A NEW DIGITAL DROPLET PCR ASSAY FOR THE INTESTINAL SCHISTOSOMIAS DIAGNOSIS

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In Brazil, intestinal schistosomiasis is caused by the *Schistosoma mansoni* and its laboratory diagnosis is performed by the Kato-Katz technique, as recommended by WHO. However, this technique presents limitations on sensitivity when applied in low parasite burden individuals. Droplet digital PCR (ddPCR) has been presenting high accuracy for diagnosis of other diseases and appears as a choice for intestinal schistosomiasis. The aim of this study was to standardize and validate a ddPCR assay for the intestinal schistosomiasis diagnosis. For that, the total DNA was extracted from adult worms and 248 samples of individuals living in the municipality of Conde in Bahia, Brazil, an area of moderate endemicity for the disease, using QIAamp® Power Fecal Pro DNA® Kit, as recommended by the manufacturer. The ddPCR assay was performed using primers and probes targeting a 90 bp from Sm1-7 region of the *S. mansoni* genome. To define the best laboratory conditions for the assay, reactions were performed at different annealing temperatures, primers and probes concentrations and total DNA amount, using ddPCR consumables and reagents from Bio-Rad Laboratories. Repeatability was performed using 5 samples repeated 6 times, reproducibility using 7 samples repeated 5 times and analytical sensitivity using DNA extracted from adult worms. The conditions were defined as an annealing temperature of 56,5° C, primer concentration of 700 nM, probe concentration of 150 nM and total DNA amount of 40 ng. The ROC curve indicated a cut-off value of 10 copies/µL and the analytical sensitivity demonstrated a limit of detection (LOD) of 0.1 pg of DNA. Repeatability showed a coefficient of variation (CV) ranging from 6.8% to 11.1%, while reproducibility exhibited a CV ranging from 5.4% to 20.34%. The positive rate detection was 51.61%, compared to 51.21% for Kato-Katz combined with Helmintex. Thereafter, the results from ddPCR will be compared to those from qPCR and LAMP using the same DNA samples.

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