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Molecular diagnosis of intestinal schistosomiasis: An overview of current protocols and what is needed, highlighted using data from a *S. mansoni* and *S. haematobium* co-endemic area

Intestinal schistosomiasis is typically diagnosed using Kato-Katz faecal-egg microscopy. Whilst this method is relatively inexpensive and can be carried out at the point-of-care, it is considered low-throughput and lacks sensitivity, particularly when attempting to diagnose individuals harbouring low-intensity infections. Reliably measuring the prevalence of *S. mansoni* infections in areas of low disease endemicity can therefore be extremely difficult using this method alone. For these reasons, a variety of immunological rapid diagnostic lateral flow tests (RDTs) have also been developed to diagnose infection with *S. mansoni*, of which the most widely used is the urine-based point-of-care circulating cathodic antigen RDT (CCA-RDT). This assay, however, can also lack sensitivity, as well as specificity, when attempting to diagnose individuals harbouring low-intensity infections or when assessing individuals co-infected with both *S. mansoni* and *S. haematobium*.

Whilst both Kato-Katz and CCA-RDT assays are valuable diagnostic tools in certain settings, highly sensitive molecular assays, such as endpoint and real-time/quantitative PCR, are also needed for effective and impactful disease diagnosis, monitoring, and surveillance. PCR, however, is currently unsuited for use in most schistosomiasis-endemic settings. Both PCR itself, as well as the essential preliminary steps needed to isolate DNA from faecal material, require expensive and sophisticated equipment, specialised personnel, and reliable laboratory infrastructure seldom available in endemic areas. In addition, whilst a standardised diagnostic real-time PCR protocol exists to detect a genus-specific locus of the *Schistosoma* internal transcribed spacer 2 (ITS2) region, there is currently no standardised diagnostic PCR assay that is routinely used to differentiate between the various human-infecting *Schistosoma* species. As such, in areas where multiple *Schistosoma* species are endemic, molecular assays capable of detecting and distinguishing these species are needed.

Isothermal (single and constant temperature) DNA amplification methods offer an alternative to PCR-based amplification and are better suited for use in resource-poor settings as they require minimal equipment and can be highly portable and user-friendly. The most widely used isothermal DNA amplification method is loop-mediated isothermal amplification (LAMP). Again, however, no standardised and routinely used *S. mansoni*-specific LAMP assay capable of reliably diagnosing intestinal schistosomiasis is currently available. In addition, an isothermal and portable *S. mansoni*-specific Recombinase Polymerase Amplification (RPA) assay has also been recently developed, however, this has not yet been fully validated using human faecal material.

Here, we present an overview of current protocols available for the molecular diagnosis of intestinal schistosomiasis, inclusive of methods needed to isolate DNA from faecal material, PCR, and isothermal DNA amplification approaches. We also outline what is currently needed to develop, standardise, and deploy highly sensitive and specific molecular diagnostic tools that can be used at the point-of-care in low-endemicity areas, highlighted using data recently generated from a *S. mansoni* and *S. haematobium* co-endemic area.