SHORT REPORT

Comparison of dacron and nylon-flocked self-collected vaginal swabs and urine for the detection of *Trichomonas vaginalis* using analyte-specific reagents in a transcription-mediated amplification assay

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ABSTRACT

Objectives To compare self-collected vaginal swab (SCVS) types and first-catch urine (FCU) to diagnose *Trichomonas vaginalis* using analyte-specific reagents designed to be used in a transcription-mediated amplification assay.

Methods A total of 241 women (group A) collected a FCU and a SCVS using a dacron swab (APTIMA collection kit). A second group of 289 women (group B) collected two SCVS using one dacron swab and one nylon-flocked swab.

Results Of 75 young women (street youth) determined to be infected with *T vaginalis* only seven reported symptoms of vaginal discharge or irritation. Using a cutoff of 50 000 relative light units, the sensitivity and specificity was 97.2% and 97.6%, respectively for dacron SCVS compared with 41.7% and 100% for FCU in group A; 92.3% and 98.8% for dacron SCVS and 92.3% and 99.2% for flocked-nylon SCVS in group B. The assay tested 96 samples in 6 h.

Conclusions Dacron and nylon-flocked SCVS performed equally well and significantly better than FCU using analyte-specific reagents in the APTIMA transcription-mediated amplification assay. Either swab type could be used for self-collection.

Women infected with *Trichomonas vaginalis* are at an increased risk of acquiring HIV and having increased HIV viral shedding.1 *T vaginalis* infection doubles the risk of persistent human papillomavirus infection in women2 and has also been associated with pelvic inflammatory disease,3 premature delivery and low birth weight.5 Molecular methods and less invasive sampling are required to identify asymptomatic infections, which are reported to be substantial.6–8

The study objectives were to compare self-collected vaginal swabs (SCVS) and first-catch urine (FCU) using analyte-specific reagents designed to be used in a transcription-mediated amplification assay. Analyte-specific reagents can be purchased to build an assay for a specific analyte using APTIMA general purpose reagents. The package insert contains guidelines to be used by the operator, who sets the cut-off for positivity in the test. These methods are different from US Food and Drug Administration-cleared kits in the USA.

METHODS

The study was approved by St Joseph’s Healthcare Research Ethics Board. From April to September 2007, 550 women attending a street youth clinic or community health centre for a regular health examination were invited to participate in the study. After a nurse explained the study, patients signed consent and provided two self-collected samples. In group A, 241 women collected a dacron swab taken from an APTIMA collection kit (Gen-Probe Inc, San Diego, California, USA) and a FCU (first 20 ml). The swab was placed into a tube containing Gen-Probe APTIMA specimen transportation media. In group B, 289 women collected a dacron swab and a nylon-flocked swab (Copan Italia, Brescia, Italy). Both swabs were transported individually in APTIMA specimen transportation media tubes.

Specimens were processed within 48 h. For the transcription-mediated amplification test, APTIMA general purpose reagents (Gen-Probe Inc) were used and the assay was performed on a semi-automated direct tube sampling platform. The lyophilised enzyme from the analyte-specific reagent package was suspended with enzyme reconstitution buffer. Three amplification solution (75 ml) was added to the hybridisation reagent. Acidinium ester-labelled probe (50 ml) was added to the hybridisation reagent. Target capture (50 ml) was added to the 250 test kit capture reagent. Acidinium ester-labelled probe (50 ml) was added to the hybridisation reagent. Target capture reagent (100 ml) and 400 ml of specimen or control were added to each reaction tube. The tubes were incubated at 62°C for 30 min followed by 50 min at room temperature. Reaction tubes were placed onto the magnetic base for 5–10 min. The solution was aspirated, discarded and replaced with 1 ml of wash solution (10 mM HEPES buffer). The reaction tubes were magnetised again for 5–10 min and the wash solution was aspirated and discarded. Oligonucleotide-spiked reconstituted amplification solution (75 ml) together with 200 ml of oil reagent was added to each tube and vortexed for 30 s before incubating the tubes at 62°C for 10 min followed by 5 min at 42°C. While still in the 42°C water bath, 25 ml of
the enzyme reagent was added to each tube, vortexed, then incubated at 42°C for 60 min. Oligonucleotide-spiked probe hybridisation reagent (100 µl) was then added to each tube and incubated at 62°C for 20 min, followed by room temperature for 5 min. Selection reagent (250 µl) was then added and incubated at 62°C for 10 min, followed by room temperature for 15 min. Reactions were read using a LEADER HC+ luminometer and a positive cut-off value of 50,000 relative light units (RLU) was used. The time to assay 96 samples was 6 h, which includes the technologist’s time. An alternative transcription-mediated amplification research use only (RUO) assay (Gen-Probe Inc) targeting a different region of ribosomal RNA was used with a cut-off value of 200,000 RLU on discordant samples. Blinded panels were sent to Gen-Probe for testing of discordant samples. Cut-offs were recommended by the manufacturer based on previously published data and personal communication. Patients were considered positive if the analyte-specific reagent assay was positive on two different samples or if a single analyte-specific reagent-positive sample was confirmed in the alternative assay. The McNemar test was used to calculate p values on pairwise comparisons, which were interpreted as significant at p<0.05.

The overall prevalence of T vaginalis infection was 14.2% (75/530, 95% CI 11.2 to 17.1); 14.9% (36/241) in group A and 13.5% (39/289) in group B. Only 9.3% (7/75) of infected women had clinical symptoms of trichomoniasis as reflected in a questionnaire that asked each patient to check a box for symptoms (vaginal discharge, irritation or itching), or a second box for no symptoms. In group A (table 1), the sensitivity and specificity of the dacron SCVS was 97.2% and 97.6% compared with the FCU, which was 41.7% and 100%, respectively (p<0.001).

Comparison of the two types of swabs in group B are summarised in table 1. Both the dacron and nylon-flocked SCVS demonstrated a sensitivity of 92.3% with specificities of 98.8% and 99.2%, respectively. Three samples were positive in the dacron swab and negative in the nylon-flocked swab, and another three were positive in the nylon-flocked swab and negative in the dacron swab. These six single positives were confirmed positive by the alternative transcription-mediated amplification RUO test. However, three dacron swabs and two nylon-flocked swab samples were negative in the alternative RUO test, reducing the specificity from 100%.

DISCUSSION

For the 75 women infected with T vaginalis, only 9.3% recorded having symptoms. Most studies and reviews discuss asymptomatic rates in T vaginalis-infected women to be greater than 50%. The higher rates in the current study may be due to a predominance of street youth clients, who have multiple partners, many infections and may perceive the presence of some symptoms to be normal. The results of using the analyte-specific reagents are similar to those reported in recently published studies, in which high sensitivities and specificities were achieved compared with traditional assays. The sensitivity (41.7%) of FCU testing by transcription-mediated amplification analyte-specific reagents in the present study was lower than that reported previously by transcription-mediated amplification and for PCR testing of urine (64.2%). PCR and transcription-mediated amplification methods reported no false positives when testing FCU. Agreement between dacron SCVS and confirmed positives was 97.9% (283/289) (κ=0.91, 0.84–0.98), between nylon-flocked SCVS and confirmed positives was 98.3% (284/294) (κ=0.95, 0.86–0.99), p=1.00.

Although the reference standard for comparing newer diagnostics for T vaginalis has been culture, our study compared transcription-mediated amplification analyte-specific reagents, which have been shown to identify virtually all culture-positive patients in other studies and found similar sensitivity and specificity values close to 100%. The assay is robust, has high sensitivity, specificity and predictive values can be achieved using various cut-off values; 50,000 RLU in this study compared with 30,000 or 100,000 used in other studies. SCVS have been shown to be effective and desirable for the diagnosis of sexually transmitted infections. This study demonstrated that nylon-flocked swabs that trap and release increased amounts of analyte for amplified diagnostic assays, performed equally well to dacron swabs for the diagnosis of T vaginalis. With high predictive values, both swab types could be used for self-collection and testing with the transcription-mediated amplification analyte-specific reagent protocol detailed in detail here, which required 6 h to test 96 samples.

Table 1  Sensitivity, specificity and predictive values of dacron or nylon-flocked SCVS and FCU using analyte-specific reagents in a transcription-mediated amplification assay

<table>
<thead>
<tr>
<th>Study group</th>
<th>Sample</th>
<th>% Sensitivity</th>
<th>% Specificity</th>
<th>% PPV</th>
<th>% NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>Dacron SCVS</td>
<td>97.2 (35/36)</td>
<td>97.6 (200/205)</td>
<td>87.5 (35/40)</td>
<td>99.5 (200/201)</td>
</tr>
<tr>
<td></td>
<td>FCU</td>
<td>41.7 (15/36)</td>
<td>100 (205/205)</td>
<td>100 (15/15)</td>
<td>90.7 (205/226)</td>
</tr>
<tr>
<td>Group B</td>
<td>Dacron SCVS</td>
<td>92.3 (36/39)</td>
<td>98.8 (247/250)</td>
<td>92.3 (36/39)</td>
<td>98.8 (247/250)</td>
</tr>
<tr>
<td></td>
<td>Nylon-flocked SCVS</td>
<td>92.3 (36/39)</td>
<td>99.2 (248/250)</td>
<td>94.7 (36/38)</td>
<td>98.8 (248/251)</td>
</tr>
</tbody>
</table>

SCVS versus FCU in group A p<0.001.

* A total of 36 true positives determined by both analyte-specific reagent results positive in SCVS and FCU, or a single analyte-specific reagent positive confirmed by an alternative RUO assay.

FCU, first-catch urine; NPV, negative predictive value; PPV, positive predictive value; RUO, research use only; SCVS, self-collected vaginal swabs.

Key messages

- There was a high rate of asymptomatic T vaginalis infections in female street youth.
- Transcription-mediated amplification analyte-specific reagents have high sensitivity and specificity for detecting T vaginalis.
- Self-collected vaginal dacron or flocked nylon swabs performed equally well.
REFERENCES

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