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Comparison of urine with urethral swabs for the detection of *Chlamydia trachomatis* in men attending an STD clinic

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Urethral swabs and first-catch urine specimens for the detection of *Chlamydia trachomatis* were collected from 370 black men with urethritis who attended a sexually transmitted disease (STD) clinic in Pretoria. An enzyme immunoassay (EIA) for *C. trachomatis* was carried out on all urethral swabs and urine specimens. Chlamydial culture and a direct immunofluorescent antibody (DFA) test (Imagen, Dako, UK) were also carried out on urethral swabs; DFA was used for confirmation of urine EIA positives.

Based on culture and/or DFA, *C. trachomatis* was detected in 96 (26%) urethral swab specimens. The sensitivity of urine EIA investigation was 94% and the specificity 99%, compared with those of urethral swab EIA which were 97% and 99% respectively. The positive and negative predictive values for urine were 96% and 98% compared with 96% and 99% respectively for urethral swabs. Urine examination was therefore sensitive and specific compared with urethral swab examination in these STD patients. In view of this, the advantage of urine as an alternative to urethral swabs for *C. trachomatis* detection is that sampling is non-invasive and non-traumatic.

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Chlamydia trachomatis is the most common sexually transmitted micro-organism in industrialised Western countries.[1,2] Although *Neisseria gonorrhoeae* is the commonest cause of sexually transmitted disease (STD) in many developing countries, including those in Africa, genital chlamydial infection has, in recent years, been found to constitute an important health problem.[3,4]

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In men with suspected urethritis, the taking of endo-urethral swabs has been the traditional way of collecting specimens for detection of *C. trachomatis* by culture. Although cell culture has been regarded as the 'gold standard' for *C. trachomatis* detection, it has been shown that the sensitivity of culture, without passage in tissue culture, is less than 100%.[5,6] In addition, cell culture is labour-intensive, and requires specialised equipment and skilled staff; it is therefore expensive.

The use of urine specimens has been investigated as a non-invasive, non-traumatic, more acceptable alternative to urethral swabbing. In 1975, Smith and Weed[7] compared urethral swab with urine culture, found the sensitivity of the latter to be low and concluded that urine was not a suitable specimen for recovery of *Chlamydia* by culture. More recently, antigen-detection methods have been tested on urine samples, with varying results in men with urethritis.[8-11] Caul *et al.*[9] screened early morning urine samples by enzyme immunoassay (EIA) and found the method more reliable than testing urethral swabs for *C. trachomatis*, since 10 - 20% of swabs could turn out to be inadequate.[9] Chernesky *et al.*[11] detected *C. trachomatis* antigens with two different EIAs in 81,6 - 86,8% of first void urine, compared with 86,8% by urethral swab culture. However, Hay *et al.*[8] found that examination of urine by EIA (IDEIA III, Dako Diagnostic Ltd, UK) was a little less sensitive (89%) than by urine direct immunofluorescent antibody (DFA) test which was in turn a little less sensitive (82%) than urethral swab DFA.[8] Paul and Caul[12] used the direct visualisation of elementary bodies in urine deposits by direct immunofluorescence as a 'gold standard', because of loss of chlamydial infectivity in urine samples on culture. IDEIA III yielded the best results of the three EIAs.

The examination of urine has been found by some workers to be less reliable in women,[9,13] although Lebar *et al.*[14] obtained a sensitivity of 93,8% and a specificity of 99% in urine specimens using a third-generation EIA (IDEIA III), compared with tissue culture. In an attempt to establish the value of this non-invasive method in our patients, we used IDEIA III with urethral swab culture and/or DFA as reference to compare urine samples with urethral swabs from symptomatic men with urethritis who attended an STD clinic.

Patients and methods

A group of 370 black men with urethritis, who attended the STD clinic in Pretoria and had not received treatment in the past month, were investigated for the presence of *C. trachomatis.* Their ages ranged from 17 to 58 years with a mean of 30. Urethritis was defined as the presence of urethral discharge and urethral leukocytosis, on the basis of more than four polymorphs per high-power field.[15] This group of patients was also studied for the presence of *N. gonorrhoeae.*

Three endo-urethral swabs were collected from each patient for *C. trachomatis* EIA, DFA and culture by insertion of the swab 2 - 4 cm into the urethra so that samples for each test were taken first, second and third an equal number of times. Specimens for EIA were collected with Dacron swabs on aluminium shafts with prescored plastic handles. Swabs were immersed and broken off in 1 ml EIA transport media, conveyed to the laboratory and stored at

4°C if they were to be tested within 7 days; if not they were stored at –20°C until testing. The test was carried out according to the manufacturer's instruction.

For DFA (Imagen TM chlamydia, Dako, UK) the swab was made on a well area in a glass slide, air-dried, fixed in acetone for 5 minutes and kept at 4°C until stained within 5 days. The presence of ⩾ 5 elementary bodies was regarded as the positive cut-off point.

The urethral swabs for chlamydial culture were immersed in 2 ml standard transport medium (sucrose phosphate buffer 2-SP).[16] Culture was performed on cycloheximide McCoy cells by standard methodology.[17]

Urine was obtained from patients after urethral swabbing.[10] About 15 - 20 ml of first voided early morning urine — failing this, the patient was required to have retained urine for 1 - 2 hours prior to collection — were collected in a sterile container and conveyed to the laboratory. Urine specimens were centrifuged at 3 500 rpm in a bench-top centrifuge (Rotor Uni II) for 15 minutes and the supernatant was discarded. Urine EIA was done according to the manufacturer's instruction.

Because of the multiple tests carried out on each patient, we chose to regard specimens as positive when urethral swab culture and/or urethral swab DFA was positive. The sensitivities and specificities, together with positive and negative predictive values of urine testing by EIA compared with urethral swabs, were investigated and calculated according to published procedures.[18]

Results

Table I shows the expanded 'gold standard' of tissue culture and/or DFA on urethral swabs compared with urethral swab EIA and urine EIA. The incidence of *C. trachomatis* based on the above standard was found to be 96/370 (26%). The sensitivity and specificity of urethral swab EIA (Table II) were 97% and 99% respectively, with positive and negative predictive values of 96% and 99% respectively. In comparison, the sensitivity and specificity of urine EIA were 94% and 99% respectively, with positive and negative predictive values of 96% and 98%. The sensitivity of urine EIA was thus only 3% lower than that of urethral swab EIA and the same specificity was found in both tests.

Table I. Tissue culture and DFA on urethral swabs compared with urethral swab EIA and urine EIA (*N* = 370)

		Urethral swab EIA		Urine EIA	
		+	–	+	–
Culture and/or DFA	+	93	3	90	6
on urethral swabs	–	4	270	4*	270

* Two of the 4 were confirmed by DFA as positives.

Table II. Sensitivities, specificities and predictive values of urethral swab EIA and urine EIA with chlamydial culture and/or DFA as reference standard

	Swab EIA positive		Urine EIA positive	
	No.	(%)	No.	(%)
Sensitivity	93/96	97	90/96	94
Specificity	270/274	99	270/274	99
Positive predictive value	93/97	96	90/94	96
Negative predictive value	270/273	99	270/276	98

Confirmatory DFA carried out on the urine deposits of 94 patients who were positive on urine EIA yielded 92 positives, but 2 of 4 deposits that were positive on urine EIA only were not confirmed.

Culture alone detected 79/94 (84%) positives detected by DFA and 79/97 (81%) of those detected by urethral swab EIA. Since the majority (14/15) of those positive on urethral swab DFA were also positive on urethral swab EIA, it was evident that culture was not sensitive, necessitating the use of culture and DFA as 'gold standard'. We also found that 312 (84%) patients were positive for *N. gonorrhoeae,* of whom 234 (63%) had *N. gonorrhoeae* without *C. trachomatis* and 78 (21%) had both *N. gonorrhoeae* and *C. trachomatis* isolated. Therefore only 58 (16%) of the patients tested had non-gonococcal urethritis, of whom 18 (31%) had *C. trachomatis* infection.

Discussion

The EIA used to detect *C. trachomatis* antigens in urine in this study yielded sensitivity and specificity rates which were almost as high as those when the same EIA was used on urethral swabs. The results of the third-generation test were superior to those given by the earlier generation EIAs tested by other authors such as Hay *et al.*,[8] Caul *et al.*[9] and Chernesky.[11] Our results compared well with or were better than EIAs from other manufacturers used on urine specimens.[10,11,19-21]

The results of culture of urethral swabs were unsatisfactory in this study, with culture detecting only 84% and 81% of the positives detected by DFA and EIA respectively. The reasons for this may be that chlamydial viability tends to decrease during transport and by approximately 20% during storage at –70°C,[22] the order of specimen collection, lack of multiple blind passage as well as toxicity to monolayers during culturing. It was therefore necessary to expand the 'gold standard' to include specimens positive on DFA, i.e. by direct visualisation of fluorescing chlamydial elementary bodies. Because of the difficulties and expense of culture we agree that culture should best be carried out in selected reference laboratories and that antigen detection methods, particularly EIA, should be encouraged in symptomatic patients. The majority of patients in our study (about 6 out of the 7 with urethritis) were also suffering from gonorrhoea, indicating that gonococcal urethritis remains a serious problem in black patients in South Africa.[23,24]

Of the 4 patients in our study who tested positive for *C. trachomatis* in urine but negative on urethral swab, 2 were confirmed positive by DFA on urine. Various reasons may explain urine-positive samples which were urethral swab-negative. A sample positive on urine only may also indicate an infection higher up in the tract rather than in the distal portion of the urethra. The taking of invasive samples from the urethra may be painful and traumatic, with the result that some samples may not be taken in an optimal way and could therefore falsely be regarded as negative.[10,25] Some patients may bleed when urethral swabs are taken (as was the case with 3 of our STD patients, 1 of whom was urine-positive only), thus impairing the quality of the specimen.

In conclusion, the use of urine as a specimen for diagnosis of *C. trachomatis* in symptomatic men can be recommended because it is almost as reliable as examination of urethral swabs. The advantage to the patient of submitting a urine specimen is that this procedure is non-invasive and non-traumatic.

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