Analytical evaluation of microscopy and molecular diagnostic tools for male genital schistosomiasis using spiked semen samples

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Background: Male genital schistosomiasis (MGS) occurs when eggs laid by *Schistosoma haematobium* become trapped within the male reproductive organs. Microscopy of semen samples for the detection of *Schistosoma* eggs is typically utilized for diagnosis, however this technique suffers from low sensitivity and no standardized protocol currently exists. Molecular diagnostic tools such as PCR may help overcome these limitations, however cost and infrastructure requirements are prohibitive to their use in resource-constrained settings. In addition, the high amount of DNA present in semen and robustness of *Schistosoma* eggs presents a challenge for DNA extraction. Isothermal diagnostic tools with lower resource requirements such as recombinase polymerase amplification (RPA) may be more suitable for use in these settings, yet none have been validated for use with semen samples. The aim of this study was to evaluate the performance of different diagnostic tests for MGS (microscopy, qPCR, RPA) using spiked semen samples, and optimize their usage for lower resource settings.

Methods: Aliquots of pooled human semen from healthy donors were spiked with known quantities of *Schistosoma haematobium* eggs (range: 1-20). For microscopy evaluation, two sample concentration techniques were compared (filtration and centrifugation). Probit regression was used to calculate the limit of detection for each method. Molecular diagnostics were assessed using aliquots of semen spiked with a single egg. Four commercial DNA extraction kits were compared and DNA quantified using the Qubit 2.0 fluorometer and a *Schistosoma* ITS2 qPCR assay. The performance of an existing *S. haematobium* RPA assay (Sh-RPA) was evaluated compared to *Schistosoma* ITS-2 qPCR as the reference standard.

Results: The limit of detection for semen microscopy with filtration was calculated to be 2 eggs, while the limit of detection for centrifugation was 5 eggs. The most effective DNA extraction kit was observed to be the QIAGEN DNeasy extraction kit with the addition of a bead-beating step. Two rapid, crude DNA extraction kits (SwiftX [Xpedite Diagnostics] and Extracta DNA Prep [Quantabio]) were able to extract *Schistosoma* DNA from semen samples, albeit in lower quantities. The Sh-RPA demonstrated a sensitivity of 95.0% (95% CI: 83.1-99.4) for samples spiked with 1 egg.

Conclusion: This study presents the first laboratory evaluation of diagnostic tools for MGS using spiked samples and identifies optimized protocols for semen microscopy and DNA extraction. We show that bead beating of samples prior to extraction is an important step in the diagnostic pipeline, and that filtration of samples prior to microscopy demonstrates higher sensitivity compared to centrifugation. The Sh-RPA demonstrated high sensitivity when tested on spiked semen samples and presents a promising opportunity for use in resource-constrained settings. Further work is needed for evaluation of each diagnostic tool with clinical samples.