IVD_08 - Prototype of an ELISA on the HRP system using a chimeric polyprotein for the diagnosis of chronic *Trypanosoma cruzi* infection

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Introduction: The absence of a gold standard test for chronic Chagas disease (CD) complicates its diagnosis which is currently time-consuming, complex, expensive and sometimes inconclusive. Our group developed a chimeric protein containing 10 epitopes specific to *T. cruzi*, named PlatCruzi V1, where the first phase of testing showed 100% sensitivity and specificity in alkaline phosphatase ELISA.

Objective: Evaluate the performance of PlatCruzi V1 in ELISA tests in the peroxidase system against the gold standard criteria.

Methodology: Recombinant PlatCruzi V1 was produced in *E. coli* strain BL21 (DE3) and purified from inclusion bodies by affinity chromatography on an Aktapurifier system. HRP ELISA tests were performed in Nunc MaxiSorp® microplates, USA, with 200 ng of the recombinant protein per well, using samples in duplicate (the IS in triplicate). The ELISA cutoff was determined using 216 serological samples (70 positive for *T. cruzi* and 146 negative) that were tested for chronic CD by ELISA (Wiener 3.0) and Indirect Immunofluorescence (IFI-Chagas-Bio-Manguinhos Fiocruz-Brasil kit). The sensibility was determined with International Standards of Biological References (IS)-WHO, from endemic areas (Mexico, Brazil and Chile) predicted for the TcI and TcII strains. Specificity was determined using a panel of patient sera with other diseases/infections. Graphs of the optical densities, reactivity index (RI) and ROC curve were generated by GraphPad Prism v8.1.

Results: PlatCruzi V1 presented 93% sensitivity and 97% specificity. The results of the assay with IS in HRP ELISA demonstrated that PlatCruzi V1 exceeded the recommended dilution of 1:64 four-fold to >1:256.

Conclusion: PlatCruzi V1 is a promising target for the detection of chronic *T. cruzi* infection by the HRP ELISA method, with high sensitivity and specificity per study region and reactivity presented in all dilutions with the IS from the Biological References, suggesting a differentiated tool to commercialized serological diagnostic tests. Patents submitted (BR 10 2019 017792 6).

Keywords: Chronic Chagas disease; Gold standard test; Chimeric protein