Quantification of parasites load in sera samples of patients with distinct clinical manifestations during the chronic phase of Chagas disease

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FIOCRUZ

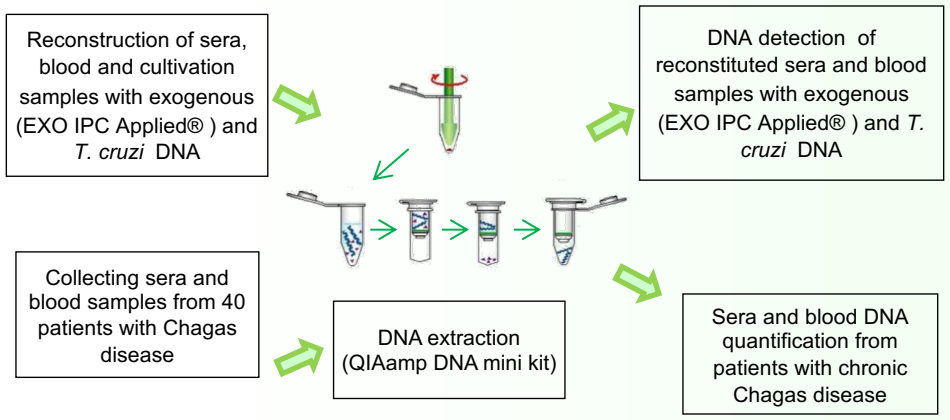
The work hypotheses: Is possible detect *T. cruzi* DNA from sera samples from patients with chronic Chagas diseases?

INTRODUCTION

Inconclusive results of serological tests for Chagas disease produce an impact on blood banks worldwide, resulting in a high number of discarded bags or risk of transmission by blood transfusion. Molecular techniques such as quantitative Real-Time PCR (qPCR) have been used to support the diagnosis and monitoring of parasitic load from peripheral blood samples. One promising perspective for the molecular diagnosis of Chagas disease is the possibility to detect *T. cruzi* DNA from the same serum samples used for the serological tests.

METHODS

In this work, a multiplex qPCR assay was employed to quantify and compare *T. cruzi* parasite load from serum and blood samples of 40 chronic Chagas Disease patients presenting distinct clinical manifestations, recruited from the Ambulatório de Doença de Chagas of the Hospital Universitário Oswaldo Cruz at the Pernambuco state, Brazil. We used the QIAamp DNA mini kit (QIAGEN) to extract all samples.



RESULTS AND DISCUSIONS

1) *T. cruzi* DNA detection from reconstituted sera and blood samples

Three reconstituted samples with CL Brener epimastigotes forms (sera, blood and cultivation medium) were subjected to qPCR to evaluate the detection of parasites after DNA extraction, and thus the technique performance. We observed a high threshold cycle (Ct) in sera samples, due to the lower DNA quantity after the coagulation processes, with statistical differences between the other samples ($p<0,001$) (figure 1). On the other hand, the RNaseP Ct in blood sample was higher than the others ($p <0,001$).

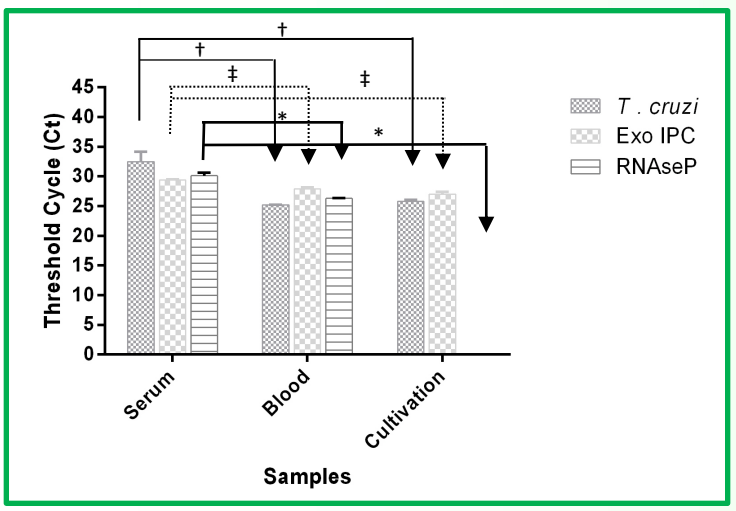


Figure 1. *T. cruzi* DNA detection of tree different samples. Results are expressed as the media of the Ct and represent the average of triplicate samples. † - difference significant between the detection of the *T. cruzi* target; ‡ - difference significant between the detection of the RNAseP target and * - difference significant between the detection of the EXO IPC target

2) *T. CRUZI* DNA quantification from patient's samples

Tree standard curve were constructed to compere the efficiency of DNA quantification. All standard curves started with 10^5 parasites equivalent / mL, Cl Brener epimastigotes were dilutaded in non-infected human blood or serum. We also constructed a standard curve with cultivation parasites in the same concentration of the serum and blood curves. The serum curve showed higher Ct levels from the same parasite load of the other two curves. Even different curves slopes, the dynamic ranges for the primer sets were adequate for use in absolute *T. cruzi* DNA quantification, when the sample was in the same condition of the standard curve. Furthermore, it is important to mention that a high amplification efficiency in conjunction with high intercept values are crucial for an accurate measurement of parasite burden in patients with chronic Chagas disease. A panel of 40 human blood and serum sample was analyzed using the qPCR strategy, all the patients are in the phase chronic Chagas. The patient's samples are quantified in duplicate and we used the serum and blood standard curves as described above. The parasite load detection was similar in both samples (serum and blood) ($p>0.05$) (figure 2). *T. cruzi* quantification of the patients was 1.125 and 1.230 median of parasite equivalents/mL for serum and blood samples respectively.

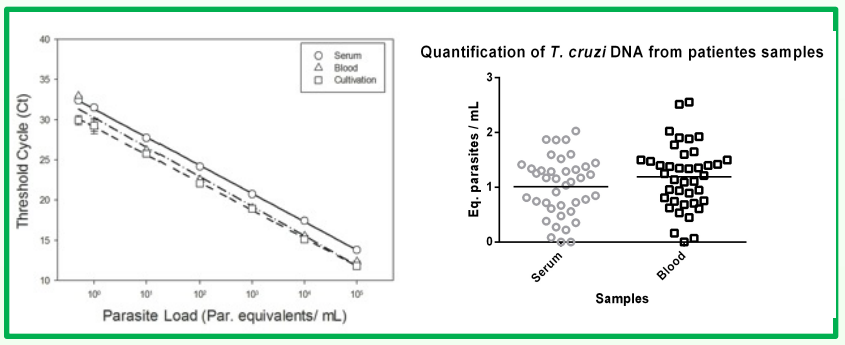


Figure 2. Parasites load in serum and blood sample of the positive patients. **A.** The range of the *T. cruzi* satellite DNA based qPCR and **B.** The DNA quantification form patients' sera and blood samples. Results are expressed as the number of parasites per milliliter of blood and represent the average of duplicate samples. Dynamic range: 0.5–105 p/mL. Parameters curves: serum y = -3,51x + 31,34; R2 = 0,99; blood y = -3,68x + 30,26; R2 = 0,99; cultivation y = -3,46x + 29,10; R2 = 0,99.

The clinical forms are related to the parasitemia of the host; therefore we evaluated the parasite load among the different clinical forms of the patients. The cardiac form showed higher *T. cruzi* quantification with 1.172 and 1.387 median of parasites equivalents / mL for serum and blood samples, however no significant difference was observed among the clinical forms compared with each other and among the serum and blood samples (figure 3)

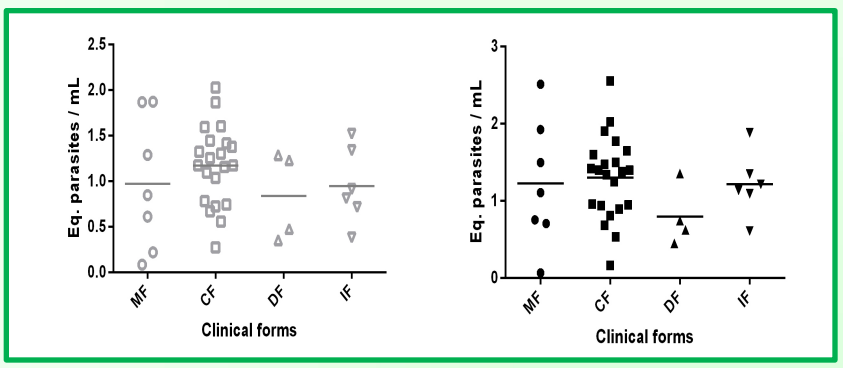


Figure 3. Parasites load in the different clinical forms from serum and blood sample of the positive patients. Results are expressed as the number of parasites per milliliter of blood and represent the average of duplicate samples. The parasite load median of the Mix form (MF) = 0,847; Cardiac form (CF) = 1.172; Digestive form (DF) = 0.853 and Indeterminate form (IF) = 0.863 from serum samples, and for the MF = 1.107; CF = 1.387; DF = 0.684 and IF = 1.175 from blood sample.

CONCLUSION

Moreover, using a serological test (recombinant ELISA and conventional ELISA) as gold standard, qPCR sensitivities for *T. cruzi* detection were 95.0% and 97.5%, for serum and blood samples, respectively, and specificities were 100% for both samples. Taken together, our data suggest the potential of serum samples for the molecular diagnosis and parasite load quantification by qPCR, to be applied in reference laboratories of Chagas disease diagnosis.

Financial support: Capes, CNPq, FAPERJ, Fiocruz