Introduction: A need still exists for a serodiagnostic test that can definitively diagnose Chagas disease in the chronic phase. Caused by the protozoan *Trypanosoma cruzi*, commercially available tests suffer from low accuracy leading to a diagnostic requirement from the Ministry of Health for two independent, concordant assays that delays results and increases cost. To overcome the limitations of previous approaches, the recombinant protein DxCruziV3 was developed to serve as a diagnostic test’s antibody capture reagent that consists of ten epitopes, exclusive to *T. cruzi*, inserted into the beta barrel structure of fluorescent proteins.

Objectives: Develop a commercially promising indirect ELISA test based on one of two forms of DxCruziV3 to confidently identify chronic Chagas disease patients through the detection of anti-*T. cruzi* IgG antibodies.

Methodology: Recombinant protein was purified from bacteria as soluble protein (sV3) or insoluble protein (iV3) that were utilized to prepare two versions of an indirect ELISA. After optimization, a panel of 212 sera (126 positive and 86 negative) was applied to calculate assay specificity and sensitivity along with an analysis of production conditions.

Results: The data show that the ELISA with iV3 presented a sensitivity of 97.66% and a specificity of 98.81%. With sV3, the sensitivity was 99.21% and 100% for specificity. During preparation of the recombinant proteins, the process for the soluble form involved multiple additional steps than the insoluble form that required more time and resources.

Conclusion: The performance of the two diagnostic ELISA tests developed for chronic Chagas disease presented excellent sensitivity and specificity. Considering the lower demands for time and resources during protein preparation, the results suggest that iV3 would be the best candidate to continue product development. In addition to diagnosis, the ELISA’s high performance could also be used to accompany loss of antibody titer during treatment. Therefore, a complete solution could be delivered in the future to chronic patients.

Keywords: Diagnostic test; Chagas disease; *Trypanosoma Cruzi*