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TITLE

DROPLET DIGITAL PCR ASSAY (DDPCR) FOR THE DIAGNOSIS OF INTESTINAL SCHISTOSOMIASIS CAUSED BY SCHISTOSOMA MANSONI

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ABSTRACT

Intestinal schistosomiasis is a disease caused by the Schistosoma mansoni parasite in Brazil. The laboratory diagnosis recommended by the WHO for active search of cases of the disease is based on the Kato-Katz parasitological technique. However, this technique present low sensitivity when applied in stool samples from individuals with low parasite burden, what compromises the estimative of the real prevalence of the disease. Droplet digital PCR (ddPCR) allows direct quantification of the target sequence contained in the sample and, it presents better sensitivity and specificity compared to other diagnostic techniquesThe ddPCR assay appears as an option for diagnose S. mansoni infection, giving its sensitivity and specificity, in view of the low concentration of parasite DNA and the heterogeneity of the stool samples. The aim of this study was to standardize a ddPCR assay for the diagnosis of the intestinal schistosomiasis caused by S. mansoni. Total DNA was extracted from three S. mansoni egg-positive and three egg-negative stool samples, using the commercial QIAamp® Power Fecal Pro® Kit (Qiagen GmbH), as recommended by the manufacturer. The ddPCR assay was performed using sense primer 5′ -CCG ACC AAC CGT TCT ATG A-3′ , antisense primer 5′ -CAC GCT CTC GCA AAT AAT CTA AA-3′ and probe 5′ -6[FAM]/TCG TTG TAT CTC CGA AACCAC TGG ACG/[3BHQ1] which amplify a 90 bp fragment of the Sm1-7 repetitive region of the S. mansoni genome (GenBank: M61098). To determine the best laboratory conditions for the assay reactions were performed for a final volume of 22 μL, at different primers annealing temperatures, different primers and probes concentrations and different concentrations of the total DNA, using supermix for probes (no dUTP) as hydrolysis probe detection reagent (Bio-Rad Laboratories). The droplets were generated in the Auto Droplet Generation, the amplification reaction was performed using the C1000 Touch Thermal Cycler and the fluorescence reading was performed in the QX200 Droplet Reader all from Bio-Rad Laboratories. The results analysis was performed using the QuantaSoft Analysis Pro software, version 1.0.596 (Bio-Rad Laboratories). The best laboratory conditions were defined as an annealing temperature of 56,5° C, primer concentration of 700 nM, probe concentration of 150 nM and total DNA concentration of 40 ng. Analytical sensitivity performed using DNA extracted from adult worms indicated a limit of detection (LOD) of 0.1 pg of DNA. The ddPCR results are preliminary and will undergo validation using DNA extracted from stool samples of 249 individuals from the municipality of Conde in Bahia, Brazil, an area of moderate endemicity for the disease. The ddPCR can constitute a alternative test for intestinal schistossomiasis diagnosis. However, larger studies are still needed to confirm ddPCR superior performance compared to conventional diagnostic techniques.

KEYWORDS

Schistosoma mansoni; Intestinal Schistosomiasis; Laboratory Diagnosis; Digital PCR Droplets; ddPCR.

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