Evaluation of Presto^{plus} Assay and LightMix Kit *Trichomonas vaginalis* Assay for detection of *Trichomonas vaginalis* in dry vaginal swabs

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ABSTRACT

This is an evaluation study of the Presto^{plus} Assay for *T. vaginalis* by comparing to the TIB MOLBIOL LightMix Kit *Trichomonas vaginalis* Assay using 615 dry collected vaginal and rectal swabs. Discordant samples were analyzed by the Qiagen® Microbial DNA qPCR for TV Assay. Both assays showed comparable performances (McNemar $p > 0.05$).

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Highlights

- Comparison study for Prestoplus versus TMB LightMix Kit T. vaginalis using dry swabs
- Comparable performances were found between Prestoplus and TMB LightMix Kit T. vaginalis.
- The largest comparison study for detection of T. vaginalis to date
- Prestoplus detects three pathogens in one assay providing new diagnostic insights.

Trichomonas vaginalis causes the most common non-viral sexual transmitted infection (STI) with annually 248 million new cases worldwide (1). *T. vaginalis* occurs often as an asymptomatic infection and is in most cases untreated. When symptomatic, women may experience itch, changes in vaginal discharge, lower and upper reproductive tract disease syndromes, including vaginitis, cervicitis, increased risk for tubal pathology, and pelvic inflammatory disease (PID) (2,3). Untreated *T. vaginalis* infection affects sexual, reproductive and obstetric health and may facilitate transmission of other STIs, including an HIV infection. *T. vaginalis* infections are more likely for HIV acquisition, an increased risk of preterm labor, an increased risk of PID, and an association is found between a *T. vaginalis* infection with a concurrent Chlamydia infection (4,5). A *T. vaginalis* infection is a curable STI, which is why diagnostic testing remains important to prevent complications.

Microscopic examination of wet mount smear and/or culture are the methods for detection of *T. vaginalis* and are currently most commonly used. However, wet mount is described to be insensitive compared to culture (53%) (6). Another disadvantage of wet mount microscopy is that should be read within ten minutes of collection (6). A disadvantage of culture is that *T. vaginalis* is undetectable for months after treatment with metronidazole in HIV positive women (7,8).

More sensitive techniques are DNA or RNA amplification tests to detect *T. vaginalis*: PCR-based methods report 40% more positive samples than culture (9). To facilitate implementation of these molecular tests in the routine microbiology laboratory, combination tests have been developed that combine multiple pathogens (*e.g. Chlamydia trachomatis*, *Neisseria gonorrhoeae,* and *T. vaginalis*) in a single assay. The Presto Assay

(Microbiome Ltd., Houten, The Netherlands) is a dual detection assay for *C. trachomatis* and *N. gonorrhoeae.* The Presto^{plus} CT/NG/TV assay (Microbiome Ltd., Houten, The Netherlands) is a triple detection assay, with an additional *T. vaginalis* detection, which can be used on a variety of open PCR systems. The Presto Assay has already been compared to the Roche cobas® 4800 CT/NG assay, and showed comparable results (10). In the current study, we evaluated the Presto^{plus} CT/NG/TV assay for evaluation of *T. vaginalis*. Due to the unavailability of a Roche *T. vaginalis* Assay, we compared it to the TIB MOLBIOL (TMB) LightMix Kit *Trichomonas vaginalis* Assay run in the open channel of the Roche Z480 using dry collected vaginal and rectal swabs.

Samples for this evaluation were obtained from a cross-sectional study that was conducted at primary healthcare facilities across the Mopani District, South Africa (11). All women (n=615) aged 18 to 49 years who reported to have been sexually active during the last 6 months were eligible; patient information was provided and written consent obtained (11). Healthcare worker-collected vaginal and rectal dry swabs (Copan Diagnostics, Brescia, Italy) were obtained and frozen at -20°C for storage until further processing. The study was approved by the Human Ethics Research Committee of the University of the Witwatersrand, South Africa (Ref. M110726)

Material was transported on dry ice to Amsterdam, The Netherlands for processing. Samples were resuspended in 1 ml of sterile phosphate-buffered saline (PBS), vortexed and diagnostically tested for *C. trachomatis*, *N. gonorrohoeae*, and *T. vaginalis infection*. 200 microliters of each sample were used for DNA extraction and the rest was stored at −20°C.

For the Presto^{plus} Assay, DNA was isolated using the High Pure PCR Template Preparation (HPPTP) Kit (Roche Diagnostics, Basel, Switzerland) as per manufacturer's instructions prior to *T. vaginalis* DNA detection was performed by the Presto^{plus} Assay according to the manufacturer's instructions. Detection was done on the LightCycler II (Roche Diagnostics, Basel, Switzerland).

For the TMB LightMix Kit *Trichomonas vaginalis* (Berlin, Germany) assay, DNA was isolated on the Roche X480 and detection on the Roche Z480.

Samples with discordant results between the Presto^{plus} and LightMix Kit *Trichomonas vaginalis* assay were again isolated by the chemagic MSM I system (PerkinElmer, Inc., Waltham, MA, USA). Samples were analyzed by the Qiagen Microbial DNA qPCR kit for *T. vaginalis* (Hilden, Germany) on the LightCycler II. The alloyed gold standard (12) was defined as concordant results for the Presto^{plus} and LightMix Kit *T. vaginalis* assays, or, for the samples with discordant results between these tests, as the concurring result of either test and the Qiagen test.

Performances (sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV)) were assessed for both assays and discrepancy analyses were performed with a third assay, the Qiagen Microbial DNA qPCR kit for *T. vaginalis*.

Of the 615 samples tested, 98 (16%) had a positive result with the Presto^{plus} Assay and 109 (18%) with the LightMix Kit *T. vaginalis* assay. Of these samples 96 were concordant positive. A total of 504 samples had concordant negative *T. vaginalis* results. Fifteen samples had discordant results between Presto^{plus} and LightMix Kit *T. vaginalis* assay. These were analyzed by the Qiagen Microbial DNA qPCR assay. Qiagen Microbial DNA qPCR assay confirmed the two Presto^{plus} positive / LightMix Kit *T. vaginalis* negative to be false negative (compared to the alloyed gold standard), eight samples to be false positive (compared to the alloyed gold standard) for LightMix Kit *T. vaginalis*, and five samples to be false negative for Presto^{plus}. See figure 1.

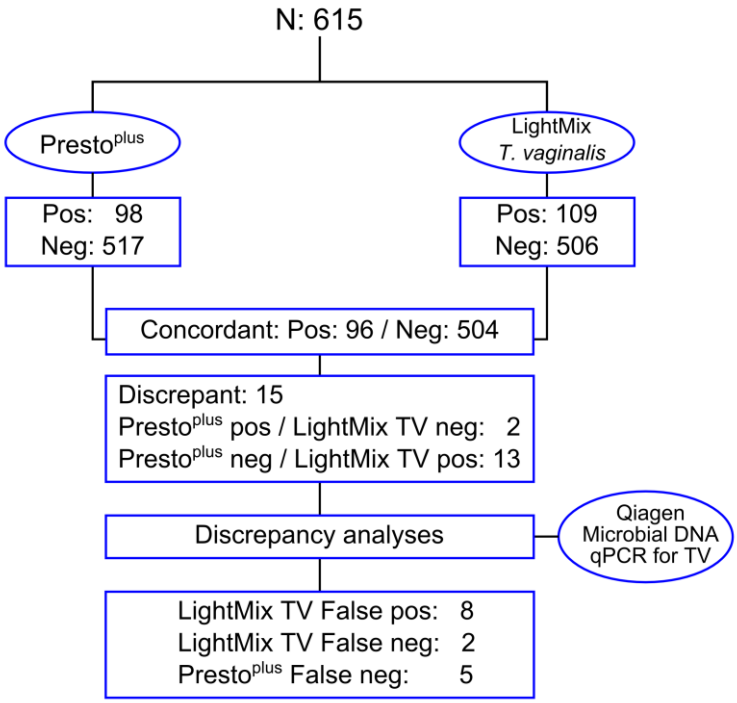

***Figure 1**: Flow diagram of the results of the vaginal samples for T. vaginalis. The 615 samples, tested by Presto^{plus} and LightMix Kit T. vaginalis resulted in concordant and discrepant results. The Qiagen assay was used for discrepant samples and the alloyed gold standard was defined as two concurring results. Sensitivity, specificity, PPV, and NPV were calculated.*

Sensitivity, specificity, and positive and negative predictive values of the Presto^{plus} and LightMix Kit *T. vaginalis* assays were calculated against the alloyed gold standard and are comparable (table 1). Sensitivity and specificity for Presto^{plus} and LightMix Kit *T. vaginalis* were 95.2% and 98.1%, respectively. Further details can be seen in table 1. Presto^{plus} and LightMix Kit *T. vaginalis* showed comparable results compared to the alloyed gold standard (McNemar $p = 0.06$ and $p = 0.1$, respectively). In addition, discrepant results were compared based on the protozoan load (Cp (crossing point)-value). LightMix Kit *T. vaginalis* false positive results show a significantly lower load in comparison to LightMix Kit *T. vaginalis* true positives (40.2 *vs*. 32.5; $p = 0.0002$).

Table 1: *Sensitivity, specificity, positive predictive value, and negative predictive value for vaginal TV for the Presto^{plus} assay and the* LightMix Kit *Trichomonas vaginalis assay.*

	Sens %	*95%CI*	*Spec %*	*95%CI*	*PPV%*	*95%CI*	*NPV%*	*95%CI*
Presto^{plus}	*95.28*	*0.93 – 0.97*	*100.0*	*0.99 – 1.00*	*100.0*	*0.99 – 1.00*	*99.0*	*0.98 – 0.99*
LightMix Kit *Trichomonas vaginalis*	*98.1*	*0.97 – 0.99*	*98.4*	*0.97 – 0.99*	*92.7*	*0.90 – 0.94*	*99.6*	*0.99 – 1.00*

SENS, sensitivity; CI, confidence interval; SPEC, specificity; PPV, positive predictive value; NPV, negative predictive value
The alloyed gold standard was a concurring result between the Presto^{plus} and LightMix Kit *T. vaginalis* assay, or when these were discrepant, a concurring result between either Presto^{plus} or LightMix Kit *T. vaginalis* assay and the Qiagen Microbial DNA qPCR kit for TV. Sensitivity, specificity, PPV, and NPV for both assays and both anatomical sites were calculated against the alloyed gold standard.

An often used method for detecting a *T. vaginalis* infection in women is culture of vaginal specimens. Culture, however, has a low sensitivity compared to the most currently introduced PCR methods and requires microscopic evaluation which is very time consuming and standardization of the interpretation of positivity is ambiguous (13). In the last decade, several PCR tests have been developed and demonstrated to have a higher sensitivity and specificity than culture. Limited studies have been performed comparing culture and PCR for the detection of *T. vaginalis*. PCR has been compared to culture and sensitivity of 84% and 78% were obtained, respectively (14). For that study, the Papanicolaou smear was used as the golden standard, which may lower the sensitivity.

Several studies compared PCR assays for the detection of *T. vaginalis*. For instance, the performance of Gen-Probe's transcription-mediated amplification (TMA) assay for *T. vaginalis* has been compared to the BTUB FRET PCR and showed comparable results: TMA showed sensitivity and specificity of 98.6% and 99.1% (15). One other study investigated the usefulness of several multiplex PCR assays for the detection of *T. vaginalis.* This study included the following tests: Anyplex™ II, Seeplex®, and AmpliSens®. The sensitivity for all three assays was 100.0%. The specificity was 99.9%, 100%, 99.4%, respectively. The PPV was evidently lower for two of their three assays than in our current study, which may be due to the low number of positive samples (n=8). The samples that were used in those studies included female swabs, female urine, and male urine (16). Detection of *T. vaginalis* by PCR in urine specimens is described to be not appropriate in women, whereas detection by PCR in vaginal swabs is a more sensitive method (17).

This is the first comparison study for detection of *T. vaginalis* with a high number of true positive samples (n=103) after calculation against the alloyed standard. In the current study we observed 17% positive samples, after discordancy analysis.

Although this is the largest comparison study for detection of *T. vaginalis* to date, our number of samples could have been higher, but that has been balanced by the high amount of positive samples found. This makes both tests suitable for detecting a *T. vaginalis* infection at one infection site. Both assays performed comparably on this study population with a high amount of positive samples and are therefore suitable for detection of *T. vaginalis.*

The evaluation of dry and wet swabs has been described in detection of *C. trachomatis* and *N. gonnorrhoeae*. That study concludes that the dry swab was as accurate as the wet swab (18). A study by Eperon *et al.* concluded that swabs can be successfully transported in a dry state at ambient temperature without greatly altering specimen integrity (19). The performances of dry swabs was previously been studied in comparison with wet swabs. Dry swabs performed as accurately as wet swabs for trichomonas at low, moderate, and high concentrations (20). Due to the performance of the dry swabs in several other studies, we chose to use dry swabs.

We previously compared the dual detecting Presto assay with the currently widely used cobas® 4800 CT/NG test for the detection of *C. trachomatis* and *N. gonorrhoeae* using dry collected vaginal and rectal swabs with the same study population (10). For *C. trachomatis* we detected 13% positive vaginal samples and 6.7% positive rectal samples. In the current study, we did not include rectal samples, due to only one positive *T. vaginalis* sample. The *C.*

trachomatis and *N. gonorrhoeae* results from the triple detecting Presto^{plus} assay were comparable with the results from the Presto assay (data not shown), so dry swabs appear to perform well and are useful for *C. trachomatis* and *N. gonorrhoeae.*

In conclusion, good diagnostics are essential for prevention of further spreading of STI in the healthy population. Therefore diagnostic tests should display high sensitivity whereas false-positives have to be excluded at any time. The Presto^{plus} assay combines multiple pathogens in a single assay, which provides new diagnostic insights and cost effectiveness.

Competing interests

SAM, employed by the VU University Medical Center has been involved in the technical development of the Presto^{plus} CT-NG-TV assay (Marketed by Goffin Molecular Technologies, Houten, The Netherlands) via Microbiome Ltd, a spin-in company of the VU University Medical Center, Amsterdam, the Netherlands.

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