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Evaluation of an Enzyme Immunoassay for Detection of *Chlamydia trachomatis* in Urine of Asymptomatic Men

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In a study of 1,486 men attending two sexually transmitted disease clinics, of whom 891 had no symptoms of urethritis, we compared an enzyme immunoassay (EIA) (Baxter-Bartels, formerly Northumbria AntigEnz) of urine sediment to urethral culture for the detection of *Chlamydia trachomatis*. *C. trachomatis* prevalence by culture alone was 7.7% in asymptomatic men and 10.9% in symptomatic men. Discrepant results between EIA of urine and urethral culture were evaluated by direct fluorescent-antibody staining (DFA) for elementary bodies in urine sediment or in culture transport media. When chlamydial infection was defined as either a positive urethral culture or positive EIA confirmed by DFA, chlamydia prevalence increased to 8.9% in asymptomatic men and 11.6% in symptomatic men. The urine EIA sensitivity, specificity, and positive and negative predictive values for chlamydia detection in asymptomatic men were 84.8, 99.3, 91.8, and 98.5%, respectively, with nearly identical results for symptomatic men. The sensitivities of urethral culture alone compared with the combination of urethral culture and urine EIA (with DFA confirmation) were 87.3 and 94.3% for asymptomatic and symptomatic men, respectively. The present EIA of urine sediment is both highly sensitive and specific for the detection of *C. trachomatis* in asymptomatic men, thus providing a noninvasive screening method for chlamydia infection in asymptomatic men attending sexually transmitted disease clinics.

Chlamydia trachomatis is the most common bacterial sexually transmitted pathogen in the United States, accounting annually for four million infections, including one million cases of nongonococcal urethritis and 400,000 cases of pelvic inflammatory disease. It causes neonatal conjunctivitis and pneumonia in 35 and 15%, respectively, of infants born to infected mothers (16). In selected populations, 3 to 9% of asymptomatic men have been documented to have *C. trachomatis* urethral infection (14, 20, 23). Currently, for asymptomatic men who could contribute to transmission of infection, there is no official recommendation for screening, at least in part because of logistical problems associated with the discomfort of a urethral swab, as well as the expense, complexity, and time requirement of urethral culture (3, 4). As an alternative screening test, the urine sediment enzyme immunoassay (EIA) for *C. trachomatis* genus-specific lipopolysaccharide antigen has the advantages of being noninvasive, rapid (4 h), and potentially less expensive than culture. Previous studies of urine EIA tests, for which sensitivities ranged from 42 to 85%, have been primarily conducted with symptomatic men (6, 7, 11, 13, 18, 19). Previous studies of urine EIA compared with urethral chlamydia culture for asymptomatic men have reported sensitivities, specificities, and positive and negative predictive values of 60 to 71, 99, 92, and 98% for 752 men (Syva Microtrak Chlamydia EIA, Syva Co., Palo Alto, Calif.) (6, 10) and 30 to 67, 98 to 99, 67 to 86, and 94 to 98%, respectively, for 799 men (Chlamydiazyme EIA; Abbott Laboratories, Abbott Park, Ill.) (6, 18). We evaluated a new urine EIA (Baxter Bartels, formerly Northumbria AntigEnz) comparing results with those of culture and urine leukocyte esterase (LE) testing as a means of screening asymptomatic men for *C. trachomatis* urethral infection.

MATERIALS AND METHODS

Subjects. Of 1,486 subjects, 891 males over 13 years of age with no symptoms of urethritis attending two Baltimore City Health Department sexually transmitted disease (STD) clinics were consecutively enrolled between September 1991 and June 1992. For purposes of comparison, 595 men with urethritis symptoms were enrolled during the first and last months of the study. Asymptomatic men were defined as men who denied the presence of discharge, dysuria, testicular pain, or inguinal adenopathy. Subjects were excluded if they had received antibiotics within the preceding two weeks or were being seen for follow-up of chronic urethritis. Subjects were requested not to void after arriving in the clinic. Forty (4%) men without urethritis symptoms and 32 (5%) men with urethritis symptoms were recorded to have voided within 2 h and were included in the analyses.

Procedures. After giving informed consent, the subjects underwent a brief, standardized interview and genital exam, which included stripping the urethra and inspecting the urethral meatus. Two urethral swab specimens were obtained by using cotton-tipped aluminum swabs (supplied by the manufacturer). Each swab was inserted 2 cm or more into the urethra, rotated, and withdrawn. The first swab specimen was used for Gram stain and plated directly onto modified Thayer-Martin medium for *Neisseria gonorrhoeae* culture. Nongonococcal urethritis was defined as ≥5 poly-

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morphonuclear leukocytes per high-power field. The second swab was placed directly into liquid chlamydia culture transport medium containing sucrose phosphate buffer, 2% fetal bovine serum, and antibiotics (mycostatin, gentamicin, and vancomycin). The specimen was refrigerated at 4°C and transported to the chlamydia laboratory at Johns Hopkins Hospital within 24 h.

LE. Fresh unspun urine was tested with Nephur-Test plus leuco test strips (Boehringer Mannheim, Indianapolis, Ind.) for the presence of LE and recorded by level (negative, 10 to 25, ca. 75, or ca. 500 leukocytes per μl). A positive result was defined as 10 to 25 or more leukocytes per μl).

***C. trachomatis* culture.** Inoculated transport media (100 μl) for each subject was placed into duplicate wells of 96-well microtiter plates containing McCoy cell monolayers pretreated with 30 μg of DEAE-dextran per ml for 30 min at 35°C. The inoculated cultures were centrifuged at 35°C for 60 min at 800 × *g* and incubated for 30 min at 35°C. After aspiration of the supernatant, 0.2 ml of medium containing 0.1 mg of cycloheximide per ml was added to each well. After 48 h of incubation at 37°C in 5% CO_2, cultures in two wells were fixed with methanol. One well was stained with a fluorescein-conjugated monoclonal antibody against *C. trachomatis* major outer membrane protein (Syva Microtrak, Syva Co.), and the duplicate well was stained with fluorescein-conjugated antilipopolysaccharide antibody (Sanofi, Chaska, Minn.) and read for the presence of inclusion bodies by fluorescence microscopy. A second passage was performed for all negative and toxic cultures and those containing fewer than three inclusions. The presence of one inclusion body was considered to be a positive culture.

Urine EIA. (i) Urine processing. A 15-ml aliquot of urine was centrifuged at 2,500 × *g* for 20 min. The pellet obtained was resuspended in one vial (1 ml) of EIA transport medium (Baxter Bartels, Bellevue, Wash.) and stored at 2 to 8°C up to 8 days prior to EIA testing. A 200-μl aliquot of the resuspended pellet was frozen at −70°C for later analysis of discrepant results between culture and EIA. Transport medium consisted of minimum essential medium containing fetal bovine serum, amphotericin B, streptomycin, and vancomycin.

(ii) EIA. Conjugate (sheep anti-murine immunoglobulin G [IgG] conjugated to horseradish peroxidase in buffered fetal bovine serum; 25 μl) was added to each microtiter well (in plates containing strips of eight wells). Patients' specimens, as well as two negative and one positive control, were boiled at 100°C for 10 min, allowed to cool, and vortexed for 15 s. The processed specimens or controls (200 μl) were added to each well (containing conjugate), 50 μl of murine monoclonal antibody (specific for chlamydial lipopolysaccharide) was added, and the mixture was incubated at 37°C for 1 h. Following incubation, plates were handwashed five times with 0.9% NaCl–0.05% Tween 20 (rinse solution). The substrate (tetramethyl benzidine in dimethyl sulfoxide and methanol; 200 μl) was then added to each well, and the plates were incubated in the dark at room temperature for 30 min, at which time the reaction was stopped by adding 50 μl of 2 N H_2SO_4. After the reaction was stopped, A_{450} was measured within 10 min with an EIA spectrophotometer blanked against air. Results were considered positive if the absorbance was greater than the threshold, defined as 0.1 optical density unit above the average of the results for two negative controls.

(iii) Blocking procedure. Specimens yielding positive results were retested by using a blocking assay. Conjugate (25 μl) and 50 μl of a patient's specimen to be retested were added to each of two wells. Blocking antibody (25 μl of rabbit anti-chlamydia serum) was added to one well, and control reagent (25μl of normal rabbit serum) was added to the other well. The EIA procedure was then repeated. A specimen was considered positive if the net absorbance in the unblocked well exceeded the calculated threshold and the net absorbance in the blocked well was 50% or less of the absorbance for the unblocked specimen (net absorbance = absorbance − average absorbance of negative controls).

TABLE 1. Prevalence of *C. trachomatis* and selected sexually transmitted infections

Infection or result	% *C. trachomatis* infection (no. of men positive/no. tested)		
	Asymptomatic[b]	Symptomatic	*P*
C. trachomatis[a]	8.9 (79/891)	11.6 (69/595)	0.11
N. gonorrhoeae	0.8 (7/890)	32.2 (191/593)	<0.001
Co-infection[b]	0.1 (1/890)	2.5 (15/593)	<0.001
≥5 PMNs/×1,000 field[c]	32.8 (289/882)	47.3 (190/402)	<0.001
Discharge on exam	27.0 (238/880)	70.4 (419/595)	<0.001

[a] *C. trachomatis* infection was defined by positive urethral culture or urine EIA confirmed by DFA.
[b] Coinfection, both *C. trachomatis* and *N. gonorrhoeae* infections.
[c] Gonococcal infections were excluded.
[d] Asymptomatic, without urethritis symptoms (discharge, dysuria, testicular pain, adenopathy).

Resolution of discrepant results by direct fluorescent-antibody staining (DFA). When discrepancies between *C. trachomatis* culture and urine EIA results were noted, the culture transport media and urine sediment were examined by fluorescence microscopy for the presence of chlamydial elementary bodies (EBs). To prepare the specimens, the 200-μl stored urine aliquot was centrifuged at 10,000 × *g* for 15 min. Urine (185 μl) was then removed and replaced with phosphate-buffered saline, and the mixture was recentrifuged. For culture specimens the vial was centrifuged at 10,000 × *g* for 15 min, and the supernatant was decanted. Pellets from both urine and culture vials were then placed on microscope slides, air dried, fixed with methanol, and stained with fluorescein-conjugated monoclonal antibody to chlamydial major outer membrane protein (Syva Microtrak). Slides were examined for the presence of EBs by fluorescence microscopy by an experienced technician. If the EIA was positive and either urine or transport medium contained EBs, the subject was considered to be a true positive and to have a chlamydial infection for purposes of data analysis.

Statistical analyses. Sensitivity, specificity, and predictive values were calculated by conventional methods. Chi square and Fisher's exact test, where appropriate, were used for significance testing of proportional data.

RESULTS

Study population. Subjects were predominantly African-American (95%) and heterosexual (97%) men, with a mean age of 27 years (range, 13 to 83 years). In men without urethritis symptoms (*n* = 891), the reason for clinic visit was symptoms other than urethritis (31.4%), STD contact (32%), or check-up (34%). Of 595 men with symptoms of urethritis, 12% also had symptoms other than urethritis and 7% reported STD contact.

Disease prevalence. The prevalences of *C. trachomatis* and *N. gonorrhoeae*, as detected by culture, and nongonococcal urethritis, defined as ≥5 polymorphonuclear leukocytes per

TABLE 2. Performance of urine EIA, urethral culture,and LE tests for detection of *C. trachomatis* in men[a]

Subject and test (no. of men tested)	% (no. of positive specimens/no. tested)			
	Sensitivity	Specificity	PPV	NPV[b]
Asymptomatic				
EIA (891)	84.8 (67/79)	99.3 (806/812)	91.8 (67/73)	98.5 (806/818)
Culture (891)	87.3 (69/79)	100.0 (812/812)	100.0 (69/69)	98.8 (812/822)
LE (881)	57.3 (39/68)	70.2 (571/813)	13.9 (39/281)	95.2 (571/600)
Symptomatic				
EIA (595)	82.8 (58/70)	99.2 (521/525)	93.5 (58/62)	97.7 (521/533)
Culture (595)	94.3 (66/70)	100.0 (525/525)	100.0 (66/66)	99.2 (525/529)
LE (591)	73.4 (47/64)	44.7 (234/523)	13.9 (47/337)	91.4 (234/256)

[a] The reference standard chlamydial infection was defined as a positive urethral chlamydia culture or a positive urine EIA confirmed by DFA for chlamydial EBs.

[b] NPV, negative predictive value.

high-power field are listed in Table 1. The prevalence of *C. trachomatis* in asymptomatic men by culture was 7.7% and by positive culture or positive EIA (confirmed by DFA) was 8.9%. In symptomatic men the prevalence was 10.9% by culture and 11.6% by culture or EIA (confirmed by DFA). The prevalences of chlamydia infection in asymptomatic and symptomatic men (8.9 and 11.6%, respectively) were not significantly different ($P = 0.11$). In contrast, *N. gonorrhoeae* was rare in asymptomatic men, with a prevalence of 0.8% but present in 32.2% of symptomatic men ($P < 0.001$). Similarly, coinfection was unusual, occurring in 0.1% of asymptomatic men and 2.5% of symptomatic men ($P < 0.001$).

Test performance. The test performance of urine EIA, urethral culture, and urine LE compared with either a positive urethral culture or positive EIA (confirmed by DFA) as the reference standard are listed in Table 2. In asymptomatic men the sensitivity of urine EIA was 84.8%, compared with 87.3% for urethral culture. The specificity of EIA was 99.3%, and the positive predictive value (PPV) was 91.8%. The EIA sensitivity for symptomatic men was similar to that for asymptomatic men (82.8% versus 84.8%, $P > 0.25$), whereas the sensitivity of urethral culture for symptomatic men was higher than that for asymptomatic men (94.3% versus 87.3%, $P < 0.001$). Urine LE was insensitive for both asymptomatic and symptomatic men (sensitivity, 57.3 and 73.4%, respectively), nonspecific (specificity, 70.2 and 44.7%, respectively), and poorly predictive (PPV, 13.9%) for *C. trachomatis* infection.

Analysis of discrepant results. There were 48 samples with discrepant results, 28 for asymptomatic men and 20 for symptomatic men. There were 15 patients with negative cultures but with positive EIA results confirmed by DFA of urine sediment or culture transport medium (false-negative culture). Of these 15 men, EBs were found in culture transport media for 14 specimens and urine sediment for 3, with a median of eight EBs (range, 2 to 26). Eleven of these 15 false-negative cultures occurred in samples from asymptomatic men. Culture detected 23 infections that were not detected by urine EIA. Of these, EBs were found in only two urine sediment specimens with three and seven EBs, respectively.

DISCUSSION

There is a need for a convenient, accurate, noninvasive method of screening asymptomatic men for urethral infection due to *C. trachomatis* (15–17, 23). LE testing of first-void urine has been advocated as a means of screening asymptomatic men for urethritis (1). In our study LE testing of fresh urine was insensitive (57.3%) and poorly predictive of chlamydial infection in asymptomatic men (PPV = 13.9%), although its sensitivity has been reported to be as high as 72% in asymptomatic adolescents for the detection of either gonorrheal or chlamydial infection (21).

The use of traditional diagnostic methods for screening asymptomatic men, such as urethral culture or DFA staining of a urethral smear for chlamydial EBs, are precluded by the invasiveness of urethral swabbing and the relative insensitivity in the latter (22). The use of fluorescein-labelled species-specific monoclonal antibodies to *C. trachomatis* major outer membrane protein has made identification of extracellular EBs in infected urethral secretions or urine sediment feasible, but the technique is labor intensive and highly dependent on the skill of a trained technician, and the criteria for a positive result vary between laboratories. Similarly, the use of EIA tests of urethral specimens is invasive and insensitive in asymptomatic men (50%) (22). PCR and ligase chain reaction tests of urine sediment also offer the potential for noninvasive, species-specific detection of chlamydial DNA (2, 8, 12), but few data are available and only one of these tests is commercially available.

It is likely that a substantial proportion of males with chlamydial infection are asymptomatic and therefore unlikely to seek screening for infection. The availability of noninvasive diagnostic tests holds promise as a means for identifying a proportion of such men and, therefore, may play an important role in chlamydial infection control efforts. Urine sediment EIA has the advantage of being noninvasive, requires less technical expertise, accommodates multiple specimens, has an objective endpoint (optical density), and is less expensive than culture. Its disadvantages are the inability to monitor specimen quality and potential for sampling variation by timing of urine collection. Comparison of different EIA tests has been difficult because of the lack of standardization of test evaluations (17).

In this study we found that the Baxter-Bartels EIA showed a high sensitivity, specificity, and PPV for detection of *C. trachomatis* infection in the urine of men without urethritis symptoms attending STD clinics. The addition of a blocking step to confirm positive EIA results allowed for increased sensitivity while maintaining a high degree of specificity. The low numbers of EBs seen by DFA of samples with discrepant results indicate that decreased sensitivity of either urine EIA or urethral culture is more

likely to occur with low-grade infection. Of the 15 culture-negative chlamydial infections identified by urine EIA only (DFA confirmed), 11 were in asymptomatic men. Discrepant results may be due to sampling variation whereby EBs or free lipopolysaccharide antigen could be present in either urine or culture transport medium alone. This is plausible because specimens are obtained from somewhat different infectious sources (urine versus urethral squamo-columnar cells in exudate). Although it has been speculated that urethral sampling prior to urine collection might explain the lower apparent sensitivity of urine EIA, this did not affect our results (18). Urethral culture for *C. trachomatis* was performed prior to urine collection in this study, and the sensitivity of EIA was high. It is possible that obtaining urine samples prior to urethral culture would have reduced sampling error even further and improved the EIA sensitivity. Only 5% of men were recorded as having voided within 2 h of sampling.

These data should be viewed as preliminary findings suggesting the utility of urine testing for chlamydia diagnosis in asymptomatic men. However, men attending STD clinics (the participants in this study) are not the population for whom such tests hold the most promise since these men are, by definition, at increased risk and would be expected to a have a higher prevalence of infection. Rather, having demonstrated the feasibility of such an approach, further studies are now indicated in more-appropriate target populations of asymptomatic sexually active males outside of STD clinics or emergency room settings. If our findings can be verified in such studies, the Baxter-Bartels test as used to detect infection in urine specimens from asymptomatic males holds considerable promise as a supplement to current chlamydial infection control efforts.

In summary, urine EIA appears to offer an accurate noninvasive means of screening asymptomatic men for chlamydial infection in this population. Since its predictive value will be lower in a lower prevalence population, this and other urine EIAs should be further evaluated before widespread use in that setting.

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