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Research paper

Usefulness of FC-TRIPLEX Chagas/Leish IgG1 as confirmatory assay for non-negative results in blood bank screening of Chagas disease

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ABSTRACT

A relevant issue in Chagas disease serological diagnosis regards the requirement of using several confirmatory methods to elucidate the status of non-negative results from blood bank screening. The development of a single reliable method may potentially contribute to distinguish true and false positive results. Our aim was to evaluate the performance of the multiplexed flow-cytometry anti-*T. cruzi/Leishmania* IgG1 serology/(FC-TRIPLEX Chagas/Leish IgG1) with three conventional confirmatory criteria (ELISA-EIA, Immunofluorescence assay-IIF and EIA/IIF consensus criterion) to define the final status of samples with actual/previous non-negative results during anti-*T. cruzi* ELISA-screening in blood banks. Apart from inconclusive results, the FC-TRIPLEX presented a weak agreement index with EIA, while a strong agreement was observed when either IIF or EIA/IIF consensus criteria were applied. Discriminant analysis and Spearman's correlation further corroborates the agreement scores. ROC curve analysis showed that FC-TRIPLEX performance indexes were higher when IIF and EIA/IIF consensus were used as a confirmatory criterion. Logistic regression analysis further demonstrated that the probability of FC-TRIPLEX to yield positive results was higher for inconclusive results from IIF and EIA/IIF consensus. Machine learning tools illustrated the high level of categorical agreement between FC-TRIPLEX versus IIF or EIA/IIF consensus. Together, these findings demonstrated the usefulness of FC-TRIPLEX as a tool to elucidate the status of non-negative results in blood bank screening of Chagas disease.

1. Introduction

Chagas disease, caused by *Trypanosoma cruzi*, remains a serious public health problem, especially in Latin America. The intensive reduction of domestic triatomines in regions with control efforts leads blood transfusion to have great epidemiological relevance (Dias, 2009). Thus, the regular and effective serological screening of donor candidates is being improved in most endemic countries (Moya-Salazar et al., 2017) in parallel with awareness and also focal screening in non-endemic countries (Assal and Corbi, 2011). Indeed, the key point for the safety of blood supply for Chagas disease is the maintenance of donor control as well as the desirable improvement of serological tests in terms of sensitivity and specificity (Dias, 2009).

The conventional serologic tests for *T. cruzi* undergo from variable sensitivity and specificity (Santos et al., 2016), the latter being due to cross-reactions with other pathogens (Ferreira, 1992). Therefore, the results obtained by different methods or considering even the same methodology, frequently display discordance (Slavov et al., 2017; Malan et al., 2006; Pirard et al., 2005; Blejer et al., 1999). Samples with low-level reactivity and inconclusive *T. cruzi* antibody results are frequently found in large scale screening, especially when parallel testing with 2 or more assays are performed (Remesar et al., 2015; Otani et al.,

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2009; Remesar et al., 2009; Salles et al., 1996). Such samples present challenges not only for donor counseling but also when evaluating the performance of new tests or estimating prevalence or incidence rates for national or regional epidemiological surveillance (Sabino et al., 2010).

Indeed, Araujo and Berne (2013) during a comparative analysis in Brazil with distinct commercial tests for Chagas disease diagnosis demonstrated that 34,1% of the samples presented discordance among the tests performed. All techniques failed at sensitivity and specificity when compared with TESA-blot (Trypomastigote excreted-secreted antigen-blot) confirmatory assay. Moreover, the proportion of blood units discarded in Brazil is approximately 10–20%, mainly due to non-negative results on the screening tests for infectious diseases (Salles et al., 2003). In fact, Chagas disease comprehends the major reason of unfitness for blood donors. Besides, candidates with clinical or serological diagnosis for Chagas, even if treated or asymptomatic, are permanently excluded (MS, 2004).

In this context, several studies have been developed in order to improve the performance of confirmatory assay that can be used routinely in blood banks, aiming the safety of serological profiles of patients and blood donors (Ferreira-Silva et al., 2010; Flores-Chávez et al., 2010; Sabino et al., 2010). Our group has developed a set of flow cytometry-based assays (Martins-Filho et al., 1995, 2002; Vitelli-Avelar et al., 2007; Matos et al., 2010), including the FC-TRIPLEX-IgG1, for simultaneous measurement of anti-*T. cruzi*, anti-*L. braziliensis* and anti-*L. chagasi* IgG1 antibodies displaying high performance for all-in-one classification of inconclusive Chagas tests (Teixeira-Carvalho et al., 2015).

Thus, the goal of this work is to evaluate the performance of the multiplexed flow-cytometry anti-*T. cruzi/Leishmania* IgG1 serology/ (FC-TRIPLEX Chagas/Leish IgG1) with three conventional confirmatory criteria (ELISA-EIA, Immunofluorescence assay-IIF and EIA/IIF consensus criterion) to define the final status of samples with actual/previous non-negative results during anti-*T. cruzi* ELISA-screening in blood banks.

2. Patients and methods

2.1. Study population

The Pro-Sangue Foundation blood center and HEMOMINAS Foundation performed the *T. cruzi* antibody screening that consist of the conventional serological methods as ELISA (EIA) and Immunofluorescence (IIF), as previously described (Sabino et al., 2010). Based on the blood screening, a total of 239 inconclusive (INC) sera samples were available from eligible donors for this study. The Fig. 1 shows the experimental design flowchart where this inconclusive (INC) samples were tested for EIA and IIF. In the present work, three different confirmatory criteria, #1 (anti-*T. cruzi* EIA testing); #2 (anti-*T. cruzi* IIF testing) and #3 (CONSENSUS of anti-*T. cruzi* Testing (EIA/IIF)), were used. The Supplementary Fig. 1 shows anti-*T. cruzi* reactivity in EIA, IIF and EIA/IIF consensus applied to define the study groups. After this screening consensus of tests, the samples were evaluated by FC-TRIPLEX-IgG1 candidate test and compared afterwards.

This study was conducted according to the Brazilian national guidelines for research with human subjects (resolution no. 466/2012). This study was approved by the Ethical Committee at the HEMOMINAS Foundation (no. 157/2007). Informed written consent was obtained from all participants before enrollment in the study.

2.2. Serological assays

The samples were tested previously by conventional EIA and IIF at Pro-Sangue Foundation blood center, according manufactures. Parasites preparation and FC-TRIPLEX-IgG1 protocol was performed

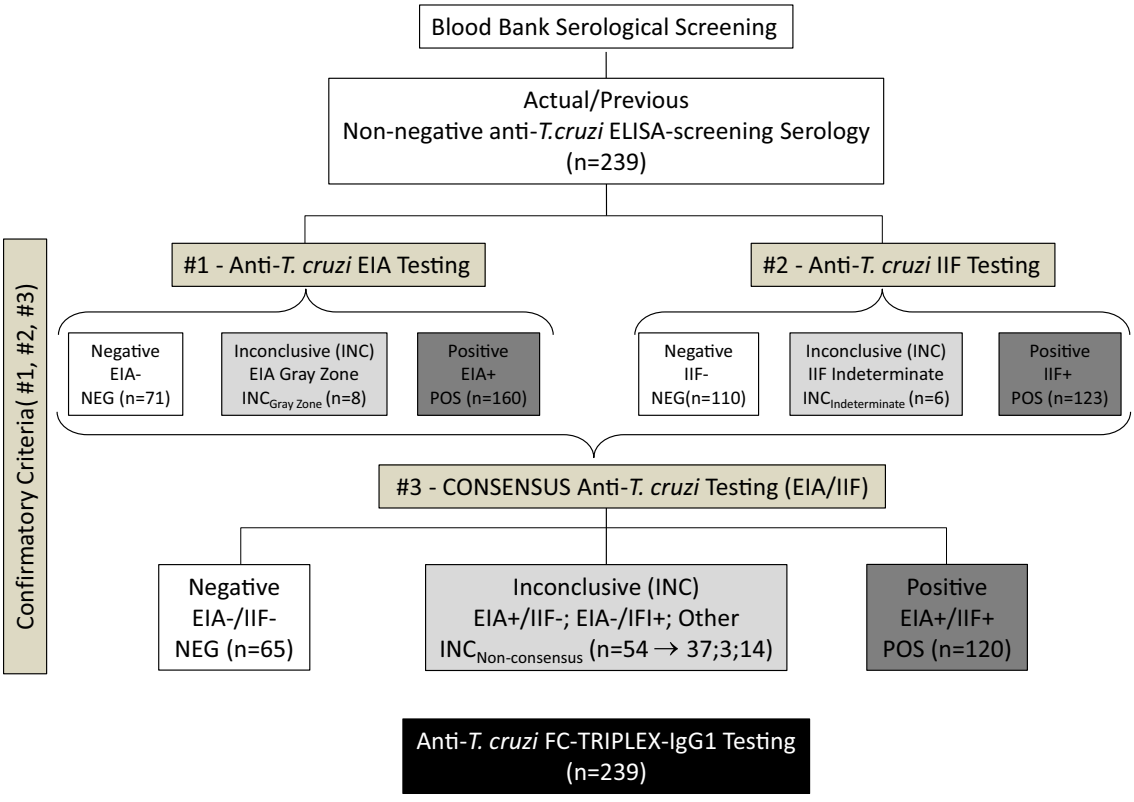

Fig. 1. Experimental design flowchart where inconclusive (INC) samples were tested for EIA and IIF. A total of 239 inconclusive (INC) sera samples were available from eligible donors for this study. Three different confirmatory criteria, #1 (anti-*T. cruzi* EIA testing); #2 (anti-*T. cruzi* IIF testing) and #3 (CONSENSUS of anti-*T. cruzi* Testing (EIA/IIF), were used. After this screening consensus of tests, the samples were performed and compared afterwards with the FC-TRIPLEX-IgG1 candidate test.