

ENDOCERVICAL AND VAGINAL SPECIMENS ARE COMPARABLE FOR DETECTION OF *T. VAGINALIS* USING TRANSCRIPTION-MEDIATED AMPLIFICATION (TMA)

Marcia M. Hobbs, Kimberly D. Rich,
Dana M. Lapple, Karen Lau, Sara Sousa
and Arlene C. Seña



THE UNIVERSITY
of NORTH CAROLINA
at CHAPEL HILL, USA

Objective: Compared to screening for gonococcal (GC) and chlamydial (CT) infections, testing for *T. vaginalis* (TV) remains inadequate in most settings. TV detection in endocervical specimens used for GC/CT testing could lead to improved diagnosis and treatment of trichomoniasis. We compared TV detection in endocervical and vaginal swabs.

Methods: We recruited a convenience sample of 381 women in an STD clinic in North Carolina, US and obtained 1 endocervical and 3 vaginal swabs. Vaginal swabs were tested by wet mount (WM) and InPouch TV culture (Biomed). Vaginal and endocervical swabs were tested by TMA using TV analyte specific reagents (ASR, Gen-Probe, Inc). Endocervical swabs were also tested for GC/CT by APTIMA Combo2 (Gen-Probe). We used nonparametric receiver-operating characteristics (ROC) analysis to assess TV ASR performance in vaginal swabs compared to culture.

Results: TV was detected by WM in 14.6%, by culture in 20.7%, and by TMA in 23.7% of vaginal swabs. Area under the ROC curve for TV ASR was >0.98, indicating excellent test performance. A cutoff of 30,000 RLU jointly maximized sensitivity (100%; 95% CI: 95-100%) and specificity (97%; 95%CI: 94-98%). TV was detected by TMA in 25.2% of endocervical swabs. Agreement between vaginal and endocervical swabs was high (Kappa = 0.95; 95% CI: 0.92-0.98). All women with TV detected in a vaginal swab also had a positive endocervical swab. In 7 women (7.3% of those with any positive TV test), TV was detected only in the cervix. *Women with TV detected only in the cervix were as likely to be symptomatic as women with positive vaginal and cervical swabs (P = 0.270, Fisher exact).*

Conclusions: Although trichomoniasis is widely considered a vaginal infection, we detected TV in the endocervix of all infected women. TV testing from endocervical swabs used for GC/CT nucleic acid amplification testing is feasible. The Gen-Probe ASR-based TMA TV assay performs well with endocervical and vaginal swab specimens.

Table 1. Performance characteristics^a of *T. vaginalis* diagnostic tests with vaginal and endocervical specimens.

Specimen	Test	% (95% confidence interval)	
		Sensitivity	Specificity
Vaginal swab	Wet mount	61.8 (51.7, 71.9)	99.7 (99.0, 100)
	Culture	88.8 (82.2, 100)	100.0 (100, 100)
Endocervical swab	TMA	100.0 (100, 100)	100.0 (100, 100)
	TMA	100.0 (100, 100)	97.7 (96.0, 99.4)

^aSensitivity and specificity were calculated by latent class analysis of all test results, which assumes tests are conditionally independent.² Results from all *T. vaginalis* tests were available for 376 women.

TMA using TV ASR is sensitive and specific for detection of *T. vaginalis* in endocervical and vaginal specimens. Agreement between the two specimens was high with Kappa = 0.95 (95% CI: 0.92-0.98). In this study, TMA from vaginal swabs detected 92.8% of cases, and TMA from endocervical swabs detected 100% of 97 *T. vaginalis* infections.

Table 2. Demographic and clinical characteristics of women with and without vaginal or cervical *T. vaginalis* infection.

Characteristic	Number (%) of Women			P value ^a	
	Without TV (N = 279)	With TV detected in vagina and endocervix (N = 90)	endocervix only (N = 7)		
Age (median, yrs.)	25	30	20	0.143	< 0.001
Black Race	245 (87.8)	83 (92.2)	6 (85.7)	0.363	NS
Symptomatic ^b	212 (76.0)	78 (86.7)	5 (71.4)	0.059	NS
Vaginal Inflammation					
> 25 WBC/hpf	64 (22.9)	30 (33.3)	2 (28.6)	0.069	NS
Cervical Inflammation					
≥ 30 WBC/hpf	162 (58.1)	62 (68.9)	3 (42.9)	0.152	NS
Co-infections ^c					
<i>C. trachomatis</i>	33 (11.8)	14 (15.6)	1 (14.3)	0.149	NS
<i>N. gonorrhoeae</i>	15 (5.4)	7 (7.8)	0 (0.0)	0.650	NS
<i>M. genitalium</i>	57 (20.1)	14 (15.6)	0 (0.0)	0.280	NS
Bacterial Vaginosis	107 (38.4)	11 (12.2)	2 (28.6)	< 0.001	NS

Abbreviations: TV, *Trichomonas vaginalis*; V, vaginal swab; C, endocervical swab; WBC/hpf, white blood cells per high powered field; NS, not significant.

^aRank Sum test for age, Chi-square or Fisher exact test for other variables.

^bSymptoms included vaginal irritation, dysuria, abnormal vaginal or cervical discharge.

^c*N. gonorrhoeae* and *C. trachomatis* detected in endocervical specimen using Gen-Probe APTIMA Combo 2; *M. genitalium* detected in vaginal or endocervical specimen using Gen-Probe transcription-mediated amplification research assay; BV diagnosed based on clinical criteria from the CDC STD Treatment Guidelines 2006.

METHODS

Reference tests for detection of viable trichomonads. Wet mount microscopy was performed on vaginal swab preparations by trained clinicians or medical technologists as part of routine clinical care. Observation of motile trichomonads defined a positive result.

In-PouchTV (Biomed Diagnostics) cultures were inoculated with vaginal swabs. Pouches were examined daily by a trained microscopist up to 5 days after inoculation or until a positive result was obtained.

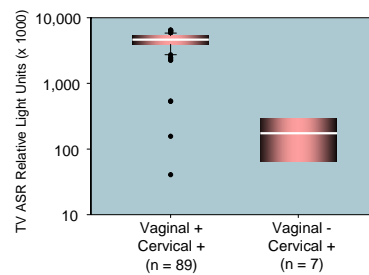


Figure 1. *T. vaginalis* detected by TV ASR in cervical specimens. All women with TV detected in vaginal specimens (n = 89) also had positive endocervical specimens. Seven women (7.3% of all positives) had TV detected only in the endocervical specimen. In the box plot above, assay output in relative light units (RLU x 1000) from endocervical specimens is compared among women with positive and negative vaginal specimens. Horizontal lines indicate medians, boxes indicate 25th and 75th percentiles, whiskers indicate 10th and 90th percentiles and outliers are plotted as individual points. Values from cervical specimens from women with negative vaginal specimens were significantly lower (P < .001, Mann-Whitney Rank Sum Test) than corresponding values from cervical specimens from women with positive vaginal specimens, suggesting a lower organism burden in women with *T. vaginalis* detected only in the endocervix.



Transcription-mediated amplification (TMA) for detection of *T. vaginalis* nucleic acids. Vaginal and endocervical swabs were processed using APTIMA vaginal swab and unisex swab specimen collection kits, respectively, according to the manufacturer's instructions. Processed specimens were tested using Gen-Probe *T. vaginalis* analyte-specific reagents with instruments in a DTS402 system as previously described.¹ Results greater

than 30,000 relative light units (RLUs) were considered positive based on receiver-operating characteristics (ROC) analysis to jointly maximize sensitivity and specificity of the test compared to a composite reference defined by a positive vaginal wet-mount or culture.

ACKNOWLEDGEMENTS. We thank Victoria Mobley for clinical data entry, Bill Miller for performing latent class analysis and Gen-Probe, Inc for providing TV ASR, *Mycoplasma genitalium* research assay reagents and APTIMA General Purpose Reagents. This work was supported by the NC Sexually Transmitted Infections and Topical Microbicides Cooperative Research Center grant (U19-AI031496) funded by the US National Institute of Allergy and Infectious Diseases.

CONCLUSIONS

- Using 30,000 RLU as the cutoff, Gen-Probe TV ASR were 100% sensitive and 97.7% specific for detection of *T. vaginalis* in endocervical swabs, compared to wet mount, culture and TMA from vaginal swabs, as determined by latent class analysis.
- Using TMA, *T. vaginalis* was detected as often in endocervical specimens as in vaginal specimens from women with trichomoniasis.
- Despite lower TV ASR assay output units, women with *T. vaginalis* detected only in the endocervix were as likely to present with symptoms as women with vaginal trichomoniasis.
- Women with TV detected only in the endocervix were significantly younger than women without TV or with vaginal TV.
- TV testing from endocervical swabs used for GC/CT nucleic acid amplification testing is feasible and may identify more infections than testing from vaginal specimens only.

REFERENCES

- Huppert JS, Mortensen JE, Reed JL, Kahn JA, Rich KD, Miller WC and Hobbs MM. 2007. Rapid antigen testing compares favorably to transcription-mediated amplification assay for detection of *T. vaginalis* in young women. Clin. Infect. Dis. 45:194-198.
- Torrance-Rynard VL, Walter SD. 1997. Effects of dependent errors in the assessment of diagnostic test performance. Stat Med16: 2157-2175.