Comparison of DNA Probe, Monoclonal Antibody Enzyme Immunoassay, and Cell Culture for the Detection of Chlamydia trachomatis

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A total of 201 endocervical specimens were obtained from patients with a clinical or epidemiological history suggestive of chlamydial infection. These specimens were tested by DNA probe (Gen-Probe, San Diego, Calif.) and the IDEIA III (Boots-Celltech, Berkshire, United Kingdom) monoclonal antibody enzyme immunoassay and compared with cell culture for detection of Chlamydia trachomatis. Discrepancies between cell culture and antigen detection methods were resolved by direct fluorescent-antibody testing. In a population with a 17.4% prevalence, the sensitivities and specificities of these assays were 82.8 and 99.4%, respectively, for the DNA probe assay and 97.1 and 98.1%, respectively, for the IDEIA III.

For DNA probe specimens, specificities were 100%; for IDEIA III, the values were calculated, and the sensitivities were more than 97% for both assays. All specimens collected in 2.0 ml of 2SP transport medium. These specimens were transported to the laboratory, where they were processed or stored at 2 to 4°C for no longer than 24 h. The transports were vortexed, and 200 μl of specimen was added to each of two shell vials containing McCoy cell monolayers on cover slips. The vials were centrifuged at 3,000 × g for 1 h at 35°C. The supernatant was aspirated, and 0.0 ml of chlamydial culture medium with cycloheximide was added. Vials were incubated at 35°C for 48 h, after which a cover slip from one of the vials was fixed in absolute methanol and stained with fluorescent-labeled monoclonal antibodies directed against genus-specific chlamydial antigens (Kallestead, Austin, Tex.). The cover slips were examined at 100× magnification, and typical inclusions were confirmed at 400×. A culture was considered positive if one or more inclusions were present. If the first vial was negative, the second vial was used as inoculum for blind passage of the specimen. All specimens were stored at −70°C for further reference.

EIA. The IDEIA III uses a genus-specific monoclonal antibody and an alkaline phosphatase substrate with an alcohol oxidoreductase-diaphorase amplification system for the detection of C. trachomatis from genital sources (6). All specimens used in this study were collected in 2SP transport medium. Upon receipt, transports were vortexed, and 200 μl of specimen was withdrawn and placed into a vial containing 1.0 ml of Boots-Celltech chlamydial transport medium. Fifty microliters of concentrated transport medium was then added, and specimens were stored as above. The EIA procedure was performed as follows. Specimens were placed in a boiling-water bath for 15 min. After cooling, 200 μl of specimen was incubated in antibody-coated wells at room temperature for 2 h. (A positive and three negative controls were included with each batch of specimens.) Fifty microliters of enzyme-conjugated monoclonal antibody was then added and incubated for 1 h. After the wells were washed, 100 μl of substrate was added and incubated for 40 min. To complete the procedure, 100 μl of amplifier was added for 10 min, and then 50 μl of stopping solution was added. The AS92 of each well was read with a Bio-Tek EL 307 spectrophotometer (Bio-Tek Instruments, Inc., Winooski, Vt.). The cutoff value was calculated by adding 0.05 to the mean value for the negative control. For a

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Methods for the diagnosis of genital Chlamydia trachomatis infection are no longer limited exclusively to cell culture. Direct detection of antigens may now be accomplished by a variety of methods, including enzyme immunoassay (EIA), direct fluorescent antibody (DFA), immunocytochemistry, and DNA probes (2, 5, 7, 11; V. Jonas, J. J. Hogan, K. M. Young, and R. N. Bryan, Abstr. Annu. Meet. Am. Soc. Microbiol. 1988, C-232, p. 370). Although the sensitivity of various cell culture methods is generally accepted to be less than 100%, they remain the standard by which immunologically based direct antigen tests and other nonculture methods are based (8, 9). Attempts at resolution of discrepancies between antigen-positive and -negative results have included repeat cultures and antigen tests, chart review for clinical or epidemiological information, and retesting by another growth-independent system for chlamydial testing, usually direct fluorescent microscopy (4, 7).

The purpose of this study was to compare two growth-independent non-culture-based tests, the IDEIA III (EIA) amplified monoclonal immunoassay (Boots-Celltech, Berkshire, United Kingdom) and an isotopic DNA probe (Gen-Probe, San Diego, Calif.) with cell culture for the detection of genital C. trachomatis infection.

MATERIALS AND METHODS

Patient population. Patients evaluated in this study consisted of females seen in the Emergency Department or Gynecology Clinic at Providence Hospital who presented with a clinical or epidemiological history suggestive of C. trachomatis infection. There were 201 patients enrolled in this study.

Specimens. The exocervix was cleansed to remove excess mucus and exudate with a cotton swab. Routine bacterial cultures were taken at this time if requested. Duplicate endocervical cultures were then collected with Dacron swabs. One swab was placed in the Gen-Probe (GP) transport system for use in the DNA probe assay, and the other was placed in 2-sucrose phosphate (2SP) chlamydial transport medium for use in the EIA and cell culture procedures. The swabs were placed into the transport systems at random.

**Note:** The asterisk (*) in the author's name indicates the corresponding author.
specimen to be considered positive, it had to demonstrate an absorbance value greater than the cutoff value plus 0.015 absorbance units.

DNA probe. The Gen-Probe *Chlamydia trachomatis* Rapid Diagnostic System (GP) was used in this study. This system uses a 125I-labeled single-stranded DNA probe complementary to the rRNA of the target organism (3). Endocervical specimens were placed in the GP transport system and delivered to the laboratory, where they were stored at 2 to 4°C for no longer than 72 h prior to testing. Specimens were vortexed, and swabs were then rotated against the side of the tube to express as much fluid as possible. Fifty microliters of specimen was added to a reaction tube, followed by 100 μl of probe solution. This mixture was incubated for 2 h in a 60°C water bath. One positive and three negative controls were included with each batch of specimens processed. Two milliliters of separation suspension was added to each tube and, after mixing, incubated for 5 min at 60°C. The tubes were then placed on a magnetic separation rack for 2 min and decanted. The tubes were washed three times with 1 ml of wash solution, vortexed, decanted, and blotted. Each tube was counted for 1 min in a single-well gamma counter (Kemble Instruments, Hamden, Conn.), and the counts per minute were recorded. The results of the assay were calculated based on the percentage of net counts added to each reaction tube. A specimen was considered positive if the net sample count was greater than the calculated cutoff (positive cutoff = [total cpm − background cpm] × 0.0007).

Resolution of discrepant results. Specimens which produced a positive result by GP or IDEIA and were negative by culture had an additional nonculture procedure performed to resolve the discrepant results. The original 2SP culture transports were centrifuged at 3,000 × g for 10 min. The supernatant was removed, the pellet was suspended, and two slides were prepared for DFA staining with a species-specific *Chlamydia trachomatis* fluorescein-labeled monoclonal antibody (Difco Laboratories, Detroit, Mich.). Specimens were considered true positives if at least four elementary bodies were seen per smear in conjunction with the positive GP or IDEIA result.

RESULTS

Two hundred and one specimens were examined by cell culture, IDEIA, and GP for the presence of *C. trachomatis*. Thirty-two of the 201 specimens tested were positive initially or upon blind passage by the culture methods used. Results of culture and GP are compared in Table 1. Twenty-eight of 32 specimens positive by culture were also positive by the GP assay, resulting in a sensitivity and specificity of 87.5 and 98.8%, respectively. Two of the four culture-positive, GP-negative specimens were detected by passage, and one negative GP was near the positive calculated cut-off value.

Table 2 compares IDEIA and culture results. Thirty-one of 32 culture-positive specimens were detected by the IDEIA procedure, for a sensitivity of 96.8% and specificity of 96.4%. The culture-positive, IDEIA-negative specimen contained greater than 50 inclusions in the culture and was also negative in the GP assay.

Six samples produced a positive result by GP or IDEIA and negative culture results. Three of these six samples were considered positive after demonstration of elementary bodies by DFA staining. The GP assay was positive for one of three of these samples, for a sensitivity of 83% and specificity of 99.4% (Table 1). Three of three samples were detected by the IDEIA, resulting in an adjusted sensitivity and specificity of 97.1 and 98.1%, respectively (Table 2). With the recalculated data, positive and negative predictive values were 96.6 and 96.4%, respectively, for the GP assay and 91.8 and 99.3%, respectively, for the IDEIA procedure.

DISCUSSION

This study compared cell culture with DNA probe and IDEIA for the detection of *C. trachomatis* in women with clinical or epidemiological evidence of infection. Previous studies have demonstrated the IDEIA to have a sensitivity ranging from 68 to 94% and specificity of 94 to 99% (1, 12). These studies were carried out in populations in which the prevalence of infection was 8 to 17.5%. Tjiam et al. have reported the sensitivity of the assay to be greater in individuals with inflammatory symptoms, such as urethritis and cervicitis, than in asymptomatic individuals (12). The prevalence of infection in our study was 17.4%, and the IDEIA demonstrated a high sensitivity in this group of patients with suspected infection.

Jonas et al. evaluated the isotopic DNA probe assay and found the test to have a sensitivity of 86.4% and specificity of 96.6%; however, no mention was made as to whether these were symptomatic or asymptomatic individuals (Jonas et al., Abstr. Annu. Meet. Am. Soc. Microbiol. 1988). A chemiluminescent probe has also been evaluated and demonstrated sensitivity and specificity comparable to those of the isotopic test (S. C. Putreba, F. A. Meier, B. A. Johnson, R. R. Brookman, and H. P. Dalton, Abstr. Annu. Meet. Am. Soc. Microbiol. 1988, C-234, p. 371). These results do not differ significantly from those obtained in this study.

The sensitivity of cell culture is accepted to be less than 100%, and when comparing a nonculture test with high sensitivity with culture tests having sensitivities of 80 to
90%, the data must be interpreted with caution (1, 8, 9). In this situation, positive nonculture results for negative cultures will invariably be classified as false positives rather than the culture result being considered a false negative (1, 10). We attempted to resolve the issue by use of another test performed on the same specimen as a reference. If one accepts the number of true positives to be the sum of specimens positive by culture and positive by a nonculture method with an alternate confirmatory procedure on discrepant samples, then the GP assay with a sensitivity of 83% and the IDEIA with a sensitivity of 97% perform as well as culture, with its expected sensitivity of 80 to 90% in this patient population.

In comparing DNA probe and IDEIA with cell culture, no one of the three tests was able to detect all specimens positive for C. trachomatis. The IDEIA proved to be the more sensitive of these two nonculture methods. However, with a positive predictive value of 92%, a small percentage of patients would be treated unnecessarily. The GP assay, although less sensitive than the IDEIA procedure, demonstrated essentially equivalent specificity in this patient population.

The issue of test sensitivity is an important consideration in a patient group with a moderately high prevalence of disease. The low sensitivity, i.e., false-negative tests, would have to be evaluated closely in the population presenting with evidence of infection. Nonculture methods for diagnosis of chlamydial infection offer the advantage of speed, labor savings, and objective result interpretation. In evaluating these methods, emphasis must be placed not only on the considerations, but also, most importantly, on patient care. The number of both false-positive and false-negative tests which may result in needless therapy or sequelae from resulting disease must be considered before decisions about the appropriateness of test protocols are made.

This study has demonstrated that both the GP assay and IDEIA produce results comparable to those of single-passage tissue culture in a patient population with a relatively high (17.4%) prevalence of disease. The greater than 91% positive predictive values and greater than 99% negative predictive values for both of these assays fulfill the requirements for a suitable diagnostic test.

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