PLOS NEGLECTED TROPICAL DISEASES

RESEARCH ARTICLE

Validation of the isothermal *Schistosoma haematobium* Recombinase Polymerase Amplification (RPA) assay, coupled with simplified sample preparation, for diagnosing female genital schistosomiasis using cervicovaginal lavage and vaginal self-swab samples

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Abstract

Background

Female genital schistosomiasis (FGS) is a neglected and disabling gynecological disease that can result from infection with the parasitic trematode *Schistosoma haematobium*. Accurate diagnosis of FGS is crucial for effective case management, surveillance and control. However, current methods for diagnosis and morbidity assessment can be inaccessible to those at need, labour intensive, costly and unreliable. Molecular techniques such as PCR can be used to reliably diagnose FGS via the detection of *Schistosoma* DNA using cervicovaginal lavage (CVL) samples as well as lesser-invasive vaginal self-swab (VSS) and cervical self-swab samples. PCR is, however, currently unsuited for use in most endemic settings. As such, in this study, we assessed the use of a rapid and portable *S. haematobium* recombinase polymerase amplification (Sh-RPA) isothermal molecular diagnostic assay, coupled with simplified sample preparation methodologies, to detect *S. haematobium* DNA using CVL and VSS samples provided by patients in Zambia.

Methodology/Principal findings

VSS and CVL samples were screened for FGS using a previously developed Sh-RPA assay. DNA was isolated from VSS and CVL samples using the *QIAamp Mini kit* (n = 603

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and 527, respectively). DNA was also isolated from CVL samples using two rapid and portable DNA extraction methods: 1) the *SpeedXtract Nucleic Acid Kit* (n = 223) and 2) the *Extracta DNA Tissue Prep Kit* (n = 136). Diagnostic performance of the Sh-RPA using VSS DNA extacts (*QIAamp Mini kit*) as well as CVL DNA extracts (*QIAamp Mini kit*, *SpeedXtract Nucleic Acid Kit* and *Extracta DNA Tissue Prep Kit*) was then compared to a real-time PCR reference test.

Results suggest that optimal performance may be achieved when the Sh-RPA is used with PuVSS samples (sensitivity 93.3%; specificity 96.6%), however no comparisons between different DNA extraction methods using VSS samples could be carried out within this study. When using CVL samples, sensitivity of the Sh-RPA ranged between 71.4 and 85.7 across all three DNA extraction methods when compared to real-time PCR using CVL samples prepared using the *QIAamp Mini kit*. Interestingly, of these three DNA extraction methods, the rapid and portable *SpeedXtract* method had the greatest sensitivity and specificity (85.7% and 98.1%, respectively). Specificity of the Sh-RPA was >91% across all comparisons.

Conclusions/Significance

These results supplement previous findings, highlighting that the use of genital self-swab sampling for diagnosing FGS should be explored further whilst also demonstrating that rapid and portable DNA isolation methods can be used to detect *S. haematobium* DNA within clinical samples using RPA. Although further development and assessment is needed, it was concluded that the Sh-RPA, coupled with simplified sample preparation, shows excellent promise as a rapid and sensitive diagnostic tool capable of diagnosing FGS at the point-of-care in resource-poor schistosomiasis-endemic settings.

Author summary

Female genital schistosomiasis (FGS) is a neglected gynecological disease that can result from infection with the parasitic trematode *Schistosoma haematobium*. In addition to disabling FGS-associated pathologies, *S. haematobium* infection has also been associated with sexually transmitted infections (STIs), cervical dysplasia and HIV transmission. Accurate diagnosis of FGS is crucial for effective case management, surveillance and control. However, current methods for diagnosis and morbidity assessment can be inaccessible to those at need, labour intensive, costly and unreliable. Molecular techniques such as PCR can be used to reliably diagnose FGS via the detection of *Schistosoma* DNA using cervicovaginal lavage (CVL) samples as well as lesser-invasive vaginal self-swab (VSS) and cervical self-swab samples. PCR is, however, currently unsuited for use in most endemic settings. As such, in this study, we assessed the use of a rapid and portable *S. haematobium* recombinase polymerase amplification (Sh-RPA) molecular diagnostic assay, coupled with simplified sample preparation methods that can be carried out in resourse-poor settings, to diagnose FGS using CVL and VSS samples. Performance of the Sh-RPA was excellent when compared to real-time PCR across all comparisons, even when using simplified sample preparation methods. Of note, optimal performance of the Sh-RPA may be achieved when used with the lesser-invasive VSS samples. The Sh-RPA therefore shows