

Rapid, On-Site Diagnosis of Chlamydial Urethritis in Men by Detection of Antigens in Urethral Swabs and Urine

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First-void urine (FVU) sediments of 240 men were tested for *Chlamydia trachomatis* antigens by two enzyme immunoassays, TestPack Chlamydia (15 min) and Chlamydiazyme (3.5 h), and the results were compared with urethral swab culture results. The sensitivity and specificity on FVU sediment for TestPack Chlamydia were 76.2% (32 of 42 specimens) and 95.5% (189 of 198 specimens) versus 81.0% (34 of 42 specimens) and 96.5% (191 of 198 specimens) for Chlamydiazyme, respectively. Rapid, on-site detection of chlamydial antigen in male FVU would shorten the infectious period by hastening diagnosis and treatment.

The substantial burden of illness in women caused by *Chlamydia trachomatis* (8) propels research for ways to interrupt its spread by diagnostic and screening maneuvers that are both effective and efficient. The detection of chlamydial antigens in first-void urine (FVU) sediments of men is a feasible and accurate test for chlamydial urethritis (2, 5, 7), and it is more acceptable than urethral swabbing (1). We evaluated a rapid (15-min) enzyme immunoassay (EIA) (4) for chlamydial antigen in male FVU sediments and urethral swabs by comparing its performance and that of a conventional EIA (with blocking confirmation) on FVU sediments with results of culture of urethral swabs.

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From January to December 1989, 240 men over the age of 15 years attending our sexually transmitted disease clinic consented to the collection of two urethral swabs and 25 to 50 ml of FVU. The men usually presented with symptoms of urethritis or because of contact with a partner with *C. trachomatis* or *Neisseria gonorrhoeae* infection. Symptoms were present in 72.1% of the men, and 96.2% of the men were heterosexuals. None of the men had received antimicrobial agents in the preceding 2 weeks. Each man signed a consent form which had been approved by a hospital ethics committee. Each of two urethral specimens was collected by inserting a swab 2 to 3 cm into the urethra and rotating it. The first, a cotton-tipped aluminum swab, was used to prepare a urethral smear for Gram stain before inoculation into a universal transport medium (minimal essential medium, 5% sorbitol, 3% fetal bovine serum, 2 mM glutamine) for *C. trachomatis* and *N. gonorrhoeae* cultures. The smear was examined for the presence of intracellular gram-negative diplococci and the number of polymorphonuclear leukocytes (PMNs) per oil-immersion field. The STD-PEN male swab transport kit (Abbott Laboratories, North Chicago, Ill.) was used to collect the second urethral swab for TestPack Chlamydia (Abbott Laboratories). A FVU specimen was collected before urethral swabbing during the first half of the study and after urethral swabbing during the second half of

the study. Specimens were shipped on wet ice and arrived at the laboratory within 24 h. All FVU samples were split into three 15-ml conical tubes, and sediments were collected by centrifugation at 3,000 rpm (2,000 × g) for 20 min at 4°C (2). Isolation of *C. trachomatis* was performed as described previously (3) by using cycloheximide-treated McCoy cells and iodine staining in a microculture system with one blind passage after 72 h. Standard methods were followed for the isolation of *N. gonorrhoeae* (6).

Urethral swabs were tested by TestPack Chlamydia (Abbott Laboratories), a 15-min solid-phase EIA (4). For TestPack Chlamydia testing of FVU specimens, the sediment remaining after centrifugation was resuspended in 1 dropperful of reagent A and 2 drops of reagent B and then tested (4). Another FVU sediment sample was resuspended in 1 ml of specimen dilution buffer and assayed by Chlamydiazyme (Abbott Laboratories). Positive Chlamydiazyme urine sediments were tested by using a confirmatory blocking assay (4). The "gold standard" for the presence of chlamydia was cell culture isolation from the urethra.

The sensitivity and specificity of TestPack Chlamydia on FVU sediment are shown in Table 1. The performances of TestPack Chlamydia and Chlamydiazyme on FVU sediment were comparable. Although the assay sensitivities on urine specimens ranged from 76 to 81% compared with those of culture, the assay specificity on FVU was not as high as that on urethral swabs. As shown in Table 2, there was complete agreement among the tests for 28 culture-positive and 185 culture-negative men. Of the 13 men with negative cultures and discordant results, 7 were positive by blocked Chlamydiazyme on FVU sediment and 3 of these were positive by TestPack Chlamydia. The other six men were only positive by TestPack Chlamydia. The prevalences of chlamydial infection were 6.2% (4 of 65 specimens) in asymptomatic men, 21.7% (38 of 175 specimens) in symptomatic men, and 17.5% (42 of 240 specimens) overall. Chlamydia-positive men were significantly ($P < 0.05$) younger and had higher rates of multiple sexual partners, a partner change in the past 6 months, dysuria, urethral discharge, and increased numbers of PMNs in urethral smears (>4 PMNs per field). There were no significant differences between chlamydia-negative and -positive men in prevalences of cohabitation, condom use, a partner change within the preceding 2 months, ciga-

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TABLE 1. Comparison of the performances of TestPack Chlamydia and Chlamydiazyme on urine sediments with that of TestPack Chlamydia on urethral swabs^a

Test, specimen	%			
	Sensitivity	Specificity	PPV ^b	NPV ^c
TestPack Chlamydia, urine	76.2 (32/42)	95.5 (189/198)	78.0	95.0
Chlamydiazyme, urine	81.0 (34/42)	96.5 (191/198)	82.9	96.0
TestPack Chlamydia, swab	81.0 (34/42)	99.0 (196/198)	94.4	96.1

^a Results of culturing of the urethral swab were used as the standard of positivity.

^b PPV, Positive predictive value.

^c NPV, Negative predictive value.

rette smoking, compliance with follow-up visit, or history of sexually transmitted disease. *N. gonorrhoeae* was isolated from 8.9 and 4.0% of the chlamydia-positive and -negative men, respectively ($P = 0.764$).

The prevalence of chlamydial infection shown by urethral swab culture was 9.2% (9 of 98 specimens) when FVU was collected first but 23.2% (33 of 142 specimens) when swabs were collected first. Performances of TestPack Chlamydia on urine did not fluctuate greatly when the order of collection was changed. The sensitivity and specificity of the TestPack assay on urine sediment were 77.8% (7 of 9 specimens) and 92.1% (82 of 89 specimens) when the FVU was collected before urethral swabbing and was 75.8% (25 of 33 specimens) and 98.2% (107 of 109 specimens) when the FVU was collected after urethral swabbing. The sensitivity and specificity of Chlamydiazyme on urine sediment were 67.6% (6 of 9 specimens) and 95.5% (85 of 89 specimens) when FVU was collected before swabbing and 84.9% (28 of 33 specimens) and 97.3% (106 of 109 specimens) when FVU was collected after swabbing.

This study shows that the TestPack Chlamydia assay used on FVU sediments performed nearly as well as when it was used on urethral swabs. TestPack Chlamydia assay of FVU sediments has the advantages of ease of specimen collection and the ability to be done rapidly, without laboratory facilities. Although TestPack Chlamydia has not yet been approved for performance on urine, it has a promising sensitivity, but the specificity warrants further study. This is the first reported use of the blocking reagent to confirm positive urine specimens by Chlamydiazyme, and this also has not

yet been approved. Although we used culture of the urethra as the primary "gold standard" to assess the performance of EIA on urine, it is apparent from the data in Table 2 that some of the 13 culture-negative but EIA-positive men may have been infected. The rapid turnaround time of TestPack Chlamydia is important since this permits detection, treatment, and education during the same visit, which may shorten the infectious period for the patient and his contact(s).

More research needs to be done with asymptomatic subjects, especially in primary-care settings, to determine risk predictors and the probably low prevalence in such populations. For a test to be useful in screening and case-finding, sensitivity and specificity should be high enough that the few cases present are not missed and that the false-positive rate is minimized. Application of appropriate criteria to select men for testing would increase both the efficiency and positive predictive value of a test by "boosting" the prevalence of infection. Investigation of an approach that uses sequential testing of a urethral swab is needed if a FVU sediment is negative by TestPack Chlamydia.

The time since previous voiding may affect the sensitivity of tests based on FVU sediment, and this is being examined in an ongoing study. Treatment of the urine in an attempt to expose more antigen is also being investigated. Our limited observations, according to whether urine was collected before or after urethral swabbing, suggest that the presence of urine in the urethra at the time of swabbing coincided with a decreased prevalence of infection. This might be due to a loss of sensitivity of the urethral swab culture, which is compatible with our previous findings that urine may be inhibitory to isolation in cell culture (2). Although our patient numbers were small, the order of specimen collection did not appear to affect the performance of either EIA in a consistent fashion.

Criteria to be used for selective testing may vary from one population to another, but the use of a rapid EIA on FVU promises to be an efficient and highly acceptable detection method for chlamydial urethritis in symptomatic and asymptomatic men.

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TABLE 2. Comparison of cell culture and antigen detection in urethral swabs with antigen detection in urine samples for the diagnosis of *C. trachomatis* in men

Culture	Urethral swab		FVU sediment		No. of men
	TestPack Chlamydia	TestPack Chlamydia	TestPack Chlamydia	Chlamydiazyme ^a	
+	+	+	+	+	28
+	+	+	+	-	1
+	+	-	+	+	1
+	+	-	-	-	4
+	-	+	+	+	3
+	-	-	-	-	3
+	-	-	+	+	2
-	+	+	+	+	2
-	-	+	+	+	1
-	-	+	-	-	6
-	-	-	+	+	4
-	-	-	-	-	185

^a All positive sediments were confirmed with the Chlamydiazyme blocking reagent.

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