MAJOR ARTICLE

# The Use of Specimens from Various Genitourinary Sites in Men, to Detect *Trichomonas vaginalis* Infection

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**Variations in estimates of prevalence of trichomoniasis in men may reflect true differences in the burden of disease but are also affected by the performance of diagnostic methods and the type of specimen tested. In this study, men were evaluated at baseline and at follow-up, to evaluate syndromic management of urethritis and the effects of human immunodeficiency virus and trichomoniasis, in Lilongwe, Malawi. First-void urine specimens and urethral swabs were obtained at enrollment, for *Trichomonas vaginalis* culture; semen specimens were also obtained at follow-up. The sensitivities of testing methods using urine specimens and urethral swabs were equal; 67% of cases were identified by use of either specimen, and, in 47% of cases, both specimens tested positive. When semen specimens were included, all 3 specimens tested positive in only 19% of cases. Semen was the most sensitive single specimen, and, in 25.6% of cases, only semen specimens tested positive. Thus, prevalence of *T. vaginalis* infection in men is underestimated if only 1 specimen is tested.**

Although *Trichomonas vaginalis* infection is a recognized cause of morbidity in women, trichomoniasis in men is not as well understood. In men, trichomoniasis is associated with urethritis [1–5] and prostatitis [5–7]. In addition, infection with *T. vaginalis* may increase HIV transmission [3, 8, 9]. Recent estimates of prevalence of trichomoniasis for men range from 6% of asymptomatic men [10] to 20% of men with urethritis [3], in Africa, and from 4% to 17% of men attending sexually transmitted disease (STD) clinics in the United States [11–13]. The reliability of estimates of prevalence, understanding the natural history and sequelae of trichomoniasis, the role of men as vectors for high prevalences in women, and the efficacy of treatment are all affected by difficulties with diagnosing trichomoniasis in men. Sensitivity of microscopic examination of wet mounts or cultures ranges from 60% to 80% [1]. Nucleic acid amplification tests are more sensitive [11, 12, 14] but are not yet in routine use. For men, the type of specimen tested may be as important as the method used to detect *T. vaginalis*. Organisms have been isolated from the urethra, prostate, urine, and external genitalia [1, 15]. The use of specimens from multiple sites may increase the sensitivity of testing [1].

The locations of *T. vaginalis* during infection in the male urogenital tract may also affect treatment effectiveness. Metronidazole is the currently accepted treatment and is considered to be highly effective, but resistant isolates have been described and may be increasing in number [16]. It is not clear whether the drug is equally successful in eliminating infections in all urogenital sites in men, since most studies use only a single specimen for detection of trichomonads, and no study has clearly documented the effectiveness of

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metronidazole in clearing parasites from seminal or urinary structures. In addition, observations documenting the self-limited nature of *T. vaginalis* infection have not included detection of organisms from multiple sites. In the present study, we cultured multiple specimens from >1300 Malawian men for *T. vaginalis,* to determine the relative sensitivities of different urogenital specimens and to examine the consequences of using a single specimen for testing.

## SUBJECTS AND METHODS

***Study population.*** Study subjects were enrolled from the STD and dermatology clinics of Lilongwe Central Hospital, Malawi, between 27 January 2000 and 28 June 2001. This study was conducted in the context of 2 other studies. One was a clinical trial of the addition of metronidazole to syndromic treatment of urethritis in men [17]. For the clinical trial, men presented with symptoms of urethritis and were randomized to receive either metronidazole or placebo in an effort to improve rates of persistent urethritis. The second study was a longitudinal study of HIV and *T. vaginalis* infection in men. For the longitudinal study, in addition to men with urethritis enrolled from the STD clinic, HIV-positive men from the dermatology clinic were enrolled as control subjects. For the present study, men evaluated for the clinical trial or the longitudinal study who were eligible and were ⩾18 years old were requested, and agreed, to return to the clinic for follow-up visits for up to 5 weeks after enrollment, and all subjects provided written, informed consent to participate.

The study was approved by the University of North Carolina at Chapel Hill School of Medicine Institutional Review Board and by the Health Sciences Research Committee in Lilongwe, Malawi. The study was explained by study personnel in Chichewa, the local language, and consent forms were provided in English and Chichewa.

***Baseline clinic visit.*** At enrollment, all subjects were administered a standardized questionnaire that included questions about symptoms, sexual behavior, current medications, and a medical history. Specimens were obtained during a standardized physical exam. Blood specimens were obtained for HIV and syphilis serologic testing. HIV testing was performed by use of the Capillus HIV-1/HIV-2 rapid test (Cambridge Diagnostics Ireland); reactive specimens were confirmed by use of EIA (Genetic Systems). Syphilis serologic testing was performed by use of the Rapid Plasma Reagin test (Becton Dickinson). Urethral swabs were obtained for *T. vaginalis* culture and Gram stain. Urethritis was defined as ⩾4 white blood cells/high-power field on Gram stain. Infection with *Neisseria gonorrhoeae* was diagnosed by the presence of gram-negative intracellular diplococci in the urethral swabs. After the physical exam, subjects were requested to provide 20–30 mL of first-void urine for *T. vaginalis* culture.

***Follow-up clinic visits.*** All subjects returned to the clinic for diagnostic test results 1 week after enrollment. Men enrolled from the dermatology clinic who were HIV seropositive with no diagnosis of any other STD and men enrolled from either clinic who were infected with *T. vaginalis* at baseline were invited to continue in the study. All subjects continuing in the study were administered a standardized questionnaire, provided specimens, and were requested to attend the clinic for 1 or 2 additional visits over a period of up to 4 weeks.

Three urogenital specimens were obtained at follow-up. Urethral swabs were obtained for Gram stain and *T. vaginalis* culture. First-void urine specimens were obtained for *T. vaginalis* culture, and subjects were asked to provide a semen specimen for *T. vaginalis* culture. All men diagnosed with *T. vaginalis* were treated with 2 g of metronidazole orally and were asked to return to the clinic 1–2 weeks later for additional cultures, to confirm treatment effectiveness. Subjects infected with *T. vaginalis* who failed to return to the clinic for follow-up were contacted at their homes or places of business by a study nurse and were requested to return to the clinic or were treated on-site.

**T. vaginalis *culture.*** Urethral swabs were used to inoculate the InPouch TV culture system (Biomed) immediately after collection. For urine specimen culture, 10 mL of urine was centrifuged at 1500 *g* for 10 min, and 50 μL of sediment was used to inoculate a culture pouch. Semen was allowed to liquefy at ambient temperature for up to 1 h before processing. Liquefied semen was centrifuged at 2000 *g* for 10 min, and ~50 μL of sediment was used to inoculate a culture pouch. Cultures were incubated in room air at 37°C. A trained microscopist examined all cultures for a minimum of 1 min each, on days 2 and 5 after inoculation; however, most cultures were examined daily for 5 days or until a positive result was obtained. A positive culture was defined as the visualization of parasites with morphology and motility characteristic of *T. vaginalis.* No motile parasites were observed in negative cultures at any reading.

***Data management and analysis.*** Data were double-entered into databases in Microsoft Excel 2000. Discrepancies were resolved by review of original laboratory data forms. Data were analyzed using Stata (version 7.0; StataCorp) and SAS (version 7; SAS Institute).

Cultures of urine specimens, urethral swabs, and semen specimens were compared for each subject at each clinic visit. Infection with *T. vaginalis* was defined as the presence of motile trichomonads in at least 1 of the specimen cultures. The sensitivity of each specimen type was calculated relative to the other specimen types (for example, the proportion of cases detected by use of urine specimen culture was compared with the proportion of cases detected by use of urethral swab or semen specimen culture). To account for repeated visits and

subsequent specimen collection from some subjects, the sensitivity estimates of each type of specimen and 95% confidence intervals (CIs) were calculated by use of logistic regression, using generalized estimating equations with robust variance estimates and an independent correlation matrix. In multivariate analysis, covariates included HIV serostatus and the presence or absence of urethritis. For the difference in proportions of *T. vaginalis* cases with accompanying *N. gonorrhoeae* and urethritis, by use of only specimens obtained at baseline, a Mantel-Haenszel $\chi^2$ test was used.

## RESULTS

***Study population.*** During the study period, 1361 subjects were enrolled from Lilongwe Central Hospital, including 929 from the STD clinic (850 were evaluated in the context of the clinical trial [17]) and 431 from the dermatology clinic. Subjects ranged in age from 18 to 63 years (mean ± SD, 26.2 ± 6.1 years). Most subjects reported current employment, and approximately one-half were married at the time of enrollment (table 1). The number of sex partners during the 4 previous weeks reported at enrollment ranged from none to >30, with >80% reporting ⩽1 sex partner during the previous 4 weeks.

Of the 929 study participants enrolled from the STD clinic, 43.7% ($n = 406$) were infected with *N. gonorrhoeae,* 46.7% ($n = 434$) were seropositive for HIV, 6.8% ($n = 63$) had positive syphilis serologic test results, and 9.0% ($n = 84$) had culture-proven *T. vaginalis* infection at the initial clinic visit. Of the 432 subjects enrolled from the dermatology clinic, 0.2% ($n = 1$) were infected with *N. gonorrhoeae,* 27.6% ($n = 119$) were seropositive for HIV, 4.4% ($n = 19$) had positive syphilis serologic test results, and 3.3% ($n = 14$) had culture-proven *T. vaginalis* infection at the initial clinic visit.

At follow-up visits, semen specimens were requested from subjects identified to have *T. vaginalis* infection at the initial clinic visit and from HIV-positive persons from the dermatology clinic (on the basis of the requirements for the longitudinal study of HIV and trichomoniasis). Subjects who provided at least 1 semen specimen at a clinic visit tended to be slightly older and had fewer signs/symptoms of STDs than the overall study population (table 1).

***Comparison of urine specimen and urethral swab cultures.*** We obtained and cultured 1701 sets of urine specimens and urethral swabs at baseline and at all follow-up visits. At least 1 specimen from each of 143 sets of specimens tested positive for *T. vaginalis.* The sensitivities of urine specimen and urethral swab culture were equal, with each correctly diagnosing 95 cases of trichomoniasis (figure 1*A*). One-third of cases were identified by use of the urine specimen culture only, one-third of cases by use of the urethral swab culture only, and, in the remaining cases, cultures using both specimens were positive. The sensitivities of urethral swab and urine specimen cultures were 66.3% (95% CI, 57.6%–73.8%) and 66.4% (95% CI, 56.6%–73.8%), respectively. The slight differences in the point estimates and CIs are due to the lack of independence of some specimens.

**Table 1. Characteristics of study participants at enrollment**

| Characteristic | All subjects ($N = 1361$) | Subjects who provided semen specimens ($n = 146$) |
|---|---|---|
| Age, range, years | | |
| 18–22 | 411 (30.2) | 24 (16.4) |
| 23–27 | 509 (37.4) | 48 (32.9) |
| 28–32 | 264 (19.4) | 40 (27.4) |
| 33–37 | 96 (7.1) | 20 (13.7) |
| 38–42 | 53 (3.9) | 11 (7.5) |
| >42 | 28 (2.1) | 3 (2.1) |
| Married | 597 (43.9) | 78 (53.4) |
| Currently employed | 1070 (78.6) | 117 (80.1) |
| Sex partners during previous 4 weeks | | |
| 0 | 298 (21.9) | 39 (26.7) |
| 1 | 773 (56.8) | 81 (55.5) |
| 2 | 210 (15.4) | 18 (12.3) |
| ⩾3 | 67 (4.9) | 8 (5.5) |
| Signs/symptoms | | |
| Complaint of urethral discharge | 515 (37.9) | 42 (28.8) |
| Genital ulcer disease[a] | 534 (39.3) | 49 (33.6) |
| Dysuria | 623 (45.8) | 45 (30.8) |
| Observed urethral discharge | 530 (38.9) | 44 (30.1) |
| Urethritis[b] | 593 (43.6) | 56 (38.4) |
| STD[c] | | |
| *T. vaginalis* infection | 100 (7.4) | 67 (45.9) |
| *N. gonorrhoeae* infection | 407 (30.0) | 16 (11.0) |
| HIV infection | 553 (40.6) | 110 (75.3) |
| *T. pallidum* infection | 82 (6.0) | 12 (8.2) |

**NOTE.** Data are no. (%) of subjects. STD, sexually transmitted disease.
[a] Observed by clinician.
[b] Defined as ⩾4 white blood cells/high-power field.
[c] *Trichomonas vaginalis* infection diagnosed by use of culture of urethral swab or urine specimen; *Neisseria gonorrhoeae* infection diagnosed by the presence of gram-negative intracellular diplococci on Gram's stain; HIV infection diagnosed by use of Capillus rapid test and EIA; and presumed *Treponema pallidum* infection diagnosed by use of rapid plasma reagin test.

Infection with *N. gonorrhoeae* was diagnosed and treated in all subjects at their initial clinic visit. Among cases of trichomoniasis, gonorrhea was not associated with trichomoniasis detected by use of either the urine specimen culture ($P = .67$, $\chi^2$ test) or urethral swab culture ($P = .61$, $\chi^2$ test) at baseline.

***Comparison of urine specimen, urethral swab, and semen specimen cultures.*** We obtained 288 complete sets of urine specimens, urethral swabs, and semen specimens from 146 subjects (mean, 2.0 visits/subject) at follow-up visits. At least 1

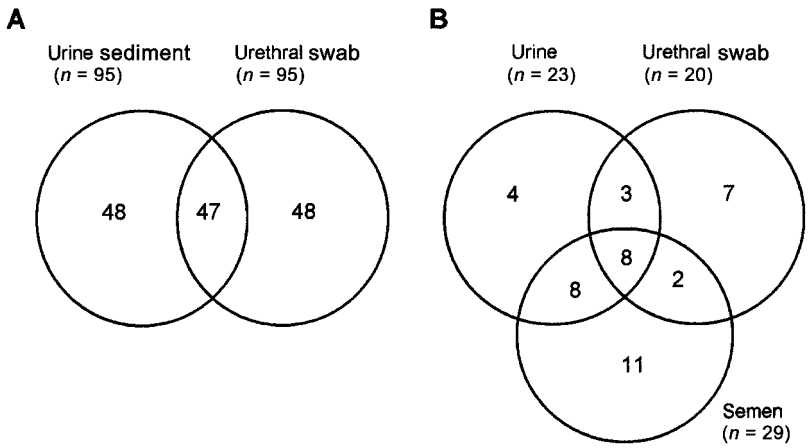


**Figure 1.** Detection of *Trichomonas vaginalis* in different urogenital specimens from men. *A,* Cases detected by use of urine specimen culture and urethral swab culture. A total of 143 infections were identified from a total of 1701 complete sets of specimens. *B,* Cases detected by use of urine specimen, urethral swab, and semen specimen culture. A total of 43 infections were identified from a total of 288 complete sets of specimens.

specimen from each of 43 sets of specimens (obtained from 35 subjects) tested positive for *T. vaginalis*. Of the 43 sets of specimens for which at least 1 specimen tested positive, 20 included a positive urethral swab, 23 included a positive urine specimen, and 29 included a positive semen specimen (figure 1*B*). All 3 specimens tested positive in only 18.6% ($n = 8$) of cases. Accounting for the lack of independence of the observations, the sensitivity of urethral swab culture was 62.2% (95% CI, 53.6%–69.8%), that of urine specimen culture was 61.6% (95% CI, 53.0%–69.3%), and that of semen specimen culture was 66.7% (95% CI, 49.6%–79.7%). Eleven cases (25.6%) were detected by use of semen specimen culture only.

Subjects were stratified by HIV serostatus (figure 2*A*) and by the presence or absence of urethritis (figure 2*B*). Urethral swab culture appeared to be most sensitive among HIV-positive subjects. Semen specimen culture was slightly, but not significantly, more sensitive among HIV-negative subjects. All 3 specimens had similar sensitivities among subjects with urethritis. Semen specimen culture was, again, slightly, but not significantly, more sensitive among subjects without urethritis.

***Longitudinal variation in specimen positivity.*** There were 92 subjects who provided a urethral swab, urine specimen, and semen specimen on >1 visit. Among subjects who were diagnosed with *T. vaginalis* infection on at least 2 occasions, the type of specimen(s) that tested positive changed at least once for 59.5%. In some cases, an individual specimen type was positive on >1 visit, but an additional specimen type was positive at a later or earlier visit. In other cases, the positive specimen(s) appeared to change completely between visits.

## DISCUSSION

The results of the present study reemphasize the importance of obtaining multiple specimens for detection of *T. vaginalis* infection in men. In the larger sample set, which contained paired urine specimens and urethral swabs, the use of each specimen was equally likely to detect culturable trichomonads; however, the use of urine specimen or urethral swab culture alone would have identified only 67% of cases detected by use of both specimens. Among subjects with trichomoniasis for whom urine specimens, urethral swabs, and semen specimens were cultured, semen was the most sensitive specimen, and all 3 specimens (urine, urethral swab, and semen) tested positive for *T. vaginalis* for only a few subjects.

The positive specimens may, but do not necessarily, reflect the actual site of *T. vaginalis* infection in the male urogenital tract. Specimens were always obtained in the same order: urethral swab, urine, and semen. Cases in which trichomonads were detected in only the semen specimen suggest infection higher in the genitourinary tract than cases in which only the urethral swab tested positive. A positive urine specimen may represent infection of the bladder or may contain parasites washed from the proximal urethra. The sensitivities of semen and urine specimen cultures calculated in the present study were probably underestimated. Had the semen or urine been the only specimen obtained, more cultures may have been positive by virtue of passing through an undisturbed urethra.

The present results are consistent with those of previous studies. Krieger et al. [18] documented recovery of *T. vaginalis* from the urethra, first-void urine, external genitalia, and semen in a study of 50 men with trichomoniasis in 1993. In an early study of husbands of women with trichomoniasis, by Watt and Jennison [19], *T. vaginalis* was detected in urine and urethral specimens and prostatic fluid, with prostatic fluid being the most sensitive single specimen. In that study, the same specimens were positive on repeat examination for fewer than half of the subjects [19]. Changes in the type of specimen that tested

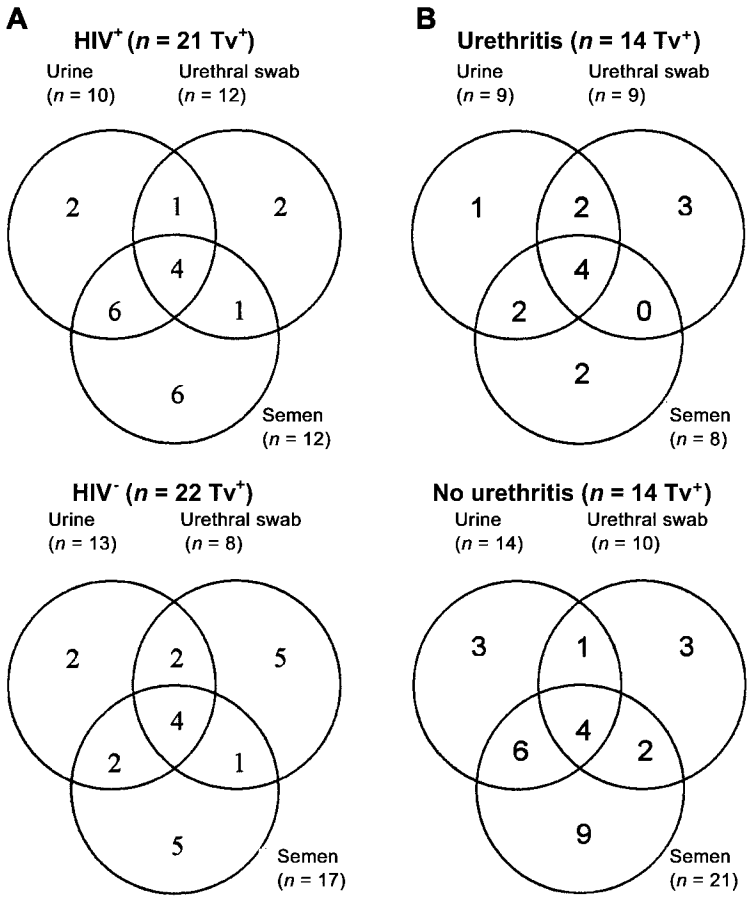


**Figure 2.** Effect of HIV status and urethritis on *Trichomonas vaginalis* (Tv) culture using different urogenital specimens from men. *A,* Comparison of HIV-positive (HIV$^+$) and HIV-negative (HIV$^-$) subjects. There were 232 complete sets of specimens for HIV$^+$ subjects and 56 complete sets of specimens for HIV$^-$ subjects. *B,* Comparison of subjects with urethritis (>4 polymorphonuclear cells/high-power field) and those without urethritis. There were 40 complete sets of specimens for subjects with urethritis and 246 complete sets of specimens for subjects without urethritis.

positive at different times may have several possible explanations. Specimens may not accurately reflect the anatomical site of the infection, or the location of trichomonads within the urogenital tract may change at different times during infection. Alternatively, culture may be insufficiently sensitive to consistently detect *T. vaginalis* in all specimens.

In the present study, laboratory-confirmed urethritis was associated with the type of specimen that tested positive. At enrollment, detection of trichomonads in urine specimens and urethral swabs was equally likely in the presence or absence of urethritis. However, at follow-up visits, for subjects from whom all 3 specimens were obtained and cultured, urethral swabs and urine specimens were more sensitive among subjects with urethritis than among subjects without urethritis. This may have been due to treatment of gonorrhea during the initial visit. At follow-up visits, a higher proportion of urethritis would have been trichomonal urethritis, with potentially more organisms, and, thus, would have been better detected in urethral swab or urine specimen cultures. Semen appeared to be more sensitive among subjects without urethritis, compared with subjects with urethritis.

Regardless of where *T. vaginalis* infects men, subjects with culturable *T. vaginalis* in semen represent a clinically and epidemiologically important group. Since these infections are likely to be transmitted to partners through sexual intercourse, they may be the most important cases to diagnose correctly. The location of *T. vaginalis* colonization in the male reproductive tract deserves further investigation, to identify the most appropriate specimens with maximal sensitivity for detection. In addition, studies of the efficacy of treatment with metronidazole in men, by use of specimens from multiple urogential compartments, are needed to substantiate or improve existing treatment regimens.

The improvement in detection of *T. vaginalis* infections, resulting from culturing multiple specimens, is critically important for the design and interpretation of research studies. When fewer specimens are obtained, reported prevalences of trichomoniasis in men are likely much lower than the true prevalences

in the populations studied. In the present study, if only urethral swabs had been cultured at the initial visit, we would have observed a 4.7% prevalence of trichomoniasis (64/1361 subjects). Using both urine specimen and urethral swab cultures, the prevalence was 7.3% (99/1361 subjects). Using results of the semen specimen culture and extrapolating to the entire study population, the resulting estimate of the prevalence of trichomoniasis was 10.9% (148/1361 subjects). The use of multiple specimens increased the number of cases by 31% (20/64 subjects). On the basis of the assumption that these phenomena are not restricted to this population, other reported estimates of *T. vaginalis* infection in men may be underestimated by a similar magnitude.

Culture is generally acknowledged to be insensitive for the diagnosis of trichomoniasis, especially in men. Nucleic acid amplification tests provide increased sensitivity, and several polymerase chain reaction (PCR) assays have been described for the detection of *T. vaginalis* in men [11, 12, 14]. Nucleic acid amplification tests may be sufficiently sensitive to detect trichomonads from a single specimen in circumstances in which culture of multiple specimens would be required. Studies are in progress to examine the detection of *T. vaginalis* in multiple specimens from men by use of PCR.

In the present study, urine specimens, urethral swabs, and semen specimens together identified the greatest number of cases of trichomoniasis. The omission of any 1 of the 3 specimens would have resulted in missed identification of cases. Urine specimens or urethral swabs are routinely used to detect *T. vaginalis.* In many situations, the collection of semen specimens may be problematic because of logistical issues, budget constraints, and reluctance of subjects to provide the specimen. In many busy clinic settings, thorough microscopic examination of >1 specimen/subject may be prohibitively time-consuming. Since routine collection of multiple specimens for detection of *T. vaginalis* is unlikely, recognition of the potential for serious underestimation of the prevalence of this infection in men is crucial. The development of new diagnostic testing methods able to identify more infections by use of a single specimen is critical to advancing our understanding of the importance of *T. vaginalis* infection in men.

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