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Evaluation of Chlamydiazyme Enzyme Immunoassay for Detection of *Chlamydia trachomatis* in Urine Specimens from Men

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Paired first-voided urine and urethral swab specimens were collected from 540 men attending sexually transmitted disease clinics in three geographic locations. Urine specimens were tested for the presence of *Chlamydia trachomatis* by commercial enzyme immunoassay (Chlamydiazyme), and the results were compared with those of urethral swab cultures. Overall prevalence of urethral *C. trachomatis* by culture was 14%, and the Chlamydiazyme assay had an overall sensitivity of 83%, a specificity of 96%, a positive predictive value of 76%, and a negative predictive value of 97%. Sensitivity was greater (94%) in those culture-positive samples with a high antigen load (≥20 inclusion-forming units per coverslip) than those with a lower antigen load (68%). Assay of urine specimens from men attending sexually transmitted disease clinics by Chlamydiazyme appears to be a reliable, noninvasive method of detection of *C. trachomatis* infection, and further evaluation of its performance in asymptomatic and low-prevalence populations is indicated.

Chlamydia trachomatis is one of the most frequent sexually transmitted infections worldwide, with an estimated 4 million new cases annually in the United States (15, 16). Chlamydia infection causes a broad spectrum of outcomes ranging from asymptomatic colonization to infections such as urethritis, cervicitis, epididymitis, pelvic inflammatory disease, neonatal conjunctivitis and pneumonia, and lymphogranuloma venereum (34). Symptomatic infections usually come to clinical attention and are treated with antichlamydial therapy. However, asymptomatic infection may go unrecognized, creating a potential for ongoing transmission and development of asymptomatic sequelae such as salpingitis, which can result in infertility and ectopic pregnancy (34). In women, up to 80% of genital infections are asymptomatic, and in men up to 26% of those infected will have no signs or symptoms of infection (2, 22, 28, 31, 40). A recent study found that 25% of the *C. trachomatis*-positive men who visited a sexually transmitted disease (STD) clinic had no clinical indications for anti-chlamydial therapy (28). Without specific testing for chlamydia, such men are usually not treated and may resume sexual activity under the assumption that they are infection-free. Thus, in order to prevent sexual transmission and the ensuing morbidity, it is important to have a sensitive, specific, and acceptable diagnostic test for *C. trachomatis* available to screen sexually active men. Until recently, the only method of sampling for genital chlamydia in men was by urethral swabbing, requiring insertion of a swab 2 to 4 cm into the urethra. Often this is uncomfortable and unacceptable to the patient, especially for those who are asymptomatic with little or no exudate to act as lubricant. The development of a noninvasive diagnostic test to screen for genital chlamydia in men has been needed. In this study, the use of first-voided urine specimens tested by enzyme immunoassay (EIA) with the Chlamydiazyme (CZ) assay (Abbott Laboratories, North Chicago, Ill.) was assessed as a noninvasive diagnostic test for the detection of chlamydial infection in men by comparison with the standard of urethral swab culture.

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MATERIALS AND METHODS

Population. The study population consisted of men attending STD clinics in Denver, San Francisco, and Hamilton, Ontario, who were undergoing urethral culture for gonorrhea and who had not been seen at the clinic for at least 6 weeks. Patients who had received antibiotics within the previous 2 weeks or who had urinated within the past hour were excluded from the study. In Denver, 200 patients were evaluated (60% symptomatic); in San Francisco, 202 patients were evaluated (85% symptomatic); and in Hamilton, 138 patients were evaluated (with history of symptomatology not recorded), for a total of 540 subjects. Patients were considered symptomatic if they had a history of discharge and/or dysuria.

Sample collection. Urethral swab samples for culture of *Neisseria gonorrhoeae* were obtained from all patients, followed by paired first-voided urine and urethral swab specimens for *C. trachomatis* testing. In Denver, the urethral swab sample for cell culture was obtained before the urine sample; in San Francisco, the order was reversed; and in Hamilton, both sequences were used, with the swab sample obtained first in 29 men and second in the remaining 109 men.

First-voided urine specimens consisted of 10 to 20 ml of urine collected in a sterile container. Urethral swab specimens for chlamydia culture were obtained by inserting a sample collection swab (calcium alginate or cotton) 2 to 4 cm into the urethra and gently rotating.

Cell culture. Urethral swabs were placed into sucrose-

TABLE 1. Evaluation of urethral *C. trachomatis* infection in men: urine CZ versus urethral culture

Group of patients (*n*)	Prevalence by culture (%)	CZ performance [no. with result/no. tested (%)]			
		Sensitivity	Specificity	Positive predictive value	Negative predictive value
Denver (200)	21 (11)	16/21 (76)	170/179 (95)	16/25 (64)	170/175 (97)
San Francisco (202)	32 (16)	29/32 (91)	161/169 (95)	29/37 (78)	161/164 (98)
Hamilton (138)	23 (17)	18/23 (78)	112/115 (97)	18/21 (86)	112/117 (96)
Total (540)	76 (14)	63/76 (83)	443/463 (96)	63/83 (76)	443/456 (97)

phosphate transport medium and refrigerated at 5°C for no longer than 24 h. If cultures could not be processed within that time, specimens were stored at −70°C until inoculation. Specimens were inoculated into duplicate vials of McCoy cells (Denver and San Francisco) or into four microtiter wells containing McCoy cells (Hamilton). *C. trachomatis* was isolated at the three sites by previously described methods (5, 7, 30). At the two sites with isolation by vials, cell cultures were stained with fluorescent anti-chlamydial antiserum (Microtrak; Syva Co, Palo Alto, Calif.) after 48 h of incubation. All negative cultures were passaged once and restained after 48 to 72 h. In Hamilton, cell cultures were stained with iodine after 48 to 96 h of incubation, passaged, and restained. A culture with no inclusions was considered negative, and cultures with inclusions were considered positive; the number of inclusion-forming units (IFU) per coverslip or well was recorded.

EIA evaluation. Urine was centrifuged at 2,000 to 3,000 × *g* for 20 min at room temperature, and the supernatant was removed. Pellets were stored at 2 to 8°C for less than 72 h before processing and then resuspended in 1 ml of specimen dilution buffer and assayed by CZ according to package insert instructions. Further analysis of specimen results discordant between cell culture and CZ (cell culture negative and CZ positive) consisted of a blocking assay (Chlamydiazyme Blocking Reagent; Abbott Laboratories) and a repeat of the CZ assay. Following this evaluation, a patient was considered to have confirmed chlamydial infection if the cell culture was positive or if the CZ blocking assay produced a reduction in signal of >50% on a CZ-positive specimen.

RESULTS

Seventy-six out of 540 men had positive urethral swab cultures for *C. trachomatis*, for an overall prevalence of 14%. The sensitivity, specificity, and predictive values of urine CZ testing compared with those of urethral culture are shown in Table 1. The overall sensitivity of CZ was 83%, with performance by site ranging from 76 to 91%; overall positive predictive value was 76%, ranging by site from 64 to 86%. Overall, specificity was 96% and negative predictive value was 97%, with little site-to-site variation.

In an effort to evaluate the different sensitivities among sites, CZ results were analyzed in relation to the number of IFU found in cell culture of positive samples for the two sites using the same laboratory methods (Denver and San Francisco) (Table 2). A difference in CZ sensitivity was found when results were arbitrarily grouped by the criteria of ≥20 or <20 IFU per coverslip (94 versus 68%, respectively). In Denver, 43% of positive samples contained <20 IFU per coverslip with a urine CZ sensitivity of 56%. In contrast, for the positive samples containing ≥20 IFU per coverslip, urine CZ had an overall sensitivity of 92%. In San Francisco, 31% of positive results were in the lower IFU range with a urine CZ sensitivity of 80%; 69% contained ≥20 IFU per coverslip, with a urine CZ sensitivity of 96%.

To determine whether clinical presentation influenced the performance of urine CZ, results for symptomatic and asymptomatic men in Denver and San Francisco were compared (Table 3). Overall, CZ sensitivity was similar among culture-positive men who were asymptomatic (7 of 8 [88%]) and symptomatic (33 of 38 [87%]). However, among symptomatic men, sensitivity tended to be greater for those with high inclusion counts (94%) than for those with lower counts (50%). Among asymptomatic men, too few were culture positive to allow a similar comparison.

Samples from 33 patients had discrepant urethral culture and urine CZ results. Thirteen patients were culture positive and CZ negative and were considered CZ false negative. The blocking confirmatory assay was used in an attempt to evaluate the 20 culture-negative/CZ-positive discrepancies. Nineteen had sufficient samples to allow repeat of the CZ and performance of the blocking assay. Fourteen of the 19 (74%) were blocked and considered to have probable true-positive urine CZ results. Two which did not block and three which were below the CZ cutoff when retested were considered false-negative CZ results.

DISCUSSION

The development of antigen detection methods has been a major breakthrough in the diagnosis of infection with *C. trachomatis*. Testing formerly limited to a relatively small number of centers with tissue culture capacity is now within the reach of most clinical laboratories. Although the potential clinical and epidemiologic benefits of screening for chlamydial infection in young sexually active men have been recognized (16, 17, 25), a drawback of this approach is the perceived and/or actual discomfort of inserting a swab 2 to 4 cm into the urethra. Patients may regard this with anxiety, and the discomfort experienced, which can on occasion persist for several hours after sampling, may deter some patients from return visits (14, 23, 44). Thus, use of a

TABLE 2. Sensitivity of urine CZ by urethral culture inclusion count

Group of patients (*n*)	Samples with <20 IFU/coverslip		Samples with ≥20 IFU/coverslip	
	No. (%)	EIA sensitivity[a]	No. (%)	EIA sensitivity
Denver (21)	9 (43)	5/9 (56)	12 (57)	11/12 (92)
San Francisco (32)	10 (31)	8/10 (80)	22 (69)	21/22 (96)
Combined sites (53)	19 (36)	13/19 (68)[b]	34 (64)	32/34 (94)

[a] No. of positive samples/total no. of samples (% sensitivity).

[b] $P < 0.05$, χ^2 test, samples with <20 IFU/coverslip versus samples with ≥20 IFU/coverslip.

TABLE 3. Sensitivity of urine CZ by symptomatology[a] and inclusion count

Clinical status	No. of CZ-positive samples/no. of culture-positive samples (% culture-positive)		
	<20 IFU/coverslip	≥20 IFU/coverslip	All
Asymptomatic	4/5 (80%)	3/3 (100%)	7/8 (88%)
Symptomatic	3/6 (50%)	30/32 (94%)	33/38 (87%)

[a] Patient histories were unavailable for three chlamydia culture-positive samples and are not included in the calculations.

noninvasive, easily obtained sample such as urine offers an attractive alternative. While attempts to culture urine have resulted in unacceptably low sensitivity rates of only 4 to 24%, possibly due to the presence of inhibitory factors (4, 38), the development of direct antigen tests has allowed a reconsideration of urine sampling, and a number of recent studies have evaluated urine EIA for detection of *C. trachomatis* infection in men. Reported sensitivity has varied, ranging from 42 to 100%, although it has generally been ≥75%; specificity has been less varied (94 to 100%) (4, 9, 12, 14, 19, 21, 23, 24, 33, 36, 37, 42).

Our study of urine CZ assay of first-voided urine confirms these prior results, with an overall sensitivity of 83% and specificity of 96%. Furthermore, in contrast to previous reports, our multicenter study design allowed us to assess performance across sites. Sensitivity was ≥75% and specificity was ≥95% in all three locations. In the relatively small number of asymptomatic men we evaluated, sensitivity was similar to that seen in symptomatic men, 88% versus 87%; this finding is important because the former is the group in whom noninvasive screening techniques are likely to be of greatest importance in chlamydia control programs. This issue requires more extensive evaluation. At least one recent study of urine EIA for detection of *C. trachomatis* in men has reported a higher sensitivity in patients with urethritis (80%) than in asymptomatic men (59%) (14), while another found equivalent sensitivities in symptomatic (79%) and asymptomatic (81%) men (33), similar to our results.

We found that sensitivity was correlated with the number of IFU present, consistent with previous studies reporting that most false-negative antigen detection results occur in specimens with low antigen levels (27, 35, 36, 39, 40). Factors which influence the antigen load are numerous and diverse, including specimen collection, transport, storage, and the sensitivity of the cell culture methods used. Host factors which have been suggested include immune response (3), symptomatology (8, 13), cervical ectopy (13, 18), recent sexual intercourse (18), sex of the patient (18), use of oral contraceptives (1, 11, 41), age (13), current and past STDs (1, 25), and ethnicity (1). It has also been suggested that *Chlamydia* strains may vary in their ability to infect genital sites (18). Because of the dependence of the sensitivity of direct antigen tests on antigen load, further studies are needed to clarify the role of these and other factors in the number of IFU. Direct-antigen tests may not be appropriate for patients likely to be shedding low numbers of *Chlamydia* organisms.

The specificity of CZ found in this study, 96% in comparison to cell culture, was excellent. Overall specificity may be even higher, since we did not routinely evaluate culture-negative/CZ-positive specimens by an independent assay such as the direct fluorescent antibody test, a procedure which can help define such discordant specimens as true positives (14, 20). False-positive CZ reactions have been reported to occur in the presence of group A (26) and group B (43) streptococci, *Neisseria gonorrhoeae* (43), *Proteus vulgaris* (10), *Acinetobacter calcoaceticus* (32, 43), *Escherichia coli* (6), *Gardnerella vaginalis* (43), *Staphylococcus aureus* (29), *Streptococcus faecalis* (31), *Salmonella* spp. (32), *Klebsiella pneumoniae* (6), and excessive mucus (35). When testing urine, it may be particularly important that a confirmatory test be performed on all positive specimens, since concentrations of 10^5 to 10^7 organisms per ml, counts easily found in patients with urinary tract infections, are reported to give false-positive CZ results (6). Identification of false-positive results is a special concern when screening low-risk patients outside STD clinics, since an erroneous positive result may cause medical, legal, and social problems for patients and their sexual partners.

Although use of the blocking assay may improve the overall performance of CZ (20), the utility of this procedure has not yet been as extensively evaluated for urine as it has for cervical specimens (20, 21). In this procedure, monoclonal antibody to a chlamydia-specific lipopolysaccharide (LPS) epitope blocks the reactivity of the polyclonal anti-chlamydia LPS antibody used in CZ. Chlamydial LPS has a second epitope which is shared with the LPS of cross-reacting bacteria; this reaction is not blocked (20). When the subset of our CZ-positive samples with discordant results (culture negative and CZ positive) was evaluated by this test, sensitivity increased to 87% and specificity increased to 99%. However, if the CZ blocking assay were used as a confirmatory test under routine laboratory conditions, all CZ-positive samples would be tested by blocking, not just a subset; thus, we were not able to determine overall sensitivity and specificity as defined by CZ with blocking assay confirmation.

In summary, assay of urine specimens from men attending STD clinics by CZ appears to be a rapid, noninvasive method of detecting urethral *C. trachomatis* infection. Further evaluations of this promising technique are needed to clarify reported variations in sensitivity and to assess its performance in asymptomatic and lower-prevalence populations of men. Should these evaluations confirm the favorable performance characteristics found by us and others, EIA-based screening of urine specimens may become an important component of chlamydia control efforts.

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