IVD.07 - Two innovative multi-epitope recombinant proteins for the diagnosis of chronic *T. cruzi* infections

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Introduction: Chagas disease is a neglected tropical disease endemic to 21 Latin American countries caused by a chronic infection of the parasite *Trypanosoma cruzi.* Approximately 28,000 new cases are reported every year with 12,000 to 14,000 deaths. In 2018, outbreaks were reported in the Brazilian States of Amazonas, Pará and Maranhão. Diagnosis of the disease in the chronic phase is through the detection of anti-T. cruzi IgG antibodies in patient sera mainly through immunological assays. Yet, no current assay serves as a gold standard and multiple tests are normally performed to confirm a diagnosis. With the severity of the disease and the rash of new cases, there is an urgent need for high performance diagnostic reagents to generate a diagnostic assay that delivers high confidence results from a single test at a low-cost and with a capacity to be used throughout all endemic areas.

Objective: We present the results on an innovative approach to create two chimeric proteins, named PlatCruzi V1 and PlatCruzi V2, that carry multiple epitopes from a variety of *T. cruzi* proteins and can serve as a target in serological tests.

Methodology: Individual linear B epitopes previously identified experimentally, both in our laboratory and described in the literature, were selected for incorporation *in silico* into a core proteinaceous sequence and synthesized as DNA fragments. After transfer to the pET-28a expression vector, proteins were easily expressed in *E. coli* at high levels and purified to near homogeneity from exclusion bodies. To evaluate their diagnostic potential, immunoassays were performed through an indirect ELISA technique using a variety of serological samples that included the International Biological Reference Standards pre-measured by the World Health Organization, sera of patients with chronic Chagas' disease from the states of Amazonas, Ceará, Maranhão and Sergipe, as well as sera from persons with other active and/or previously resolved infections: visceral and cutaneous leishmaniasis, dengue, malaria and tuberculosis along with samples of people who did not present any known infections at the time collection.

Results: Both recombinant proteins displayed excellent performance to detect persons infected by *T. cruzi* based on Receiver Operating Characteristics: PlatCruzi V1 (100% sensitivity and specificity) and PlatCruzi V2 (96.72% sensitivity and 100% specificity). From the recommended dilution series of the International Biological Reference Standards, each had a calculated index reactivity >1.0.

Conclusion: The results suggest that both chimeric proteins were successfully engineered to represent *T. cruzi* for use in ELISA system and are potential targets to use in point-of-care systems in endemic and non-endemic areas. Highlight the core protein that shows great promise of technological innovation to generate immunological mimics of different pathogens through the insertion of pathogen specific epitopes, which can potentially permit the bioengineering of a wide range of immunological reagents for diagnostic, therapeutic and vaccine purposes.

Keywords: Chagas? disease; Serological diagnosis; Chimeric proteins