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: *T. vaginalis* [in-Pouch™TV+, BioMed Diagnostics] was propagated 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 [RUO] TMA test directed against alternate targets from a different rRNA region [ALT TMA]. Both of the *T. vaginalis* -TMA tests use APTIMA® general purpose reagents. A cutoff value of 50,000 RLU was used to define a positive TMA result.

Results: *T. vaginalis* diluted in STM was detected by the ASR to a dilution of 10^{-8}. The *T. vaginalis* prevalence was 18.2% [45/247]; 20.1% [35/174] at the street youth clinic and 13.6% [10/73] at the community health centre. The percent sensitivity and specificity of the ASR-TMA test performed on self-collected vaginal swabs was 93.3 [42/45] and 97.5 [197/202] compared to wet mount which was 20.0 [9/45] and 100 [202/202].

Conclusions: This *T. vaginalis*-TMA test using ASR was easy to perform and yielded clear results with self-collected vaginal swabs. A run of 98 samples was processed in approximately 6 hours. The sensitivity of wet mount makes it unacceptable as a screening test.

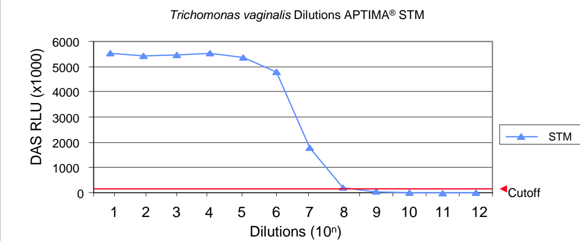
OBJECTIVES

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

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

ENDPOINT DETERMINATION TV-ASR



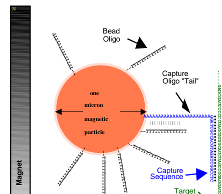
TV organisms diluted in AC2 STM were detected in the TV TMA assay to a dilution of 10^{-8}.

Patient Recruitment:
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%).

Gen-Probe TV-ASR Specimen Testing Protocol:
(The assay was performed using the APTIMA® General Purpose Reagents (GPR)

- The lyophilized Enzyme Reagent was reconstituted with the Enzyme Reconstitution Reagent.
- The lyophilized Primer-less Amplification Reagent was reconstituted with the Amplification Reconstitution Buffer.
- 50 µl of amplification Oligo 1, amplification Oligo 2 , and 50 µl of 1/10 000 dilution of Oligo 3 were added to the reconstituted 250µl test amplification reagent.
- 50 µl of Target Capture Oligo was added to the 250 test Target Capture Reagent (TCR).
- 50 µl of AE-labeled Probe Oligo was added to the Hybridization Reagent.
- 100 µl of the spiked TCR and 400 µl of specimens/controls were added to each reaction tube.
- Specimens were incubated at 62°C ± 1°C for 30 min and then vortexed for 1 min and incubated for 30 min at RT.
- The reaction tubes were placed on the magnetic base for 5 to 10 min, the solution was aspirated and discarded. 1 ml of wash solution (10 mM HEPES) was added to the reaction tubes.
- The reaction tubes were magnetized again for 5 to 10 min, and the wash solution was aspirated and discarded.
- 75 µl of Oligo spiked reconstituted amplification solution, and 200 µl of oil reagent was added to each tube, vortexed for 30 s.
- 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.
- 100 µl of the Oligo spiked hybridization reagent was added to each tube, vortexed and incubated at 62°C ± 1°C for 20 min.
- The reaction tubes were then incubated at RT for 5 min.
- 250 µl of Selection Reagent was added to each tube and incubated at 62°C ± 1°C for 10 min followed by a RT incubation for 15 min.
- The reactions were read using the LEADER HC+ luminometer, and a cut-off value of 50,000 RLUs was used.
- The time to run 98 samples was 6 hours.
- The GPR-based alternate TV TMA (ALT-AMP) is a Research Use Only (RUO) test targeting a different region of the ribosomal RNA (rRNA) and was used on discordant patient samples with a similar cutoff value.

Sample Processing: Magnetic Particle Based Target Capture



Sample lysis from 400 µl of specimen

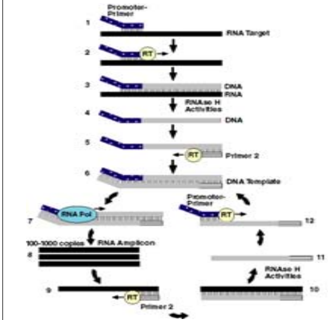
Form hybrids between: nucleic acid and capture oligomer to bind target to magnetic particle

Wash away cellular debris, non-specific DNA/RNA, protein and plasma and potentially inhibitory substances

Ready to amplify

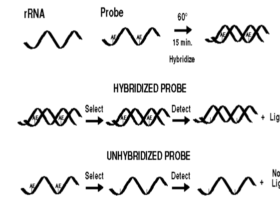
Rapid and automatable: process 200 specimens in < 2 hours

Transcription-Mediated Amplification (TMA)



Utilizes two enzymes: Reverse Transcriptase and T7 RNA Polymerase
Amplifies RNA and DNA
Produces RNA amplicon
Exponential, > billion fold amplification in less than one hour
Isothermal, simplifies automation

HPA
Hybridization Protection Assay



- Utilizes Acridinium Ester (AE) labeled probes
- Reaction Steps:

1. Hybridization
   AE-labeled probe hybridizes to target RNA in solution
2. Selection
   Label on unhybridized probe is hydrolyzed, label on hybridized probe is protected
3. Detection
   Label on protected hybridized probe is detected by chemiluminescence

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. Combined the prevalence was 18.2% (45/247)

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

Test	%Sensitivity	%Specificity	%PPV	% NPV
ASR-TMA	93.3 (42*/45)	97.5 (197/202)	89.4 (42/47)	98.5 (197/200)
Wet-mount	20.0 (9/45)	100 (202/202)	100 (9/9)	81.8 (202/247)

- *Of the 33 VS positive by ASR and negative by wet-mount, 19 were confirmed by the VS being positive in the ALT-AMP test, 6 were positive in the ALT-AMP test and had a FCU positive by TV-ASR, 8 had a positive CS by TV-ASR and/or ALT-AMP.

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.