Leukocyte esterase urine strips for the screening of men with urethritis—use in developing countries

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Abstract

**Background and Objectives**—The leukocyte esterase (LE) strip is a useful tool for the screening of men with urethritis. In developing countries, where laboratory facilities are limited, and sexually transmitted diseases endemic, simple and inexpensive diagnostic tests which perform well, would be of great value.

**Methods**—Men presenting with urethritis to a referral clinic for sexually transmitted diseases in Nairobi, Kenya participated in this cohort analytical study. First-void urine was collected for LE dipstick testing as part of the diagnostic work-up. The results of the dipstick measurement were compared with the laboratory detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae*.

**Results**—Of 200 men with symptoms of urethritis, 33 (17%) had a pathogen detected from the urethra or the urine. Chlamydia was detected in urine by PCR in 22 (11%), and gonorrhoea was cultured from the urethra in 11 (6%). Esterase activity (trace or greater) had a sensitivity of 76%, a specificity of 90%, a positive predictive value of 42% and a negative predictive value of 94% for the presence of chlamydia or gonorrhoea.

**Conclusions**—The use of the LE dipstick for the screening of men with symptomatic urethritis can improve diagnostic accuracy and reduce the amount of empiric antimicrobial therapy. The low detection rate of chlamydia in these men with a clinical diagnosis of nongonococcal urethritis needs further study.

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**Introduction**

The diagnosis and treatment of sexually transmitted diseases (STDs) has traditionally been a neglected health issue in developing countries. A major limitation of STD control initiatives has been the lack of accurate diagnostic testing. As a result, many STD patients in developing countries receive empiric treatment, which leads to poor clinical outcomes and the inappropriate use of antibiotics. Simple and inexpensive diagnostic tests are needed to improve the clinical diagnosis of common STDs in resource poor countries. The Leukocyte Esterase (LE) urine dipstick is an example of such a test. The dipstick is inexpensive, easy to use, and could greatly facilitate the evaluation of urethritis in settings where diagnostic facilities are unavailable.

The LE dipstick has been evaluated primarily for the screening of asymptomatic men who are carriers of *C. trachomatis* or *N. gonorrhoeae*. It has great potential in identifying the large population of asymptomatic individuals who are the major transmitters of chlamydial infections. It has also been evaluated as an initial screening tool for men who present with symptoms of urethritis or are at high risk for STDs. The sensitivity of the LE dipstick test has been consistently high in both the low and high prevalence groups.

This study was conducted to evaluate the performance of the LE dipstick in Nairobi, Kenya, where STDs are common and resources are limited. The study was also intended to document the prevalence of *C. trachomatis* in this population, by optimising detection with the use of Chlamydiazyme enzyme immunoassay and polymerase chain reaction (PCR).

**Methods**

**Setting**—The study was conducted at the Nairobi City Commission Special Treatment Clinic, which serves as a large referral centre for STD patients from Nairobi and the surrounding areas. Men with STD complaints are routinely evaluated by a physician who makes a clinical diagnosis and assigns treatment. During the month of June 1992, two clinic physicians were asked to refer only those men with clinical findings consistent with non-gonococcal urethritis (NGU). A clinical algorithm was used which identified only those men with non-purulent urethral discharge or symptoms of dysuria with no evidence of urethral discharge. This was done to exclude men with obvious purulent discharge, consistent with *N. gonorrhoeae*, who received alternative treatment. The study was approved by the University of Nairobi’s Scientific and Ethical Review Committee.

**Subjects**—Two hundred consecutive men with clinical symptoms of NGU were referred for study enrollment by the clinic physicians. All men were between the ages of 17 and 40 years, otherwise thought to be in good health, and not currently taking antibiotics. The study protocol was explained to each patient, which included pre-test counselling for HIV-1. There were no patients who refused
to participate in the study following referral. Each patient gave verbal informed consent, including three men who agreed to participate in the study, but refused HIV testing. A brief questionnaire dealing with demographic information, sexual behaviour, past STDs and current symptoms was administered by a trained research assistant. The men were then asked to provide 25 ml of first-void urine in a pre-marked plastic container. A genital examination was performed to document the presence of urethral discharge, genital ulcers or inguinal swelling. Two urethral swabs were obtained from each patient. The first swab was streaked directly onto a plate containing modified Thayer-Martin media, and the second swab was transported in the Chlamydiazyme transport tube. Blood was then drawn for HIV-1 testing. All men received empiric treatment for chlamydial infection with a 3 g dose of tetracycline, to be taken 4 times daily for 7 days. Follow-up was arranged at 10 to 14 days, to assess the response to treatment, to give information about STD prevention and condom use, and to receive HIV-1 test results. All patients with positive HIV-1 results received individual counselling and were offered continued follow-up at the clinic.

Laboratory assessment—Specimens collected at the clinic were transported to the laboratory for processing within 4 hours. All specimens were assessed by individual technicians who had no knowledge of the other test results. The specimens for gonorrhoea culture were incubated at 35°C and inspected at 48 hours for colony formation. N gonorrhoeae was identified by characteristic oxidase-positive gram negative diplococci. The second urethral swab was processed by Chlamydiazyme (Abbott Laboratories, North Chicago, IL).

The leukocyte esterase test strip (Chemstrip 2 LN, Boehringer Mannheim Corp., Indianapolis, IN) was immersed in uncentrifuged, unspun specimens in the clinic, and the enzymatic activity quantified as negative, trace, 1 + or 2 +, using a colorimetric chart provided by the manufacturer. All dipsticks were read at 2 minutes. The urine specimens were then concentrated by centrifugation at 2000 × g for 20 minutes at 4°C. The urine sediment was resuspended in 2 ml of Chlamydiazyme diluent, and the specimen divided into two 1-0 ml vials. One vial was processed by Chlamydiazyme in Nairobi and the other frozen at −20°C for subsequent confirmatory Chlamydiazyme and PCR testing in Canada.

The frozen specimens were subsequently thawed for repeat Chlamydiazyme testing and testing for C trachomatis by a plasmid-based polymerase chain reaction (PCR). The DNA target for amplification was a 241 base pair (bp) sequence of the genetically conserved cryptic plasmid. KL1 and KL2 primers amplified plasmid DNA from all C trachomatis serovars, and yielded Hind III digestion products of 167 and 74 bp that were resolvable on 5% polyacrylamide gels. A total of 10 μl of the Chlamydiazyme treated urine sediment was amplified in a 100 μl reaction as described previously. PCR positive specimens were confirmed by a second confirmatory PCR by using primers T1 and T2 which amplify a 517 bp fragment of plasmid DNA. PCR with primers T1 and T2 was performed as described, with the addition of 5 × 10^-4 M tetramethylammonium chloride to enhance specificity, using 35 cycles of amplification.

Serological testing for HIV-1 was performed using an enzyme-linked immunosorbent assay (HIV-1 ELISA. Organon, Geneva). Positive specimens were confirmed using a second synthetic peptide based HIV-1 ELISA (HIV-1 ELISA. Behring). Indeterminate tests were resolved by Western Blot as described (Biorad).

Statistical analysis—Univariable analyses were performed with the chi square, Fisher's exact test and standard nonparametric statistical tests as appropriate. A significance level, alpha, was chosen to be 0.05 (two-tailed).

Results

Study Group—The demographic characteristics and sexual activity was recorded for the study participants. The mean age was 27.4, SD 6.8 years. Eighty-eight (44%) of the men were married. The mean number of different sex partners reported in the preceding 3 months was 1.5, SD 1.2, in the past one year was 3.3, SD 4.5 and lifetime was 18.5, SD 24.9. The presumed source of the current infection was reported to be from a woman working as a prostitute by 62 (31%), from a "pick-up" or a woman previously unknown by 66 (33%), from a girlfriend by 48 (24%), from a spouse by 14 (7%), and the source was unknown in 10 (5%) cases.

A history of past STDs was common. Prior treatment for urethral discharge was reported by 100 (50%) of the men. Of those with a past discharge, 34% reported one previous episode, 34% reported two previous episodes, 14% reported three previous episodes and 18% reported more than three past episodes of urethral discharge. A history of genital ulceration was reported by 55 (28%). HIV-1 seropositivity was detected by ELISA in 15 (8%).

Symptoms at presentation—The severity of symptoms at presentation were recorded for dysuria and urethral discharge. Dysuria was described as none, occasional or regular and urethral discharge as none, light or heavy. Men with occasional dysuria or light discharge were considered to have mild symptoms and men with regular dysuria or heavy discharge were considered to have severe symptoms. A pathogen was subsequently identified in 16 (13%) of 120 men with mild symptoms and 18 (23%) of the 80 men with severe symptoms (p = 0.13; OR 1-9, 95% CI 0.8-4.2).

Aetiology of symptoms—The aetiology of the urethritis was confirmed in 33 (17%) of the
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The performance of the LE dipstick in this study, was similar to previous studies which have consistently shown high sensitivity and specificity. The performance, however, has been somewhat dependent on the population being studied and the diagnostic method chosen for comparison. A study of 55 men attending an STD clinic in Tanzania reported the sensitivity of the LE test in detecting urethral infection to be 94% and the specificity 53%. This group included 32 men with urethral discharge, nine men with urethral symptoms without evidence of discharge, and 14 men with other complaints. Although the performance of the LE test was similar in this population, differences in the diagnostic methods and in the selection of patients prevent direct comparisons with our study. In a group of asymptomatic adolescent male detainees the sensitivity was 100% and the specificity 83% when compared with culture.

In a group of symptomatic men attending an STD clinic, the sensitivity was 100% and the specificity 55% when compared with microscopy. A large study of asymptomatic young men with low prevalence of disease, reported a sensitivity of 88-9% and a specificity of 97-7% when compared to chlamydial PCR. The men in our study are a unique group, in that despite their symptoms, the detection of a causative pathogen was found in only 17%. The reasons for this low infection rate are unclear.

The clinical screening process was intended to select men with chlamydial infections. At the time of the study an algorithm for urethral discharge was being evaluated at the clinic which separated men into two groups. Those with purulent urethral discharge were treated for gonococcal urethritis and those with dysuria and/or non-purulent urethral discharge were treated for chlamydial infection. Although such a clinical decision may lead to unacceptably high misclassification, the anticipated effect would be to exclude the majority of men with gonococcal infections and include men with chlamydial infections. The chance of detecting chlamydia, however, was optimised, not only by strict inclusion criteria, but also by rigorous diagnostic testing. The decision to forego simpler diagnostic tests such as Gram stain and a two glass urine test in this study was based on two considerations. Firstly, past experience at the clinic had illustrated the difficulty in performing and maintaining these test procedures and secondly, with the availability of ELISA and PCR which could be performed outside the clinic, it was decided that the simpler tests would add little to the diagnosis. This decision does not minimise the potential use of these simpler tests in future clinic routines where ELISA and PCR are not available. The use of urine PCR as the gold standard was chosen because of its excellent previous performance and the convenience of using PCR on stored urine samples. The PCR appeared to perform well, as it confirmed all of the Chlamydiazyme positive urine specimens and detected four additional samples. The four urine specimens not

### Table 1 Performance of the Urinary Leukocyte Esterase Strip by Activity Level (N = 200)

<table>
<thead>
<tr>
<th>Leukocyte esterase strip reactions</th>
<th>≥ trace</th>
<th>≥ 1+</th>
<th>2+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlamydia or Gonorrhoea Detection*</td>
<td>(+)</td>
<td>(-)</td>
<td>(-)</td>
</tr>
<tr>
<td>Esterase Activity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>32</td>
<td>111</td>
<td>25</td>
</tr>
<tr>
<td>Negative</td>
<td>1</td>
<td>56</td>
<td>8</td>
</tr>
<tr>
<td>Esterase (+)%, %</td>
<td>72</td>
<td>30</td>
<td>15</td>
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<tr>
<td>Sensitivity, %</td>
<td>97</td>
<td>76</td>
<td>36</td>
</tr>
<tr>
<td>Specificity, %</td>
<td>36</td>
<td>80</td>
<td>90</td>
</tr>
<tr>
<td>Predictive Value, %</td>
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<td>41</td>
</tr>
<tr>
<td>Positive</td>
<td>98</td>
<td>94</td>
<td>88</td>
</tr>
<tr>
<td>Negative</td>
<td></td>
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</tbody>
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*Positive for Neisseria gonorrhoeae by culture or Chlamydia trachomatis by PCR.

200 study participants. Eleven men had a positive culture for N gonorrhoeae and 22 men had C. trachomatis identified in the urine by PCR. There were no dual infections. Initial Chlamydialysis testing in Nairobi found 18 specimens to be positive on both the urine and urethral specimens, and an additional two specimens to be positive on the urethral swabs only. Repeat Chlamydialysis testing on all 200 of the stored urine specimens, confirmed the 18 positive samples, but was negative for the two positive urethral specimens. The PCR confirmed all 18 of the positive Chlamydialysis specimens, and was negative for the two urethral swab samples which had only been positive in Nairobi. There were also four additional urine specimens which were positive by PCR only and all four were confirmed with the second set of PCR primers. For the purpose of the analysis the two patients with positive urethral swab tests were considered negative. Both of these patients were culture positive for N gonorrhoeae.

Performance of the LE dipstick—The performance profile of the esterase dipstick is recorded in table 1, using 3 cut-off levels. The percent sensitivity, specificity and predictive values are optimal at a cut-off value of greater than or equal to 1+; 76, 80, 42 and 94 respectively.

Discussion

This study demonstrates the usefulness of the LE dipstick in the screening of men with symptomatic urethritis in settings with limited diagnostic facilities. It is evident from this study that the use of empiric therapy for the treatment of urethritis could be significantly reduced, if men without detectable urinary esterase could be appropriately excluded from receiving antibiotic treatment. In this study, if urine specimens showing esterase activity of greater than or equal to 1+ were accepted as positive, then 71% (141/200) of the men could have avoided treatment, and only eight documented STDs would have been missed. Ten of the 11 gonococcal infections would have been detected using this criterion. If the cut-off was chosen as greater than or equal to trace, 29% (57/200) of the men would not have received treatment, and only one chlamydial infection would have gone untreated.
detected by Chlamydiyzyme may be explained by the dilution of the urine sediment, as in order to produce enough specimen for PCR, the urine was diluted in 2 ml of Chlamydiyzyme diluent instead of the recommended 1 ml. The low isolation rate of chlamydia at the Special Treatment Clinic has been noted previously (unpublished), but has been attributed to biased selection of patients, poor specimen collection and inconsistent laboratory techniques. Although all of these factors may still be contributing to the low detection rate in this study, it appears C trachomatis may not be the responsible pathogen in many of these men.

If the diagnostic tests for C trachomatis were indeed optimized then other alternative explanations for the poor detection rate must be considered. It is possible that men chosen for the study had complaints of urethritis which were exaggerated; however, it is unlikely that these men would endure long line-ups and pay money to be examined for trivial symptoms. Another explanation could be previous antibiotic treatment which may influence the chlamydizyme and PCR detection rates. Although men who were currently taking antibiotics were excluded from the study, information regarding prior antibiotic use was not obtained. A recent study conducted at the same clinic in a group of 819 men with genital ulcers, found that 42% had received treatment elsewhere. It is possible that undiagnosed pathogens were responsible for the symptoms. The identification of unusual pathogens causing urethritis have largely come from studies conducted in Western countries where diagnostic facilities are available. Ureaplasma urealyticum, Trichomonas vaginalis, herpes simplex virus and Haemophilus species, are all potential candidates in the aetiology of NGU which have not been adequately explored in developing countries. The role of these and other pathogens needs to be investigated in this population. Other sexually transmitted aetiologies have also been associated with dysuria. Urinary tract infections, bacterial prostatitis, urethral stricture, schistosomiasis, tumours and chemical irritation are other potential causes of dysuria. However, in this population of sexually active men with acute symptoms and recent sexual exposure, it is unlikely that these alternative aetiologies accounted for much disease.

The HIV-1 seroprevalence of 7.5% in this group was lower than expected. A consistent finding in men attending STD clinics in sub-Saharan Africa has been the very high prevalence of HIV-1. Recent studies from the same clinic have shown a seroprevalence of 21-1% in men with genital ulcers and 12-3% among men with urethritis. This indicates that the type of STD exposure may be an important factor in contracting HIV. Although a high proportion of these men reported sexual contacts with whom they would not consider to be at risk for HIV exposure, it appears that there may be differences in the risk of contracting HIV between men who present with nonpurulent urethritis, purulent urethritis and genital ulcer disease.

The value of the LE dipstick in this population was convincingly demonstrated, despite uncertainty regarding the aetiology of the urethritis. In settings with severely limited resources and high infection rates, the distribution of antibiotics could be optimised. The LE dipstick has great potential in aiding decisions regarding antibiotic use in men with urethritis, if men without positive urine specimens are excluded. Further studies are needed to evaluate the LE strip in other similar populations, and to identify the aetiology of NGU in men without C trachomatis infections.

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