Use of Spun Urine to Enhance Detection of Trichomonas vaginalis in Adolescent Women

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Background: Diagnosis of Trichomonas vaginalis infection is traditionally performed by microscopic examination of vaginal fluid. Although this technique is relatively insensitive compared with culture, it is widely used because of its lower cost and immediate results.

Objective: To assess the utility of microscopic examination of spun urine as a means of increasing the sensitivity of microscopic diagnosis of T vaginalis.

Design and Setting: Retrospective observational study performed in a hospital-based adolescent clinic.

Subjects: Female patients enrolled between July 1995 and August 1996 into a larger study evaluating diagnosis of vaginal infections (N = 686). To be included, subjects had to have a positive culture for T vaginalis (n = 97); those who did not have a spun urine examination were excluded (n = 22).

Main Outcome Measure: Microscopic examination of vaginal fluid and spun urine for presence of motile trichomonads. Using a positive Trichomonas culture as the reference standard, the sensitivity of vaginal fluid alone was compared with vaginal fluid plus spun urine. The McNemar test for paired samples was used to test the statistical significance of the difference in sensitivities.

Results: Ninety-seven subjects had culture results positive for Trichomonas. Of these, 75 (77%) had a spun urine examination performed. Subjects were aged 13 to 22 years and all were African American. Seventy-three percent of the infections were detected by vaginal fluid specimen, 64% by spun urine, and 85% by either vaginal specimen or spun urine. The difference in sensitivity between vaginal specimen alone and vaginal specimen plus spun urine was 12% (95% confidence interval, 3%-21%; P < .005). Nine patients who would not have been diagnosed by examination of vaginal fluid alone were diagnosed with the addition of spun urine examination.

Conclusion: Microscopic examination of a spun urine specimen performed in conjunction with microscopic examination of a vaginal fluid specimen improves the detection rate of T vaginalis.


Editor's Note: I'm all for anything that would increase the detection of sexually transmitted diseases without further invasion. Catherine D. DeAngelis, MD

Trichomoniasis is a common sexually transmitted disease. Although the infection is not reportable, prevalence rates in the United States suggest that approximately 6 million women and men are infected annually.1 Trichomonas vaginalis, the causative organism, is a motile protozoan parasite that infects the urogenital tract. Typical symptoms in women include vaginal discharge, dysuria, vulvar pruritis, vulvovaginal irritation, and occasionally lower abdominal pain. Nevertheless, as many as 50% of infections are asymptomatic.2

Demonstration of motile trichomonads by microscopic examination of vaginal fluid or wet mount has long been the standard method of diagnosis, with a sensitivity of 35% to 92%, depending on the skill of the microscopist.3-7 Most investigators report an average sensitivity of 60% to 80%. Culture using Diamond medium is considered to be far superior to wet mount examination, with a sensitivity of 91% to 100%.3,7,9 However, the disadvantages of culture are that it requires 2 to 7 days to obtain a result, is more expensive than wet mount examination, and the culture media has a relatively short shelf life.
PATIENTS, MATERIALS, AND METHODS

DESIGN
We conducted a retrospective study comparing the sensitivity of microscopic examination of vaginal fluid alone to the combined sensitivity of microscopic examination of vaginal fluid and spun urine for detection of *T vaginalis*.

STUDY POPULATION
The study was conducted in the Johns Hopkins Hospital Adolescent Clinics (Baltimore, Md), which provide primary care to predominantly urban youth. Young women aged 12 to 22 years who were enrolled between July 5, 1995, and August 13, 1996, in a larger prospective study evaluating diagnosis of vaginal infections (N = 686) were eligible for inclusion. Study protocol and consent procedures for the larger study from which these data were abstracted were approved by the institutional review board of the Johns Hopkins Medical Institutions. The prospective study protocol did not specify inclusion of a urinalysis. However, many patients received urinalysis as part of their routine care. Patients were included in this retrospective study if they had had a culture positive for *Trichomonas* (n = 97). Patients were excluded if a spun urine examination was not performed as part of their usual care or the results of the examination were not available (n = 22), yielding a final sample of 75.

SPECIMEN COLLECTION, TRANSPORT, AND PREPARATION
Spun Urine Specimen
As part of routine care, many patients had a urine specimen collected some time during the course of their visit. Although no systematic criteria were used to determine who received spun urine examination, discussion with the clinic nurses indicated that patients receiving yearly physical examinations or those with genitourinary symptoms were more likely to have urine collected for microscopic examination. Urine specimens were transported to the clinic’s on-site laboratory, where the clinic laboratory technician centrifuged the specimens at 1163 g for 5 minutes. The supernatant was decanted and the sediment resuspended in the drop of supernatant remaining in the centrifuge tube. The resuspended sediment was examined under the microscope at ×400 power. In most cases, urine was examined within 30 minutes of collection. However, if the urinalysis was performed following the vaginal fluid wet mount examination, the laboratory technician may have been aware of the vaginal fluid results. If trichomonads were noted during routine urinalysis, the laboratory technician recorded this result on the clinical laboratory urinalysis form.

Vaginal Fluid Specimens for Wet Mount and Culture
Following insertion of a nonlubricated speculum, vaginal fluid specimens were collected with cotton swabs for microscopic wet mount examination and for *Trichomonas* culture. Cotton swabs for wet mount examination were placed into a test tube containing 0.25 mL of saline. Cotton swabs for *Trichomonas* culture were placed into modified Diamond medium (REMEI Inc, Lenexa, Kan).

Immediately following completion of the pelvic examination, the vaginal fluid specimens were transported to the clinic’s laboratory where a research assistant prepared vaginal fluid wet mounts, which were then microscopically examined for motile trichomonads. The research assistant was blinded to the results of the laboratory technician’s spun urine examination. The *Trichomonas* cultures were immediately placed into an incubator at 37°C. Cultures were evaluated daily, except on weekends, by microscopic examination for up to 7 days. Incubator temperature was checked and recorded daily except on weekends.

DATA COLLECTION
The larger study’s data log was reviewed, and results of the vaginal fluid wet mount examinations and *Trichomonas* cultures were abstracted. *Trichomonas* culture served as the reference standard for a diagnosis of trichomoniasis. Urinalysis reports for patients with trichomoniasis were then retrieved and results were abstracted. Of the original 686 patients, 5 had positive vaginal fluid wet mounts but cultures were negative *Trichomonas*. These patients were not included in the main analysis; however, they were incorporated into 2 of the sensitivity analyses.

STATISTICAL METHODS
The *t* test, Pearson χ² test, and Fisher exact test were used to determine if differences in patient characteristics existed between the group of patients who had urine available (included) and the group who did not (excluded). Sensitivity of microscopic examination of vaginal fluid wet mount, spun urine, and wet mount plus spun urine as compared with the reference standard was calculated. The McNemar test for paired samples was used to test for a difference in sensitivities between the vaginal fluid wet mount alone and the wet mount plus spun urine. A 95% confidence interval (CI), corrected for continuity, was constructed around the absolute difference in sensitivities.

Although other methods of detection are available, they are not widely used. This may be due to their expense, their need for highly trained technicians, or their relatively low sensitivity and specificity. Some of these other methods include Papanicolaou smear, direct immunofluorescence assay, direct enzyme immunoassay, DNA probe assay, and latex agglutination tests. Polymerase chain reaction tests for detection of *T vaginalis* are being developed but are not yet commercially available.

Trichomonads are often incidentally noted during microscopic examination of female patients’ spun urine specimens. If clinicians have access to a centrifuge, addition of a spun urine examination to routine wet mount examination might enhance detection of *T vaginalis* without adding significant expense. We sought to determine whether spun urine examination enhances detection of *T vaginalis* when combined with microscopic examination of vaginal fluid.
CHARACTERISTICS OF STUDY POPULATION

Ninety-seven patients had positive Trichomonas cultures. Of these, 75 (77%) had a spun urine examination performed and were included in the analysis. The mean age and racial distribution of the group who did not have urine available (excluded) did not differ significantly from the group who did have urine available (included). Although the proportion of patients presenting with some symptom consistent with trichomoniasis was similar between groups, 14% of excluded subjects vs 0% of included subjects (P = .01) had vaginal itch as a presenting symptom.

DETECTION OF TRICHOMONIASIS

The Table shows the number of infections detected by each method or combination of methods. Vaginal wet mount plus spun urine resulted in detection of 12% more infections than vaginal wet mount alone. This difference is statistically significant, with a 2-sided P < .005.

SENSITIVITY ANALYSES

Twenty-two (23%) of the patients with cultures positive for Trichomonas were excluded because urinalysis results were not available. A sensitivity analysis was conducted to estimate the effect that these excluded patients would have had on the results had their urine samples all been negative. Seventeen of the 22 excluded patients had positive vaginal fluid specimens. If all of these patients had negative spun urine specimens, there still would have been a 10% difference (P < .005) between vaginal fluid specimen alone and vaginal fluid specimen plus spun urine specimen.

A second sensitivity analysis was conducted to estimate the effect that the patients with cultures negative for Trichomonas but positive vaginal fluid wet mounts would have had on the results if all of their urine samples were negative. Had this been the case, there would still have been an 11% difference (P < .005) between vaginal fluid specimen alone and vaginal fluid specimen plus spun urine specimen. If the 2 sensitivity analyses are combined for a worst-case scenario in which the spun urine specimens from the 22 excluded patients and the spun urine specimens from the 5 patients who had positive wet mounts but cultures negative for Trichomonas were all negative, the difference between the 2 groups (vaginal fluid alone vs vaginal fluid plus spun urine) would still be 9% (P < .005).

Even in the hands of a very skilled microscopist, vaginal fluid wet mount is usually significantly less sensitive than culture because more organisms are needed to yield a positive wet mount than to yield a positive culture. Because T vaginalis infects the urethra as well as the vagina, urine represents another potential source for microscopic examination. In our study, we found that 9 patients with negative vaginal wet mounts had positive urine specimens. If a clinician or clinical laboratory technician has a centrifuge available, addition of a spun urine microscopic examination would significantly enhance detection of T vaginalis without substantially increasing the cost of detection. However, it should be noted that microscopic examination of vaginal fluid alone was more sensitive than microscopic examination of spun urine alone. Examination of spun urine does not replace examination of vaginal fluid but rather complements it.

This retrospective study was limited by the exclusion of almost one quarter of the eligible patients because urinalysis results were unavailable. There was no systematic bias determining who received urinalysis; however, patients with genitourinary symptoms or those receiving routine physical examinations (likely asymptomatic) were more likely to be asked for a urine specimen. Nevertheless, patient demographics and the proportion of patients having at least 1 symptom consistent with trichomoniasis was similar in both groups. Furthermore, a sensitivity analysis evaluating the effect that the excluded patients would have had on the results if all excluded patients had had negative spun urine specimens with positive vaginal fluid wet mounts demonstrated that the difference in sensitivity between the vaginal specimen alone and the vaginal specimen plus the urine specimen would not have changed significantly.

We did not attempt to obtain urinalysis results for patients who had cultures negative for Trichomonas. Consequently, it is not possible to determine the specificity of microscopic examination of urine specimens for detection of trichomonads. Nevertheless, both the reference standard and the urine specimen rely on the same outcome measure, namely, detection of motile trichomonads during microscopic examination. Therefore, the likelihood of a false-positive urine result is very low. Additionally, even if trichomonads were only present in the urethra and bladder and not present in the vagina, they would still be considered a pathogen and worthy of diagnosis and treatment. Therefore, we believe that inability to determine specificity is only a minor limitation of this study.

Sensitivity of Vaginal Fluid Wet Mount, Spun Urine, and Vaginal Fluid Wet Mount Plus Spun Urine for Detection of Trichomonas vaginalis in 75 Patients

<table>
<thead>
<tr>
<th>Specimen</th>
<th>No. (%) Detected</th>
<th>Difference in Sensitivity, % (95% Confidence Interval)*</th>
<th>McNemar Test 2-Tailed P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaginal wet mount</td>
<td>55 (73)</td>
<td>-9 (-24 to 5)</td>
<td>.01</td>
</tr>
<tr>
<td>Spun urine</td>
<td>48 (64)</td>
<td>-9 (-33 to 1)</td>
<td>.23</td>
</tr>
<tr>
<td>Vaginal wet mount plus spun urine</td>
<td>64 (85)</td>
<td>12 (3 to 21)</td>
<td>&lt;.005</td>
</tr>
</tbody>
</table>

* Compared with vaginal wet mount.
† Ellipses indicate not applicable. Vaginal wet mount used as reference for McNemar test and for difference in sensitivity.

COMMENT

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Despite these limitations, we have demonstrated that the addition of spun urine examination to routine vaginal fluid wet mount examination improves the sensitivity of microscopic examination for detection of *T vaginalis*.

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REFERENCES