynx than infants with conjunctivitis. Variation in specimen collection could be another possible cause of discordant results, especially when the titers of organism are relatively low. Two previously reported studies have evaluated the MicroTrak DFA for the diagnosis of neonatal chlamydial conjunctivitis. In these reports, the sensitivity and specificity for conjunctival specimens were 100 and 94 to 100%, respectively (1, 4). Our lower sensitivities may have been caused by variation in specimen collection; in one study, the conjunctival specimens were collected by an ophthalmologist, whereas many of ours were collected by pediatric residents. Bell et al. (1) also found the sensitivity and specificity of MicroTrak for nasopharyngeal specimens from infants with conjunctivitis to be 86 and 75%, respectively, compared with those of chlamydia culture. These specimens were also collected with swabs. We found many of the nasopharyngeal specimens very difficult to read. There were numerous artifacts, especially when there was mucus in the sample.

Generally, the concordance between the two DFAs for conjunctival specimens was good. There was one infant with conjunctivitis who on two occasions was only positive with MicroTrak. This was not due to a low titer of organisms, as there were >10 elementary bodies present in the well each time. A recent study by Cles et al. (2) comparing the staining characteristics of six commercially available monoclonal antibody reagents used for DFAs found wide variations in the degree of brightness, consistency, and specificity of staining when the reagents were used to stain purified elementary bodies from 14 *C. trachomatis* serovars. The reagents directed toward the major outer membrane protein appeared to produce brighter fluorescence, more consistent elementary body morphology, and less nonspecific staining than the monoclonal antibody reagents directed towards the lipopolysaccharide. However, even among the three anti-major outer membrane protein reagents, there was variation in staining of some of the serovars. Thus it is possible that some monoclonal DFA reagents may not detect all strains of *C. trachomatis* in clinical specimens. Thus, one may not be able to extrapolate from the performance of one reagent to another. Pathfinder and MicroTrak both use a monoclonal antibody directed towards the major outer membrane protein. An earlier comparison of Pathfinder and MicroTrak with culture for urethral and cervical specimens from sexually active men and women found no significanct difference overall in the sensitivities and specificities of both test kits (5). There were four separate centers involved in this study, and there was some interlaboratory variation in the sensitivity of the microscopy but little difference in the specificity.

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