



## RESEARCH ARTICLE

# A conventional multiplex PCR for the detection of four common soil-transmitted nematodes in human feces: development and validation

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## ARTICLE HISTORY

Received: 17 September 2021

Revised: 15 March 2022

Accepted: 15 March 2022

Published: 31 March 2022

## ABSTRACT

Soil-transmitted helminth (STH) infections, mainly caused by *Ascaris lumbricoides*, *Trichuris trichiura*, and hookworms, are among the most common intestinal parasites that infect humans. The infections are widely distributed throughout tropical and subtropical countries, including Malaysia, particularly in underprivileged communities. Microscopic and culture techniques have been used as a gold standard for diagnostic techniques. However, these methods yield low sensitivity and specificity, laborious and time-consuming. Therefore, simple, rapid, and accurate alternative methods are needed for the simultaneous detection of STH infections. Although advanced technologies such as real-time multiplex PCR have been established, the use of this technique as a routine diagnostic is limited due to the high cost of the instrument. Therefore, a single-round multiplex conventional PCR assay for rapid detection of four STH species in the fecal sample was developed in this study. To perform the single-round multiplex PCR, each pair of species-specific primers was selected from target genes, including *Ancylostoma duodenale* (Internal Transcribed Spacer 2; accession No. AJ001594; 156 base pair), *Necator americanus* (ITS 2; accession No. AJ001599; 225 base pair), *Ascaris lumbricoides* (Internal Transcribed Spacer 1; accession No. AJ000895; 334 base pair) and *Trichuris trichiura* (partial ITS 1, 5.8s rRNA and partial ITS 2; accession No. AM992981; 518 base pair). The results showed that the newly designed primers could detect the DNA of STH at low concentrations (0.001 ng/μl) with no cross-amplification with other species. This assay enables the differentiation of single infections as well as mixed infections. It could be used as an alternative and is a convenient method for the detection of STHs, especially for the differentiation of *N. americanus* and *A. duodenale*.

**Keywords:** Multiplex PCR; soil-transmitted helminths (STHs); intestinal nematodes; Malaysia.

## INTRODUCTION

Soil-transmitted helminth (STH) infections are among the most common infections worldwide that affect the poorest and most deprived communities. More than 1.5 billion people, or 24% of the world's population, are infected with STH infections worldwide (Wardell *et al.*, 2017; WHO, 2018; Han *et al.*, 2019; Kaewpitoon *et al.*, 2019). The main species that infect humans are the roundworm (*Ascaris lumbricoides*), whipworm (*Trichuris trichiura*), and hookworms (*Necator americanus* and *Ancylostoma duodenale*). The World Health Organization (WHO) and Disease Control and Prevention (CDC) have recognized STH infections as one of the most neglected tropical diseases under the same category as other infectious diseases (Hotez, 2009; Wardell *et al.*, 2017). More than a

quarter of the world's population is at risk for infection with the soil-transmitted helminths (STHs). The infections are most prevalent in tropical and subtropical areas, with the most significant numbers occurring in sub-Saharan Africa, the Americas, China, and East Asia (Pullan *et al.*, 2014; WHO, 2018; Darlan *et al.*, 2019). In Malaysia, STH infections are prevalent in the poor and socioeconomically deprived communities (Ngui *et al.*, 2015; Rajoo *et al.*, 2017; Mohd Shaharuddin *et al.*, 2018; Muslim *et al.*, 2019).

Infection with the STHs seldom causes death. Instead, the burden of disease is related less to mortality than to the chronic detrimental effect on the hosts' health and nutritional status (Hotez *et al.*, 2009). Soil-transmitted helminths (STHs) impair the nutritional status of the people they infect in multiple ways. The STHs feed on host tissues,