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Test-of-Cure Analysis by Direct Immunofluorescence for *Chlamydia trachomatis* after Antimicrobial Therapy

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The direct immunofluorescence assay (DFA) was compared with culture for test-of-cure analysis for *Chlamydia trachomatis* in patients 7 to 10 days after antimicrobial therapy was given. DFA test-of-cure results correlated with culture results in 79.5% of 39 patients. Of DFA-negative patients, 97% had negative cultures. Six of seven patients with borderline DFA results had negative culture results.

Since the introduction of the direct immunofluorescence assay (DFA) for *Chlamydia trachomatis* in genital tract specimens (8), numerous studies have shown that direct testing is highly sensitive and specific (3, 5, 7, 8) compared with culture isolation methods. Culture methods may be indicated to verify patient compliance with antimicrobial therapy and to ensure effective therapy for infection. Although the DFA has proven useful for the detection of infection, there are no data on the use of this test as a test of cure (TOC). Therefore, the purpose of this study was to compare the MicroTrak DFA (Syva Co., Palo Alto, Calif.) with isolation by cell culture as a TOC in female patients seen in our Family Planning Clinic 7 to 10 days after the cessation of appropriate antimicrobial therapy.

The study was done in the Family Planning Clinic at the Hospital of the University of Pennsylvania. The prevalence of chlamydial infection in the population in this study was approximately 14% based on culture results from our hospital laboratory. The prevalence of gonococcal infection in this population has been studied previously and was 6.9% (4). Patients seen in the clinic for their routine gynecologic examination or for diagnosis of a suspected sexually transmitted disease were tested. They were given a pelvic examination by either a nurse practitioner or a physician, and two swabs were collected for testing at that time. Although the specimens were not routinely collected in any particular order, specimen 1 was generally collected for DFA, and specimen 2 was collected for culture. We used the MicroTrak Direct Test for *Chlamydia trachomatis* as the DFA. Slides were prepared according to the instructions of the manufacturer at the time of collection, and fixed slides were sent to the Clinical Microbiology Laboratory at our hospital for examination. Results were recorded as negative, borderline (1 to 9 elementary bodies [EB] per well), or positive (light [10 to 50 EB per well], moderate [50 to 100 EB per well], or heavy [>100 EB per well]). Swabs for chlamydial culture were placed into chlamydia transport media (Bartels Immunodiagnostic Supply Co., Bellevue, Wash.), the patient sample was expressed from the swab, and the swab was removed. The tube with the sample was transported to the laboratory on ice, and the specimens were frozen at −70°C after they were received. Specimens from patients seen for the first visit were processed only if the DFA result was borderline or positive. Specimens were cultured for all patients seen for TOC analysis, regardless of the DFA result. Cultures were grown by inoculating the specimen on cycloheximide-treated McCoy cells (Bartels Immunodiagnostic Supply Co.) in duplicate by using standard methods (6). Inclusions were detected by immunofluorescence staining of cover slips with a monoclonal antibody (Syva Co.) after 48 to 72 h. If the culture was negative, a second passage was done and it was stained after 48 to 72 h of incubation.

For a patient to be eligible for this study, both the DFA and culture results from the initial visit had to be positive. After the results were available, a patient with a positive DFA and culture was recalled to the clinic to receive the appropriate antimicrobial therapy, and an appointment was scheduled for TOC testing. Except for one patient treated with erythromycin, 500 mg four times a day for 10 days, all of the patients received doxycycline, 100 mg two times a day for 7 days (1). Several patients had also received ampicillin and probenecid for concomitant gonococcal infections. TOC testing was done 7 to 10 days after the end of antimicrobial therapy.

During the study period, 866 patients were screened for chlamydial infection. Of these patients, 13.7% (119 patients) had chlamydial infections as defined by a positive DFA and culture test; 63% (75 patients) returned for follow-up visits, with 32.7% (39 patients) returning within the 7- to 10-day period after cessation of antimicrobial therapy for TOC analysis. Table 1 shows the TOC results of 39 patients

TABLE 1. TOC results by DFA in patients with chlamydial infections

TOC results (DFA/culture)	No. of patients (%)
Negative/negative	30 (76.9)
Borderline/negative	6 (16.6)
Positive/positive	1 (2.6)
Borderline/positive	1 (2.6)
Negative/positive	1 (2.6)

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analyzed in this study. The mean age of patients analyzed was 18.6 years (range, 15 to 23 years). Four patients had concomitant gonococcal infections, four had cervicitis or cystitis or both, three had trichomonas infections, and one had a herpes infection. The remaining patients were asymptomatic.

Of 39 patients, 92.3% (36 patients) were cured with antimicrobial therapy based on negative TOC culture tests. Of 36 patients, 83.3% (30 patients) had negative DFA results and 6 had boderline DFA results. Of the six patients with borderline results, one patient had a repeat DFA 60 days after the TOC tests, and it was negative. In the group with positive cultures, one patient (who tested positive in both the DFA and culture) was subsequently found to have had sexual intercourse with an infected partner during the interim between cessation of antimicrobial therapy and examination in the clinic and was excluded from further analysis. Both of the other patients with positive cultures denied having had coitus during the interval between treatment and TOC analysis.

In this study, 83.3% (30 of 36) of patients with negative culture TOC results had negative DFA results. However, six patients had borderline results. Only 1 patient of 39 analyzed (2.6%) had a negative DFA and a positive culture. Thus, the predictive value of a negative test was high in this population. The reasons for borderline DFA results and negative culture results are not known. It is reasonable to speculate that dead organisms are present after antimicrobial therapy and are detected by immunofluorescence stains. Alternatively, we used frozen specimens for culture, and it is possible that there was a loss of viability after thawing. We did not have sufficient follow up after the TOC analysis to address these possibilities.

Because the number of patients analyzed in this study was small, it is difficult to perform statistical analysis on the results. If we assume 90% sensitivity and 95% specificity on the basis of previously published studies (2, 3, 5, 7, 8), the predictive value of a negative test in this study is 96 to 97%. Of the seven patients with borderline DFA results, only one had a positive culture. Our study was based on using a borderline result as a category in reporting positive results (1 to 9 EB per well). However, some laboratories may call this category negative and depend on the experience of laboratory personnel in determining results. By categorizing borderline results as negative, there is much better correlation of the DFA with culture results.

According to Centers for Disease Control guidelines (2), follow-up tests after appropriate therapy are generally not needed when the therapy is followed as directed. However, when compliance is in question and TOC analysis is suggested, DFA may be an initial alternative to culture for rapid results. Although further studies are needed with larger numbers of patients at different risks of infection, our study suggests that the DFA may be useful in the initial testing of patients after antimicrobial therapy, particularly if cultures are not available in the laboratory. If a borderline or positive DFA result is obtained, we recommend retesting the patient by DFA or by culture, or retreating the patient.

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