Polymerase Chain Reaction Analysis of Distal Vaginal Specimens: A Less Invasive Strategy for Detection of *Trichomonas vaginalis*

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We compared polymerase chain reaction (PCR) analysis of specimens obtained from the distal vagina with wet mount microscopy and culture of specimens from the posterior vaginal fornix. One or all three techniques revealed that 61 (20.3%) of 300 women tested were positive for *Trichomonas vaginalis*. PCR analysis of distal vaginal specimens detected 56 (91.8%) of 61 infections, while wet mount microscopy and culture detected 49 (80.3%) of 61 infections. Results of this study may impact the approach to testing for *T. vaginalis* by eliminating the requirement of a vaginal speculum examination. The distal vagina is an appropriate testing site for *T. vaginalis* by PCR analysis.

*Trichomonas vaginalis* is the most common nonviral agent of sexually transmitted disease (STD) in the world; two to four million cases of *T. vaginalis* infection occur annually in the United States [1]. *T. vaginalis* is an important cause of vaginitis, and studies have demonstrated an association between trichomonas vaginitis and adverse outcomes of pregnancy, postabortion pelvic inflammatory disease, and posthysterectomy vaginal cuff cellulitis and abscess [2-5]. Recently, infection with *T. vaginalis* has been associated with an increased risk of acquisition of HIV infection [6]. Clearly, the identification and treatment of trichomoniasis have important public health benefits.

The diagnostic gold standard for *T. vaginalis* infection is culture of vaginal fluid from the posterior vaginal fornix [1]. These specimens are obtained during a vaginal speculum examination; this examination must be performed by skilled personnel, and it is an uncomfortable procedure that may limit compliance to undergo testing. Optimally, a noninvasive sampling technique would greatly enhance screening for *T. vaginalis* infection. By using PCR analysis, we have shown that the distal vagina (introitus) is the single best testing site for *Chlamydia trachomatis* [7]. The purpose of the current study was to evaluate the distal vagina as a less invasive sampling site for the detection of *T. vaginalis*.

**Methods**

Three-hundred women attending the Sexually Transmitted Diseases Clinic at the Allegheny County Health Department in Pittsburgh were nonconsecutively enrolled in the study. Regardless of symptoms, all women undergoing STD testing were eligible for enrollment if research personnel were in attendance at the clinic. No exclusion criteria were established. Before vaginal speculum examination, health care providers inserted a Dacron swab (Baxter Healthcare, Deerfield, IL) ~1 inch into the distal vagina for 10 seconds and inoculated the specimen into PCR transport media (Amplicor *C. trachomatis* Test, Roche Diagnostic Systems, Branchburg, NJ). The examination was then performed, and specimens from the posterior vaginal fornix were obtained for *T. vaginalis* testing by wet mount microscopy and culture.

*Wet mount microscopy.* Vaginal secretions from the posterior fornix were mixed in two drops of 0.9% normal saline on a clean microscope slide, and a coverslip was placed. Microscopy was performed within 5 minutes; the slide was examined at magnifications of ×100 and ×400 for the presence of motile trichomonads in at least 10 high-power fields.

*Culture.* Specimens collected from the posterior fornix were inoculated into 5 mL of Trichosel broth (Becton-Dickinson Microbiology Systems, Cockeysville, MD) supplemented with 5% horse serum and were transported to the on-site microbiology laboratory within 10 minutes. Cultures were performed on specimens from women for whom wet mount microscopy was negative for motile trichomonads. The specimens were incubated at 37°C and examined daily for the presence of motile trichomonads from incubation days 3 to 5 unless they were found to be positive by day 4 or earlier.

*PCR analysis.* Twenty-five microliters of the introitus samples was mixed with an equal volume of reaction mixture containing 10 mM Tris-HCl (pH 8.3), 0.5 U of *Taq* polymerase, 1.5 mM MgSO₄, 50 mM KCl, 200 mM deoxyadenosine triphosphate, 200 mM deoxythymidine triphosphate, 200 mM deoxyguanosine triphosphate, 200 mM deoxycytidine triphosphate, and two oligonucleotide primer pairs specific for a 102-bp region of the *T. vaginalis* genome [8]. The samples were vortexed, microcentrifuged, and subjected to 40 cycles of sequential incubations at 94°C for 1 minute and 47°C for 1 minute and 72°C for 1 minute.
1 minute that were followed by a 7-minute extension cycle at 67°C. A portion of the final reaction product was digested with the restriction endonuclease Hin fl to verify that the amplification products were true-positive results. The reaction products were subjected to electrophoresis on 6% polyacrylamide gels, stained with ethidium bromide, and visualized under ultraviolet light. A sample was considered positive for T. vaginalis if it yielded a 102-bp band that was cleaved by Hin fl to 50- and 46-bp bands [8].

Statistical analysis. The statistic \( \kappa \) was calculated to correlate the agreement between results of PCR analysis of distal vaginal specimens and results of wet mount microscopy and culture of specimens from the posterior vaginal fornix. An excellent correlation was defined as a \( \kappa \) of >.75, and a marginal correlation was defined as a \( \kappa \) of <.4.

Results

Wet mount microscopy, culture, or PCR analysis revealed that 61 (20.3%) of the 300 women who were screened had evidence of infection with T. vaginalis. No women had received antitrichomonal treatment in the 2 weeks before presentation. The results of PCR analysis and wet mount microscopy and culture are presented in table 1. The sensitivity of PCR analysis of distal vaginal samples was 91.8%, while that of wet mount microscopy and culture was 80.3%. The correlation between PCR analysis of distal vaginal specimens and wet mount microscopy and culture of specimens from the posterior vaginal fornix was excellent (\( \kappa = .80 \)).

Discussion

Noninvasive testing strategies utilizing amplification technologies are the future of STD diagnostics. First-catch urine specimens for the detection of C. trachomatis and Neisseria gonorrhoeae are the focus of numerous investigations; however, because of the anatomic separation of the urethra and cervix as well as the rigid handling criteria for urine specimens, we have focused on the distal vagina as a site for specimen collection. In this study, we found an excellent correlation between wet mount microscopy and culture of samples from the posterior vaginal fornix (the standard techniques for detecting T. vaginalis) and PCR analysis of specimens from the distal vagina near the introitus. We did not evaluate wet mount microscopy and culture of distal vaginal specimens or PCR analysis of specimens from the posterior vaginal fornix.

Results for 17 women were discrepant. There were 12 specimens that were positive by PCR analysis and negative by wet mount microscopy and culture. Our procedure for detection of amplification products includes digestion with the restriction endonuclease Hin fl that is specific for T. vaginalis, which confirms positive results. Therefore, we believe that these results represent true infections (i.e., false-negative results of wet mount microscopy and culture). This finding is not surprising as both wet mount microscopy and culture are based on organism viability and quantity, while PCR testing is not constrained by either variable.

Theoretically, infections with low levels of parasite may lead to false-negative results of culture and wet mount microscopy but to positive results of PCR analysis. Further, use of Trichosel media has been shown to be less sensitive than use of the more expensive Diamonds media (Remel Microbiology Products, Lenexa, KS) [9]. It should be stressed, however, that no culture medium is 100% sensitive for the growth of T. vaginalis.

Five specimens were positive by wet mount microscopy and culture but negative by PCR analysis; these specimens represent true infections (i.e., false-negative results of PCR analysis). Given the fact that the characteristic motility of T. vaginalis is required for confirmation, false-positive results of wet mount microscopy and culture are not encountered. Sampling error could account for the false-positive results of PCR analysis; however, protein concentrations were equivalent in the samples (this fact indirectly documents the adequacy of the specimens). It is possible that these results can be ascribed to the presence of PCR inhibitors in vaginal secretions. Inhibitors have been implicated in false-negative results of PCR analysis for C. trachomatis [10].

Noninvasive strategies for specimen collection either by the health care provider or by the patient alone are necessary to improve widespread efforts at STD screening. Eliminating the requirement of gynecologic examinations by health care personnel will likely increase patient access to STD testing, particularly in underserved regions. Further, patients reluctant to undergo a vaginal speculum examination, such as adolescents, would more likely agree to screening. Self-collection of genital specimens may also improve compliance to undergo STD testing by imparting a sense of privacy. Both health care provider-collected and self-collected vaginal swabs have been shown to be sensitive for detecting C. trachomatis by PCR analysis [7].

The present study demonstrates that PCR analysis of health care provider-collected vaginal swabs was more sensitive than the traditional methods of detection. Definitive studies evaluat-

### Table 1. Results of PCR analysis and wet mount microscopy and culture for the detection of Trichomonas vaginalis in 300 vaginal specimens.

<table>
<thead>
<tr>
<th>PCR analysis</th>
<th>Wet mount microscopy and culture</th>
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<tbody>
<tr>
<td></td>
<td>Positive</td>
</tr>
<tr>
<td>Positive</td>
<td>44</td>
</tr>
<tr>
<td>Negative</td>
<td>5</td>
</tr>
<tr>
<td>Total</td>
<td>49</td>
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NOTE. Results are given as no. of samples. \( \kappa = .80 \).
ing self-collection of distal vaginal specimens for the detection of T. vaginalis and other agents of STDs are under way. We stress that self-collection and subsequent testing should augment rather than replace the interaction between health care provider and patient. Self-testing should not replace regular gynecologic examinations, as these visits provide the opportunity for counseling on STDs, contraception, and health care maintenance as well as Pap smear testing and other essential components of women’s health care.

References