

Age and Gender-Specific Prevalence of *Trichomonas vaginalis* and Co-Detection with *Chlamydia trachomatis* and *Neisseria gonorrhoeae*

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Abstract

Trichomonas vaginalis (TV), the causative agent of trichomoniasis, is the most common parasitic protozoan isolated from humans in industrialized countries. Infection caused by this organism can be easily treated, but it is also often overlooked due to the asymptomatic nature of infection in approximately 70-80% of cases. Furthermore, the most common diagnostic test for TV, the 'wet mount', is known to be an insensitive method. Untreated infection with TV can have a significant impact on a woman's health, leading to preterm delivery, low-birth weight, pelvic inflammatory disease and an increased susceptibility to HIV infection.

The objective of this study was to establish the prevalence of TV alone and in the presence of other pathogens in patients being screened for sexually transmitted infections (STIs). Using Target Enriched Multiplex Polymerase Chain Reaction (TEM-PCR[™]) technology, 28,914 female and 6,103 male specimens submitted for testing from July 1, 2012 to September 1, 2013 were run on the STD panels (Diatherix Laboratories, Huntsville, AL). Data was compiled to determine rates of reported prevalence within populations.

The overall detection rates for TV, *Chlamydia trachomatis* (CT), and *Neisseria gonorrhoeae* (NG) were 4.93%, 6.07%, and 1.25% respectively. The prevalence of TV in females was 5.7% compared to 1.29% in males; findings that are of statistical significance given the size of the database used in the study. The rate of TV in women and men over the age of 40 was 7.61% and 3.22%, respectively, compared to 5.33% and 0.85% in younger women and men.

Co-detection rates among females were determined as CT/NG (0.35%), CT/TV (0.55%), NG/TV (0.16%) and CT/NG/TV (0.06%). Rates among males for CT/NG rose to 0.72%, while others fell for CT/TV (0.11%), NG/TV (0.05%), and CT/NG/TV (0.02%).

The use of molecular methods, such as TEM-PCR, for the diagnosis of STIs has contributed to perhaps a more accurate understanding of TV infection rates among the general population. We have observed an increased prevalence of this pathogen over culture, especially among the older adult population, likely due to the increased sensitivity and accuracy of multiplex testing. The overall rates of co-detection (particularly in males) were low but of potential clinical significance.

Introduction

- Trichomonas vaginalis* (TV) is the most common pathogenic protozoan of humans in industrialized countries and has the highest prevalence of any sexually transmitted infection worldwide.¹
- Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (NG) infections are the two most common sexually transmitted infections (STIs) in the United States with >1.3 million and 309,341 infections reported to CDC in 2010, respectively.²
- The overwhelming majority of cases of TV, CT, and NG are asymptomatic in nature, especially among women. Left untreated, these STIs can lead to preterm delivery, low-birth weight, pelvic inflammatory disease and an increased susceptibility to HIV infection.
- Until the introduction of multiplexed molecular methods, little was known of the rates of co-infection between the most common STIs.
- The purpose of this study was to determine prevalence rates of detection and co-detection of these STIs among certain populations using a laboratory-developed multiplexed molecular test, such as the STD5 TEM-PCR panel (Table 1).

Materials & Methods

Sample Collection

Genitourinary swabs and/or urine samples were collected from patients by clinicians distributed throughout the United States and transported overnight to Diatherix Laboratories (Huntsville, AL) in multi-Collect Transport Tubes (Abbott Laboratories, Abbott Park, Illinois). Specimens must be less than 5 days old to be accepted for testing.

DNA Extraction

DNA was extracted from clinical specimens on the KingFisher Flex system (Thermo Scientific, Vantaa, Finland) using a modification of the Mag-Bind[®] Viral DNA/RNA Kit from Omega Bio-tek (Norcross, GA).

TEM-PCR[™]

For the STD5 Panel, we designed primers for specific detection of five sexually transmitted pathogens: *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Trichomonas vaginalis*, Herpes Simplex Virus (HSV) Type 1 and HSV Type 2. Each pathogen target is detected by a nested set of primers which, upon specific annealing to and amplification of extracted nucleic acids, will incorporate a biotin label into the resultant PCR product (Figure 1).



Figure 1. TEM-PCR[™] Scheme. Low concentration nested gene-specific primers are (Fo – forward out; Fi – forward in; Ri – reverse in; and Ro – reverse out) designed to enrich the targets during the initial PCR cycles. Later in the procedure, a pair of universal SuperPrimers (Fs and Rs) is used to amplify all targets. The Rs primer is labeled with biotin for subsequent detection. PCR products are detected by a complementary detection probe covalently coupled to a microsphere or glass microarray substrate.

Hybridization and Detection

PCR products were hybridized to a target-specific bead mix containing detection oligonucleotides covalently coupled to carboxylated microspheres. Data were reported as median fluorescent intensity (MFI) values, and sample values above cutoff (established at 2x Limit of Blank) were reported as 'Detected'.

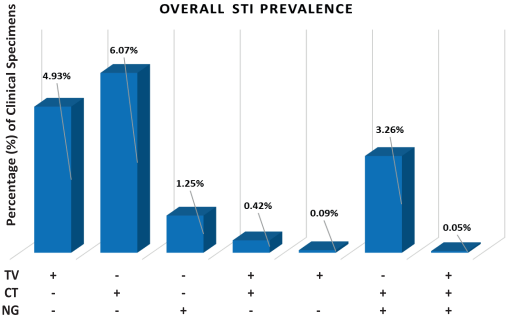
Data Compilation and Analysis

All database information from 35,018 clinical samples submitted for testing between the dates of July 1, 2012 to September 1, 2013 using the STD5 panel was archived and sorted using an internal SQL Server 2012 data warehouse.

STD5 Target	Target Name
<i>Chlamydia trachomatis</i>	CT
<i>Neisseria gonorrhoeae</i>	NG
Herpes Simplex Virus-Type 1	HSV1
Herpes Simplex Virus-Type 2	HSV2
<i>Trichomonas vaginalis</i>	TV
Sample Control	SC

Results

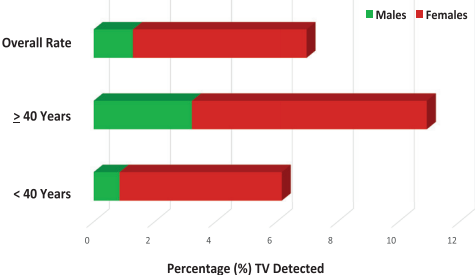
Overall STI Prevalence



Pathogen	Gender	Prevalence (%)
TV	Male	4.93%
	Female	6.07%
CT	Male	1.25%
	Female	0.42%
NG	Male	0.09%
	Female	3.26%

Figure 2. Prevalence rate of detected *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Trichomonas vaginalis* in submitted clinical samples (n=35,017).

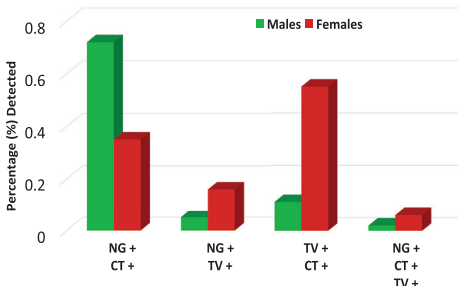
Age- and gender-specific detection of *Trichomonas vaginalis*



Age Group	Gender	Percentage (%) TV Detected
Overall Rate	Male	~1.3%
	Female	~7.6%
≥ 40 Years	Male	~3.2%
	Female	~10.8%
< 40 Years	Male	~0.8%
	Female	~6.0%

Figure 3. Age- and gender-specific detection of *Trichomonas vaginalis* in submitted clinical samples (n=35,017). The overall rate indicates the percentage of TV positive specimens detected in both male (n=6,103) and female (n=28,914) patients. The rates of TV positive specimens were further determined for male and female populations by age group; Males ≥ 40 (n=1,150), Females ≥ 40 (n=4,636), Males < 40 (n=4,953), Females < 40 (n=24,278).

Gender-specific co-detection of *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Trichomonas vaginalis*



Co-detection	Gender	Percentage (%) Detected
NG + CT +	Male	~0.75%
	Female	~0.4%
NG + TV +	Male	~0.1%
	Female	~0.2%
TV + CT +	Male	~0.15%
	Female	~0.6%
NG + CT + TV +	Male	~0.05%
	Female	~0.1%

Figure 4. Gender-specific co-detection of *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Trichomonas vaginalis* in submitted clinical samples (n=35,017).

Results (continued)

	Roche COBAS AmpliCor	BD ProbeTec ET	GenProbe Aptima Combo2	Abbott Lcx	Diatherix STD5 Panel
Technology	Polymerase Chain Reaction (PCR)	Strain Displacement Amplification (SDA)	Transcription Mediated Amplification (TMA)	Ligase Chain Reaction (LCR)	Target Enriched Multiplex Polymerase Chain Reaction (TEM-PCR)
Gene target: CT	Cryptic plasmid	Cryptic plasmid	23S rRNA	Cryptic Plasmid	Cryptic plasmid; Outer membrane protein gene
Analytical sensitivity: CT	125 EB/mL	2500 EB/mL	10 EB/mL	n/a	10 EB/mL; 300 EB/mL
Gene target: NG	Cytosine DNA methyltransferase	Multicopy pilin gene-invertin analogue	16S rRNA	Opa	porA Pseudogene
Analytical sensitivity: NG	5 cells/mL	50 cells/mL	625 cells/mL	100 cells/mL	50 cells/mL
Gene target: TV	n/a	n/a	16S rRNA	n/a	Beta-tubulin
Analytical sensitivity: TV	n/a	n/a	0.01 TV/mL	n/a	100 cells/mL

Discussion

- The ability to perform highly sensitive multiplexed testing for sexually transmitted infections enables the clinician to better understand complicated STI occurrences within commonly affected populations.
- Co-detection with *N. gonorrhoeae* and *C. trachomatis* was more prevalent than any other co-infection, especially among men.
- Co-infection rates among females was highest with *T. vaginalis* and *C. trachomatis* being the most commonly co-detected organisms.
- Interestingly, of the 19 samples co-detected with TV, CT and NG, all patients were between 15 and 25 years of age. Of the 176 samples co-detected with TV and either CT or NG, all but one (n=175) were between the ages of 15 and 40. This could be attributed to high-risk sexual behavior and/or increased screening for STIs within this age group.
- We detected a substantial increase of TV infection in the population 40 years of age or older, correlating with previously published studies. It has been suggested that hormonal effects are likely to influence these differences.¹
- The disproportional results of TV infection between genders, with males having significantly lower detection rates than females, may be due to a historical lack of testing for *T. vaginalis* in the male population.

Conclusions

- The use of molecular methods, such as TEM-PCR, for the diagnosis of STIs has contributed to perhaps a more accurate understanding of TV infection rates among the general population.
- Further analyses should be made into the prevalence rates of TV along other STIs, such as HPV and the Herpes Simplex Viruses, and/or the causative agents of Bacterial Vaginosis.

Acknowledgements

We would like to thank the Clinical Department at Diatherix Laboratories for processing all samples for this study.

References

- Poole, DN and McClelland, RS. *Global epidemiology of Trichomonas vaginalis*. Sex Transm Infect 2013; 89:418-422.
- Papp et al. Recommendations for the Laboratory-Based Detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae*-2014. *MMWR. Recommendations and Reports*. March 14, 2014/63(RR02);1-19.