Trichomonas vaginalis Detection in Female Specimens with cobas® TV/MG for use on the cobas® 6800/8800 Systems

Elizabeth M. Marlowe[1*], Peter Gohl[2], Michael Steidle[3], Rodney Arcenas[1] and Carolin Bier[4]

[1]*Roche Molecular Systems, Inc, Pleasanton, California, USA*
[2]*Bioscientia, Ingelheim, Germany*
[3]*Laborärzte Sindelfingen, Sindelfingen, Germany*
[4]*Roche Molecular Diagnostics, Rotkreuz, Switzerland*

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Trichomoniasis, a common curable sexually transmitted infection caused by the protozoan *Trichomonas vaginalis* (TV), is usually asymptomatic. However, symptomatic women may experience vaginal discharge and/or vulvar irritation. This study evaluated cobas® TV/ *Mycoplasma genitalium* (MG) (Conformité Européene marking for in vitro diagnostic medical devices [CE-IVD]) against other nucleic acid amplification tests (NAATs) for detecting TV in female urogenital specimens. Matched de-identified specimens from 412 females were collected. cobas® TV/MG results were compared against a composite reference (CR) of 3 different NAATs for TV (Aptima TV, modified S-DiaMGTV™, and a laboratory-developed test). The overall TV prevalence rate was 6.2%, based on cobas® TV/MG results. Relative to the CR, cobas® TV/MG sensitivity/specificity for the specimen types were endocervical swabs (ES) 100%/99.2%, vaginal swabs (VS) 100%/99.7%, urine (U) 100%/99.7%, and cervical specimens in PreservCyt® solution (PC) 100%/99.5%. There was no significant statistical difference between clinician-collected and self-collected VS (p = 0.28). Correlation of cobas® TV/MG vs. Aptima TV demonstrated the following positive, negative, and overall percent agreements, respectively: ES 69.0%, 98.7%, and 96.6%; VS 88.9%, 99.5%, and 98.8%; U 100%, 100%, and 100%; and PC 95.5%, 99.0%, and 98.8%. Detection of TV with cobas® TV/MG for use on the cobas® 6800/8800 systems demonstrated excellent performance in female urogenital specimens (overall sensitivity/specificity of 100%/≥99.2%).

Keywords: *Trichomonas vaginalis*, molecular diagnostics, sexually transmitted infection, cobas®

Introduction

Trichomoniasis is the most common non-viral sexually transmitted infection (STI), with an estimated 142.6 million new cases globally in 2012 [1, 2]. *Trichomonas vaginalis* (TV) primarily infects the squamous epithelial cells of the lower genital tract in females and the urethra and prostate in males [3]. Humans are the only known host for TV, and the pathogen is primarily transmitted through sexual contact. TV infections may persist for long periods (months to years) in women; however, in men, the infection is often self-limited and will clear without medical intervention [3]. Although most infections are asymptomatic, symptomatic women with TV infection will present with vaginal discharge, pruritus, and irritation. Clinical signs of infection include malodor, vaginal and vulvar edema, and/or erythema.

National routine screening programs for TV do not exist. However, the Centers for Disease Control and Prevention (CDC) does recommend that women who test positive for TV be rescreened 3 months after treatment. Further recommendations in women infected with HIV include screening for TV at the initial visit and annually thereafter [2].

Laboratory diagnosis of TV has historically relied on direct microscopic visualization of motile organisms on a wet mount preparation from the patient's discharge. Wet mount microscopy is quick and inexpensive but has limited sensitivity, which ranges from 60–70% versus culture and nucleic acid amplification tests (NAATs) [4, 5]. Since TV does not survive for long outside the body, the prompt reading of a specimen is a critical factor for quality results [6]. Rapid antigen tests are available with an overall lower sensitivity than NAATs but have demonstrated good performance in symptomatic patients [7]. Prior to NAATs, the gold standard for the laboratory diagnosis of TV was culture, using the InPouch™ TV (Biomed Diagnostics, White City, Oregon, USA). The sensitivity of the culture has been reported to be 81–94% [8, 9]. This method is time-consuming and not widely available for clinical use [6, 10].

Today, NAATs are the recognized gold standard for TV diagnosis [2] and are recommended in the UK [11] and Australian TV management guidelines [12]. Commercially available NAATs are becoming widely available for the detection of TV in women and, more recently, in men [13, 14]. The objective of this study was to evaluate the performance of cobas® TV/ *Mycoplasma genitalium* (MG) (Conformité Européene marking for in vitro diagnostic medical devices [CE-IVD]) for use on the cobas® 6800/8800 systems against other NAATs for detecting TV in female urogenital specimens.

Materials and Methods

Clinical Specimen Collection. Prospectively collected, matched de-identified specimens from 412 female subjects across all specimen types were collected in Germany, Ukraine,

*Author for correspondence: Roche Molecular Systems, Inc., 4300 Hacienda Drive, Pleasanton, California 94588 USA; E-mail: beth.marlowe@roche.com; Tel: +1-925-251-6788; Fax : +1-925-730 8388.

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and the USA. First-catch urine (U) specimens were collected first, followed by vaginal swabs (VS) (clinician-collected [CC] and self-collected [SC]), endocervical swabs (ES), and lastly, cervical specimens (collected in PreservCyt® Solution [PC]). All specimens were collected in an alternating order between cobas® PCR Media and Aptima transport medium; for VS, collections alternated between SC and CC. For Aptima TV testing, the swab, as well as U specimens, was collected and stabilized in Aptima transport medium. Matched specimens collected and stabilized in cobas® polymerase chain reaction (PCR) media were used for any other TV testing. All PC were collected in PreservCyt® Solution and processed according to manufacturer's recommendations.

The patient population group included subjects who were at risk of infection, consulted a healthcare provider for TV screening, or were found to be positive for TV during routine testing. Subjects who had already been treated with antibiotics within a time frame of 21 days before collection were excluded from the study. In addition, subjects who had a history of hysterectomy or were pregnant were not eligible for study enrollment. Furthermore, to be selected as a participant for the study, the subject had to be ≥18 years. Each specimen from a subject was included only once in the study.

NAAT Tests. Each specimen per subject was tested with 4 different NAATs. The TV NAATs utilized for testing included cobas® TV/MG (CE-IVD) (Roche Diagnostics), Aptima TV (Hologic), and 2 laboratory-developed tests (LDTs). The Aptima and the cobas® assays both target multicopy regions of the rRNA and were performed according to the respective manufacturer's instructions. The first LDT (LDT1) was a modified S-DiaMGTV™ (Diagenode) repeated sequence assay. The modified S-DiaMGTV™ test was run with the extraction automated on the cobas® 4800 system, followed by the amplification on a LightCycler480 as described in the user's manual from Diagenode. The second LDT (LDT2) used the generic reagents, extraction, and amplification of the cobas® 6800/8800 systems. The PCR mastermix was composed of the generic backbone, to which TV-specific primers and a probe were added; all other test-specific reagents were used as described in the user's manual for the cobas® Omni channel. The primers used in the LDT2 targeted the beta-tubulin gene of TV.

Testing Locations. Laboratory testing for the study was executed at 2 diagnostic laboratories in Germany and a Roche site in Switzerland. The cobas® TV/MG and LDT1 were performed at Bioscientia, Ingelheim. The Aptima TV testing was conducted at Laborärzte Sindelfingen, Sindelfingen. The LDT2 was performed at Roche Diagnostics International, Switzerland.

Data Analysis. Only complete data sets were used for data analysis. TV results in female urogenital specimens by cobas® TV/MG were correlated to Aptima TV results to determine the overall percent agreement (OPA), negative percent agreement (NPA), and positive percent agreement (PPA). A composite reference (CR) was used to determine if the TV results from the cobas® TV/MG were considered true positive or true negative to calculate the sensitivity and specificity. The CR was composed of Aptima TV and the TV result of the two LDTs. An overall TV positive result of the CR was determined if at least two out of three tests were positive for TV. Any other combination of results was considered TV negative.

Ethics. The study procedures were carried out in accordance with the Declaration of Helsinki. The Institutional Review Board of the Biomex (Germany), BioPartners (Ukraine), and Planned Parenthood clinics in Southern New England and the Gulf Coast (USA) approved the study. All subjects were informed about the study and all provided informed consent.

Results

A total of 1648 specimens across all specimen types were tested for TV. Among the tested samples, a very low number of invalids were observed for cobas® TV/MG: only 3/1651 (0.2%) reactions failed, and these were resolved by retesting. Aptima TV also demonstrated a very low number of invalids: 0.2% (4/1652); in contrast, LDT1 produced 1.7% (28/1676) invalid or failed results in eligible subjects on the first attempt. The initial test results from specimens that had a complete data set were used to evaluate assay performance.

In direct comparison to the Aptima TV result, the OPA across all female urogenital specimen types for cobas® TV/MG was 98.5%, the NPA was 99.3%, and the PPA was 87.5%. Specifically, the OPA, NPA, and PPA, respectively, were as follows: 100%, 100%, and 100% for U, 98.8%, 99.5%, and 88.9% for VS, 96.6%, 98.7%, and 69.0% for ES, and 98.8%, 99.0%, and 95.5% for PC (Table 1).

Overall, relative to the CR result, the sensitivity/specificity across all female urogenital specimen types for cobas® TV/MG was 100%/99.5% (Table 2). Sensitivity/specificity for the specific specimen types were as follows: 100%/99.7% for U; 100%/100%, 100%/99.4%, and 100%/99.7% for VS-CC and VS-SC, separately, and combined VS, respectively; 100%/99.2% for ES; and 100%/99.5% for PC. No significant difference between the VS-SC and VS-CC specimens was observed ($p = 0.28$).

Table 1. Summary of percent agreements and prevalence of TV results with cobas® TV/MG compared with Aptima TV

Specimen type	N	Ref test	PPA	NPA	OPA	Prevalence based on comparator	Prevalence based on cobas® TV/MG
U	412	Aptima TV	100% (26/26) 95% CI: 86.8–100%	100% (386/386) 95% CI: 99.0–100%	100% (412/412) 95% CI: 99.1–100%	6.3% (26/412)	6.3% (26/412)
VS	412	Aptima TV	88.9% (24/27) 95% CI: 70.8–97.6%	99.5% (383/385) 95% CI: 98.1–99.9%	98.8% (407/412) 95% CI: 97.2–99.6%	6.6% (27/412)	6.3% (26/412)
VS-CC	222	Aptima TV	93.3% (14/15) 95% CI: 68.1–99.8%	100% (207/207) 95% CI: 98.2–100%	99.5% (221/222) 95% CI: 97.5–100%	6.8% (15/222)	6.3% (14/222)
VS-SC	190	Aptima TV	83.3% (10/12) 95% CI: 51.6–97.6%	98.9% (176/178) 95% CI: 96.0–99.9%	97.9% (186/190) 95% CI: 94.7–99.4%	6.3% (12/190)	6.3% (12/190)
ES	412	Aptima TV	69.0% (20/29) 95% CI: 49.2–84.7%	98.7% (378/383) 95% CI: 97.0–99.6%	96.6% (398/412) 95% CI: 94.4–98.1%	7.0% (29/412)	6.1% (25/412)
PC	412	Aptima TV	95.5% (21/22) 95% CI: 77.2–99.9%	99.0% (386/390) 95% CI: 97.4–99.7%	98.8% (407/412) 95% CI: 97.2–99.6%	5.3% (22/412)	6.1% (25/412)
Total female	1648	Aptima TV	87.5% (91/104) 95% CI: 80.9–92.4%	99.3% (1533/1544) 95% CI: 98.8–99.6%	98.5% (1624/1648) 95% CI: 98.0–99.0%	6.3% (104/1648)	6.2% (102/1648)

Abbreviations: CC, clinician-collected; CI, confidence interval; ES, endocervical swabs; NPA, negative percent agreement; OPA, overall percent agreement; PC, cervical specimens collected in PreservCyt® Solution; PPA, positive percent agreement; SC, self-collected; TV, *Trichomonas vaginalis*; U, urine; VS, vaginal swabs.

Table 2. Summary of sensitivity and specificity of cobas® TV/MG compared with a composite reference

Specimen type	*N*	Sensitivity	95% CI	Specificity	95% CI	Prevalence based on CR
U	412	100% (25/25)	86.3–100%	99.7% (386/387)	98.6–100%	6.1% (25/412)
VS	412	100% (25/25)	86.3–100%	99.7% (386/387)	98.6–100%	6.1% (25/412)
VS-CC	222	100% (14/14)	76.8–100%	100% (208/208)	98.2–100%	6.3% (14/222)
VS-SC	190	100% (11/11)	71.5–100%	99.4% (178/179)	96.9–100%	5.8% (11/190)
ES	412	100% (22/22)	84.6–100%	99.2% (387/390)	97.8–99.8%	5.3% (22/412)
PC	412	100% (23/23)	85.2–100%	99.5% (387/389)	98.2–99.8%	5.6% (23/412)
Total female	1648	100% (95/95)	96.9–100%	99.5% (1546/1553)	99.2–99.8%	5.8% (95/1648)

Abbreviations: CC, clinician-collected; CI, confidence interval; CR, composite reference; ES, endocervical swabs; PC, cervical specimens collected in PreservCyt® Solution; SC, self-collected; TV, *Trichomonas vaginalis*; U, urine; VS, vaginal swabs.

The observed prevalence of TV within the female specimen types was between 5.3–6.3% when looking at the overall positivity rate derived from the CR (Table 2). An analysis of the differences in proportion between the detection rates in each specimen type resulted in *p*-values >0.1. Thus, no significant differences were observed between the female urogenital sample types tested for the detection of TV.

Discussion

Globally, TV is one of the most common STIs, of which women tend to bear the burden of disease. Infections are often asymptomatic or can be confused with other vaginal discharge infections, such as bacterial vaginosis, making diagnosis clinically challenging [15]. Thus, prevalence is likely to be underestimated. TV is not a reportable public health pathogen, but infections can have significant consequences for sexual health, such as vaginitis, cervicitis, premature rupture of membranes, preterm delivery, and infertility, as well as an increased risk for an HIV infection [2, 16, 17]. Accurate detection of TV is a key to providing proper treatment and preventing further transmission between sexual partners.

NAATs are recommended diagnostic methods for the detection of TV, as they provide a superior sensitivity compared to culture and microscopy [2]. Molecular STI testing strategies continue to evolve. Multiplex molecular assays for a large panel of STI pathogens are available; however, the clinical utility and health economics of panel testing for both routine STI screening and targeted populations remains to be supported by studies in the peer reviewed literature. Point of care (POC) molecular testing for TV is also of interest, and commercially available options will continue to grow for STI diagnostics. However, POC programs still require proper laboratory oversight to ensure their success and often come at a higher cost than centralized testing [18]. In this study, centralized testing with the cobas® TV/MG showed excellent overall clinical sensitivity/specificity: 100%/≥99.2%, for the detection of TV DNA in female urogenital specimens. The OPA with Aptima TV was high (≥96.6) for female specimens.

Data from this study indicate a prevalence of 5.3–6.3% for TV in all female specimen types, further highlighting the burden of female infection. When considering the optimal specimen type for TV NAAT detection, the data presented in this study suggest that any of the tested female urogenital specimens demonstrated equivalent detection of TV DNA with cobas® TV/MG. Thus, specimens already collected for routine chlamydia screening could also be tested for TV. Furthermore, if a pelvic examination is warranted, cervical or endocervical specimens could also be utilized, decreasing the burden to the patient for collection of multiple specimen types at the same time if volume allows. A limitation to this study is the statistical power of this small data set. However, the positivity rate reflected in this study would mirror many screening populations. Thus, these data demonstrate the high negative predictive value of this NAAT for detecting TV in female urogenital specimens. Additional data from a US clinical trial are forthcoming.

In summary, TV is a significant cause of disease, and diagnostic testing is warranted in targeted populations. The results of this study support the use of cobas® TV/MG for use on the cobas® 6800/8800 systems for the detection of TV in female urogenital specimens. In addition, the cobas® TV/MG offers automation and effective workflow for high-volume STI testing. Enhanced diagnostic strategies are fundamental to STI management and public health prevention policies.

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Authors' Contributions

C.B., P.G., and M.S. planned the study and performed NAAT testing. E.M.M., P.G., M.S., R.A., and C.B. performed assessment and evaluation of the data. All authors jointly wrote the paper, performed revisions, had access to the study data presented, and take responsibility for the integrity of the data and the accuracy of the data analysis.

Conflict of Interest

E.M.M. and R.A. are employees of Roche Molecular Systems, Inc., and C.B. is an employee of Roche Molecular Diagnostics. P.G and M.S. received funding from Roche Molecular Diagnostics to support the study.

Notes

cobas® TV/MG is currently available as CE-IVD and Australian IVD only. It is not available in the USA. The performance characteristics of cobas® TV/MG are currently under review by the FDA, pending *de novo* authorization.

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