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TITLE

DEVELOPMENT AND APPLICATION OF QPCR AS AN ALTERNATIVE FOR THE DIAGNOSIS AND CONTROL OF SCHISTOSOMIASIS IN AREAS OF LOW ENDEMICITY FOR THE DISEASE

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ABSTRACT

Introduction: The diagnosis of schistosomiasis is one of the major problems regarding its control and further elimination as a public health problem. The parasitological methods are limited in sensitivity, which is directly dependent on the parasite load of the host. This problem becomes evident in low-endemic areas, represented by low positivity rate and low parasite load. Facing this problem, the aim of this study is to develop and evaluate a new Real-Time PCR system for the diagnosis of schistosomiasis in areas of low endemicity for the disease. Methodology: A new qPCR system was designed to target the Sm1-7 gene. The assay limit of detection was defined in a standard curve experiment with different concentrations in a 10-fold dilution of Schistosoma mansoni DNA ranging from 1 ng/µl to 1 fg/µl. Also, the Sm1-7-qPCR specificity was evaluated in a MegaBLAST search and experimentally, using 1 ng of genomic DNA from the parasites Wuchereria bancrofti, Mycobacterium tuberculosis, Strongyloides stercoralis, Leishmania infantum, L. braziliensis and Escherichia coli. A cross-sectional study was conducted in Catamarã, a neighborhood in Jaboatão dos Guararapes (PE) to evaluate the Sm1-7-qPCR performance. All individuals who signed an Informed Consent form, approved by the Aggeu Magalhães Ethics Committee, and handed the stool samples were included in the study. After, the patients were diagnosed using the Kato-Katz (KK) method, in which two slides were examined per sample, and Hoffman (HH), in which all the sediment was analyzed. In parallel, the stool samples had their DNA extracted, then submitted to the optimized Sm1-7-qPCR, together with an Internal Positive Control of the reaction (IPC). The results were used to estimate the sensitivity, specificity, Cohen's kappa and predictive values of the qPCR. Results: The system showed high sensitivity, amplifying up to 1 fg /µl of S. mansoni genomic DNA with an average Ct of 30.737, which was the cut-off point for determining positive and negative samples. There was no amplification of the other parasites' DNA, nor was there any probable alignment with any other sequence by the MegaBlast search. A total of 351 stool samples were collected during the coproscopic investigation, of which 38 were positive for S. mansoni in KK, while 23 were positive in HH and 162 were positive for Sm1-7-qPCR. A composite reference method (CRM), including the results of the parasitological methods, resulted in 44 positive samples in total. Compared with the CRM, the sensitivity of Sm1-7-qPCR was 85.11%, the specificity was 59.61%, the positive predictive value was 24.39%, the negative predictive value was 96.32% and the Kappa was 0.218. Conclusion: The new Sm1-7-qPCR has high sensitivity and moderate specificity and may be used as a screening test for schistosomiasis in low-endemic areas. In addition, the selection of sensitive reference methods is essential for the evaluation of new diagnostic methodologies under development.

KEYWORDS

Schistosomiasis mansoni; Molecular Diagnosis; Low-endemic Areas; Parasite Load

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