Evaluation of Presto\textsuperscript{plus} assay and LightMix kit \textit{Trichomonas vaginalis} assay for detection of \textit{Trichomonas vaginalis} in dry vaginal swabs

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ABSTRACT

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

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\textit{Trichomonas vaginalis} causes the most common non-viral sexual transmitted infection (STI) with annually 248 million new cases worldwide (\textit{World Health Organization DoRHaR}, 2011). \textit{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) (McGowin et al., 2014; Soper, 2004). Untreated \textit{T. vaginalis} infection affects sexual, reproductive and obstetric health and may facilitate transmission of other STIs, including an HIV infection. \textit{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 \textit{T. vaginalis} infection with a concurrent Chlamydia infection (Mavedzenge et al., 2010; Naidoo and Wand, 2013). A \textit{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 \textit{T. vaginalis} and are currently most commonly used. However, wet mount is described to be insensitive compared to culture (53%) (Kissingier, 2015). Another disadvantage of wet mount microscopy is that should be read within ten minutes of collection (Kissingier, 2015). A disadvantage of culture is that \textit{T. vaginalis} is undetectable for months after treatment with metronidazole in HIV positive women (Gatski et al., 2010; Peterman et al., 2009).

More sensitive techniques are DNA or RNA amplification tests to detect \textit{T. vaginalis}: PCR-based methods report 40% more positive samples than culture (Caliendo et al., 2005). To facilitate implementation of these molecular tests in the routine microbiology laboratory, combination tests have been developed that combine multiple pathogens (e.g. \textit{Chlamydia trachomatis}, \textit{Neisseria gonorrhoeae}, and \textit{T. vaginalis}) in a single assay. The Presto Assay (Microbiome Ltd., Houten, The Netherlands) is a dual detection assay for \textit{C. trachomatis} and \textit{N. gonorrhoeae}. The Presto\textsuperscript{plus} CT/NG/TV assay (Microbiome Ltd., Houten, The Netherlands) is a triple detection assay, with an additional \textit{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 (de Waaij et al., 2015). In the current study, we evaluated the Presto\textsuperscript{plus} CT/NG/TV assay for detection of \textit{T. vaginalis}. Due to the unavailability of a Roche \textit{T. vaginalis} Assay, we compared it to the TIB MOLBIOL (TMB) LightMix Kit \textit{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 (Peters et al., 2014). 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 (Peters et al., 2014). 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. gonorrhoeae, and T. vaginalis infection. 200 μl of each sample were used for DNA extraction and the rest was stored at −20 °C.

For the Prestoplus 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 Prestoplus 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 Prestoplus 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 (Spiegelman et al., 1997) was defined as concordant results for the Prestoplus 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 Prestoplus 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 Prestoplus and LightMix Kit T. vaginalis assay. These were analyzed by the Qiagen Microbial DNA qPCR assay. Qiagen Microbial DNA qPCR assay confirmed the two Prestoplus 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 Prestoplus. See Fig. 1.

Sensitivity, specificity, and positive and negative predictive values of the Prestoplus and LightMix Kit T. vaginalis assays were calculated against the alloyed gold standard and are comparable (Table 1). Sensitivity and specificity for Prestoplus and LightMix Kit T. vaginalis were 95.2% and 98.1%, respectively. Further details can be seen in Table 1. Prestoplus 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).

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 (Sena et al., 2007). 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

![Flow diagram of the results of the vaginal samples for T. vaginalis. The 615 samples, tested by Prestoplus 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.](Image 313x508 to 559x741)

![Fig. 1. Flow diagram of the results of the vaginal samples for T. vaginalis. The 615 samples, tested by Prestoplus 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.](Image 313x508 to 559x741)

84% and 78% were obtained, respectively (Wendel et al., 2002). For that study, the Papanicolaou smear was used as the gold standard, which may lower the sensitivity.

Several 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% (Hardick et al., 2006). 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 (Cho et al., 2013). 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 (Lawing et al., 2000).

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. gonorrhoeae. That study concludes that the dry swab was as accurate as the wet swab (Gaydos et al., 2002). 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 (Eperon et al., 2013). The performances of dry swabs was previously been studied in comparison with wet swabs. Dry swabs
performed as accurately as wet swabs for trichomoniasis at low, moderate, and high concentrations (Gaydos et al., 2012). 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 (de Waaij et al., 2015). 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 Coffin Molecular Technologies, Houten, The Netherlands) via Microbiome Ltd., a spin-in company of the VU University Medical Center, Amsterdam, the Netherlands.

### References


### Table 1

<table>
<thead>
<tr>
<th>Sensitivity (%)</th>
<th>Sens. CI (%)</th>
<th>Specificity (%)</th>
<th>Spec. CI (%)</th>
<th>PPV (%)</th>
<th>PPV CI (%)</th>
<th>NPV (%)</th>
<th>NPV CI (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Presto®Plus</td>
<td>95.28</td>
<td>95.1–95.36</td>
<td>100.0</td>
<td>99.97</td>
<td>99.98</td>
<td>99.0</td>
<td>98.8–98.9</td>
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<tr>
<td>LightMix Kit</td>
<td>98.1</td>
<td>97.9–98.3</td>
<td>98.4</td>
<td>97.9</td>
<td>92.7</td>
<td>98.6</td>
<td>99.6–99.9</td>
</tr>
</tbody>
</table>

Sensitivity, CI, confidence interval; Specificity, Spec. CI, confidence interval; 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.