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Article

Detection of *Schistosoma mansoni* and *Schistosoma haematobium* by Real-Time PCR with High Resolution Melting Analysis

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Abstract: The present study describes a real-time PCR approach with high resolution melting-curve (HRM) assay developed for the detection and differentiation of *Schistosoma mansoni* and *S. haematobium* in fecal and urine samples collected from rural Yemen. The samples were screened by microscopy and PCR for the *Schistosoma species* infection. A pair of degenerate primers were designed targeting partial regions in the cytochrome oxidase subunit I (*cox1*) gene of *S. mansoni* and *S. haematobium* using real-time PCR-HRM assay. The overall prevalence of schistosomiasis was 31.8%; 23.8% of the participants were infected with *S. haematobium* and 9.3% were infected with *S. mansoni*.

With regards to the intensity of infections, 22.1% and 77.9% of *S. haematobium* infections were of heavy and light intensities, respectively. Likewise, 8.1%, 40.5% and 51.4% of *S. mansoni* infections were of heavy, moderate and light intensities, respectively. The melting points were distinctive for *S. mansoni* and *S. haematobium*, categorized by peaks of 76.49 ± 0.25 °C and 75.43 ± 0.26 °C, respectively. HRM analysis showed high detection capability through the amplification of *Schistosoma* DNA with as low as 0.0001 ng/μL. Significant negative correlations were reported between the real-time PCR-HRM cycle threshold (Ct) values and microscopic egg counts for both *S. mansoni* in stool and *S. haematobium* in urine ($p < 0.01$). In conclusion, this closed-tube HRM protocol provides a potentially powerful screening molecular tool for the detection of *S. mansoni* and *S. haematobium*. It is a simple, rapid, accurate, and cost-effective method. Hence, this method is a good alternative approach to probe-based PCR assays.

Keywords: *Schistosoma mansoni*; *S. haematobium*; real-time PCR; high resolution melting analysis

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1. Introduction

Schistosomiasis, one of the most common neglected tropical diseases (NTDs), is caused by parasitic trematode worms of the genus *Schistosoma*. Three medically important species known to infect humans are *Schistosoma mansoni* and *S. japonicum*, which cause intestinal schistosomiasis, and *S. haematobium*, which causes urogenital schistosomiasis. Almost 240 million people are infected worldwide and about 700 million people are at risk of this infection [1,2]. *S. haematobium* and *S. mansoni* are endemic in sub-Saharan African, Middle Eastern and North African regions, and South America, while *S. japonicum* is restricted to southern Asia [3].

The optimal effectiveness of schistosomiasis treatment regimens and control programs are usually associated with the accurate and precise diagnosis of the infection. In many low-income countries endemic with schistosomiasis, microscopy remains the gold standard for a fast, easy, and cheap method of identification of *Schistosoma* species eggs in stool and urine samples [4–7]. However, the capability of microscopy still needs repeated sampling and careful investigation to give greater sensitivity, especially when the infections are light [8]. Several antigen methods for the detection of schistosomiasis such as ELISA and dipstick platforms assays are used for the detection of circulating cathodic (CCA) and anodic (CAA) antigens in blood and urine which are captured by using monoclonal antibodies [9–11]. These methods are specific for current infections and provide estimation for infection intensity [12–14]. Likewise, schistosomiasis antibody detection methods are highly sensitive, particularly in low transmission areas; however, they cannot differentiate between current and past infections, and blood samples are not easily collected in the endemic areas [8–15].

The increasing number of travellers, immigrants and foreign workers carrying schistosomiasis from endemic areas to non-endemic countries resulted in the development of polymerase chain reaction (PCR) methods which became a crucial, sensitive and specific diagnostic tool, particularly at an early stage of infection or in selection of the optimal treatment [16–21]. Hence, the development of PCR