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TITLE

EVALUATION OF ELISA TESTS FOR THE DIAGNOSIS OF SCHISTOSOMIASIS USING A SCHISTOSOMA MANSONI CHIMERIC RECOMBINANT PROTEIN

AUTHORS

Fernandes, M.A.*[1]; Bernades, W.P.O.S.[1]; Carvalho, G.B.F.[1]; Silva-Pereira, R.A.[1]; Fonseca, C.T.[1]

AFFILIATIONS

[1] Grupo de Pesquisa em Biologia e Imunologia de Doenças Infecciosas e Parasitárias, Instituto René Rachou – Fiocruz Minas

ABSTRACT

Introduction: The Kato-Katz method has low sensitivity for detecting S. mansoni infected individuals with low parasite burden. To overcome this, the Enzyme-Linked Immunosorbent Assay (ELISA) arises as an alternative. But the applicability of the ELISA is limited by its low specificity. We hypothesize that the use of a chimeric recombinant protein, containing epitopes from different parasite antigens, in an ELISA would result in a more accurate test. Objective: The aim of this study was to obtain a chimeric recombinant protein and to standardize and evaluate an ELISA test using this antigen. Methods: The recombinant protein was constructed by combining 5 epitopes of S. mansoni proteome. The gene that encodes the protein was inserted into a vector and the expression was carried out in a prokaryotic system. After induction with IPTG, the bacteria were lysed by sonication in a denaturing lysis buffer and purified using nickel affinity chromatography. ELISA standardization was performed by testing different microplates, antigen concentrations and blocking solutions. In the microplate's evaluation 1; 2; 4; 6; 10 and 12µg/mL of antigen was tested in a Maxisorp and Corning EIA/RIA plates using an anti-his-HRP antibody (1:2000) as the detector antibody. The blocking solutions tested were 10% FBS and 3% BSA in a Phosphate Saline Buffer with tween 20. Serum from uninfected and infected individuals from an endemic region, tested by parasitological and molecular methods, was used to evaluate test's performance. The standardized test was carried out using two different batches of recombinant protein. The absorbance was measured in a microplate reader at 450nm. Arbitrary unit was calculated by dividing the absorbance of the sample by the absorbance of a calibrator. Data were analyzed using the ROC curve test. It was determined a cutoff point that prioritize the highest values for the test accuracy. Result: The recombinant protein (25kDa) was obtained with satisfactory purity and yield (4.9mg/L). ELISA standardization demonstrated that 11g/mL of recombinant protein is sufficient to saturate the well of the Maxisorp plate while the Corning plate required more antigens to do that. The solution containing 3% BSA was more effective in blocking nonspecific bonds. So, the ELISAs assays were performed in a Maxisorp plate, coated with 12µg/mL of chimeric protein, blocked with 3% BSA. Serum samples and anti-IgG conjugated to HRP were used at a 1:50 and 1:60.000 dilution respectively. TMB was used as substrate. A common cutoff point of 0.18191 was used for both batches of test. For batch 1 the sensitivity was 76% and the specificity was 38% with an Area Under the curve (AUC) of 0.63. For batch 2, the sensitivity was 83% and specificity 30% with an AUC of 0.65. Conclusion: Despite demonstrating a high sensitivity value, the chimera-Elisa test showed low specificity for diagnosing the infection in endemic areas, limiting its use to an initial screening stage.

KEYWORDS

Diagnosis; Chimeric Protein; Schistosomiasis

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