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Enzyme Immunoassay and Direct Immunofluorescence for Detection of *Chlamydia trachomatis* Antigen in Male First-void Urine

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First-void urine specimens, collected from 309 military recruits, 246 male adolescent gymnasium students and 194 patients consulting venereal disease clinics, were studied for the presence of *Chlamydia trachomatis* with the use of antigen detection tests – two enzyme immunoassays (EIA) and a direct immunofluorescence test (DIF; Syva MicroTrak). Urethral swabs were collected when discrepancies between the EIA and DIF tests were detected. The patient was regarded as positive when the culture result was positive or when two antigen detection tests corraborated one another. The Syva MicroTrak EIA and DIF tests were more sensitive than the Orion EIA, i.e. 98.5%, 99.2% and 74%, respectively. This was true when testing both low- and high-risk groups, with a prevalence of chlamydial infection ranging from 0.4% to 58.6%. All three tests were highly specific. The positive predictive values for the Syva MicroTrak EIA, the DIF and the Orion EIA were 99.2%, 100% and 100%, respectively and the negative predictive values 99.8%, 99.8% and 94.8%, respectively.

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During the past decades, *Chlamydia trachomatis* has been recognized as one of the most widely spread causative agents of sexually transmitted diseases (STDs) (1).

One reason why effective control of STDs caused by *C. trachomatis* has been hampered is the lack of rapid, inexpensive, non-invasive sampling and diagnostic methods. The diagnosis of genital chlamydial infection in males has so far generally depended on the collection of urethral swabs, a procedure which most asymptomatic males are reluctant to undergo. First-void urine (FVU) has more recently been used as a non-invasive alternative for screening males for *C. trachomatis* (2). By testing FVU for the presence of *C. trachomatis* antigen, using an enzyme immunoassay (EIA), one has found sensitivities in the range of 76 to 87% (3–5).

The purpose of this study was to evaluate the sensitivity of two commercial EIA kits in detecting *C. trachomatis* antigen in FVU, collected from asymptomatic men (in a low chlamydia prevalence population) and from outpatients attending a venereal disease clinic (in a high chlamydia prevalence population) and compare the results with those of a direct immunofluorescence (DIF) test.

MATERIALS AND METHODS

Study populations

Three hundred and nine male recruits (Group A; median age 19.7 years) and 246 male adolescent students (Group B; median age 18 years) were enrolled in the study. In addition, 194 patients (median age 29 years) attending two venereal disease (VD) clinics were recruited during April (Group C; $n = 49$) and June (Group D; $n = 145$) in 1992.

Patients were asked to collect the first 15–20 ml of their early morning urine in sterile polypropylene tubes.

Sample treatment

The urine samples were divided into three aliquots, each of which was centrifuged at 3,000 × g for 30 min. The supernatant was decanted and one of the pellets was tested by a DIF (MicroTrak, Direct Specimen Test, Syva, CA, USA). Five μl of the pellet were suspended in the liquid remaining in the tube after decantation and were then transferred onto an 8-mm well and left to air-dry. The slide was fixed in 100% methanol and stained with MicroTrak fluorescein-conjugated monoclonal antibody against *C. trachomatis*. The slides were examined under a fluorescent microscope (×400; Nikon, Diaphot-TMD, Japan).

The pellets from the other two aliquots were suspended in 1 ml of Syva MicroTrak EIA and Orion EIA specimen treatment solution, respectively. They were kept at 4°C before testing. Just before the assay was performed, the samples were vortexed and preheated for 30 min at 90°C. The assays were performed according to the instructions of the manufacturers.

From persons who had a positive DIF test only, a urethral swab was collected.

Isolation of *C. trachomatis* was performed on a culture of cyclohexiimide-treated McCoy cells, as described by Ripa & Mårdh (6). After 72 h, the cells were fixed with 100% methanol and stained with MicroTrak antibodies aimed at chlamydial inclusion detection.

A positive culture result, or two corraborating antigen detection tests, was regarded as a true positive.

Sensitivity/specificity

The denominator used for calculation of sensitivities was the sum of patients with a positive test result in at least two of the different test systems used. Specificities were calculated using patients with negative results in all tests, with a positive result in only one of the non-culture tests as the denominator.

RESULTS

Of all the 749 urine samples studied, 131 samples were considered chlamydia-positive. Out of these samples, 96 were positive in both the EIA and in the DIF test. Thirty-three were positive in the Syva MicroTrak EIA and DIF tests, but negative in Orion EIA. One sample was positive in the Syva MicroTrak EIA and two in the Syva MicroTrak as well as in the Orion EIA. All these three samples were negative in the DIF test. One sample was positive in both the Orion EIA and the DIF test, but negative in the Syva MicroTrak EIA.

Table I presents the results of the Syva MicroTrak EIA, Orion EIA and Syva MicroTrak DIF tests on FVU samples collected from the 309 military recruits (Group A), the 246 male students (Group B) and the 194 patients from VD clinics (Groups C and D).

One of the 309 samples from military recruits (Group A) was positive in all tests. In the DIF test, the FVU sediment of this patient's urine contained 10–20 EBs per well. In another case,

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Table I. *Investigation of first-void urine from military recruits (Group A), adolescent students (Group B), and patients from two venereal disease clinics (Groups C and D), performed with the use of the Orion EIA and DIF tests*

Group	Syva EIA/Orion EIA/DIF						
	+/+/+	+/−/+	+/−/−	+/+/−	−/−/+	−/+/+	−/−/−
A	1	1	1	0	0	0	306
B	1	0	0	0	0	0	245
C	33	8	0	1	1	0	6
D	61	24	0	0	0	1	59
Total	96	33	1	1	1	1	616

an agreement between the Syva MicroTrak EIA and the DIF test results (with presence of 10–20 EBs per well) was found. In yet another one case, in which the tests were repeated three times, the Syva MicroTrak EIA remained the only positive test result.

One of the 246 samples from students (Group B) was positive in all the three detection tests used. In the DIF test of the FVU sediment, 20 EBs per well were found.

As Table I shows, 43 of the 49 samples from the VD patients in Group C were positive. Of these 43 samples, 33 were positive in the Syva MicroTrak, Orion EIA and in the DIF tests. In 8 cases positive FVU sediments were found in the Syva MicroTrak EIA and DIF tests. These samples were all negative in the Orion EIA. In one case, the Syva MicroTrak and the Orion EIA were positive, but not the DIF test; in the latter case the cell culture was positive. In one case, where only the DIF was considered positive (based on the observation of two EBs in the slide well), the result was confirmed by isolating the organism from the urethra.

Of the 145 FVU samples from Group D, 86 samples proved positive. Sixty-one were positive in al three tests; 24 were positive in the Syva MicroTrak EIA and DIF tests, but negative in the Orion EIA. One sample was positive both in the Orion EIA and DIF tests.

The sensitivities and specificities of the two EIA and of the DIF test are shown in Table II.

The Syva MicroTrak EIA and DIF tests had a higher sensitivity than the Orion EIA, i.e. 98.5%, 99.2% and 74.2%, respectively. All three tests showed high specificities, i.e. 99.8%, 100% and 100%, for Syva MicroTrak, DIF and Orion EIA, respectively.

The positive predictive value (PPV) and negative predictive value (NPV) for the Syva and Orion EIA and the DIF are shown in Table III. The PPV for the Syva MicroTrak EIA, the Orion EIA and the DIF was 99.2%, 100% and 100%, respectively, while the NPV was 99.8%, 94.8% and 99.8%, respectively.

DISCUSSION

A sensitive, specific and cheap test, requiring non-invasive sampling, would be valuable in the control of *C. trachomatis* epidemics, which are still prevailing in most countries.

Larsson et al. (7) and Genç et al. (8) found the asymptomatic carrier rate of *C. trachomatis* infection to be 9.3–10%, in 19-year-old military recruits. However, Rahm et al. (9) reported a marked decrease in the prevalence of *C. trachomatis* infection during recent years among men of the same age as military recruits.

Schafer et al. (10) investigated urethral swab cultures of sexually active school boys (13–18 years old) in San Francisco and found a prevalence of *C. trachomatis* infection of 8–9%. The low prevalence in the male adolescent gymnasium students in our study is in conformity with other recent observations in Sweden (11).

In several countries, the prevalence in genital chlamydial infections in the general population has decreased during the last years, while in other countries such a change is not obvious. Marked and rapid changes in the prevalence imply difficulties in predicting which type of non-culture test should be used for population screening at a given time in a given area.

Urethral sampling may cause pain, particularly in asymptomatic men, if they have no discharge. The test of FVU overcomes that problem. Urine, as mentioned, is not a suitable type of sample for chlamydial culture (12); the sensitivity is only in the range of 20–30%, as compared to urethral culture. Urine has, however, proved a suitable sample for chlamydial antigen detection tests in males, i.e. EIA and DIF (4, 13, 14).

The usefulness of FVU for *C. trachomatis* antigen detection has been supported by several studies. Chernesky et al. (4) found *C. trachomatis* antigen in FVU sediments from 224 men in 81.6%–86.6%, as compared to 86.6% positive by urethral swab cultures. Urine specimens were positive in all patients with

Table II. *Sensitivity and specificity for the Syva MicroTrak EIA, Orion EIA and DIF in the detection of Chlamydia trachomatis antigen in first-void urine of military recruits (Group A), adolescent students (Group B), and patients from two venereal disease clinics (Groups C and D)*

Group	Sensitivity			Specificity		
	Syva EIA	Orion EIA	DIF	Syva EIA	Orion EIA	DIF
A	2/2	1/2	2/2	306/307	307/307	307/307
B	1/1	1/1	1/1	245/245	245/245	245/245
C	42/43	34/43	42/43	6/6	6/6	6/6
D	85/86	62/86	86/86	59/59	59/59	59/59
Total	130/132 (98.5%)	98/132 (74.2%)	131/132 (99.2%)	617/618 (99.8%)	618/618	618/618

Table III. *Positive (PPV) and negative (NPV) predictive values for the Syva MicroTrak EIA, the Orion EIA and DIF for first-void urine from military recruits (Group A), adolescent students (Group B) and patients from two venereal disease clinics (Groups C and D)*

Group	PPV			NPV		
	Syva EIA	Orion EIA	DIF	Syva EIA	Orion EIA	DIF
A	2/3	1/1	2/2	306/306	307/308	307/307
B	1/1	1/1	1/1	245/245	245/245	245/245
C	42/42	34/34	42/42	6/6	6/15	6/7
D	85/85	61/61	86/86	59/60	59/83	59/59
Total	130/131 (99.2%)	97/97	131/131	616/617 99.8%)	617/651 (94.8%)	617/618 (99.8%)

a positive urethral culture and in 6 out of 54 patients with negative test results (15). Similar data have been shown by Genç et al. (16), who found the sensitivity of testing FVU EIA to be greater than that of testing urethral specimens (77% vs. 62%). These results show that testing FVU samples does not only have the advantage of being a non-invasive procedure but also provides a sensitive method for the detection of *C. trachomatis* infections in men. FVU is suitable for the screening of both asymptomatic and symptomatic persons (17). The possibility for female patients to bring urine from their male partner when attending their gynecologist with genital symptoms or when subjected to screening for STDs, pregnancy testing, cancer screening etc., should be considered as well.

Sensitivity and specificity for detection of *C. trachomatis* with EIA in urine, as compared to culture from the urethra, has been reported to be in the range of 59–100% and 93–99%, respectively (18–20). Schwebke et al. (21) demonstrated, as expected, that the sensitivity of an EIA strongly correlates with the amount of antigen present in the sample as indicated by the number of inclusion-forming units found in culture. In experiments using serial dilutions of test material, the Syva MicroTrak EIA was less sensitive than the Syva DIF test. Thus dilution of 10^{-8}, as compared to 10^{-6}, proved positive. Other commercial EIAs available on the market at the time were found to be 3 log less sensitive (22). The sensitivities of various EIA kits may be due to one or more serovars of *C. trachomatis* being detected with varying efficacy (4).

In all the groups studied, the Syva MicroTrak EIA revealed more true positives than the other tests used, both when testing the low and high prevalence groups studied, i.e. with a range of 0.4% to 58.6% positives. Assuming that one non-culture test can be used to verify another test principle, we conclude that the selected commercial EIA kits performed equally well for urine and urethral swabs of the same individual (4, 23).

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