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NOV
2023



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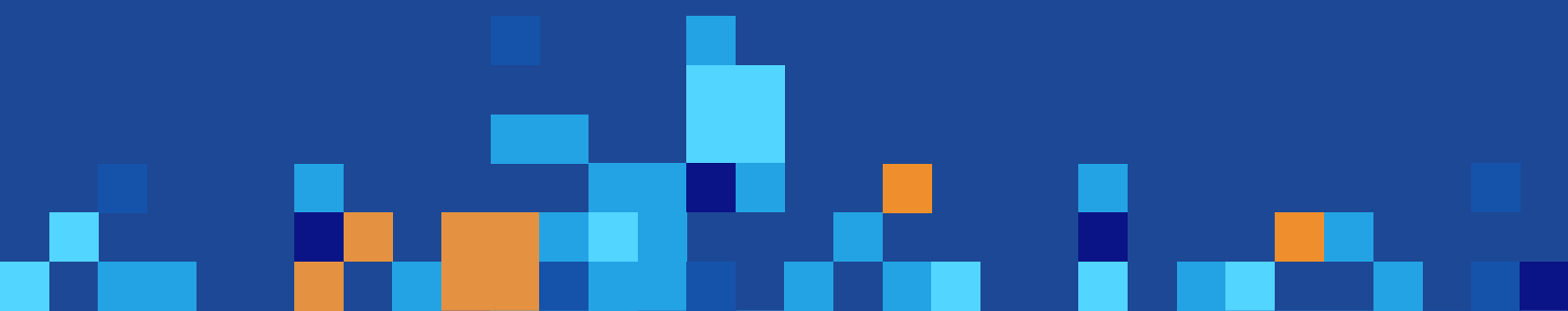


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Diagnostic performance of two domains derived from a novel *Trypanosoma cruzi* protein to chronic Chagas disease in endemic areas of Brazil

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Chronic Chagas disease (CCD), a potentially life-threatening illness, is an infection caused by *Trypanosoma cruzi* parasite. In this stage, the WHO advises using at least two distinct IgG-based tests for a reliable diagnosis, due to the lack of a "gold-standard" laboratory technique. Recently, we demonstrated the diagnosis performance of two domains (D3 and D6) derived from a hypothetical *T. cruzi* protein, named Tc323, which has no homologs in *T. brucei* or *Leishmania* genomes. For this, we used plasma of patients with CCD and non-infected donors from Argentina, Bolivia, and Paraguay. Here, we extended our work by testing plasma of individuals with CCD or infected with unrelated diseases living in endemic regions of Brazil. After the optimization of our indirect ELISA, recombinant D3 and D6 proteins were assessed by employing plasma of 405 *T. cruzi*-positive and 530 *T. cruzi*-negative individuals from distinct areas of Brazil. Additionally, 748 samples from donors with unrelated diseases were included to evaluate cross-reactivity. The receiver operating characteristic (ROC) curve was used to define the cut-off value with the best diagnostic performance for each ELISA assay using pooled plasma samples from positive and negative donors. The results were expressed as reactivity index (RI), calculated as the ratio between the optical density (OD) of the samples and

the OD of the cut-off, where RI >1.00 were considered positive. To evaluate the overall precision for each antigen, areas under the ROC curve (AUC) were performed. The AUC values were 92.89% and 92.11% for D3 and D6, respectively. Both domains exhibited 97.92% specificity while D3 was 79.51% sensitive and D6 81.48%. The accuracy of D6 was 90.8% compared to the lower value for D3 (89.95%). Assuming an inconclusive range of RI values within 1.0 ± 0.10 , 13 (3.95%), and 16 (4.86%) true positive samples tested against D3 and D6, respectively, led to false results. By analyzing the true negative samples, 4 (0.75%) fell inside the inconclusive space using D3 and only 1 (0.18%) using D6. The incidence of cross-reactivity was almost negligible: only 4 out of 748 plasmas displayed seroreactivity using D3, while 5 samples generated false positives using D6. In both cases, samples came from patients infected with *Leptospira* or *Leishmania*. However, no reactivity was detected with plasma from individuals with dengue, filariasis, HBV, HCV, HIV, rubella, schistosomiasis, syphilis, COVID, leprosy and toxoplasmosis. Our results demonstrated a noteworthy performance of D3 and D6 for the diagnosis of CCD individuals from endemic areas of Brazil. Even more, both domains showed insignificant cross-reactivity with non-related infectious diseases.