

IVD_11 - Evaluation a prototype rapid test for chronic Chagas disease in an endemic region of the Brazilian Amazon

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Introduction: Individuals infected with *Trypanosoma cruzi*, the etiological agent of Chagas disease, from the Brazilian Amazon have historically proven to be difficult to identify through serological tests due to false negatives (low sensitivity) and false positives (low specificity) that has necessitated the use of multiple assays for diagnosis.

Objectives: Develop a lateral flow rapid test employing our DxCruziV3 to diagnose *T. cruzi* infections with high confidence.

Methodology: DxCruziV3, a multi-epitope protein composed of ten *T. cruzi* specific epitopes inserted into the β -barrel of Thermal Green Protein, was produced as insoluble and soluble recombinant protein that was affinity purified by metal affinity chromatography. A performance evaluation by *in-house* ELISAs showed a sensitivity >96% and 100% specificity (no cross-reactivity with cutaneous and visceral leishmaniasis, dengue, malaria, or syphilis). Two versions of the rapid test prototypes were independently produced using either the soluble or insoluble fractions and applied to a total of 167 individuals recruited in the municipality of Barcelos, Amazonas that included residents from five riverside communities. Whole blood (10 μ L) and serum (5 μ L) were tested in both kits during a patient session. A venous blood sample (5 mL) was also collected for retesting in the lab as well as to perform confirmatory assays using an *in-house* ELISA, indirect immunofluorescence (IFI), and a commercial ELISA (BioClin).

Results: Forty-three (26%) individuals were reactive for chronic Chagas disease by both whole blood and serum samples. Three (2%) showed divergent results between the soluble and insoluble prototypes. By IFI, 83% (139) of samples gave concordant results while 75% (125) agreed with the commercial ELISA. The *in-house* ELISA (DxCruziV3) had the highest concordance (95%; 159).

Conclusion: Our multi-epitope protein DxCruzi V3 showed excellent performance as the capture molecule in a lateral flow format for the rapid testing of Chagas disease in persons living in and around the municipality of Barcelos, Amazonas, an areas endemic for Chagas disease, malaria, cutaneous leishmaniasis and other diseases.

Keywords: Chronic Chagas disease, gold standard test, chimeric protein