Noninvasive Detection of *Chlamydia trachomatis* Urethritis in Men by a Rapid Enzyme Immunoassay Test

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Background. The purpose of this investigation was to evaluate the ability of a rapid enzyme immunoassay test to noninvasively detect *Chlamydia trachomatis* urethritis in men from a urine specimen.

Methods. Urethral samples and urine from 207 patients were evaluated. Urethral and urine sediment Gram stains, leukocyte esterase dipstick tests, and enzyme immunoassay analyses of centrifuged and uncentrifuged urine were compared with urethral *C trachomatis* culture.

Results. The prevalence of infection in this population was 10.3%. Sensitivity and specificity of the enzyme immunoassay on the centrifuged urine specimen were 70% and 96%, respectively. The positive and negative predictive values were 67% and 97%, respectively. The uncentrifuged urine enzyme immunoassay sensitivity was 35.7% and specificity was 98.9%. Leukocyte esterase test sensitivity compared with that of the *Neisseria gonorrhoeae* and/or *C trachomatis* cultures was 83.3%, and specificity was 52%.

Conclusions. The rapid enzyme immunoassay clinically complemented the screening urine sediment Gram stain and the leukocyte esterase test. The judicious use of a noninvasive *C trachomatis* rapid enzyme immunoassay test to identify organism-specific urethritis may improve patient management of sexually transmitted disease.

Key words. Urethritis; immunoenzyme technics; *Chlamydia trachomatis. J Fam Pract 1991; 33:73-78.*

The noninvasive testing of urine samples to clinically screen for urethritis in men by a leukocyte esterase dipstick test has been recommended for high-risk populations.[1,2] The importance of this practice is attributed to several factors. Undiagnosed *Chlamydia trachomatis* urethritis in men can lead to prostatitis, epididymitis, and sterility.[3] Many male patients with *C trachomatis* urethritis are asymptomatic.[4–7] Asymptomatic infected male patients serve as silent carriers and can subsequently spread infection to other partners. Screening only women for *C trachomatis* has not adequately decreased the overall prevalence of chlamydial infections. Furthermore, the clinical signs of chlamydial urethritis are variable and nonspecific, and the presumptive clinical diagnosis of *C trachomatis* has been proven to be unreliable.[8]

The presence of urethral leukocytes implies urethritis but is not diagnostic for any specific organism. Urethritis can be caused by many organisms with varied antibiotic susceptibilities. The laboratory identification of the specific organism prevents inaccurate diagnosis, inappropriate or unnecessary therapy, and the exacerbation of psychological trauma and anxiety for both the patient and his or her sexual partners. The absence of a precise microbiologic diagnosis can create a dilemma in determining whether additional sexual contacts of the partners require treatment. Consequently, a nonspecific initial diagnosis may result in poor therapeutic compliance by the sexual contact. Further spread of infection to other sexual contacts may then follow.

The reference standard for diagnosing *C trachomatis* is the McCoy cell culture. Chlamydial cultures require the use of a urethral swab for sample collection.[9] Unfortunately, chlamydial cultures of urine samples are not reliable because of the urine's toxic effect on the live McCoy cells used in this culture procedure.[10] An antigen-based chlamydial test, such as an enzyme immunoassay (EIA), does not have this limitation.

It can be advantageous to obtain a screening urine from either a symptomatic or a high-risk asymptomatic patient, test for leukocyte esterase, and then use the remaining urine sample for specific testing. Only one patient sample needs to be collected. A noninvasive col-

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lection is not painful and is therefore more easily accepted by the patient than a swab collection. Furthermore, the EIA test is less expensive than a culture, and results can be obtained within 20 minutes instead of several days. Chlamydial EIA tests are now readily available and can be used in physician office laboratories. Office personnel can reliably perform EIA tests after routine training by diagnostic company representatives.[11]

The purpose of this investigation was to evaluate the ability of a rapid EIA test to noninvasively detect *Chlamydia trachomatis* urethritis in men from a urine sample.

Methods

The participants in this investigation were men of varied ethnic backgrounds, between 18 and 50 years of age. Subjects were seen and evaluated at the Richmond County Health Department (South Carolina), the Aiken County Health Department (Georgia), or the Student Health Service of the Medical College of Georgia (Augusta).

Inclusion criteria were that the subject be male, 18 years of age or older, and have symptoms of dysuria, a urethral discharge, or a history of sexual contact with a person infected with anogenital *Neisseria gonorrhoeae* or *C trachomatis*. The exclusion criteria were: the subject had used antibiotics within the previous 4 weeks, had urinated less than 1 hour before sample collection, or had urethral stenosis.

Clinicians obtained a standardized history from each patient and had each sign an informed consent form. A brief anogenital examination was then performed. Anogenital clinical findings were noted and recorded.

During the first half of the investigation, each subject obtained a 20-mL initial-stream urine sample in a 50-mL conical tube prior to urethral swabbing. The urine sample was obtained following urethral swab collection for use in the second half of the investigation. Each urine sample was split. Uncentrifuged urine and centrifuged urine sediment were tested for *C trachomatis* by an enzyme immunoassay. A Chemstrip-9 dipstick was used to test uncentrifuged urine for leukocytes, protein, and nitrite. Three urethral swabs were used to obtain samples for *C trachomatis* culture, *N gonorrhoeae* culture, and a urethral Gram stain, respectively. Specimens for chlamydial testing were kept refrigerated at 4°C, and hand-delivered in a cold pack to the respective laboratories. All laboratory tests were performed on the same day as the specimen collection. The EIA tests and the cultures were processed at separate laboratories. Therefore, clinicians and laboratorians were blinded from respective results.

A single Chemstrip-9 was dipped into each subject's freshly voided urine sample at the collection site and interpreted according to the manufacturer's directions. Urethral Gram stain analysis was done by clinicians at the sites. Gram stain smears were examined for evidence of leukocytes and recorded as greater or less than 4 leukocytes per high power field. The slide was also examined for gram-negative intracellular diplococci. The urethral chlamydial culture was performed by previously reported standard protocol.[11] The urine specimen was processed by first sampling the uncentrifuged urine with a collection swab. The remaining 15 mL of urine was centrifuged at 3000 × g for 10 minutes. The supernate was discarded and the sediment was resuspended to 1.0 mL with phosphate-buffered saline. A loopful of the resuspended sediment was used for a urine sediment Gram stain. A 0.2-mL sample of resuspended sediment was then used for *C trachomatis* culture. The final swab collected a sample of the resuspended sediment for chlamydial EIA testing.

Both the centrifuged and uncentrifuged specimens for EIA testing (Surecell Chlamydia, Eastman Kodak Company, Rochester, NY) were processed by medical technologists in the Family Practice Clinic according to the manufacturer's specifications. The manufacturer's collection kits were used to obtain samples from subjects' urine. Positive controls were performed daily for each EIA test kit used. Enzyme immunoassay positivity was defined as the appearance of a substantially darker red color in sample well No. 2 compared with the color of the negative sample in well No. 1.

During the second half of the investigation, when the swab sampling sequence was changed, an improved modified EIA test was introduced by the manufacturer and used in the study.

Specimens for *N gonorrhoeae* culture were inoculated on Thayer-Martin plates. The culture was placed into a bag containing a bicarbonate pellet and incubated at 36°C for 48 hours. Oxidase-positive colonies were Gram-stained, and definitive identification was made by the Phadabact procedure, followed by testing for penicillinase-producing *Neisseria gonorrhoeae* (PPNG).

Outcome groups (based on chlamydial culture results) were compared by race, prior sexually transmitted disease history, symptoms, and laboratory result using the chi-square test of independence, except in tables with an expected frequency of less than 5 in one or more cells, in which case Fisher's exact test was used. Demographic data of subjects with positive chlamydial cultures were compared with those of subjects with negative chlamydial cultures by the Wilcoxon rank-sum test. Sensitivity

Table 1. Centrifuged Urine *Chlamydia trachomatis* Enzyme Immunoassay (EIA) Test Results Compared with *Chlamydia trachomatis* Urethral Culture

Test Performance	EIA First Half		EIA Second Half*		EIA Total	
	No. (%)	95% CI† (%)	No. (%)	95% CI† (%)	No. (%)	95% CI† (%)
Sensitivity	8/13 (61.5)	(31.6–86.1)	6/7 (85.7)	(42.1–99.6)	14/20 (70.0)	(45.7–88.1)
Specificity	71/77 (92.2)	(83.8–97.1)	97/98 (99.0)	(94.4–100.0)	168/175 (96.0)	(91.9–98.4)
Positive predictive value	8/14 (57.0)	(28.8–82.3)	6/7 (85.7)	(42.1–99.6)	14/21 (67.0)	(43.0–85.4)
Negative predictive value	71/76 (93.0)	(85.3–97.8)	97/98 (99.0)	(94.4–100.0)	168/174 (97.0)	(92.6–98.7)

**A modified EIA test was used during the second half of the investigation.*
†Confidence interval.

and specificity for tests were compared using the McNemar test. Adjustments for multiple comparisons were made using the Bonferoni *t* technique. Confidence limits for binomial proportions were calculated.[12]

Results

During the study, 220 patients were tested for *C trachomatis* and *N gonorrhoeae*, and complete data were available for 207. Thirteen cell cultures that displayed cytotoxic effects and the corresponding patients' results were excluded from the calculations. Patient demographics revealed that 86.5% of the subjects were black and that the average age was 26.7 years. The average age of first intercourse was 14.5 years, and the average lifetime number of sexual partners was 10. Most patients were symptomatic; 64.7% reported dysuria, and 69.1% reported a urethral discharge, which was clinically confirmed by examinations in 63.4% of the subjects. Approximately 10.3% of the subjects were culture-positive for *C trachomatis*, and 42.2% of the subjects were culture-positive for *N gonorrhoeae*. Approximately 31% of patients with a positive culture for *N gonorrhoeae* also had *C trachomatis* infections. Conversely, 42.9% of patients with a positive *C trachomatis* culture also had gonorrhea.

A history of prior sexually transmitted diseases was reported by subjects as follows: 22.7% had been diagnosed as having *C trachomatis*, 53.4% as having *N gonorrhoeae*, and 10% as having syphilis. There were no statistically significant independent risk factors for disease; this was probably due to the small number of culture-positive patients identified. The relative odds of a man younger than 20 years of age with a history of more than 10 sexual partners having *C trachomatis* urethritis was 2.47. This increased risk was not statistically significant ($P = .18$), however. The relative odds of this man having *C trachomatis* urethritis with a prior history of gonorrhea was 2.75 ($P = .051$).

The results of the urine *C trachomatis* EIA test compared with chlamydial culture are listed in Table 1. The second half of the investigation results probably reflect the addition of a new modified filter membrane to the kit. The apparently improved EIA test sensitivity was 85.7% and specificity was 99.0%. Resolution of discrepant results was attempted by direct fluorescent antibody (DFA) testing of the remaining centrifuged urine samples. The marked insensitivity of the DFA test, however, precluded the use of this methodology for analysis of discordant results.

A comparison of the centrifuged with the uncentrifuged urine *C trachomatis* EIA results are found in Table 2. The sensitivity of the centrifuged urine EIA was nearly twice (61.5%) that of the uncentrifuged urine EIA (35.7%).

The leukocyte esterase test and the urethral and urine sediment Gram stains were compared with chlamydial and gonorrhea cultures (Table 3). The leukocyte esterase sensitivity (83.3%) is similar to that reported by

Table 2. Centrifuged and Uncentrifuged Urine *Chlamydia trachomatis* Enzyme Immunoassay Test Results Compared with *Chlamydia trachomatis* Urethral Culture

Test Performance	EIA Centrifuged		EIA Uncentrifuged	
	No. (%)	95% CI* (%)	No. (%)	95% CI* (%)
Sensitivity	8/13 (61.5)	(31.6–86.1)	5/14 (35.7)	(12.8–64.9)
Specificity	71/77 (92.2)	(83.8–97.1)	91/92 (98.9)	(94.1–100.0)
Positive predictive value	8/14 (57.0)	(28.8–82.3)	5/6 (83.0)	(35.9–99.6)
Negative predictive value	71/76 (93.0)	(85.3–97.8)	91/100 (91.0)	(83.6–95.8)

**Confidence interval.*

Table 3. Leukocyte Esterase, Urethral and Urine Sediment Gram Stains Compared with *Chlamydia trachomatis* or *Neisseria gonorrhoeae* Urethral Culture

Test Performance	Leukocyte Esterase		Urethral Gram Stain*		Urine Sediment Gram Stain*	
	No. (%)	95% CI† (%)	No. (%)	95% CI† (%)	No. (%)	95% CI† (%)
Sensitivity	45/54 (83.3)	(70.6–92.1)	36/48 (75.0)	(60.3–86.3)	51/54 (94.4)	(84.6–98.8)
Specificity	26/50 (52.0)	(37.4–66.3)	18/44 (40.9)	(26.4–56.8)	12/50 (24.0)	(13.1–38.1)
Positive predictive value	45/69 (65.2)	(52.8–76.3)	36/62 (58.1)	(44.8–70.5)	51/89 (57.3)	(46.4–67.8)
Negative predictive value	26/35 (74.3)	(56.8–87.5)	18/30 (60.0)	(40.5–77.4)	12/15 (80.0)	(51.9–95.7)

**Gram stain positivity is defined as >4 WBC/hpf and/or presence of gram-negative intracellular diplococci.*
†Confidence interval.

Sadof et al[2] (83%) and Shafer et al[1] (72%). The specificity (52.0%) is much lower, however. The urine sediment Gram stain sensitivity (94.4%) was better than the urethral Gram stain sensitivity (75%). The sensitivity of the leukocyte esterase test compared with the *C trachomatis* culture was 71.4%, and compared with the *N gonorrhoeae* culture was 89.6%. The specificities were 34.8% and 51.9%, respectively.

During the first half of the study, 12 EIA test samples were observed to develop into a thick mucoid specimen following the extraction step. The specimen could not pass through the membrane filter. Retrospectively, all 12 patients were confirmed to have *N gonorrhoeae* by culture. It is postulated that these excessively purulent urine sediment samples, which were associated with a gonorrhea infection, overwhelmed the protease enzyme extraction process. This complication was not observed with the modified test in the second half of the investigation.

Discussion

The intent of this investigation was to evaluate the feasibility of a rapid enzyme immunoassay test to detect *C trachomatis* antigen in a urine sample collected from men. Before the development of antigen-based tests, urine and urinary sediment were known to be poor specimens for identifying *C trachomatis* infection of the urethra. Smith and Weed[9] compared the isolation of *C trachomatis* by culture from urethral swabs, urine, and urinary sediment specimens from male patients with urethritis. Cultures from urethral swabs contained five times more chlamydial inclusions than positive cultures from urine or urinary sediment. Poor urine sediment culture isolation results were also obtained in our investigation.

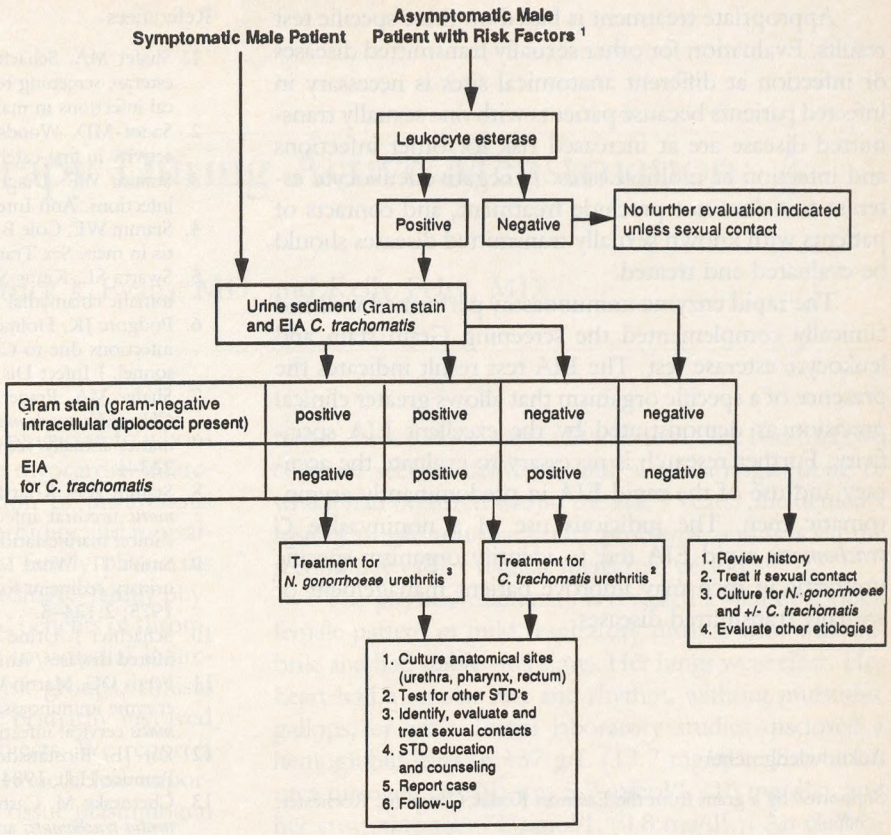
Thereafter, several investigations[1,2] demonstrated the ability of the urinary leukocyte esterase test to predict urethritis in men. This discovery is important because it allows noninvasive sampling, is useful for inexpensive screening purposes, and is fairly specific (93% to 100%) for identifying urethritis. This test, however, is not organism specific, and test sensitivities (72% to 83%) are marginal even when symptomatic male patients are evaluated.

Chernesky et al[13] compared two EIA tests and one direct immunofluorescence test with urethral *C trachomatis* culture. The culture positivity rate (in the study population of 224 men) was 14.8%, slightly higher than that reported in this investigation. The ability of each test to detect *C trachomatis* antigen from urine sediment was reported. Chlamydiazyme had a 86.8% sensitivity; IDEIA had a 81.6% sensitivity, and Microtrak (DFA testing) had a 86.8% sensitivity. All were reported to have a 100% specificity ("true positivity" defined as having a positive culture or two of three antigen tests that are positive). Urine collection order was switched halfway through that study. In contrast to the current investigation, no difference was seen when the urine specimen was collected before or after urethral swab sampling. Yet, just as documented in this investigation, centrifugation of the urine specimen resulted in a vastly improved assay.

Paul and Caul[14] compared three enzyme immunoassay tests (Chlamydiazyme, IDEIA, and Pharmacia chlamydia ELISA) with a direct immunofluorescence test (Syva Microtrak). In this study of 62 men, 30.6% were positive for *C trachomatis* by DFA. Sensitivities and specificities for the assays were reported, respectively, as follows: Chlamydiazyme, 84.2% and 76.7%; IDEIA, 100% and 95.3%; and Pharmacia chlamydia ELISA, 42.1% and 100%.

Both of the previously cited investigations concluded that noninvasive isolation of *C trachomatis* antigen was equal or superior to a urethral-swab-collected specimen for culture. Each study demonstrated the ability of ELISA and DFA tests to isolate *C trachomatis* antigen from a urine specimen. However, none of these high-volume, reference laboratory tests are suitable for a physician office laboratory where skilled laboratorians or special equipment are generally not located.

The rapid test results available from the office labo-



1 Risk factors include: multiple sexual partners, new sexual partner, age less than 25 years, sexual partner of a patient with an STD, and history of prior STDs.

2 Treatment for *C. trachomatis* urethritis
Doxycycline 100 mg BID X 7 days or
Tetracycline 500 mg QID X 7 days or
Erythromycin 500 mg QID X 7 days

3 Treatment for *N. gonorrhoeae* urethritis
Ceftriaxone 250 mg IM plus
Doxycycline 100 mg BID X 7 days or
Spectinomycin 2 gm IM plus doxycycline or
If not penicillin-resistant gonorrhea,
Amoxicillin 3 gm PO with probenecid 1 gm, plus doxycycline.

Figure 1. Evaluation and management of urethritis in men.

ratory can simplify and improve patient management, increase the efficiency and productivity of the clinician and the nursing staff, and help alleviate worry for a patient who would otherwise have to wait several days for test results. In addition, the inherent problems with specimen transport to a reference laboratory are avoided. A rapid, noninvasive EIA for *N gonorrhoeae* would complement this chlamydial EIA and allow urine specimen testing for the two major causes of urethritis in men.

A practical approach to the noninvasive evaluation and management of urethritis in men is outlined in Figure 1. Asymptomatic men at high risk for infection should be screened noninvasively with the leukocyte esterase test. Risk factors for asymptomatic men include a history of multiple sexual partners, a recent new heterosexual partner, history of prior sexually transmitted disease, a sexual contact of a patient with disease, and age less than 25 years. A positive leukocyte esterase result implies urethritis. Noninvasive testing for *C trachomatis* with an EIA test, and a urine sediment Gram stain for *N gonorrhoeae* should follow. Physicians may wish to confirm a positive EIA test result with a culture, especially in a population with a low prevalence of *C trachomatis* (less than 5%). The presence of gram-negative intracellular diplococci should also be followed by a culture for *N gonorrhoeae*, which will identify antibiotic-resistant organisms.

Appropriate treatment is based on these specific test results. Evaluation for other sexually transmitted diseases or infection at different anatomical sites is necessary in infected patients because patients with one sexually transmitted disease are at increased risk for other infections and infection at multiple sites. A negative leukocyte esterase test does not preclude treatment, and contacts of patients with known sexually transmitted diseases should be evaluated and treated.

The rapid enzyme immunoassay performed well and clinically complemented the screening Gram stain and leukocyte esterase test. The EIA test result indicates the presence of a specific organism that allows greater clinical precision as demonstrated by the excellent EIA specificity. Further research is necessary to evaluate the accuracy and use of the rapid EIA in predominantly asymptomatic men. The judicious use of a noninvasive *C trachomatis* rapid EIA test to identify organism-specific urethritis in men may improve patient management of sexually transmitted diseases.

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