Evaluation of Three Immunoassays for Detection of *Chlamydia trachomatis* in Urine Specimens from Asymptomatic Males

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The performances of three commercially available immunoassays (Chlamydizyme/Antibody Blocking Assay [Abbott Diagnostics, Abbott Park, Ill.], IDEIA [Analytab Products, Plainview, N.Y.], and Microtrak EIA [Syva Co. Palo Alto, Calif.]) were evaluated for the detection of *Chlamydia trachomatis* in urine specimens from asymptomatic males. Assay results were compared with direct specimen immunofluorescence (DFA) analysis of urine sediment (Syva Microtrak; Syva Co.), which was chosen as the study confirmation assay. An overall *Chlamydia* prevalence of 7% (24 of 340) was found in our study population, with peak incidences occurring in the adolescent (8 of 93 specimens) and young adult (11 of 146 specimens) age groups. Sensitivity and specificity data among the Chlamydizyme, IDEIA, and Microtrak enzyme immunoassays (EIAs) were determined to be 79.1 and 99%, 91.7 and 98%, and 95.8 and 99%, respectively. The Microtrak EIA and IDEIA products demonstrated sensitivities and specificities equal to or greater than those claimed for urine specimens. The diagnostic accuracies of these assays on asymptomatic subjects, along with the ease of this collection method, suggest a role for these products as screening tools. The sensitivity of the Chlamydizyme assay was lower than that claimed previously in symptomatic patients, with 5 of 24 positive specimens demonstrating false-negative results. In those cases, centrifugation of the original immunoassay aliquot material and then DFA examination confirmed specimen positivity. Urine immunoassay screening in combination with DFA confirmation (which was chosen because it has antibody epitopic specificity different from that of the primary assay) provides a high degree of diagnostic precision. The use of noninvasive collection methods could result in greater testing compliance among asymptomatic males and, subsequently, could reduce the incidences of both symptomatic and silent chlamydial infections.

*Chlamydia trachomatis* is an obligate intracellular pathogen and has been recognized as a major etiologic agent of sexually transmitted disease (6). Genital chlamydial infections in females have been associated with cervicitis, endometritis, salpingitis, pelvic inflammatory disease, and infertility (6, 12). Infections in males may include nongonococcal and postgonococcal urethritis, epididymitis, and prostatitis (2, 3). In addition to overt clinical symptoms, a silent or asymptomatic response can occur (2, 3, 5, 18).

The prevalence of *C. trachomatis* is highest among sexually active adolescents, with an asymptomatic carriage rate ranging between 6 and 15% (2, 3, 5, 10, 17, 18). The significant health problems and staggering health care costs associated with this large asymptomatic reservoir of infection have resulted in calls for the institution of diagnostic screening programs (2, 13). Compliance with testing among asymptomatic males has been problematic, in part because of the traumatic, invasive nature of the techniques used for specimen collection (2, 10, 18).

Noninvasive techniques, including those that demonstrate the presence of pyuria, by cytometric and microscopic methods have generally produced unfavorable or incomplete results when compared with urethral swab cultures (2, 3). Measurement of urinary leukocyte esterase, while having been shown to be procedurally simple and cost-effective, has a reported sensitivity that ranges from 72 to 100% (10, 14, 17, 18). Although these methods offer noninvasive approaches to specimen collection, failure to specify etiologic causation limits their value in diagnostic evaluation.

Direct specimen immunofluorescence (DFA)-monoclonal antibody examination of urine sediment provides a highly accurate, noninvasive *Chlamydia* identification method (8, 9, 15). The morphologic detection of noninfectious elementary bodies by DFA offers advantages over culture isolation methods, which require organismic viability (8, 9). Unfortunately, the labor intensity and technical skills required for DFA limit its value as a routine screening tool.

The detection of *C. trachomatis* in male urine specimens by enzyme immunoassays (EIAs) has recently become possible (4, 11, 16). These assays are rapid, cost-effective, and relatively non-labor-intensive tools which specify chlamydial infections. Comparison of these methods with culture isolation has demonstrated similar detection frequencies in males; however, a great reduction in the sensitivities of the EIAs occurs in female urine specimens (1, 11). The performance findings between the sexes is not surprising, because anatomical differences can additionally allow for cervical and endocervical chlamydial involvement. Infection at those sites, potentially without any urethral involvement, has precluded urine testing in females.

Previous investigations that compared enzymatic urine analysis with urethral culture isolation have demonstrated significant variations in the performance of individual assay...
products (4, 11, 16). Those early studies must be carefully evaluated because the establishment or modification of manufacturers’ procedures (including the use of confirmation assays) may have occurred subsequent to their publication.

The purpose of this investigation was to evaluate the product performance of three commercially available immunoassays for the detection of C. trachomatis by using urine specimens from asymptomatic males. EIA sensitivity and specificity with respect to this group and specimen type were incomplete, despite recommendations of health professionals for the institution of screening regimens designed with the use of noninvasive methods.

**MATERIALS AND METHODS**

**Study population.** A total of 340 asymptomatic male volunteers was recruited from patient clinics (Adolescent Medicine, Employee’s Health, Infectious Diseases) at the Nassau County Medical Center and the Nassau County Department of Health facilities. Following an interview with a physician, an informed consent containing both age data and a result notification option (if so desired) was signed prior to enrollment.

Potential donors were excluded if they gave no prior history of sexual intercourse, if they had received antimicrobial therapy in the previous month, or if symptoms consistent with urethritis were present. Approval of the study was granted by the Medical Center institutional Grants and Research Committee.

**Specimen collection and handling.** A urine specimen (50 to 60 ml) was collected in a sterile container, refrigerated, and processed within 48 h. Participants had not urinated for at least 1 h prior to urine donation.

Bulk urine specimens were vigorously vortexed for at least 30 s prior to being divided into aliquots (15 ml) among three centrifuge tubes. The remaining urine specimen (at least 5 ml) was placed in a fourth tube for testing by DFA. Specimens containing less than a 50-ml volume were excluded from testing.

Following aliquot centrifugation (3,000 × g for 30 min), the supernatants were removed and the appropriate assay specimen dilution buffer was added to each tube. Sediment from the DFA tube was washed in phosphate-buffered saline (PBS), recentrifuged as described above, and resuspended in a 0.2- to 0.5-ml volume of PBS (11). A drop of this suspension was placed onto a Teflon-coated slide (Carlson Scientific, Poitine, Ill.), air dried, and fixed in methanol for 5 min.

**EIA procedures.** Three commercially available immunoassays (Chlamydiazyme/Antibody Blocking Assay; lot 51022 M100 [Abbott Diagnostics, Abbott Park, Ill.]; IDEIA; lot 115101 [Analytab Products, Plainview, N.Y.]; and Microtrak EIA; lot 56919-D29 [Syva Co., Palo Alto, Calif.]) approved for testing for C. trachomatis in male urine were evaluated. All assays contained genus-specific antibodies directed against the lipopolysaccharide component of C. trachomatis. The IDEIA product incorporates monoclonal-monoclonal antibody capture technology, whereas the Syva and Chlamydiazyme assays use polyclonal antibody capture methods. Assay procedures were done by the protocol for each product, which included the use of a blocking assay to confirm Chlamydiazyme-positive specimens. Spectrophotometric results were obtained by using equipment supplied for each assay (Quantum II for Chlamydiazyme, EL312e Bio Tek reader for IDEIA, and Microtrak autoreader for Microtrak EIA). A recently approved 90-min protocol was used for IDEIA.

Assay testing was done without knowledge of participant age, clinic source, or the results of tests by the other products being compared in order to eliminate these potential sources of bias.

**Immunoﬂuorescence assay.** DFA testing of urine sediment was chosen as the study confirmation assay, as described previously (11). A fluorescent-labeled monoclonal antibody (Microtrak, lot 8H149-C2D; Syva Co.) directed against a different epitope (major outer membrane protein) than those used in the assays under investigation was selected. A slide was prepared for each test specimen. In addition, direct specimen testing of the original immunoassay aliquot was done when discrepancies among products arose.

Slide wells were scanned at a ×400 magnification by using a Nikon Labophot episcopic microscope equipped with a 460- to 490-nm immunoﬂuorescence excitation filter (Nikon Inc., Garden City, N.Y.). The presence of elementary bodies was confirmed under oil immersion. Specimen and control slides were coded to prevent potential bias of the results. Specimens containing at least two Chlamydia elementary bodies demonstrating both proper morphology and immunoﬂuorescence intensity were judged to be positive.

**RESULTS**

The incidence of C. trachomatis among our study population is given in Table 1. An overall prevalence of 7% (24 of 340) was obtained, with the highest infection rate (8.6%) found among adolescents (ages 13 to 20 years). A similar incidence (7.5%) was observed in young adults (ages 21 to 30 years) but was reduced in older men. Age data for 20 subjects, including one positive study participant, could not be ascertained.

Comparative immunoassay performances are summarized in Table 2. Measurements of the sensitivities and specificities of the Chlamydiazyme, IDEIA, and Microtrak products were found to be 79.1 and 99%, 91.7 and 98%, and 95.8 and 99%, respectively.

Immuoassay results were routinely compared with DFA findings and demonstrated a high degree of correlation. On one occasion, a positive result was recorded by all immunoassays but failed confirmation by DFA. This specimen was considered positive, and the DFA finding was thought to be attributable to a possible sampling error.

In situations in which discrepancies in results among products arose, portions of the original immunoassay aliquot were also prepared and analyzed by DFA. This examination proved valuable, because the original immunoassay aliquot material from seven of eight patients with reportedly negative immunoassay results demonstrated elementary bodies available for antigen detection. The specimen from the
remaining patient was also considered a true positive specimen, because this result was seen in the DFA and the other two immunoassays. Aliquots from false-positive specimens were never demonstrated to have elementary bodies by DFA. The process of aliquot boiling used by two products (IDEIA, Microtrak EIA) did not appreciably alter elementary body morphology or fluorescence.

The IDEIA and Microtrak assays demonstrated performances equal to or better than those claimed by the literature for the respective products. The sensitivity of Chlamydiazyme was below product claims for symptomatic males, with 5 false-negative results occurring among 24 confirmed positive specimens. In those cases, spectrophotometric absorbances were higher than those found for true-negative specimens, but they failed to reach positive cutoff values.

DISCUSSION

A Chlamydia screening program must use a diagnostic approach which is simple to perform, highly accurate, inexpensive, and noninvasive (15). In this study we examined the ability of commercially available EIAs to satisfy these criteria. Our results demonstrate the effectiveness of the Microtrak EIA and IDEIA in detecting C. trachomatis in urine specimens from asymptomatic males.

The IDEIA product has previously demonstrated sensitivities and specificities of 100 and 100%, and 81.6 and 100% in studies involving 62 and 224 symptomatic men, respectively (4, 11). A similar investigation involving 348 symptomatic men reported sensitivities and specificities of 87 and 97%, 76 and 97%, and 71 and 98% for the Microtrak EIA, IDEIA, and Chlamydiazyme products, respectively (7). The resulting assay performance rank for symptomatic men was identical to that found in this study of asymptomatic individuals.

Evaluation of the Chlamydiazyme product resulted in a 79.1% sensitivity. This value is situated between numerous reported sensitivities ranging from 42 to 88% when male urine specimens are used (1, 4, 7, 11, 14–16). The poor sensitivity obtained for the Chlamydiazyme product (42%) in one study led investigators to suggest that urine immunoassay methods may be unsuitable for screening asymptomatic individuals (14). One explanation for this significant reduction in sensitivity may result from the methodology used to obtain study material. The collection of two urethral swabs prior to urine donation may have erroneously biased the available detection material (elementary bodies and antigen) in favor of the culture isolation method. Although a reduced sensitivity of the Chlamydiazyme assay compared with the microtrak and IDEIA assays was also found in the current investigation, the performances of the Microtrak and IDEIA assays suggest their potential for use as screening tools.

A basic difficulty encountered in comparative product investigations is the potential for specimen collection bias, which may obscure the accuracy of study results (11). The use of urine eliminated errors associated with multiple and/or improper sampling and essentially presented each test product with an equal amount of detectable antigen. This specimen source also allowed a clear determination of how each assay would perform under noninvasive screening conditions.

Under these conditions, we found a 7% C. trachomatis carriage rate among asymptomatic individuals in our study population. This infection rate is lower than that described previously (2, 3, 5, 18); however, in the present investigation, we did not place any age restrictions on study participants. The observed infection rate in young adults (ages 21 to 30 years) was relatively equal to that in adolescents (ages 13 to 20 years). Although the target screening groups for C. trachomatis have traditionally been adolescents, the findings presented above suggest that young adults should also be included.

In summary, our results demonstrate the effectiveness of the Microtrak EIA and IDEIA immunoassay products for the accurate detection of C. trachomatis in asymptomatic men and suggest their possible role as screening tools. The use of this noninvasive specimen source could result in greater testing compliance in Chlamydia screening and could reduce this pathogen’s spread in adolescents and young adults.

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REFERENCES


