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Comparison of Enzyme Immunoassay and Culture for Diagnosis of Chlamydial Conjunctivitis and Respiratory Infections in Infants

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The efficacy of Chlamydiazyme (Abbott Laboratories, North Chicago, Ill.) in detecting neonatal conjunctival and respiratory infections caused by *Chlamydia trachomatis* was determined by comparison of this enzyme immunoassay (EIA) with the method of isolation of chlamydiae in tissue culture. The sensitivity and specificity of Chlamydiazyme for detecting *C. trachomatis* in conjunctival specimens from infants with conjunctivitis were 98 and 94%, respectively. For nasopharyngeal infection in infants with conjunctivitis, the sensitivity and specificity were 87 and 92%, respectively. There were nine nasopharyngeal specimens that were Chlamydiazyme positive and culture negative. All of these specimens demonstrated the presence of typical fluorescing chlamydial elementary bodies when pellets of the original specimens were examined with a fluorescein-conjugated monoclonal antibody. When the EIA was performed on nasopharyngeal specimens from infants with suspected chlamydial pneumonia, 6 culture-positive and 10 culture-negative specimens were correctly identified.

Chlamydia trachomatis is the most common identifiable cause of neonatal conjunctivitis and an important cause of pneumonia in young infants. Many infants with chlamydial conjunctivitis also have chlamydial infection present in their nasopharynx. In 1985, we compared Chlamydiazyme (Abbott Laboratories, North Chicago, Ill.), an enzyme immunoassay (EIA), with the culture method for the diagnosis of chlamydial conjunctivitis. The EIA had a sensitivity of 93% and a specificity of 97% (3). Chlamydiazyme was later modified to provide maximum sensitivity and specificity, and subsequently, we reevaluated the test for both ocular and nasopharyngeal infection in infants.

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 presented to the emergency room or neonatal service at Kings County Hospital Medical 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, (iv) peripheral eosinophilia (>300/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 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, 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. For the EIA, specimens were collected with swabs (STD-PEN; Abbott Laboratories) 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 cyclohexmide-treated McCoy cells grown in 96-well microtiter plates. After 48 to 72 h of incubation, the wells were fixed and stained with fluorescein-conjugated monoclonal antibody (Microtrak; Syva Co., Palo Alto, Calif.) (6). We did not perform blind passages, as in our previous study (3), because we found that results of single passage with fluorescent antibody staining were equivalent to those of blind passage with iodine staining.

EIA. Chlamydiazyme was performed according to the instructions of the manufacturer; reagents and equipment were supplied by Abbott Laboratories. Specimen dilution buffer (1 ml) was added to each tube that contained a specimen swab. After 10 min specimens were vortexed for three cycles of 15 s each. Samples (0.2 ml) of the diluted specimens and positive and negative controls, which were supplied with the kit, were placed in wells in plastic plates and reacted in turn with a treated polystyrene bead (1 h), rabbit antibody to *C. trachomatis* (1 h), and horseradish peroxidase-conjugated antibody to rabbit immunoglobulin G (1 h) according to the instructions of the manufacturer. The substrate *o*-phenylenediamine was added, and after 30 min of incubation the reaction was stopped by the addition of 1 N sulfuric acid.

The A_{492} was determined in a spectrophotometer (Quantum II; Abbott Laboratories). A result was considered positive if the optical density exceeded the mean of the three negative controls plus 0.10.

Specimen confirmation test with fluorescein-conjugated monoclonal antibody. Clinical samples which were Chlamydiazyme 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 to

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TABLE 1. Comparison of *C. trachomatis* detection by culture and EIA in conjunctival specimens from infants with conjunctivitis

EIA result	No. of culture results[a]:		
	Positive	Negative	Total
Positive	47	5	52
Negative	1	85	86
Total	48	90	

[a] Sensitivity was 98%, specificity was 94%, and the predictive values for the positive and negative tests were 90.4 and 99%, respectively.

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 Co.) and examined for the presence of apple green fluorescing elementary bodies. Positive and negative controls were examined at the same time.

RESULTS

From February through November 1986, 138 paired (culture and EIA) conjunctival and 131 paired nasopharyngeal specimens were obtained from 124 infants with conjunctivitis. Paired nasopharyngeal specimens were also obtained from 16 infants with possible chlamydial pneumonia. The majority of the specimens were collected by the pediatric house staff of the hospital. Of the 124 infants with conjunctivitis, 48 (39%) had positive conjunctival cultures for *C. trachomatis*. Of these infants, 23 (48%) also had positive nasopharyngeal cultures, and 6 (38%) of the infants with suspected chlamydial pneumonia had positive nasopharyngeal cultures.

Of the conjunctival specimens tested, 47 (34%) were both EIA and culture positive; 1 specimen was EIA negative and culture positive and 5 were EIA positive and culture negative (Table 1). One of these infants was partially treated with erythromycin at the time that the specimens were obtained. All five discordant eye specimens were examined by fluorescent antibody staining; three specimens were negative. The remaining two specimens had typical fluorescing elementary bodies on smears of the pellets.

Twenty of the nasopharyngeal specimens from infants with conjunctivitis were both EIA and culture positive; three were EIA negative and culture positive and nine were EIA positive and culture negative (Table 2). All of the EIA-positive, culture-negative specimens were from infants who had positive conjunctival cultures for *C. trachomatis*. All nine discordant nasopharyngeal specimens were evaluated with by fluorescent antibody staining. Smears of the pellets of all nine contained typical fluorescing elementary bodies.

A total of 6 (38%) of the infants with suspected chlamydial pneumonia were both EIA and culture positive; there were no discordant specimens. Of the 6 positive nasopharyngeal specimens from infants with pneumonia, 5 had >100 inclusions per well, as compared with only 2 of 12 positive nasopharyngeal specimens from infants with conjunctivitis.

Results of the comparison of the EIA with the cell culture method are summarized in Tables 1 and 2. The EIA demonstrated a sensitivity of 98% and a specificity of 94% for conjunctival specimens. The predictive value of a positive and negative test were 90.4 and 99%, respectively. For nasopharyngeal specimens from infants with conjunctivitis, the sensitivity was 87% and the specificity was 92%. The predictive value of a positive test was 70%, while the predictive value of a negative test was 97%. For infants with pneumonia, the sensitivity and specificity of EIA for nasopharyngeal specimens were 100%.

DISCUSSION

Overall, Chlamydiazyme appears to be both a sensitive and a specific test for the detection of *C. trachomatis* in conjunctival and nasopharyngeal specimens from infants with chlamydial conjunctivitis and pneumonia. The results of this study are comparable to those we obtained in our previous study (3) of the use of Chlamydiazyme for the diagnosis of neonatal chlamydial conjunctivitis. The present study was conducted with the kit that is now commercially available. These results are also comparable to those seen with urethral and endocervical specimens from adults (2).

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 EIA-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 a fluorescein-conjugated anti-chlamydial monoclonal antibody. Therefore, the specificity and predictive value of a positive nasopharyngeal EIA would be higher than originally calculated. The chlamydiae may have been nonviable or too scarce to be detected by our culture methods.

Although our test sample size was small, no EIA-positive, culture-negative specimens occurred in infants with chlamydial pneumonia. These infants tended to have higher titers of *C. trachomatis* present in their nasopharynx. Prior antibiotic therapy may also result in false-positive results, since the EIA can detect nonviable organisms (3). This may have been responsible for one of the EIA-positive, culture-negative conjunctival specimens. Another cause of discordant results may be variation in specimen collection. The majority of the specimens were obtained by the house staff of the hospital without specific instructions.

The direct immunofluorescence assay (DFA; Microtrak) has also recently been evaluated for the diagnosis of neonatal chlamydial infections. Bell et al. (1) found the sensitivity and specificity of the DFA compared with those of culture for the diagnosis of chlamydial conjunctivitis to be 100%. Recently, Rapoza et al. (5) reported a sensitivity of 100% and a specificity of 94% in their evaluation of 100 infants with conjunctivitis. Bell et al. (1) also found the sensitivity and specificity for nasopharyngeal specimens from infants with conjunctivitis to be 86 and 75%, respectively, compared with the values for cultures. The specimens were collected with swabs. In another recent study of 125 infants with suspected

TABLE 2. Comparison of *C. trachomatis* detection by culture and EIA in nasopharyngeal specimens from infants with conjunctivitis

EIA result	No. of culture results[a]:		
	Positive	Negative	Total
Positive	20	9	29
Negative	3	99	102
Total	23	108	

[a] Sensitivity was 87%, specificity was 92%, and the predictive values for the positive and negative tests were 70 and 97%, respectively.

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chlamydial pneumonia (4), the DFA had a sensitivity of 93% and a specificity of 98% compared with the values for culture. Unlike the prior study by Bell et al. (1), the nasopharyngeal secretions were collected by aspiration. They hypothesized that the combination of better specimen collection methods and the higher titers of chlamydia seen in infants with pneumonia resulted in higher sensitivity and specificity. However, the use of aspirates requires an additional step in specimen preparation in comparison with the use of swabs. We found that specimens obtained from the nasopharynx with STP-PEN swabs were adequate for the EIA.

The EIA and DFA both have advantages and disadvantages. Specimens for the EIA can be refrigerated for up to 5 days. The test is semiautomated and suitable for the processing of large numbers of specimens, and the results are objective. On the negative side, the EIA requires special equipment, and the adequacy of the specimen cannot be evaluated. The EIA takes about 5 h to run; thus, it cannot be considered a rapid test for the evaluation of samples immediately after receipt in the laboratory. Specimens for the DFA can be processed and read within 20 min, but it requires a fluorescence microscope and a very well trained microscopist. There is a large subjective component in designating some samples as positive or negative, and the cutoff is ambiguous, i.e., 1 to 10 elementary bodies.

Since the overall performance of the EIA and DFA for the diagnosis of neonatal chlamydial conjuctivitis and pneumonia are similar, the use of one method or the other depends on the requirements of the institution.

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