# Detection of Trichomonas Vaginalis in Various Transport Systems and Clinical Specimens Using Analyte Specific Reagents in a Transcription Mediated Amplification Test

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## REVISED ABSTRACT

Background: *Trichomonas vaginalis* (TV) infections are sexually transmitted and are associated with reproductive health complications. New, sensitive nucleic acid amplification tests have been developed and may eventually serve as screening tools to detect and treat TV infections. The objectives were to determine the ability of analyte specific reagents (ASR) in a transcription mediated amplification (TMA) test (Gen-Probe Inc.) to detect TV from two liquid Pap transport (LPT) media (PreservCyt and SurePath), Gen-Probe sample transport media (STM) from the Gen-Probe APTIMA Combo 2 assay and M40 Amies transport media.

Methods: To determine the endpoints of detection in various transport systems, 10-fold serial dilutions of TV, propagated in Diamond's media, were made in LPT for PreservCyt, SurePath as well as STM. TV in clinical samples were detected with the TMA-ASR as previously described. Wet mount slides of vaginal swabs collected in M40 (Copan) collection tubes were read within 6 hours of collection to facilitate optimal conditions for comparison. To assess the ability of the TV-ASR to detect retrospectively TV in the M40 media, 166 selected clinical specimens (131 negative and 35 positive) were tested from Gamma-Dynacare Medical Laboratories. We also compared the TV-ASR assay to wet mount on cervical, vaginal, urine and LPT samples from 259 women.

Results: TV organisms diluted in AC2 STM were detected in the TV TMA assay to a dilution of $10^{-8}$ compared to the PreservCyt ($10^{-7}$) and SurePath ($10^{-6.5}$) LPT. Testing of 166 vaginal swabs collected into M40 media showed 100% concordance with wet mount positives (n= 35) and negatives (n= 131) which had been tested previously then stored at 4°C for several weeks. In a group of 174 women self collecting vaginal swabs into STM (Gen-Probe Inc.) and M40 media, and also submitting a first catch urine (FCU), all eight wet mount positive women were also positive by TMA (7 in both VS and FCU), and an additional 32 women were TMA positive initially (24 by VS only, 7 by VS and FCU and 1 by FCU only). Testing with alternate primers confirmed 25/31 VS positives available for retesting. In an additional study group of 73 women tested, 13 women were TMA positive by CS, 4 by LPT, and 3 by FCU, with 1, 2, or 3 specimen types positive.

Conclusions: The Gen-Probe TV TMA-ASR is easy to use, detecting low levels of TV nucleic acids in various sample diluents. The test demonstrated versatility in detecting positive women from multiple samples types including CS, VS, FCU and LPT.

## INTRODUCTION

- On a worldwide scale, *Trichomonas vaginalis* (TV) is the most common sexually transmitted disease. WHO in 1999, estimated there are 174 million new cases diagnosed each year.
- Complications include premature labor, low birth weight infants, PID, ectopic pregnancy, TFI, adverse pregnancy outcome and potentially increased risk of acquisition/transmission of other STD's including HIV, *Chlamydia trachomatis* and *Neisseria gonorrhoeae*.
- Although the 2007 Bethesda system recommends reporting the presence of *T. vaginalis* in the conventional Pap test, the sensitivity and specificity range from 44-79% and 83-99% respectively.
- The adaptation of the liquid Pap media for the collection/transportation of cytology samples has shown to improve the diagnosis of squamous lesions, reduce artifacts with better fixation and separation.
- Liquid Pap test (LPT) residual media has shown to be a compatible medium for molecular testing namely HPV, *C. trachomatis* and *N. gonorrhoeae*.

## OBJECTIVES

1. To determine the ability of analyte specific reagents (ASR) in a transcription mediated amplification (TMA) test (Gen-Probe Inc.) to detect *T. vaginalis* from two liquid Pap transport (LPT) media (PreservCyt and SurePath), Gen-Probe APTIMA swab transport media (STM).
2. To determine whether the routine wet-mount preparation microscopy vaginal swab specimens collected in the M40 Amies collection tube could be used in the APTIMA swab transport system and tested in the TV-ASR assay.
3. To determine the sensitivity and specificity of the TV-ASR assay on self-collected vaginal and FCU samples.
4. To determine the sensitivity and specificity of the TV-ASR assay on physician-collected LPT, cervical, vaginal and patient collected FCU samples.

## 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 the LPT media (PreservCyt® and SurePath™) and STM. Dilutions were tested in the TV-ASR assay.

**Samples:**

*Study Group I (n=166):* vaginal swab specimens were collected (May–July 2006) in the M40 Amies transport system from patients attending a family doctor's office for routine culture and sensitivity and sent to Gamma-Dynacare Medical Laboratories (GDC). The specimens were prepared for wet-mount microscopy to identify inflammatory cells, Candida, fungal or trophozoite organisms. Positive and negative TV specimens were shipped to SJH for TV-ASR testing. M40 swabs were transferred into the APTIMA® STM and swirled to remove residual material and tested in the TV-ASR.

*Study Group II (n=174):* cross-sectional, informed consent from patients attending an inner-city street-youth clinic aged 14-25 years old. Patients were asymptomatic (>80%), sexually assaulted at an early age (>50%), had a previous STD (>50%) and had multiple sex partners in the past two months. Patients consented to self-collection of two swabs (M40 and STM) and FCU (20-30mL). Two mL of FCU was transferred into the Gen-Probe urine transport tubes.

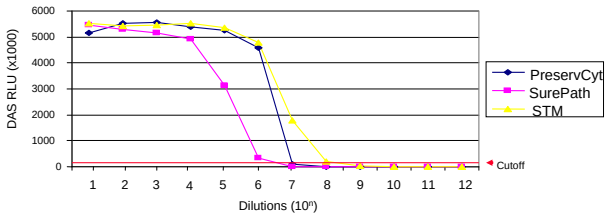
*Study Group III (n=73):* cross-sectional, informed consent from patients attending the Hamilton Community Health Centre. Each patient had a SurePath® (TriPath) sample collected for Pap testing, cervical swab (CS) and two vaginal swabs (M40 and STM) and a FCU. All specimen except the Pap samples were tested at SJH. The Pap samples tested at GDC were processed according to the established procedure. One mL was transferred into the STM and transported to SJH for TV-ASR testing.

**Specimen Testing:**

TV-ASR Testing: The TMA assay was performed using the APTIMA® General Purpose Reagents (GPR) and *T. vaginalis* analyte-specific reagents (TV-ASR, Gen-Probe Inc.) as described previously (Getman et al. 2005; Scheveningen Molecular Meeting Poster). The GPR based alternate TV TMA (ALT-AMP) which is a Research Use Only (RUO) assay was used for discordant testing which targets a different region of the ribosomal RNA (rRNA). A cut-off value of 50,000 RLU was used to define a positive TV-ASR result. A patient was considered infected if the wet-mount was positive or if two specimen types were positive or if two or more tests found a single specimen positive.

**ENDPOINT DETERMINATION TV-ASR**

*Trichomonas vaginalis* Dilutions in PreservCyt®, SurePath™ and APTIMA® STM




TV organisms diluted in AC2 STM were detected in the TV TMA assay to a dilution of $10^{-8}$ compared to the PreservCyt ($10^{-7}$) and SurePath ($10^{-6.5}$) LPT.

## RESULTS

**STUDY GROUP I**

Table 1. Comparison of TV-ASR and Wet Mount M40 Amies Swab Specimens Collected from a Family Doctor's Office

| | | Wet Mount | |
|---|---|---|---|
| | | Positive | Negative |
| TV-ASR | Positive | 35 | 0 |
| | Negative | 0 | 131 |

There was 100% concordance between wet-mount and TV-ASR.

**STUDY GROUP II**

Table 2. Comparison of Results According to Specimen Type From 174 Patients Attending a Street-Youth Clinic

| | TV-ASR | | ALT-AMP | | | |
|---|---|---|---|---|---|---|
| Wet-mount | VS | FCU | VS | FCU | n | Infection Status |
| - | - | - | ND | ND | 134 | Uninfected |
| + | + | + | + | + | 7 | Infected |
| + | + | - | + | + | 1 | Infected |
| - | + | + | + | + | 6 | Infected |
| - | + | + | - | - | 1 | Infected |
| - | + | - | + | - | 18 | Infected |
| - | + | NA | + | NA | 1 | Infected |
| - | - | + | + | - | 1[a] | Infected |
| - | + | - | - | - | 5 | Uninfected |

Table 3. Sensitivity, Specificity and Predictive Values of Various Methods on Different Specimen Types

| Method | Specimen | Sensitivity% | Specificity% | PPV | NPV |
|---|---|---|---|---|---|
| Wet mount | VS | 22.9 (8/35) | 100 (139/139) | 100 (8/8) | 83.7 (139/166) |
| TV-ASR | VS | 97.1 (34/35) | 96.4 (134/139) | 87.2 (34/39) | 99.3 (134/135) |
| TV-ASR | FCU | 44.1 (15/34)[a] | 100 (139/139) | 100 (15/15) | 88.0 (139/158) |

- [a]One patient was TV-ASR and ALT-AMP positive of the vaginal swab (STM) but could not void a FCU sample
- The prevalence of *T. vaginalis* infection in the street-youth population was 20.1% (35/174)

**STUDY GROUP III**

Table 4. Comparison of Results According to Specimen Type From 15 TV Positive Patients

| | | TV-ASR | | | | ALT-AMP | | |
|---|---|---|---|---|---|---|---|---|
| Specimen ID | Wet Mount | VS | LPT | CS | FCU | LPT | CS | FCU |
| 070 | - | - | + | - | - | - | + | ND |
| 119 | - | - | - | + | - | - | + | - |
| 138 | - | - | - | + | - | - | + | - |
| 139 | - | + | ND | + | ND | ND | ND | ND |
| 144 | - | + | - | + | - | + | + | - |
| 155 | - | ND | + | + | - | ND | ND | ND |
| 169 | - | + | - | - | - | - | - | + |
| 173 | - | + | - | + | - | - | + | - |
| 175 | - | - | - | + | - | + | + | - |
| 176 | - | - | + | + | + | ND | ND | ND |
| 178 | - | + | - | + | - | - | + | - |
| 181 | - | - | - | + | + | ND | ND | ND |
| 182 | + | + | + | + | + | ND | ND | ND |
| 322 | - | + | - | + | - | + | + | - |
| 336 | - | + | - | + | - | - | + | - |

Table 5. Sensitivity, Specificity and Predictive Values of Various Methods on Different Specimen Types

| Method | Specimen | Sensitivity% | Specificity% | PPV | NPV |
|---|---|---|---|---|---|
| Wet Mount | VS | 6.7 (1/15) | 100 (58/58) | 100 (1/1) | 80.6 (58/72) |
| TV-ASR | VS | 57.1 (8/14) | 98.3 (58/59) | 88.9 (8/9) | 90.6 (58/64) |
| TV-ASR | LPT | 28.6 (4/14) | 100 (59/59) | 100 (4/4) | 85.5 (59/69) |
| TV-ASR | CS | 86.7 (13/15) | 100 (58/58) | 100 (13/13) | 96.7 (58/60) |
| TV-ASR | FCU | 21.4 (3/14) | 100 (59/59) | 100 (3/3) | 84.3 (59/70) |

## CONCLUSIONS

- Swab specimens collected in the M40 Amies transport collection tubes for wet mount/culture can be used for TV-ASR.
- Vaginal and cervical swabs and FCU TV-ASR detected significantly more TV infections than conventional wet-mount microscopy.
- Vaginal and cervical swabs detected significantly more TV infections than FCU testing.
- The lower sensitivity of FCU and LPT (SurePath) in the TV-ASR assay may be due to sample degradation (Shafir et al).
- Further studies using an expanded gold standard determined by maximum number of test and specimen types should help determine the usefulness of TMA testing for *T. vaginalis*.