Detection of Trichomonas vaginalis in Canadian Women Using Transcription Mediated Amplification Analyte Specific Reagents on Self-Collected Vaginal Swabs

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REvised Abstract

Objective: To determine the prevalence of T. vaginalis by testing self-collected vaginal swabs by wet mount and a transcription mediated amplification (TMA) test which uses Gen-Probe analyte specific reagents (ASR).

Methods: 7,894 [n=7,894] ± 7,894. BioMed Diagnostics was grown in Diamond’s medium and ten-fold dilutions were made in sample transport medium [STM] before testing to determine the endpoint of detection using the T. vaginalis-ASR reagents [Gen-Probe Incorporated]. A cross-sectional study of 247 women attending a street youth clinic [n=174] and a community health centre [n=73] self-collected 2 vaginal swabs. The first dacron swab was collected and placed into an M40 tube [Copan] for wet mount examination within 24 hours. The second swab was placed into an STM tube for T. vaginalis ASR. Extra samples included a first catch urine [FCU] or a cervical swab [CS]. Patients were considered infected if the vaginal swab was positive in both wet mount and ASR or by wet mount or ASR only, but confirmed by having a CS or FCU positive by ASR or by a second research use only [RUSO] TMA test directed against alternate targets from a different reference region (ALT TMA). Both of the T. vaginalis - TMA tests use APTIMA® analyte specific reagents. A cutoff value of 150,000 RLU was used to define a positive TMA result.

Results: T. vaginalis detected in STM was detected by the ASR to a dilution of 10⁻¹. The 7.894 ± 7,894 prevalence was 18.3% [4,042/21.5% [371/45] at the street youth clinic and 13.4% [27/202] at the community health centre. The percent sensitivity and specificity of the ALT TMA test performed on self-collected vaginal swabs was 93.3% [4,245] and 97.5% [197/202] compared to wet mount which was 20.0% [9/45] and 100% [202/202].

Conclusions: This T. vaginalis-STM test using ASR was easy to perform and yielded clear results with self-collected vaginal swabs. A total of 174 patients from Evergreen Health Centre and 73 Hamilton Community Health Centre self collected two vaginal swabs (VS). One swab was placed into Gen-Probe sample transport media (STM) for T. vaginalis Analyte Specific Reagent (ASR) testing and the other swab was placed in Copan M40 transport media for wet mount microscopy to identify inflammatory cells, Candida, fungal or trophozoite organisms. All patients had either a cervical swab collected (90%) or a first catch urine (70%).

Endpoints Determination: a positive culture (in-Pouch™ TV+, BioMed Diagnostics) was grown in Diamond’s media.

Detection of Detection of M. Chernesky Gen-Probe TV-ASR Specimen Testing Protocol:

- The lyophilized Enzyme Reagent was reconstituted with the Enzyme Reconstitution Reagent.
- The lyophilized Primer-less Amplification Reagent was reconstituted with the Amplification Reconstitution Buffer.
- The lyophilized Oligo 1, primers Oligo 2 , and 50 µl of 1/10 000 dilution of Oligo 3 were added to the reconstituted amplification Oligo 1 and 50 µl of amplification Oligo 2 were added to the reconstituted amplification Oligo 2 .
- 50 µl of Target Capture Oligo was added to the 250 test Target Capture Reagent (TCR).
- 100 µl of the spiked TCR and 400 µl of specimens/controls were added to each reaction tube.
- The reaction tubes were magnetized again for 5 to 10 min, and the wash solution was aspirated and discarded.
- The reaction tubes were incubated at 62°C ± 1°C for 10 min followed with an incubation at 42°C ± 1°C for 5 min.
- While the tubes were still in the 42°C water bath 25 µl of the reconstituted enzyme reagent was added and then incubated at 42°C ± 1°C for 60 min.
- The reaction tubes were incubated at 62°C ± 1°C for 10 min followed with an incubation at 42°C ± 1°C for 5 min.
- The reaction tubes were incubated at 62°C ± 1°C for 10 min followed by a RT incubation for 5 min.
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- The reaction tubes were incubated at 62°C ± 1°C for 10 min followed with an incubation at 42°C ± 1°C for 5 min.

RESULTS

The prevalence of T. vaginalis infection was 20.1% [35/174] at the street youth clinic and 13.6% (10/73) at the community health centre. A run of 98 samples was processed in approximately 6 hours. The sensitivity of wet mount makes it unacceptable as a screening test.

OBJECTIVES

The objective of this study was to determine the prevalence of T. vaginalis by testing self-collected vaginal swabs by wet mount and a transcription mediated amplification [TMA] test which uses Gen-Probe analyte specific reagents [ASR].

METHODS

Sample Processing: Magnetic Particle Based Target Capture

Transcription-Mediated Amplification (TMA)

EndPoint Determination: a positive culture (in-Pouch™ TV+, BioMed Diagnostics) was grown in Diamond’s media. Test dilutions were determined and 10-fold serial dilutions were made in STM. Dilutions were tested in the TV-ASR assay.

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CONCLUSIONS

This T. vaginalis-TMA test using ASR was easy to perform and yielded clear results with self-collected vaginal swabs; the sensitivity was very high at 93.3% with few false positives and acceptable predictive values. A run of 98 samples was processed in approximately 6 hours. The sensitivity of wet mount makes it unacceptable as a screening test.

Sensitivity, Specificity and Predictive Values of the TV-ASR-TMA Test and Wet-Mount Microscopy on Self Collected VS

<table>
<thead>
<tr>
<th></th>
<th>TV-ASR Test</th>
<th>Wet-Mount Microscopy</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>%Sensitivity</td>
<td>%Specificity</td>
</tr>
<tr>
<td>TV-ASR-TMA</td>
<td>93.3 (42/45)</td>
<td>97.5 (197/202)</td>
</tr>
<tr>
<td>ALT-AMP</td>
<td>97.5 (197/202)</td>
<td>100 (202/202)</td>
</tr>
</tbody>
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Utilizes two enzymes: Reverse Transcriptase and T7 RNA Polymerase

- Amplifies RNA and DNA
- Exponential, > billion fold amplification in less than one hour
- Rapid and automatable: process 200 specimens in < 2 hours
- Ready to amplify
- Produces RNA amplicon
- Protein and plasma and potentially inhibitory substances
- Amplify by exponential, > billion fold amplification in less than one hour
- Rapid and automatable: process 200 specimens in < 2 hours
- Ready to amplify
- Produces RNA amplicon
- 0-10000

- Cutoff
- 1. Hybridization
- 2. Selection
- 3. Detection

- Target hybridized probe is detected by chemiluminescence
- Label on unhybridized probe is hydrolyzed, label on hybridized probe is protected
- Label on unhybridized probe is hydrolyzed, label on hybridized probe is protected
- Target hybridized probe is detected by chemiluminescence

Utilizes Acridinium Ester (AE) labeled probes

1. Hybridization
2. Selection
3. Detection

- Target hybridized probe is detected by chemiluminescence