Tropical Medicine and International Health doi:10.1111/tmi.12144

VOLUME 18 NO 9 PP 1103–1109 SEPTEMBER 2013

Performance of different *Trypanosoma cruzi* antigens in the diagnosis of Chagas disease in patients with American cutaneous leishmaniasis from a co-endemic region in Argentina

A.F. Vega Benedetti[1,2,3], Rubén O. Cimino[1,2], Pamela S. Cajal[2], Marisa Del Valle Juarez[2], Carlos A. Villalpando[2], José F. Gil[2,5], Iván S. Marcipar[4,5], Alejandro J. Krolewiecki[2,3,5] and Julio R. Nasser[1,2]

1 *Cátedra de Química Biológica, Facultad de Ciencias Naturales, Universidad Nacional de Salta, Salta, Argentina*
2 *Instituto de Investigaciones de Enfermedades Tropicales, Sede Regional Orán, Universidad Nacional de Salta, Salta, Argentina*
3 *Instituto de Patología Experimental, Facultad de Ciencias de la Salud, Universidad Nacional de Salta, Salta, Argentina*
4 *Laboratorio de Tecnología Inmunológica, Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral, Santa Fe, Argentina*
5 *Consejo Nacional de Investigaciones Científicas y Técnicas, Buenos Aires, Argentina*

Abstract

OBJECTIVE To determine the ability of recombinant antigens to detect cases of infection with *Trypanosoma cruzi* among cases of infection with *Leishmania* spp. by serological methods.
METHODS Sera from 41 patients infected with *Leishmania* spp. were evaluated with ELISA using single (FRA, CP1 and TSSAVI) or pooled (commercial Rec-ELISA) recombinant proteins or homogenate antigens (commercial H-ELISA). As there is no gold standard antigen to discriminate Chagas disease from leishmaniasis, the correlation of results between defined antigens and the homogenate was made with Kappa Index (KI), the level of correlation considered being used as a criterion of specificity.
RESULTS Single recombinant antigens and Rec-ELISA showed good correlation (KI > 0.8). A low correlation (KI < 0.66) was observed between the results from single recombinant antigens or the commercial recombinant kit and H-ELISA.
CONCLUSIONS The highly correlated results between *T. cruzi* single or pooled recombinant proteins are indicative of the usefulness of recombinant antigens for Chagas diagnosis. Our results also indicate that in the city of Oran in Argentina, between 12% and 17% of patients with leishmaniasis are also infected with Chagas disease. The high KI values between TSSAVI and the other recombinant proteins suggest that in these patients, the infection may be caused by *T. cruzi* II and/or V and/or VI lineages.

keywords recombinant antigen, *Trypanosoma cruzi*, leishmaniasis

Introduction

The endemic areas of *Trypanosoma cruzi* and *Leishmania* spp. infections in Latin America overlap in many regions. This is especially true for Argentina and specifically for the province of Salta (Gil *et al.* 2011). Because both species exhibit close phylogenetic relationships, it is difficult to discriminate between Chagas disease and American cutaneous leishmaniasis (ACL) in overlapping areas due to antigenic cross-reactivity (Chiaramonte *et al.* 1996; El-Sayed *et al.* 2005).

A pitfall of conventional serological methods to diagnose Chagas disease is the potential cross-reactivity with antibodies from patients infected with *Leishmania* spp. In an attempt to overcome this limitation, efforts were made to develop ELISA with parasite-specific antigens for Chagas disease. The importance of this non-conventional serological test lies in its feasibility, flexibility and low cost (WHO 2007; Brasil *et al.* 2010).

Some *T. cruzi* recombinant antigens are as follows: shed acute-phase antigen (SAPA) (Affranchino *et al.* 1989), flagellar repetitive antigen (FRA) (Krieger *et al.* 1992; dos Santos *et al.* 1992) and trypomastigote small surface antigen (TSSA) (Di Noia *et al.* 2002; Cimino *et al.* 2011; De Marchi *et al.* 2011; Risso *et al.* 2011). Another alternative is the construction of chimeric

proteins, such as CP1 (Camussone *et al.* 2009). Nowadays, recombinant antigens have been incorporated in commercial kits of important companies, such as ABBOT (PRISM Chagas assay), Omega Diagnostics (Pathozyme Chagas) and Wiener Lab (Chagatest Rec v3.0) (Oelemann *et al.* 1998; Caballero *et al.* 2007; Otani *et al.* 2009). In particular, Chagatest Rec v3.0 is a kit widely used in Argentina and is prepared with a mixture of recombinant antigens (antigens 1, 2, 13, 30, 36 and SAPA) (Sanchez Negrette *et al.* 2008).

In the province of Salta, Oran and San Martin departments are endemic for ACL and have the highest prevalence values. Oran is the city with the greatest incidence of ACL in Argentina. According to the 1995–2007 database, the prevalence was 6.73% in children <15 years of age (Gil *et al.* 2010). Serological studies performed with *T. cruzi* antigens not related to *Leishmania* spp. determined that more than 35% of patients with symptomatic cutaneous leishmaniasis were infected with *T. cruzi* (Frank *et al.* 2003; Gil *et al.* 2011). Therefore, it is important to differentiate these infections because treatment and clinical follow-up are largely different and seroepidemiological studies can underestimate or overestimate the prevalence of these parasitic diseases (Gil *et al.* 2011).

Previously, antigens used to discriminate between Chagas and leishmaniasis were assessed by considering other immunochemical techniques or epidemiological criteria. However, there is still no gold standard to differentiate between infections caused by *T. cruzi* and those caused by *Leishmania* spp.; hence, the results have to be confirmed. Taking into account the difficulty to define infection status in the study population, in the present work, we tried a new approach to evaluate the reliability of *T. cruzi* recombinant antigens to determine Chagas disease in a group of patients with leishmaniasis from a co-endemic area. We analysed the correlation among ELISA assays performed with FRA, CP1, TSSAVI, a commercial kit that contains a pool of six recombinant proteins (Chagatest Rec v3.0) and a commercial kit prepared with homogenate from the parasite [Chagatest ELISA (H-ELISA)]. Using this criterion, we confirmed that recombinant antigen has higher specificity than *T. cruzi* parasite homogenate. These results also allowed us to estimate the proportion of Chagas disease in patients with leishmaniasis and the specific *T. cruzi* strains responsible for these infections.

Materials and methods

Human sera

In 2009, 64 ACL cases were recorded at the Instituto de Investigaciones de Enfermedades Tropicales (IIET), a regional reference centre for the diagnosis of leishmaniasis located in Oran (Figure 1). Of those cases, 41 (64%) were analysed in this work. All patients with ACL lived in Oran and San Martin departments and presented classic cutaneous lesions confirmed by the observation of amastigotes in Giemsa-stained smears. In previous studies, among patients with ACL, *Leishmania (Viannia) braziliensis* was the most prevalent species of the three *Leishmania* species known to occur in northern Argentina (Marco *et al.* 2012; Krolewiecki *et al.* 2013).

T. cruzi recombinant antigens

We worked with the recombinant purified antigens CP1, TSSAVI and FRA, produced according to the specifications described elsewhere (Camussone *et al.* 2009; Cimino *et al.* 2011; Valiente-Gabioud *et al.* 2011). These antigens show high specificity and reactivity in human sera.

Serological studies

All sera were analysed by conventional serology [Chagatest ELISA (H-ELISA) and Chagatest ELISA *recombinante v. 3.0* (Rec-ELISA), Wiener Lab, Rosario, Argentina], following the manufacturer's guidelines.

For the non-conventional serology, assays were standardised by evaluating the following factors: (i) carbonate buffers (pH = 9.6) and PBS (phosphate-buffered saline, pH = 7.2); (ii) optimal antigen concentration (0.25 and 0.5 μg per well/100 μl); (iii) different serum dilutions (1:20,1:40, 1:50 and 1:100) and (iv) conjugate dilutions (1:5000 and 1:10000). The optimal conditions were those that better discriminated between negative and positive control sera. The washing buffer used was 0.1% Tween in PBS. The reaction was developed using tetramethylbenzidine (TMB) (Invitrogen) in H O . The reaction was stopped with 0.5N H SO , and the absorbance was measured with an ELISA reader (Biotek ELx800) with 450-nm filter. All samples were measured in duplicate.

For the FRA-ELISA, polystyrene microplates were coated with 0.25 μg per well/100 μl in carbonate buffer. The serum dilution was 1:100, and peroxidase-conjugated anti-human IgG (Sigma, St. Louis) was 1:10000.

For the CP1-ELISA, polystyrene microplates were coated with 0.5 μg per well/100 μl in carbonate buffer. The serum dilution was 1:40, and peroxidase-conjugated anti-human IgG (Sigma, St. Louis) was 1:10000.

TSSAVI-ELISA, protocol described by Cimino *et al.* (2011).

We standardised ELISA technique with 18 negative control sera (non-chagasic) from Salta city, a non-endemic area for leishmaniasis. Fourteen serum samples were used

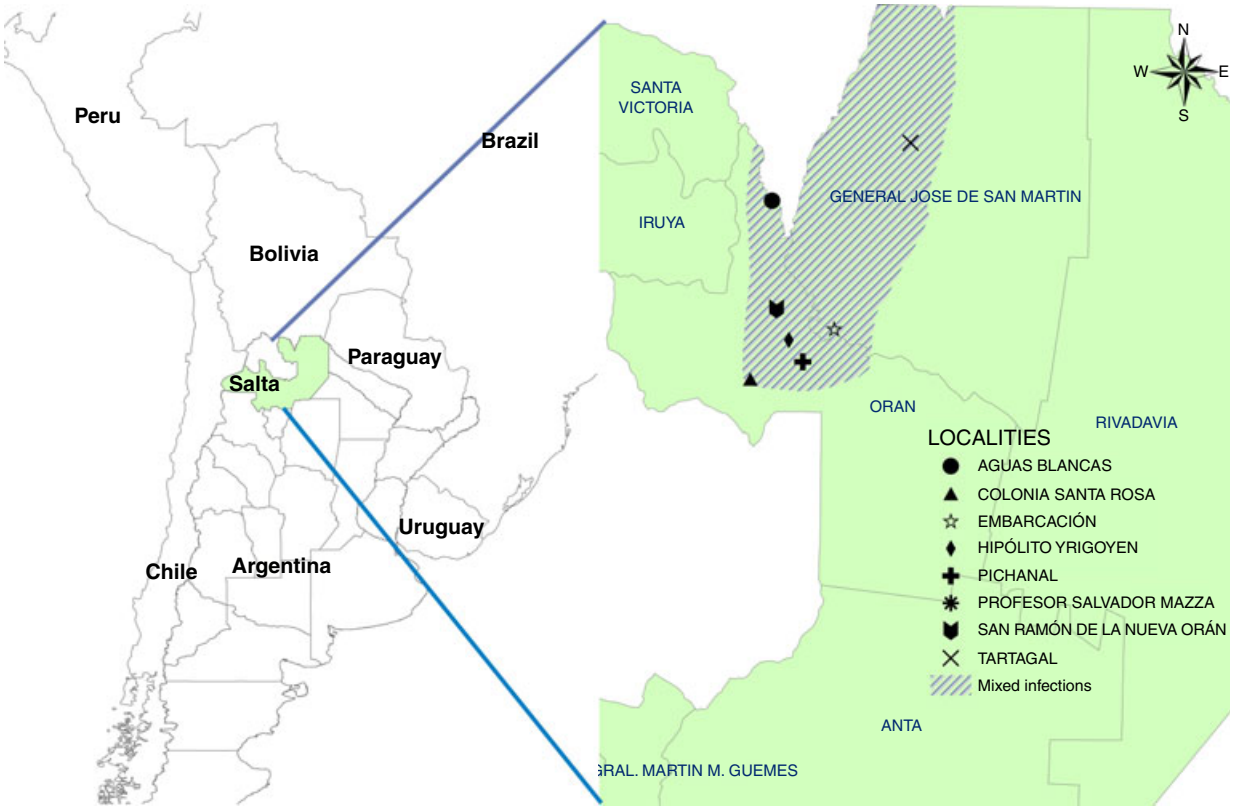

Figure 1 Geographic location of San Ramon de la Nueva Oran and San Martin departments, province of Salta, Argentina. The map shows different localities in these departments and highlights the overlapping area of Chagas disease and leishmaniasis.

as positive controls. These samples were confirmed by conventional serology (HAI and Chagatest Rec-ELISA), both from Wiener Lab, Rosario, Argentina.

Data analysis

The analysis was performed with Graph Pad Prism v.5.0 (San Diego, CA). Relative optical density (ROD) was calculated and defined as OD/cut-off, where optical density (OD) is the mean value of each serum sample and cut-off was determined for each assay as the mean OD of all negative control serum samples plus 3 standard deviations. Indeterminate samples are considered those with absorbance within the indetermination zone (cut-off ± 10%). Kappa Index (KI) was calculated using EPIDAT v3.1 software.

Results

We analysed 41 sera from patients with ACL, 9.7% women and 90.3% men, aged between 10 and 70 years. 11 (27%) of these patients were from Hipolito Irigoyen, 16 (39%) from San Ramon de la Nueva Oran, 4 (10%) from Pichanal and 10 (24%) from San Martin department (Figure 1). Of all the samples analysed, 75.6% (31/41) had a ROD lower than the cut-off. A total of 11 samples were positive for at least one assay (Figure 2). Of the sera reactive to Rec-ELISA, 71.4% (5/7) were also positive for the other assays (Table 1).

The highest KI, which indicates the best agreement found in this procedure, was Rec-ELISA *vs*. FRA-ELISA and FRA-ELISA *vs*. CP1-ELISA, with a KI = 0.91. The agreement between the recombinant antigen assays was high, with values of KI > 0.8. TSSAVI-ELISA showed an important agreement with FRA-ELISA (KI = 0.89). The greatest disagreement was observed between H-ELISA and the remaining assays, with a KI < 0.7 (Table 2).

Discussion

Although multicentric studies have determined that the sensitivity of parasite antigen homogenate is highly useful to diagnose *T. cruzi* infection with ELISA assays, a lack of specificity is observed in patients with leishmaniasis

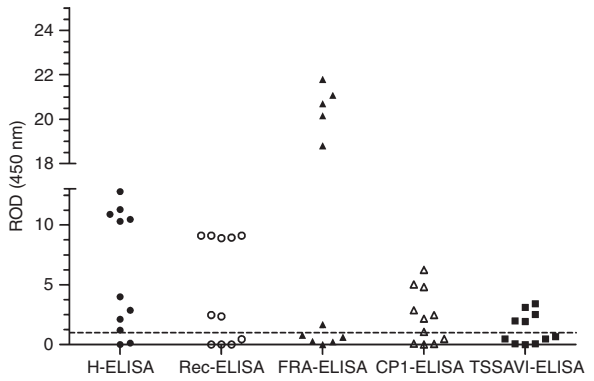

Figure 2 Relative OD distribution obtained from the 11 sera of the patients with cutaneous leishmaniasis that were positive for at least one of the assays with *Trypanosoma cruzi* recombinant antigens or homogenate. H-ELISA, homogenate ELISA; Rec-ELISA, recombinant ELISA; ROD, relative optical density. The horizontal line at 1.0 ROD represents the cut-off.

(Caballero *et al.* 2007; Remesar *et al.* 2009). When recombinant proteins of *T. cruzi* are assessed to determine their specificity, a panel of negative sera from patients with *Leishmania* spp. infection is usually obtained from a Chagas-free area to ensure, using epidemiological criteria, that study participants are not infected with *T. cruzi* (Umezawa *et al.* 1999, 2004; Aguirre *et al.* 2006). This criterion was used because there is no gold standard to discriminate *T. cruzi* infection from *Leishmania* spp. infection or from a mixed infection. This methodological restriction leads to biased results because variables (prevalence of different parasites, concomitant infections, nutritional habits) that determine cross-reactivity in individuals from an area are generally different from those present in other areas. To overcome this weakness of traditional assessment, we calculated the concordant performance of different antigens using a population of patients with leishmaniasis from a co-endemic area.

The recombinant antigens that were assessed correspond to antigens that were previously described to be experimentally specific for determination of Chagas disease (Umezawa *et al.* 1999; Camussone *et al.* 2009). This specificity can be explained when the identity of the sequence is analysed, that is, the best identity of FRA sequence within *Leishmania* genome (taxid: 38568) is of 62% but the identity with sequences from the genome from the phylogenetic distant *T. cruzi* CL Brener strain (discrete typing unit, DTU TcVI) and *T. cruzi* Silvio

Table 1 Positive cases for at least one of the assays

No. of sera	Rec-ELISA	H-ELISA	FRA-ELISA	CP1-ELISA	TSSAVI-ELISA
HS1	+	+	+	+	+
HS2	+	+	+	+	+
HS3	+	+	+	+	+
HS4	+	+	+	+	+
HS5	+	+			
HS6	+		+	Id	
HS7	+	+	+	+	+
HS8				+	
HS9		+			
HS10		+			
HS11		+			

HS, human sera; H-ELISA, homogenate ELISA; Rec-ELISA, recombinant ELISA. Human sera 6 (HS6) has an absorbance for CP1 within the indetermination zone (Id).

Table 2 Kappa Index for all the different pairs of assays

	H-ELISA	Rec-ELISA	FRA-ELISA	CP1-ELISA	TSSAVI-ELISA
H-ELISA	–				
Rec-ELISA	0.69	–			
FRA-ELISA	0.59	0.91*	–		
CP1-ELISA	0.54*	0.83	0.91*	–	
TSSAVI-ELISA	0.66	0.81	0.89	0.81	–

*There are statistically significant differences in the KI comparison (0.9 and 0.54) with a value of $P < 0.05$.

strain (DTU TcI) reaches 100 and 91%, respectively. It is therefore expected that serum reactivity against this antigen be mainly due to *T. cruzi* infection. Recently, a differential evolution of repetitions has been described for this molecule in *T. cruzi* and *Leishmania* species, this branching being coincident with the absence of B epitope cross-reactivity between them (Galetovic *et al.* 2011). However, cross-reactivity against a single antigen cannot be ruled out due to the presence of antibodies developed against other microorganisms. By contrast, the occurrence of this cross-reactivity against more than one protein is very unlikely. Therefore, the concordance of reactivity against specific recombinant proteins seems to be a reliable criterion to confirm a specific infection when a gold standard is not available.

The different assays using the recombinant antigens presented a good agreement compared with Rec-ELISA commercial assay, which uses a mixture of six recombinant antigens. The recombinant antigen FRA has 97% identity with Ag1 from the kit Wiener Lab (Camussone *et al.* 2009). The greatest disagreement was observed in the comparison of the results obtained with the recombinant antigens to results of H-ELISA commercial assay (protein mixture from epimastigotes). The use of a mixture of unknown proteins from the parasite makes antigenic cross-reaction possible because, as *T. cruzi* and *Leishmania* spp. are phylogenetically closely related, they have highly conserved proteins.

FRA recombinant antigen is a cytoskeleton-associated protein with a high performance used for the diagnosis of chronic infections (Krieger *et al.* 1992; da Silveira *et al.* 2001). The high reactivity against FRA shows that these patients with ACL might be in the chronic phase of Chagas disease. Moreover, the high agreement (KI $\geq$ 0.9) of FRA-ELISA with Rec-ELISA, TSSAVI-ELISA and CP1-ELISA is noticeable.

The results obtained with CP1 confirm previous results (Camussone *et al.* 2009) with respect to antigen specificity in sera of patients with ACL. In this work, when 15 sera of patients with ACL were analysed, CP1 showed a good performance, 93% of sensitivity and a higher reactivity than that obtained by peptide mixture. Our evaluation of sera from an endemic region of northern Argentina (Salta province) provides a novel contribution. The use of this antigen for the diagnosis of *T. cruzi* infection allowed us to confirm cases of *Leishmania* spp. – *T. cruzi* mixed infections, reported by previous studies carried out by our group (Gil *et al.* 2011). With this protein, however, an odd result was obtained with the HS8 sample, which was positive for this antigen and negative for the others. The difference between CP1 and FRA is the presence of SAPA epitopes, and this sequence might be responsible for the reactivity. However, the negative result of this serum with Chagatest, which has this protein within a mixture of recombinants, is not consistent with CP1 reactivity. This result might be attributed to the structural configuration of the chimeric antigen, which might establish an unspecific conformational epitope.

We used most of the sensitive and specific recombinant proteins that have been assessed to diagnose Chagas disease up to now: single proteins FRA, CP1, TSSAVI and a mixture of antigens 1, 2, 13, 30, 36 and SAPA in Chagatest Rec. Indeed, most of these proteins have been assessed in multicenter studies and their reactivity was found to be slightly different when using sera from regions where predominant DTUs are TcV and TcVI, as in Brazil or Argentina, or TcI, as in Colombia or Ecuador (Marcipar & Lagier 2012). This difference can be attributed to genetic variability in the parasites for these sequences. However, this lower reactivity was not translated into a lower positive detection index of the antigens in different regions. Indeed, these results are consistent with slight variations of amino acids in the sequences of these antigens within *T. cruzi* species. In the case of FRA, all of the 67 aa of the repetitive sequence blasted with FRA from CL Brener strain, whereas 60 aa blasted with FRA from Sylvio strain. In the case of SAPA antigen, corresponding to the C-terminal fraction from CP1 (the N-terminal fraction is FRA), the identity of the sequence with the one present in CL Brener and Silvio strains corresponds to 12/12 aa and 11/12 aa, respectively.

Contrary to the proteins described above for diagnostic purposes, TSSAVI have been proposed to differentiate patients infected with *T cruzi* lineages TcII, TcV and TcVI, but not with lineages TcI, TcIII and TcIV, based on the lineage specificity of this peptide (Bhattacharyya *et al.* 2010). Our result with TSSAVI reactivity (5 of 11 sera positive for at least one of the assays) suggests infections mainly with TcII and/or TcV and/or TcVI. This is the first description of the circulating *T. cruzi* lineages in the north of Salta province using serological methods. The present results are consistent with previous reports from Argentina, where the more prevalent lineages in humans are TcII, TcV and TcVI, whereas TcI and TcIII are less frequent (Cura *et al.* 2012). In fact, patients HS1, HS2, HS3, HS4 and HS7 would be infected with parasites belonging to TcII and/or TcV and/or TcVI, and patients HS5 and HS6 are probably infected with TcI and/or TcIII and/or TcIV.

In summary, this work highlights the importance of non-conventional serology for the diagnosis of Chagas disease. Further studies will be necessary to determine specificity of each recombinant antigen and whether these proteins can be used as a confirmatory test for *T. cruzi* infection in a larger number of cases of diverse geographic origin.

Conclusion

Non-conventional ELISA is a low-cost, simple-to-use technique. *T. cruzi* recombinant antigens have good correlations, as indicated by Kappa Index. This highlights the importance of non-conventional serology for the diagnosis of Chagas disease. TSSAVI reactivity proposes the presence of *T.cruzi* TcII, TcV and/or TcVI lineages in the north of Salta province, where no description of the circulating *T. cruzi* strains has been provided using serological methods.

Acknowledgements

This work was supported by Consejo de Investigación de la Universidad Nacional de Salta (CIUNSA), Number 2001 and 2056. We are grateful to Wiener Laboratories, Rosario, Argentina, for providing the commercial Chagatest kits.

References

Affranchino JL, Ibanez CF, Luquetti AO *et al.* (1989) Identification of a *Trypanosoma cruzi* antigen that is shed during the acute phase of chagas' disease. *Molecular and Biochemical Parasitology* **34**, 221–228.

Aguirre S, Silber AM, Brito ME, Ribone ME, Lagier CM & Marcipar IS (2006) Design, construction, and evaluation of a specific chimeric antigen to diagnose chagasic infection. *Journal of Clinical Microbiology* **44**, 3768–3774.

Bhattacharyya T, Brooks J, Yeo M *et al.* (2010) Analysis of molecular diversity of the *Trypanosoma cruzi* trypomastigote small surface antigen reveals novel epitopes, evidence of positive selection and potential implications for lineage-specific serology. *International Journal for Parasitology* **40**, 921–928.

Brasil PE, De Castro L, Hasslocher-Moreno AM, Sangenis LH & Braga JU (2010) ELISA versus PCR for diagnosis of chronic Chagas disease: systematic review and meta-analysis. *BMC Infectious Diseases* **10**, 337.

Caballero ZC, Sousa OE, Marques WP, Saez-Alquezar A & Umezawa ES (2007) Evaluation of serological tests to identify *Trypanosoma cruzi* infection in humans and determine cross-reactivity with *Trypanosoma rangeli* and *Leishmania* spp. *Clinical and Vaccine Immunology* **14**, 1045–1049.

Camussone C, Gonzalez V, Belluzo MS *et al.* (2009) Comparison of recombinant *Trypanosoma cruzi* peptide mixtures versus multiepitope chimeric proteins as sensitizing antigens for immunodiagnosis. *Clinical and Vaccine Immunology* **16**, 899–905.

Cimino RO, Rumi MM, Ragone P *et al.* (2011) Immuno-enzymatic evaluation of the recombinant tssa-ii protein of *Trypanosoma cruzi* in dogs and human sera: a tool for epidemiological studies. *Parasitology* **138**, 995–1002.

Cura CI, Lucero RH, Bisio M *et al.* (2012) *Trypanosoma cruzi* discrete typing units in chagas disease patients from endemic and non-endemic regions of Argentina. *Parasitology* **139**, 516–521.

Chiaramonte MG, Zwirner NW, Caropresi SL, Taranto NJ & Malchiodi EL (1996) *Trypanosoma cruzi* and *Leishmania* spp. *American Society of Tropical Medicine and Hygiene* **54**, 271–273.

da Silveira JF, Umezawa ES & Luquetti AO (2001) Chagas disease: recombinant *Trypanosoma cruzi* antigens for serological diagnosis. *Trends in Parasitology* **17**, 286–291.

De Marchi CR, Di Noia JM, Frasch AC, Amato Neto V, Almeida IC & Buscaglia CA (2011) Evaluation of a recombinant *Trypanosoma cruzi* mucin-like antigen for serodiagnosis of chagas' disease. *Clinical and Vaccine Immunology* **18**, 1850–1855.

Di Noia JM, Buscaglia CA, De Marchi CR, Almeida IC & Frasch AC (2002) A *Trypanosoma cruzi* small surface molecule provides the first immunological evidence that chagas' disease is due to a single parasite lineage. *The Journal of Experimental Medicine* **195**, 401–413.

dos Santos CN, Krieger MA, Almeida E, Lafaille JJ, Goldenberg S & Galler R (1992) *Trypanosoma cruzi* flagellar repetitive antigen expression by recombinant baculovirus: towards an improved diagnostic reagent for chagas' disease. *Biotechnology (N Y)* **10**, 1474–1477.

El-Sayed NM, Myler PJ, Blandin G *et al.* (2005) Comparative genomics of trypanosomatid parasitic protozoa. *Science* **309**, 404–409.

Frank FM, Fernandez MM, Taranto NJ *et al.* (2003) Characterization of human infection by *Leishmania* spp. In the northwest of Argentina: immune response, double infection with *Trypanosoma cruzi* and species of Leishmania involved. *Parasitology* **126** (Pt 1), 31–39.

Galetovic A, Souza RT, Santos MR *et al.* (2011) The repetitive cytoskeletal protein h49 of *Trypanosoma cruzi* is a calpain-like protein located at the flagellum attachment zone. *PLoS One* **6**, e27634.

Gil J, Cimino R, Lopez Quiroga I, Cajal S *et al.* (2011) [reactivity of gst-sapa antigen of *Trypanosoma cruzi* against sera from patients with chagas disease and leishmaniasis]. *Medicina (B Aires)* **71**, 113–119.

Gil JF, Nasser JR, Cajal SP, Juarez M *et al.* (2010) Urban transmission of American cutaneous leishmaniasis in Argentina: spatial analysis study. *American Society of Tropical Medicine and Hygiene* **82**, 433–440.

Krieger MA, Almeida E, Oelemann W *et al.* (1992) Use of recombinant antigens for the accurate immunodiagnosis of chagas' disease. *American Society of Tropical Medicine and Hygiene* **46**, 427–434.

Krolewiecki AJ, Gil JF, Quipildor M *et al.* (2013) Restricted outbreak of American tegumentary leishmaniasis with high microfocal transmission. *American Society of Tropical Medicine and Hygiene* **88**, 578–582.

Marcipar IS & Lagier CM (2012) Advances in serological diagnosis of chagas' disease by using recombinant proteins. In: *Current Topics in Tropical Medicine*, (ed AJ Rodriguez-Morales) INTECH, Croatia, pp. 273–298.

Marco JD, Barroso PA, Mimori T *et al.* (2012) Polymorphism-specific PCR enhances the diagnostic performance of American tegumentary leishmaniasis and allows the rapid identification

of *Leishmania* species from Argentina. *BMC Infectious Diseases* **12**, 191.

Oelemann WM, Teixeira MD, Verissimo Da Costa GC *et al.* (1998) Evaluation of three commercial enzyme-linked immunosorbent assays for diagnosis of chagas' disease. *Journal of Clinical Microbiology* **36**, 2423–2427.

Otani MM, Vinelli E, Kirchhoff LV *et al.* (2009) Who comparative evaluation of serologic assays for chagas disease. *Transfusion* **49**, 1076–1082.

Remesar MC, Gamba C, Colaianni IF *et al.* (2009) Estimation of sensitivity and specificity of several *Trypanosoma cruzi* antibody assays in blood donors in Argentina. *Transfusion* **49**, 2352–2358.

Risso MG, Sartor PA, Burgos JM *et al.* (2011) Immunological identification of *Trypanosoma cruzi* lineages in human infection along the endemic area. *American Society of Tropical Medicine and Hygiene* **84**, 78–84.

Sanchez Negrette O, Sanchez Valdez FJ, Lacunza CD *et al.* (2008) Serological evaluation of specific-antibody levels in patients treated for chronic chagas' disease. *Clinical and Vaccine Immunology* **15**, 297–302.

Umezawa ES, Bastos SF, Camargo ME *et al.* (1999) Evaluation of recombinant antigens for serodiagnosis of chagas' disease in South and Central America. *Journal of Clinical Microbiology* **37**, 1554–1560.

Umezawa ES, Luquetti AO, Levitus G *et al.* (2004) Serodiagnosis of chronic and acute chagas' disease with *Trypanosoma cruzi* recombinant proteins: results of a collaborative study in six Latin American countries. *Journal of Clinical Microbiology* **42**, 449–452.

Valiente-Gabioud AA, Veaute C, Perrig M, Galan-Romano FS, Sferco SJ & Marcipar IS (2011) Effect of repetitiveness on the immunogenicity and antigenicity of *Trypanosoma cruzi* fra protein. *Experimental Parasitology* **127**, 672–679.

WHO, 2007. World Health Organization (WHO) special programme for research and training in tropical disease (tdr). In: *Report of Scientific Group in Chagas Disease*, (eds F Guhl & JK Lazdins-Helds) WHO, Buenos Aires, Argentina, pp. 1–97.

Corresponding Author **Rubén Oscar Cimino**, Facultad de Ciencias Naturales, Universidad Nacional de Salta, Av. Bolivia 5150, 4400 Salta, Argentina. Tel.: +54 387 4255594; E-mail: rubencimino@gmail.com