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Rapid Diagnosis of Chlamydial Infections with the MicroTrak Direct Test

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A direct test on clinical specimens, using fluorescein-labeled monoclonal antibodies, for *Chlamydia trachomatis* (MicroTrak [Syva Co.]) was evaluated for the rapid diagnosis of chlamydial infections. Asymptomatic females attending pregnancy or planned parenthood clinics were tested by the direct test and by a cell culture method. Of 401 paired, endocervical specimens, 398 (99.3%) gave identical direct and culture results. The overall sensitivity of the direct test was 96.3% (26 of 27), and the specificity was 99.5% (372 of 374) as compared with that of culture. More than four-fifths of the direct smears were read within ca. 2 min. In this study population, the performance of the direct specimen test was comparable to that of cell culture methods. Rapid turnaround time and elimination of the need for cell culture make the direct test a practical method for the specific diagnosis of chlamydial infections.

Chlamydial infections of the cervix, urethra, and rectum are the most prevalent sexually transmitted diseases in the United States today, frequently leading to serious complications, including pelvic inflammatory disease, epididymitis, and proctitis. In men, *Chlamydia trachomatis* accounts for ca. 40% of nongonococcal urethritis and 60% of postgonococcal urethritis (9). The majority of chlamydial infections in women are asymptomatic, with sexually transmitted disease clinics reporting asymptomatic carrier rates of over 20% (4, 8) and screening studies in family planning and prenatal clinics revealing rates of 5 to 10% (5, 10). Further, infants can acquire the infection during passage through an infected birth canal, with ca. 50% developing inclusion conjunctivitis and 20% developing pneumonia (6).

Although classified as a bacterium because of its cell wall chemistry, *C. trachomatis* is an obligate intracellular parasite and requires a cell culture (CC) host system for its isolation. Currently, isolation in a cycloheximide-treated McCoy cell monolayer is the most common diagnostic procedure (2). This technique is based on the detection of characteristic inclusion bodies, which are visualized after 2 to 3 days with immunofluorescent, iodine, or Giemsa staining procedures. If passage is required, the procedure can take up to 6 days. Since chlamydial culture procedures are not widely available, diagnosis and treatment of chlamydial infections are frequently based on the clinical syndrome alone (3). However, presumptive therapy for symptomatic patients does not control the expanding reservoir of asymptomatic infections in women, who risk the most serious complications of chlamydial infection and pass the infection to their newborns.

A rapid, less expensive diagnostic technique has been a major goal in efforts to control chlamydial infections. The MicroTrak *Chlamydia trachomatis* Direct Specimen Test (jointly developed by Syva Co. and Genetic Systems Corp.) is a rapid alternative method for the detection of *C. trachomatis* and is commercially available (5, 11, 12; T. C. Quinn, P. Warfield, E. W. Kappus, M. B. Barbacci, and M. R. Spence, Int. Conjoint STD Mtg., Montreal, Canada, abstr. no. 105, 1984; W. E. Stamm, H. R. Harrison, E. R. Alexander, L. D. Cles, B. Cole, M. R. Spence, and T. C. Quinn, Ann. Intern. Med., in press). The test uses fluorescein-labeled monoclonal antibodies to detect individual elementary and reticulate bodies in direct smears (DSs). We report on a comparison of the direct procedure with the traditional culture method in patients attending planned parenthood and pregnancy clinics.

MATERIALS AND METHODS

Patient population. Specimens were obtained from asymptomatic pre- and postabortal patients attending either a pregnancy clinic in Stockton, Calif., or a planned parenthood clinic in Monterey, Calif. Specimens were transported to the laboratory for MicroTrak and culture testing.

Specimen collection. More than 400 endocervical specimens were evaluated by both the direct specimen test and a CC method. Specimens were collected, using the MicroTrak *Chlamydia trachomatis* Specimen Collection Kit which includes two Dacron swabs (one large and one small), a microscope slide with a single 8-mm well, a vial of acetone fixative, instructions, and a cardboard transport container. Before collection, excess mucus and exudate were removed from the exocervix with a cotton or Dacron swab. The large MicroTrak swab was then used to remove intact cuboidal or columnar epithelial cells or both from the endocervix. The specimen was applied directly to the collection kit slide by firmly rolling the swab within the well perimeter. Immediately after smearing, the same swab was placed into 2 ml of *Chlamydia* transport medium in a 15-ml conical centrifuge tube (Bartel's Immunodiagnostic Supplies, Inc., Bellevue, Wash.) and refrigerated. The smear was air dried and then fixed with acetone for 1 min. The smear and transport tube were delivered to the laboratory in an ice chest within 72 h of collection.

MicroTrak procedure. The direct specimen test uses monoclonal antibodies against the major outer membrane protein present in all 15 known human serovars of *C. trachomatis*. The antibodies are labeled with fluorescein isothiocyanate. When viewed under a fluorescence micro-

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scope, MicroTrak-stained positive specimens exhibit apple-green elementary or reticulate bodies contrasted by the red counter-stained cells. The MicroTrak *Chlamydia trachomatis* Direct Specimen Test consists of the antibody conjugate reagent containing Evans Blue counterstain, a mounting medium containing an agent to retard photobleaching (pH 9.0), and control slides. Each control slide contains two wells: one well contains mammalian cells and elementary bodies (EBs), approximating the appearance of a positive cervical or urethral specimen, and the other well contains mammalian cells only, approximating the appearance of a negative specimen.

When fixed smears arrived in the laboratory, they were overlaid with 30 μl of reconstituted antibody reagent and incubated in a moist chamber at room temperature (22 to 28°C) for 15 min. The slides were rinsed in distilled water and air dried, and cover slips were applied, using the MicroTrak mounting fluid. We used a Zeiss standard microscope equipped with a vertical illuminator, a quartz halogen 12-V, 100-W light source, and filter systems for fluorescein isothiocyanate examination. The smears of the patients and the control slides were screened at ×400 magnification (oil), and EBs were confirmed at ×1,000 magnification (oil). Chlamydial EBs are ca. 350 nm in diameter and at ×1,000 magnification appear round with smooth edges and even fluorescence. Forms intermediate between reticulate (initial) bodies and EBs appear two to three times the size of an EB and often have a peripheral halo. All other particles, which were both highly variable in size and white, yellow, or red in color were considered artifacts. The testing of a positive and negative control slide with each batch of patient specimens assured proper performance of test reagents and facilitated the recognition of EBs. The time required to interpret the slides was recorded, and positive slides were graded as follows: light, <5 organisms per high-power field (×400 magnification [oil]); moderate, 5 to 20 organisms per high-power field; heavy, >20 organisms per high-power field. The presence of at least two chlamydial organisms in the entire smear was considered diagnostic of chlamydial infection. Smears with fewer than two organisms were reported as negative, provided that the smear showed adequate numbers of cuboidal and columnar epithelial cells.

Cell culture method. McCoy cell monolayers seeded on cover slips in 1-dram (3.89-g) shell vials with maintenance medium were received weekly from Bartel's, incubated at 35°C for 24 to 48 h, and then held at room temperature. Monolayers were screened for culture suitability with an inverted microscope upon receipt and before inoculation. The isolation protocol followed recommended procedures (2). Cells were prepared for inoculation by removing the maintenance medium and rinsing each vial twice with 0.5 to 1.0 ml of DEAE-dextran in phosphate-buffered saline. The specimen was processed on the day of receipt by removing the swab from the transport tube, vortexing the tube for 1 min, and centrifuging for 3 min at 600 × *g*. A Pasteur pipette was used to transfer 0.2 to 0.5 ml (10 drops) of the cell pellet to the monolayer. Vials were then centrifuged at 2,500 × *g* for 1 h in a temperature-controlled (maximum temperature, 35°C) Beckman model 6JB centrifuge. After centrifugation, the medium was removed, and the inoculated cells were rinsed twice with phosphate-buffered saline (pH 7.0). One milliliter of refeeding medium containing cycloheximide was added to each vial. After incubation at 35°C for 48 h, cultures were fixed with ethanol. Cover slips were then removed from the vials, placed in 24-well microtiter plates, and stained with MicroTrak *Chlamydia trachomatis* Culture Confirmation Reagent (Syva Co.). Positive control cultures were stained with each batch of specimen-inoculated cultures. Under the fluorescence microscope, infected cells were identified by the presence of brightly glowing apple-green inclusion bodies. The positive smears were graded as follows: light, <10 inclusions per cover slip; moderate, 10 to 50 inclusions per cover slip; heavy, >50 inclusions per cover slip.

RESULTS

Four hundred and four specimens were originally submitted for this study. Three specimens were rejected due to either insufficient cellular material on the DS or bacterial contamination or toxicity on the CC. The results of the 401 acceptable paired (DS and CC) specimens are shown in Table 1, where it can be seen that 398 of 401 (99.3%) specimens gave identical results, whereas 3 (0.8%) showed a discrepancy. The overall sensitivity was 96.3% (26/27), with a specificity of 99.5% (372/374). The predictive value of a positive result was 92.9% (26/28), and the predictive value of a negative result was 99.7% (372/373). The comparison of degrees of positivity between DS and CC results for which grading was recorded is shown in Table 2. The majority of smears (67%) were examined and scored in ca. 2 min. Of the smears, 19%, including all of those graded as heavy, were evaluated within 1 min. Most of the remaining smears took 3 to 5 min to examine, depending on the quality of the smear. Uneven distribution of cells or the presence of artifacts on the slide increased the time required for interpretation, but very rarely did it require more than 5 min.

DISCUSSION

The MicroTrak *Chlamydia trachomatis* Direct Specimen Test was compared to a culture method, using McCoy cell monolayers from Bartel's stained with fluorescein-conjugated antibody from Syva Co. Both the Bartel's cells (1) and the Syva culture confirmation antibody (7) have been shown to be superior to other methods; thus, they provided the optimal system with which to compare the direct specimen test.

Our study was limited to asymptomatic females who were either pre- or postabortal patients. In this population, we found excellent agreement between DS and CC samples (99.3%). Discrepancies were noted with only three specimens, which we attributed to random uneven distribution of chlamydial bodies between the DS and CC samples. Overall, the direct specimen test was found to be easy to perform and interpret. Although the directions of the manufacturer currently suggest considering any specimen that has fewer than 10 chlamydial organisms per well (assuming an adequate smear) as negative, we found that after completing the customer training program we could reliably identify as few as two EBs. This lower criterion for positive diagnosis has been adopted by other experienced investigators as well (A. M. Rompalo, R. J. Suchland, C. B. Price, and W. E. Stamm, Int. Conjoint STD Mtg., Montreal, Canada, abstr.

TABLE 1. Comparison of DS with CC

Result		No.
DS	CC	
Negative	Negative	372
Positive	Positive	26
Positive	Negative	2
Negative	Positive	1

TABLE 2. Comparison of degrees of positivity between DS and CC

Degree of positivity in[a]:		No.
DS	CC	
Heavy	Heavy	10
Moderate	Moderate	3
Light	Light	2
Heavy	Moderate	1
Heavy	Light	1
Light	Heavy	1
Light	Moderate	1

[a] See the text for grading.

no. 216, 1984; Stamm et al., in press). Although we occasionally make a positive diagnosis on specimens with between two and nine organisms per well in our routine practice, no specimens in this study were in this range, so even if the current recommendation of the manufacturer was followed, results would not have been affected. We also noticed that the time required for interpretation of smears decreased rapidly with experience.

In conclusion, results of the MicroTrak *Chlamydia trachomatis* Direct Specimen Test correlated highly with CC techniques in diagnosing chlamydial infections in this study population. Studies of the efficacy of this method in other populations are warranted. The direct specimen test offers results within 30 min of specimen receipt in the laboratory and makes routine screening for chlamydial infections more practical. The results of our study indicate that the direct specimen test will contribute significantly to the early diagnosis of chlamydial infections.

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