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TITLE

DEVELOPMENT OF A CRISPR-BASED DIAGNOSTIC TOOL FOR SCHISTOSOMIASIS

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ABSTRACT

Objectives: Effective diagnostics are needed urgently for rapid mapping of schistosomiasis transmission sites and in monitoring control programs, but many current diagnostic procedures lack the requisite sensitivity, portability and cost-effectiveness needed for use in resource-poor settings. Our study aims to develop an ultra-sensitive, field-friendly, potable and affordable point-of-care CRISPR-based diagnostic tool for detecting schistosomiasis in endemic countries.
Methodology: For the first time, we developed a CRISPR-based diagnostic platform SHERLOCK (Specific High-Sensitivity Enzymatic Reporter UnLOCKing) for schistosomiasis diagnosis, by combining recombinase polymerase amplification (RPA), which can be performed at a single temperature at 37• , with CRISPR-Cas13 detection, the results can be visualized either through fluorescent or colorimetric readout on a lateral flow strip. We present the novel SHERLOCK-based diagnostic assays for the detection of Schistosoma japonicum and S. mansoni infection. The assays were validated using faecal samples obtained from a mouse model infected with S. japonicum or S. mansoni, as well as clinical faecal and serum samples obtained from an S. japonicum-endemic area in Northern Samar, the Philippines and an S. mansoni-endemic area in Mayuge, Uganda. We further simplified the SHERLOCK system by combining the two-step process into a one-pot reaction, making it more user-friendly for field applications. We then compared the sensitivity of the one-pot reaction to the original two-step SHERLOCK process.
Results: The results we will present show that the CRISPR-based system is specific, sensitive, cost-effective and user-friendly. Although the assay does not require the specialized equipment or expertise necessary for real-time PCR detection, it provides a similar level of sensitivity. We found sensitivity of one-pot SHERLCOK was slightly, but not significantly, reduced compared to two-step reaction.
Conclusion: This novel schistosomiasis diagnostic test provides the basis for the development of accurate and field-friendly CRISPR-based diagnostics for other parasitic neglected tropical diseases, both as powerful point-of-care tests and as surveillance tools.

KEYWORDS

CRISPR-Based Diagnosis; SHERLOCK, Schistosomiasis, Point-of-Care, Helminth Coinfection

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