Further optimisation of the *Schistosoma haematobium* Recombinase Polymerase Amplification assay: moving towards point of care use in endemic settings

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Background

Schistosomiasis is a neglected tropical disease (NTD) affecting millions of people globally, estimated to cost 1.6 million disability-adjusted life years (DALYs) with its combined morbidity and mortality (1). *Schistosoma haematobium* causes urogenital schistosomiasis, and while diagnostics already exist for this disease, improvements are essential to move beyond control of disease morbidity and towards the goal of elimination as a public health problem (2), which the WHO has declared a priority in their NTD roadmap (3). Recombinase Polymerase Amplification (RPA) offers significant promise as a sensitive, specific, and portable point-of-need diagnostic for *S. haematobium* (4), but needs further optimisation before clinical use in the field.

Methods

S. haematobium RPA (Sh-RPA) was performed on synthetic DNA standards, *Schistosoma* adult worm and egg genomic DNA, and clinical urine samples containing *S. haematobium* eggs. Key aspects of the reaction, such as betaine content (which reduces secondary structure formation) and sample volume, were altered to ascertain conditions for maximal sensitivity, specificity, and reaction efficiency. Advanced primer/probe combinations were designed and tested, with a view to improving analytical specificity. Additionally, four simple and field applicable sample preparation kits were compared for use in Sh-RPA by extracting DNA from a single *S. haematobium* egg, with the intention of filling a critical gap in knowledge for the potential use of DNA based diagnostics in low resource settings.

Results

Overall, Sh-RPA performed best in reactions containing 0.5µL of betaine and with the addition of the maximal sample volume possible, which did not hinder assay performance.

The advanced primer and probe design proved robust, with 100% analytical specificity, obviating the need for betaine and thereby simplifying reaction set up. The limits of detection were $1x10^2$ copies of synthetic Dra1 DNA and 1pg of genomic DNA. Two simple and rapid DNA extraction methods proved optimal for the preparation of DNA from single *S. haematobium* eggs, each of which enabled 100% sensitivity and specificity for the Sh-RPA. These required only 1-2 steps using simple lysis or magnetic bead methodology, without the need for lab-based equipment. Of note, addition of *S. haematobium* eggs directly to the reaction yielded strongly positive results, suggesting promise for direct addition of unextracted urine in the field, although this requires evaluation with clinical samples.

Conclusions

Previous studies have shown that the Sh-RPA is a promising diagnostic for field use, but further optimisation was needed. Here, Sh-RPA's optimal analytical specificity has been achieved, using a simpler assay with no loss of sensitivity. Additionally, sample preparation methods with minimal equipment/resources have proven suitable for the Sh-RPA assay, with a robust lower limit of detection of a single *S. haematobium* egg. This research has greatly advanced the Sh-RPA towards field use, with clinical sample testing the next vital step.

References

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