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Use of a Urine Enzyme Immunoassay as a Diagnostic Tool for *Chlamydia trachomatis* Urethritis in Men

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We collected first-voided urine specimens from 659 males attending a sexually transmitted disease clinic and performed both enzyme immunoassay (EIA) for detection of chlamydial antigen and leukocyte esterase testing on these urine samples. The overall prevalence of chlamydial urethritis in the study population as determined by culture of urethral swabs was 11%. However, 46% of all men in the study had no symptoms of urethritis. Compared with urethral cultures for chlamydiae, the urine EIA had a sensitivity of 42% and a specificity of 99%. The sensitivity of the EIA strongly correlated with the amount of antigen present in culture as assessed by numbers of inclusion-forming units. The sensitivity of the leukocyte esterase test compared with that of chlamydia culture was 88%. We conclude that in this population of men, which included many patients without symptoms of urethritis, the urine EIA was a relatively insensitive means of screening for chlamydial infection.

Effective control of sexually transmitted infections caused by *Chlamydia trachomatis* has thus far been hampered by the lack of a rapid, inexpensive, and accurate means of diagnosis. Furthermore, current diagnostic techniques for males rely upon obtaining urethral swabs, a procedure which most asymptomatic males are reluctant to undergo. More recently, first-voided urine (FVU) specimens have been used as a noninvasive alternative method for screening males for urethritis. The first studies using this approach relied upon nonspecific tests, such as the urine microscopic exam for leukocytes and the leukocyte esterase (LE) test. The sensitivity of this approach (75 to 85%) has been acceptable (1, 10), but a specific etiologic agent cannot be identified. Subsequently, Chernesky et al. (1) and others (3, 6) have tested FVU specimens for the presence of chlamydial antigen by using enzyme immunoassay (EIA) and have reported sensitivities of 76 to 87%. To assess the usefulness of the EIA performed on centrifuged urine samples as a specific means of identifying *C. trachomatis* infection in men, we screened urine samples from 659 male patients attending a sexually transmitted disease (STD) clinic and compared the EIA results with those of chlamydia cultures of urethral swabs. Unlike researchers in previous studies, we found that the EIA of FVU resulted in an unacceptably low sensitivity compared with that of urethral culture.

(This work was presented in part at the 30th Interscience Conference on Antimicrobial Agents and Chemotherapy, Atlanta, Ga., 21 to 24 October 1990 [5].)

MATERIALS AND METHODS

FVU specimens were collected from male patients during their initial visits for a new problem at the Harborview STD Clinic, Seattle, Wash. Each specimen consisted of the initial 15 to 20 ml of voided urine and was not necessarily a first morning specimen. During the same visit, swabs were routinely obtained for urethral Gram stains and for cultures for gonococci and chlamydiae. All urine samples were obtained after the two urethral swabs had been collected. Dacron swabs inserted 2 to 4 cm into the urethral canal were utilized for the chlamydia cultures.

Chlamydia cultures were performed as previously described (11). In brief, cultures were inoculated onto cycloheximide-treated McCoy cells grown in 96-well microtiter plates and were stained with fluorescein-conjugated genus-specific antibodies to identify inclusions. No passage was performed. FVU was refrigerated and examined within 24 h for the presence of LE, by using the Ames Multistick (Miles, Inc., Elkhart, Ind.). A reading of trace or greater was considered positive. Urine samples were prepared for EIA within 5 days of collection. Twenty milliliters of the FVU was centrifuged at 2,000 × *g* for 15 min, and the pellet was then resuspended in 1 ml of EIA specimen dilution buffer for subsequent assay. EIA (Chlamydiazyme; Abbott Laboratories) was performed according to the manufacturer's instructions by a technologist who had no knowledge of the patient or of the culture results. Direct fluorescent-antibody testing with MicroTrak (SYVA Corporation) was performed by spotting the slide with a portion of the resuspended urine pellet when there was a discrepancy between the culture and EIA results. A direct fluorescent-antibody assay which contained greater than or equal to two elementary bodies was considered positive.

Historical data about each patient were collected by reviewing the medical record in the clinic. Included in the record was the length of time since the patient had voided prior to collection of the urine sample.

RESULTS

Characteristics of the study population. Urine specimens were collected from 659 consenting male patients. Three patients were eliminated from the study because concomitant urethral swabs had not been obtained. LE testing of 644 specimens was performed.

The prevalence of chlamydial infection in the study population as determined by urethral culture was 11.2%. The population was largely heterosexual (>92%), and the primary reason for visiting the clinic was genital symptoms. However, only 54% of the patients reported specific symp-

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TABLE 1. Characteristics of the study population

Characteristic	Value for group			P value[a]
	All patients (n = 656)	Chlamydia culture positive (n = 74)	Chlamydia culture negative (n = 582)	
Mean age (yr)	29.8	25.0	30.4	<0.0001
Race (%)				
White	53.8	51.4	54.1	
Black	37.1	41.7	36.5	NS[b]
Other	9.1	7.0	9.4	
Sexual preference (%)				
Heterosexual	92.3	98.6	91.5	
Homosexual	4.7	1.4	5.2	NS
Bisexual	3.0	0	3.3	
Reason for visit (%)				
Symptoms, any	73.6	75.3	73.4	
Dysuria and/or urethral discharge	54.0	71.6	52.2	NS
Contact with STD	14.9	17.8	14.5	NS
Screening	11.5	6.8	12.1	
Gonococcal infection (%)	6.8	8.2	6.6	<0.0001
Presence of urethral discharge on exam (%)	48.0	90.1	42.7	<0.0001
≥5 PMNs/1,000× field (%)	44.1	87.5	38.5	<0.0001

[a] *C. trachomatis* positive versus negative; comparisons by *t* test.
[b] NS, not significant.

toms of urethritis (dysuria and/or urethral discharge) (Table 1). Men infected with chlamydiae were younger than those not infected (mean age, 25 versus 30, respectively; $P < 0.0001$) (Table 1). The detection of chlamydiae also correlated positively with the presence of a urethral discharge and ≥5 polymorphonuclear leukocytes (PMNs) per 1,000× field upon Gram stain (Table 1). Eighty percent of the men reported voiding 2 or more h prior to the examination.

Results of LE testing and urine EIA. The sensitivities and specificities of LE testing and urine EIA of FVU compared with those of urethral cultures for diagnosing chlamydial infection are shown in Table 2. The sensitivity of the urine EIA was only 42%, while the specificity was 99%. For patients not reporting symptoms of urethritis, the sensitivity of the EIA was only 30%, compared with 45% for those men who were symptomatic. For the LE test, the sensitivity was 88% and the specificity was 58%. Table 3 shows the sensitivities of the LE test for the diagnoses of gonorrhea, chlamydial infection, and all causes of urethritis combined.

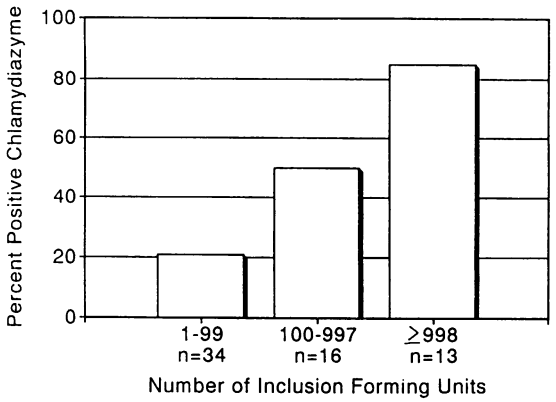

FIG. 1. Sensitivity of urine Chlamydiazyme EIA stratified by number of inclusion-forming units in cell culture.

Although the sensitivities of LE tests for gonococci and chlamydiae were equivalent (88 to 92%), they fell to 71 to 78% when nongonococcal, nonchlamydial etiologies of urethritis were included. LE testing was also more sensitive for younger patients (83% for the 15- to 19-year-olds versus 71% for the 20- to 24-year-olds).

The sensitivity of urine Chlamydiazyme testing was strongly influenced by the amount of antigen present, defined as the number of inclusion-forming units on culture (Fig. 1). Additionally, the number of inclusion-forming units strongly correlated with the optical density of the EIA ($P < 0.0001$; data not shown).

Six specimens reacted positively in the Chlamydiazyme EIA but were negative by culture. Of these, elementary bodies were noted on one of the corresponding direct fluorescent-antibody assay slides, indicating a false-negative culture. Adequate direct fluorescent-antibody assay slides were available for 29 of the specimens with negative EIA results but positive cultures. Of these, elementary bodies were seen in 38%.

The sensitivity of the urine EIA was not affected by the initial volume of urine collected. The sensitivity of the urine EIA for those specimens with >15 ml of urine was 37.5%, whereas it was 42.6% for those with a volume of <15 ml. The sensitivities of both LE testing and EIA were unaffected by variations in the length of time since the patient's last void prior to specimen collection.

DISCUSSION

Infections in asymptomatic or minimally symptomatic men probably represent an important untreated reservoir from which infections in women originate. For this reason, successful detection of chlamydial infection in asymptomatic males would be of great potential benefit in the control of sexually transmitted *C. trachomatis* infections. Because

TABLE 2. Sensitivities, specificities, and predictive values of urine Chlamydiazyme EIA and LE testing with chlamydial culture of urethral swabs as the reference standard

Test (no. of specimens)	Sensitivity (%)[a]	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
Urine Chlamydiazyme (656)	41.9 (31/74)	99.0 (576/582)	83.7 (31/37)	93.0 (576/619)
LE (644)	88.1 (59/67)	58.6 (338/577)	19.8 (59/298)	97.7 (338/346)

[a] All values in parentheses represent number of positive specimens out of total number of specimens tested.

TABLE 3. Sensitivities, specificities, and predictive values of the LE test by diagnosis

Diagnosis (cause)	Sensitivity (%)[a]	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
Gonorrhea[b]	92.3 (36/39)	56.9 (340/598)	12.2 (36/294)	99.1 (340/343)
Chlamydial infection[c]	88.1 (59/67)	58.6 (338/577)	19.8 (59/298)	97.7 (338/346)
Urethritis (any)[d]	78.4 (207/264)	57.6 (57/99)	83.1 (207/249)	50.0 (57/114)
Urethritis (all except gonococcal and chlamydial infection[e])	71.0 (115/162)	60.2 (50/83)	77.7 (115/148)	51.6 (50/97)

[a] All values in parentheses represent number of positive specimens out of total number of specimens tested.
[b] Compared with a clinical diagnosis of gonorrhea.
[c] Compared with chlamydial culture as a reference standard.
[d] Compared with a clinical diagnosis of gonorrhea, a chlamydial culture, and a microscopic diagnosis of nongonococcal urethritis on urethral Gram stain (≥5 PMNs per 1,000× field) as reference standards.
[e] Compared with urethral Gram stain (≥5 PMNs per 1,000× field).

pain upon insertion of urethral swabs deters asymptomatic men from being tested, collection of FVU has been investigated as a more acceptable means of obtaining a diagnostic specimen. While a urine sample is not a suitable specimen for chlamydial culture (8), resulting in a sensitivity of only 20 to 30% compared with that of urethral swab cultures, it may be suitable for antigen detection methods. Chernesky et al. (1) successfully used EIA in diagnosing chlamydial infection in FVU specimens from 224 men who were either symptomatic or possible sexual contacts of infected women. Using two separate EIAs, the researchers found the sensitivity, compared with that of urethral culture, to range from 81.6 to 86.8% for these largely symptomatic males. Paul and Caul (3) found the sensitivity of the urine Chlamydiazyme to be 76% for a group of largely symptomatic men but limited their analysis to first morning urine specimens. Our results were much less encouraging, with the EIA demonstrating a sensitivity of only 42% compared with that of urethral culture. Several factors may account for the lower sensitivity we observed. First, only 54% of the men we studied had symptoms of urethritis. The inclusion of many asymptomatic men, who typically have infections with lower inclusion counts and less antigen (9), provided a more rigorous test of the EIA than would the inclusion of men with overt urethral discharge. We also tested random FVU specimens, which may contain less antigen, as opposed to first morning specimens. Additionally, the numbers of patients studied were larger than in these two previous reports. An important technical difference may have been the speed of centrifugation (2,000 × *g* in our study versus 3,000 × *g* in the others). However, 2,000 × *g* has been used by other investigators who have reported sensitivities of 71 to 81% for the Chlamydiazyme EIA with FVU specimens from symptomatic patients (2, 6). In addition, we have used 3,000 × *g* in our laboratory in a subsequent study of the urine Chlamydiazyme EIA and saw no improvement in EIA performance (10). Thus, we doubt that centrifugation speed is the explanation for this discrepancy. Because we obtained the FVU after two urethral swabs had been collected from the patient, the sensitivity of the urine EIA may have been reduced because some antigen was undoubtedly removed. However, Chernesky and colleagues reported that the order of collection did not influence their results (1). Finally, the strongest predictor of a false-negative Chlamydiazyme result may be a low inclusion count in culture. We suspect that because our study included many cultures with low inclusion counts (49% had inclusion counts of <100), the EIA results were not as good. Although cell culture has been used as the standard of comparison in all other studies of urine EIA, the sensitivity of cell culture, especially in detecting infections characterized by few inclusions, varies from laboratory to laboratory. We may have detected more of such infections than had been detected in other studies, correspondingly diminishing the sensitivity of the EIA.

Although LE testing had a much higher sensitivity than urine EIA, its nonspecificity limits its value as a specific diagnostic tool. In our study, 82% of patients with a positive LE test were found to have gonorrhea (13%) or nongonococcal urethritis (69%). The sensitivity of LE testing was comparable to that previously found by others (4, 7). Thus, a positive LE test is a useful predictor of urethritis and could be used to identify patients in need of further testing. Differentiation between these two infections, however, requires a Gram-stained urethral smear and/or urethral cultures.

In summary, we found the sensitivity of the urine EIA (Chlamydiazyme) for specimens from males attending an STD clinic to be unexpectedly low. The discrepancy between our results and those of others may be due to the facts that only half of our patients had symptoms of urethritis and many had low-inclusion-count infections. Since large-scale screening efforts with the urine EIA for males would focus mainly on asymptomatic individuals, this may prove to be a serious disadvantage to this approach. More sensitive methods for the detection of chlamydiae in urine, such as polymerase chain reaction-based assays, may overcome this problem.

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