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A new rapid molecular point-of-care assay for *Trichomonas vaginalis*: preliminary performance data

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Abstract

Objective—*Trichomonas vaginalis* infection is the most prevalent treatable sexually transmitted infection (STI) in the world. An accurate point-of-care (PoC) molecular test would enable patients to be tested and treated for *T vaginalis* in a single visit to the genitourinary medicine clinic, community STI clinic, pharmacy or doctor's office. In this report, we describe a rapid prototype assay for *T vaginalis* designed for use in conjunction with the Atlas io PoC platform, and initial verification of its performance using 90 clinical samples.

Methods—A rapid prototype *T vaginalis* assay was designed. The test, featuring novel electrochemical endpoint detection, used a multi-copy region of the *T vaginalis* genome as the assay target. Ninety clinical vaginal swab samples were used to verify the performance of the prototype assay.

Results—The assay demonstrated a sensitivity and specificity of 95.5% (42/44) and 95.7% (44/46), respectively, when tested using clinical samples. Assay inclusivity was demonstrated for a number of geographically diverse *T vaginalis* isolates, and the test showed no cross-reactivity with either human DNA or a panel of DNAs isolated from common cross-reactants.

Conclusions—The sensitivity and specificity achieved using this prototype assay is comparable with that achieved for existing central laboratory nucleic acid amplification tests used for screening patients for *T vaginalis*.

INTRODUCTION

Trichomoniasis is the most prevalent curable sexually transmitted infection (STI) in the world, causing an estimated 248 million new cases of genital *Trichomonas vaginalis* infection annually.[1] The organism affects the urogenital tract of both sexes, where it can cause vaginitis in women, and non-gonococcal urethritis and prostatitis in men. Timely diagnosis and treatment is essential, but current STI screening tests suffer from lengthy

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turnaround times, exacerbating the spread of the disease through continued sexual activity during this period and the failure of patients to attend follow-up appointments where treatment can be provided. This is especially important in view of the established links between *T vaginalis* infection and an increased risk of HIV-1 acquisition,[2] and between *T vaginalis* and low birth weight and preterm delivery of neonates.[3] The treatment for trichomoniasis is a single oral dose of either metronidazole or tinidazole,[4] which, in combination with a rapid test for *T vaginalis*, would allow a 'test and treat' approach to this infection at the point of care. Point-of-care (PoC) tests currently available for *T vaginalis* include wet mount microscopy (which is a subjective test and suffers from a lack of accuracy) and immunochomatographic assays that approach the sensitivities observed using molecular testing methods.[5] To meet the need for rapid PoC diagnostics, Atlas is developing io: a platform technology capable of running molecular and immunoassays with a time-to-result of 30 min, which is within the optimum waiting period for a PoC diagnostic test result suggested in a recent study.[6] The technology comprises a cartridge to which an unprocessed clinical sample is added. The sample is fully processed on the cartridge using on-cartridge reagents, a low-cost instrument that provides fluidic and temperature control, end-point electronics and software. We have previously reported a PoC test for *Chlamydia trachomatis* using this platform.[7] In the present study, a *T vaginalis* assay was designed and tested with vaginal swab samples that had been pre-typed for *T vaginalis* using the Gen-Probe APTIMA *T vaginalis* test (Hologic Gen-Probe, San Diego, California, USA).[8]

MATERIALS AND METHODS

The published *T vaginalis* draft genome sequence has identified a superabundance of regions with repeats and transposable elements comprising up to two-thirds of the genetic material in this organism.[9] A bench-top (laboratory) version of the *T vaginalis* assay was developed using a genetic biomarker identified as being both unique to the parasite and present in multi-copy. The assay procedure was developed in a similar manner to that described for Atlas's *C trachomatis* assay.[7] Molded plastic 'subcircuits', each representative of one of the three stages of the assay (target DNA extraction, amplification and electrochemical detection) were employed. Following extraction in the presence of an internal assay control, genetic material from *T vaginalis* was amplified using PCR. Amplicons generated were subsequently detected using electrochemical detection.[10] The output of the assay is an electrochemical signal obtained using differential pulse voltammetry.[11] A high peak indicated the presence of *T vaginalis*, whereas a low (or no) peak indicated absence of *T vaginalis* in a clinical sample. Assay inclusivity was tested using a panel of 13 *T vaginalis* isolates obtained from worldwide geographical locations (see web appendix). Assay exclusivity was tested using a panel of common cross-reactants comprising genomic material from organisms generally associated with the genitourinary tract and human DNA, plus the DNA from two protists that are closely genetically related to *T vaginalis: Trichomonas tenax* and *Pentatrichomonas hominis* (see web appendix). Analytical sensitivity of the *T vaginalis* assay was determined by adding serial 10-fold dilutions of *T vaginalis* cells to aliquots of swab transport medium. Clinical sample testing was performed by similarly testing 90 self-collected vaginal swab residual samples (44 positive, 46 negative) that were available for use at the Johns Hopkins International STD Laboratory. The samples had previously been typed using the Gen-Probe APTIMA *T vaginalis* assay before being stored frozen for this evaluation. The swab used for collection was that supplied in the Gen-Probe vaginal specimen collection kit.

RESULTS

The detection limit of the *T vaginalis* assay was shown to be five *T vaginalis* cells. No cross-reactivity with the nucleic acids from organisms commonly associated with the

genitourinary tract, human DNA, *T tenax* or *P hominis* was detected, indicated by electrochemical signals below cut-off being observed for these sample types (see web appendix). All 13 *T vaginalis* isolates obtained from a range of geographical locations were successfully detected, with electrochemical peak heights above cut-off being obtained for each isolate (see web appendix). The clinical sample testing data (figure 1) demonstrated a clinical sensitivity and specificity of 95.5% (42/44, 95% CI: 83.2% to 99.2%) and 95.7% (44/46, 95% CI: 84.0% to 99.2%), respectively. The cut-off value of 51.48 nA was selected by taking the mean peak height values of the negative clinical sample plus 3 SD.

DISCUSSION

We have designed a highly sensitive assay for *T vaginalis* that detects all *T vaginalis* isolates tested and excludes common cross-reactants. A qualitative study involving STI clinic directors, clinicians and public health professionals in the USA suggests the sensitivity and specificity demonstrated by this assay is in line with expectations for a nucleic acid amplification test that could be used for detecting STIs in the PoC setting.[612] Combined with an overall duration of under 30 min, this PoC test would allow a considerable reduction in time-to-result to be realised compared to laboratory-based molecular screening tests. The speed, simplicity and robustness of the electrochemical end-point detection method are well suited to a PoC diagnostic and offer a number of benefits over fluorescence detection methods. The instrumentation involved in electrochemical analysis is simpler and more robust compared to fluorescence analysis, making the instrument easier to miniaturise, simpler to maintain and produce at a cost compatible with PoC settings. Multiple labels have been synthesised that will permit multiplex assays to be developed, meeting the requirements of clinicians and laboratory directors.[12] In this study, there was insufficient clinical material to allow the resolution of discrepant samples. This was a pilot study and in a future prospective evaluation, we would consider using a third molecular method to resolve such samples. The testing conducted in this study was achieved using moulded plastic subcircuits, each performing a single stage of the assay process. The next stage of this work will be to adapt the *T vaginalis* assay onto a fully integrated cartridge capable of being run on the Atlas io instrument. We will then complete a more detailed performance evaluation of the final PoC assay using a greater number of clinical samples.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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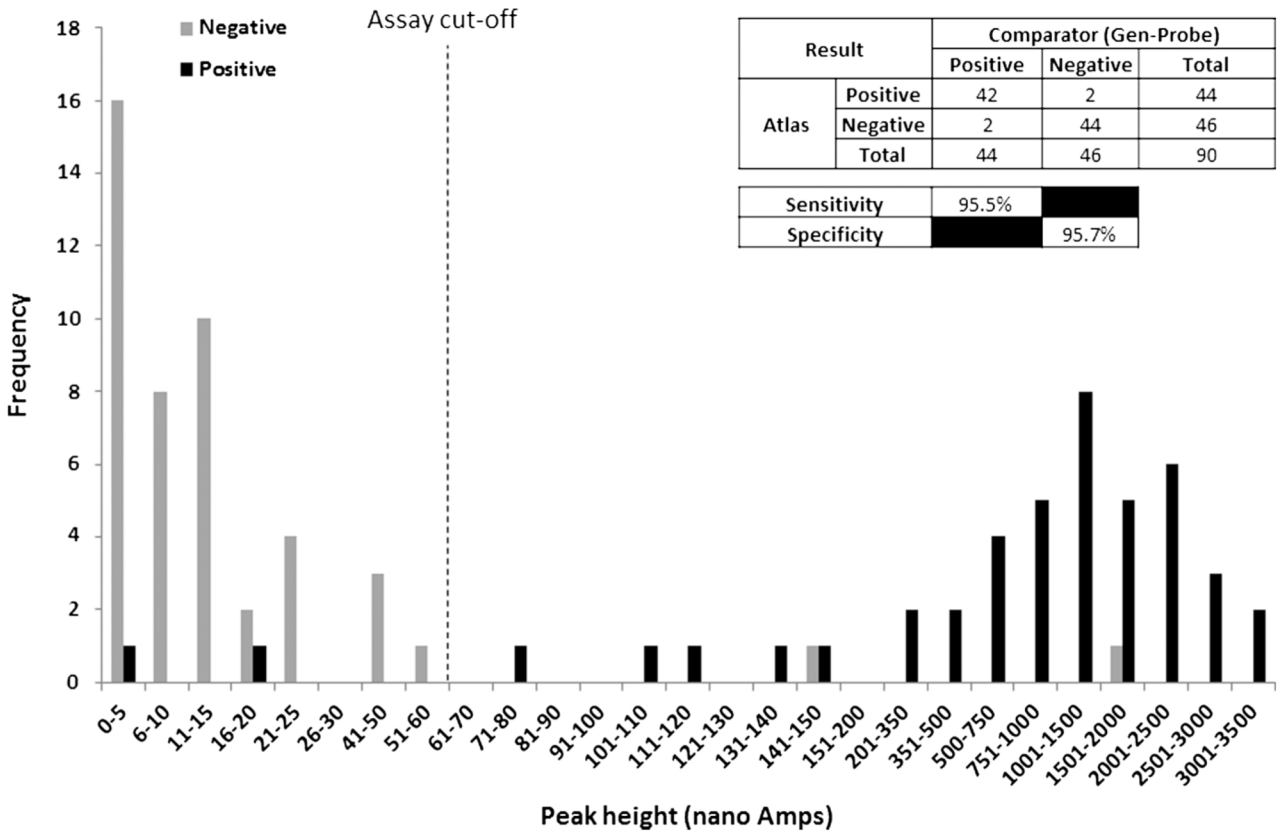

Result		Comparator (Gen-Probe)		
		Positive	Negative	Total
Atlas	Positive	42	2	44
	Negative	2	44	46
	Total	44	46	90

Sensitivity	95.5%	
Specificity		95.7%

Figure 1.
Clinical sample data.