Evaluation of a Recombinant Trypanosoma cruzi Mucin-Like Antigen for Serodiagnosis of Chagas’ Disease\textsuperscript{ν}

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Chagas’ disease is caused by the protozoan parasite Trypanosoma cruzi and is one of the most important endemic problems in Latin America. Lately, it has also become a health concern in the United States and Europe. Currently, a diagnosis of Chagas’ disease and the screening of blood supplies for antiparasite antibodies are achieved by conventional serological tests that show substantial variation in the reproducibility and reliability of their results. In addition, the specificity of these assays is curtailed by antigenic cross-reactivity with sera from patients affected by other endemic diseases, such as leishmaniasis. Here we used a highly sensitive chemiluminescent enzyme-linked immunosorbent assay (CL-ELISA) to evaluate a recombinant protein core of a mucin-like molecule (termed trypomastigote small surface antigen [TSSA]) for the detection of specific serum antibodies in a broad panel of human sera. The same samples were evaluated by CL-ELISA using as the antigen either a mixture of native T. cruzi trypomastigote mucins or an epimastigote extract and, for further comparison, by conventional serologic tests, such as an indirect hemagglutination assay and indirect immunofluorescence assay. TSSA showed ~87% sensitivity among the seropositive Chagasic panel, a value which was increased up to >98% when only parasitologically positive samples were considered. More importantly, TSSA showed a significant increase in specificity (97.4%) compared to those of currently used assays, which averaged 80 to 90%. Overall, our data demonstrate that recombinant TSSA may be a useful antigen for the immunodiagnosis of Chagas’ disease.

American trypanosomiasis, or Chagas’ disease, is a major health and economic problem in Latin America caused by the protozoan parasite Trypanosoma cruzi. It is estimated that 8 million people are currently infected and that 90 million individuals living in areas of endemicity are at risk of infection (27, 32). The parasite is transmitted to humans through the feces of infected, blood-sucking triatomine bugs, blood transfusion, or congenital contamination or by the ingestion of tainted food and fluids (35). In recent years, several efforts have been successfully undertaken to control vectorial transmission in Latin American countries, with a concomitant decrease in the actual numbers of acute infections (28). However, since T. cruzi infection is asymptomatic in most cases, chronically infected individuals can serve as parasite reservoirs throughout their lifetimes. Thus, there is a consensus that congenital T. cruzi infection will be a pressing public health problem for at least the next 20 years (30). In addition, the risk of acquiring Chagas’ disease through infected blood transfusion is becoming a problem even in areas of nonendemicity, such as the United States and Europe, and some cases have already been reported (18, 23, 32). Owing to the risk of transmission by blood transfusion and organ transplantation, most blood donations in the United States have routinely been screened in recent years (1). Nevertheless, in many developed countries, the blood supply is not yet regularly tested for anti-T. cruzi antibodies (18, 32).

Due to the low parasite levels present in the chronic phase of the disease, its detection in blood samples by direct examination, hemoculture, or xenodiagnosis is difficult and time-consuming (19). Several PCR- and real-time-PCR-based procedures have been reported that, though highly specific and sensitive, might not be appropriate for routine use in blood supplies or health centers (16, 31). Detection of anti-T. cruzi antibodies is still the most effective method for demonstrating direct exposure to the parasite. At present, the most widely used serologic methods are indirect hemagglutination assays (IHAs), indirect immunofluorescence (IIF), and enzyme-linked immunosorbent assays (ELISAs) using total parasite homogenates or semipurified antigenic fractions from epimastigotes, the noninfective parasite form present in the digestive tract of the insect vector (19). However, these tests show vari-
atations in the reproducibility and reliability of their results that can be attributed to the poor standardization of the reagents (12). The advent of recombinant DNA technology allowed the production and one-step purification of large amounts of highly pure T. cruzi immunodominant antigens, some of which were evaluated by way of multicenter trials (12, 19, 25). The use of linear and/or branched synthetic peptides spanning B-cell epitopes has also been successfully applied (20, 22, 34). Both recombinant antigens and synthetic peptides minimize the extent of specificity problems, one of the major drawbacks of immunodiagnosis of Chagas’ disease (19). As previously shown, sera from individuals with leishmaniasis, mycoses, and/or certain autoimmune disorders cross-react with crude preparations of T. cruzi antigens (6, 33).

The mucin coat that covers the surfaces of bloodstream trypanosomatids (trypanosomatid glycosylphosphatidylinositol [tGPI] mucins) is decorated with highly immunogenic α-galactosyl (α-Gal) epitopes (3, 4). Chagasic anti-α-Gal antibodies have a complement-independent lytic effect on bloodstream trypanosomatid forms (26), and more importantly, their titer is considerably reduced in benzimidazole-treated patients in the early stage of chronicity (5, 13), suggesting that they might also be useful for monitoring patients after drug treatment. The trypanosomatide small surface antigen (TSSA) is a mucin-like glycoprotein displayed on the surface of infective trypanosomatid forms (15) and potentially involved in host cell recognition (G. E. Cáñepa and C. A. Buscaglia, unpublished data). Two main isoforms of TSSA were originally recognized in the two major lineages or subgroups into which the T. cruzi species has been divided (36): one isoform present in lineage I (currently known as T. cruzi discrete typing unit I [DTU TcI]) parasite stocks and one isoform present in TcHe (now DTU TcVI) isolates. Sequence variations between the isoforms were shown to have a major impact on TSSA antigenicity, leading to negligible cross-reactivity between them (15). This property was proposed to have great epidemiological value, as it allowed for identification of the lineage of the infecting strain by simple serologic methods (9). Recent studies challenged this idea by showing that TSSA is significantly more diverse in amino acid structure than previously described (7), although the antigenic impact of these changes, if any, remains uncertain.

Here we report a thorough study of the sensitivity and specificity of TSSA which demonstrates that this recombinant antigen is a useful molecule for the immunodiagnosis of Chagas’ disease. In order to improve the sensitivity of the assay, a high-throughput chemiluminescent ELISA (CL-ELISA) was employed (3).

MATERIALS AND METHODS

Study populations. Human serum samples (n = 617) were obtained from the Hospital das Clínicas da Universidade de São Paulo (HC-USP), the Laboratório de Investigação Médica-Parasitológica, Instituto de Medicina Tropical de São Paulo (IMT), Departamento de Moléstias Infecciosas, HC-USP, and the Fundação Hemocentro de Ribeirão Preto, São Paulo, Brazil. The use of these human serum samples was approved by the institutional review boards of the IMT and the Instituto de Cieˆncias Biome´dicas, USP (ICM-USP). Some of the samples were part of a serum panel stocked by the ICM-USP. These sera had been coded upon collection, and therefore no information (e.g., name, age, sex, etc.) regarding the patient was available. They were approved for use in the current study by the institutional review boards of both the ICM-USP and the IMT. Sera were collected from clotted blood obtained by venipuncture, diluted 1:2 with high-grade glycerol for conservation, and stored at −80°C until use. Three panels of human sera were used in this work. The first panel was composed of 237 samples collected from individuals that were grouped into seropositive Chagasic samples (those rendering positive results only for conventional serology tests [n = 185]) or parasitologically positive Chagasic samples (those rendering positive results for both conventional serology tests and hemoculture [n = 52]). The second panel was composed of 200 samples from healthy, noninfected individuals (NHS) rendering negative results for T. cruzi by three independent assays (ELISA using total parasite homogenerate, IHA, and IIF) and was thus defined as the seronegative panel. The third panel, termed the specificity control panel, was composed of 180 samples collected from non-Chagasic individuals affected by unrelated diseases, as defined by the clinical and serologic diagnoses of their respective pathologies. Twenty-nine of these 180 samples were from patients infected with cutaneous leishmaniasis, 31 were from patients with visceral leishmaniasis, 4 were from histoplasmosis patients, 9 were from patients infected with Mycobacterium leprae, and 28 were from patients affected by different helminth and protozoan infections. Specifically, 4 of the last samples were from individuals infected with Schistosoma spp., 5 were from individuals infected with Giardia lamblia, 1 was from an individual infected with Hymenolepis nana, 4 were from individuals infected with Tricuris trichuara, 2 were from individuals infected with Strongylodae stercoralis, 2 were from individuals infected with Ancylostoma spp., 3 were from individuals infected with Acanthamoeba lamosa, 1 was from an individual infected with Entamoeba vermicularis, 3 were from individuals infected with Toxocara canis, and 3 were from individuals infected with Cryptococcus spp. In addition, this panel contained 79 samples from individuals afflicted by autoimmune disorders: 53 with rheumatoid arthritis and 26 with systemic lupus erythematosus.

Parasites. Cell-derived trypanosomatids from the Y strain (DTU TcI) were collected from the supernatant of Mycoplasma-laccine LLC-MK2 cells (American Type Culture Collection, Manassas, VA) grown in Dulbecco's modified Eagle medium (DMEM) containing 4.5% glucose, 10% fetal bovine serum (FBS), and antibiotics (8). After three washings in phosphate-buffered saline (PBS), parasites were hypophylized and stored at −70°C until use. Epimastigotes from the same strain were cultured in liver infusion tryptose (LIT) medium containing 10% FBS and 5% glucose (10).

Purification of parasite tGPI mucins and preparation of total epimastigote extracts. tGPI mucins from 10^5 parasites were purified from delipidated butan-1-ol-water extracts by hydrophobic interaction chromatography as described previously (4). One total epimastigote extract (EpEx) was obtained as described previously (3).

Expression and purification of recombinant GST-TSSA proteins. Genes coding for the Sylvio X-10/1 TSSA (TSSA I; GenBank accession number ACY02665.1) and the CL Brenner TSSA (TSSA VI, formerly TSSA II; GenBank accession number ACY54510) have been described (7, 15). To reduce the risk of false-positive sampling, the recombinant TSSA proteins used in this work consisted only of the predicted, full-length, mature products, i.e., without most of the endoplasmic reticulum and GPI anchor signals (Fig. 1A). Briefly, previously described glutathione S-transferase (GST)–TSSA clones were reamplified by PCR using the oligonucleotides EM555 and EM575 (15), digested with BamHI and EcoRI, and cloned into pGEX-2T vector (GE). The recombinant TSSA proteins from Escherichia coli cultures induced for 3 h at 28°C with 0.1 mM isopropyl-β-D-thiogalactopyranoside were purified by glutathione-Sepharose chromatography (15) and dialyzed against PBS. The purity of GST-TSSA samples was assessed with silver-stained SDS-PAGE gels (Fig. 1B). Some of the faint bands that can be seen in the gel correspond to multimeric forms and/or degradation products of TSSA molecules, as judged by anti-GST Western blot analysis (not shown). CL-ELISA. Polystyrene ELISA microplates (FluoroNunc; Nunc, Roskilde, Denmark) were coated with the corresponding antigen (50 µl containing 1.2 ng tGPI mucins, 2.2 ng EpEx, or 10 ng TSSA per well), diluted in 50 mM carbonate-bicarbonate buffer, pH 9.6 (CBB). After 18 h at 4°C, plates were blocked with CBB containing 0.1% bovine serum albumin (CBB-BSA) and washed 3 times with 300 µl of PBS, pH 7.2, containing 0.05% Tween 20 (PBS-T). Sera were diluted in PBS-T as follows: (i) 1:2,000 for a chemiluminescent ELISA (CL-ELISA) with tGPI mucins or EpEx and (ii) 1:2,000 for a CL-ELISA with TSSA I and TSSA VI (15). In all cases, sera were incubated for 1 h at 37°C, and plates were then washed as before and developed by the addition of biotin-labeled anti-human IgG antibody (50 µl, 1:1,000 dilution; Amersham, GE Healthcare, Piscataway, NJ), followed by streptavidin coupled to horseradish peroxidase (HRP) (50 µl, 1:2,000 dilution; Amersham, GE Healthcare). Plates were washed as before, and 50 µl of ECL reagent (Amersham, GE Healthcare) diluted 1:20 in CBB was added. Reading of the plates was carried out with a Fluoroskan Ascent FL apparatus (Thermo Labsystems, Helsinki, Finland), and values were expressed in relative luminescence units (RLU). The cutoff value for each antigen was calculated with the equation, cutoff = k(m – mC), where k is the
number of standard deviations (SD) obtained for the negative-control sera separating the maximum RLU value for the negative-control sera and the minimum RLU value for the positive-control sera, and the SD obtained for the negative-control sera separating the maximum RLU value for the negative-control sera and the minimum RLU value for the positive-control sera, and $m_n$ and $m_b$ are the mean values of results from the negative-control sera and the background (without the addition of serum), respectively. The $k$ values were estimated as 3 for the tGPI mucins and 2 for EpEx and the GST-TSSA recombinant proteins. In order to compare the results of different CL-ELISAs using distinct antigens, serological titers were calculated by dividing the RLU values of individual serum samples by the cutoff values for each antigen.

Conventional serologic tests (IHA, IIF, and ELISA). Serum samples were tested by IHA and IIF as described previously (21), using commercial reagents (Ceccon, São Paulo, Brazil). For the latter, fluorescence isothiocyanate (FITC)-coupled anti-human IgG antibodies were used (New England Biolabs, Beverly, MA). The cutoff value determined for both techniques was 1:40, and in both cases, a 1:20 titer was considered inconclusive. A commercial ELISA kit composed of a crude extract of epimastigote forms was purchased from Embrabio (São Paulo, Brazil) and used according to the manufacturer’s guidelines. For ELISA and CL-ELISA, the corresponding cutoff value ± 10% was considered inconclusive.

RESULTS

Development of the T. cruzi mucin-based CL-ELISA. The quality of the purified antigenic GST-TSSA I and GST-TSSA VI (formerly GST-TSSA II [15]) recombinant proteins was assessed with silver-stained SDS-PAGE gels (Fig. 1A). These proteins rendered a single band of the expected molecular mass (∼35 kDa) (Fig. 1B), whereas purified tGPI mucins migrated as a broad smear ranging from 40 to 250 kDa, which is barely visible upon silver staining (4, 8; not shown). This heterogeneity is the result of the coexpression of multiple and heterogeneous apomucin polypeptides subjected to different extents of posttranslational modifications, mainly the addition of O-glycans (2, 8). The optimal concentration of protein per assay and the dilution of the serum samples were determined for each antigen by antigen-serum cross-titration, and they are defined as the antigen concentration and serum dilution that gave the highest ratio between a blindly selected pool of parasitologically positive sera ($n = 10$) and a blindly selected pool of NHS from our seronegative panel ($n = 10$). For tGPI mucins and EpEx, the optimal conditions were achieved at concentrations of 1.2 and 2.2 ng/well, respectively, and in testing the samples at a 1:2,000 dilution. In the case of the GST-TSSA VI molecule, optimal results were achieved by using 10 ng/well and diluting the serum samples 1:200. Accordingly, the same conditions were used for the evaluation of the GST-TSSA I recombinant protein. The cutoff and $k$ values for each antigen were calculated as indicated in Materials and Methods by the use of (i) 52 parasitologically positive sera and (ii) 100 NHS samples which were blindly selected from our seronegative panel.

Sensitivity of the CL-ELISA using seropositive and parasitologically positive human sera. A panel of 237 samples was analyzed by CL-ELISA using EpEx, purified tGPI mucins, and recombinant GST-TSSA VI and GST-TSSA I proteins as antigens. For comparison, all the samples were analyzed by three widely used serodiagnostic techniques: IIF, IHA, and ELISA. Two groups can be defined among the tested samples: those showing positive results in conventional serology tests and hemoculture (52 parasitologically positive samples) and those showing positive results only in conventional serology tests (185 seropositive samples), which were collected either in Brazil ($n = 160$) or Chile ($n = 25$). Box-and-whisker plots expressing the results obtained in each case are shown in Fig. 2, and

![FIG. 1. Features of evaluated TSSA molecules. (A) Schematic illustration of TSSA products showing the predicted signal peptide (SP) and GPI-anchoring signals. The sequences of the TSSA I and TSSA VI regions expressed as GST fusion proteins are indicated. Variable positions between both proteins are shaded. The predicted SP cleavage site is indicated with an inverted triangle, and amino acids (aa) predicted to be O-glycosylated in the TSSA VI protein are indicated with asterisks. B-cell epitopes recognized by human Chagasic sera in the TSSA VI protein are underlined. (B) The purity of the recombinant GST fusion proteins used for the CL-ELISAs was ascertained by silver staining of an SDS-PAGE gel containing 1 μg of the indicated protein. Molecular size markers (in kDa) are indicated.](image-url)

![FIG. 2. Recognition of evaluated antigens by sera from T. cruzi- and Leishmania-infected individuals. Reactivities of sera collected from individuals infected with T. cruzi (Chagasic [Ch]) ($n = 237$) or Leishmania spp. ($n = 60$) and from healthy individuals (NHS) ($n = 200$) to the indicated T. cruzi-derived antigens were determined by CL-ELISA. Results are presented by box-and-whisker plots, indicating the median serum titer (horizontal line inside box), SD (box’s upper and lower lines), and maximal and minimal serum titers (whiskers). The serological titer for each sample was determined as described in Materials and Methods. Sera from Leishmania-infected individuals were grouped according to the manifestation of the disease. VL, visceral leishmaniasis ($n = 31$); CL, cutaneous leishmaniasis ($n = 29$).](image-url)
the raw data are summarized in Table 1. When tGPI mucins were used as antigens, 100% sensitivity was obtained for both groups of samples, in accordance with previous data (3). This value is even higher than that obtained by IHA or IIF and also higher than that obtained with EpEx, with which only 1 out of 237 samples yielded negative results. On the other hand, the overall sensitivity of GST-TSSA VI is 86.9% and thus in the range of values for other recombinant parasite antigens proposed for Chagas’ disease immunodiagnosis (12, 25). However, and according to previous results, sensitivities clearly differed between hemoculture-tested and -untested sera (15). The value for the first group was >98% (only 1 out of 52 samples produced inconclusive results), whereas a significant reduction to 83.7% was verified for the second group. No significant differences in the sensitivity indexes were observed when sera collected from Brazil and Chile were compared (83.7 and 84.0%, respectively), although the mean values recorded for the Brazilian samples were consistently higher (not shown). In line with previous results, none of the tested sera rendered positive values when evaluated by the control GST-TSSA I protein (Table 1), which could be attributed to (i) low levels of expression/antigenicity of the TSSA isoform present in DTU Tc1 parasite stocks and/or (ii) the predominance of non-DTU Tc1 infections in the domestic and peridomestic cycles of Chagas’ disease in the Southern Cone countries of Latin America (11, 15).

Specificity of the CL-ELISA. The specificity of each antigen in our CL-ELISA was analyzed by using 380 serum samples collected from non-Chagasic individuals (Table 1). Among these samples, 200 were from healthy, noninfected blood donors (NHs; seronegative panel) and 180 from individuals affected by other infectious or autoimmune diseases which might elicit cross-reactive humoral responses toward T. cruzi infections in the domestic and peridomestic cycles of Chagas’ disease in the Southern Cone countries of Latin America (11, 15).

### TABLE 1. Sensitivities and specificities of CL-ELISA with distinct T. cruzi antigens and of conventional serologic methods

| Index       | No. of samples | Result (%) using CL-ELISA with: | Result (%) using: |
|-------------|----------------|--------------------------------|-------------------|
|             |                | EpEx | tGPI mucin | TSSA VI | TSSA I | IHA | IIF | ELISA |
| Sensitivity | 237            | 99.6 | 100        | 86.9    | 0     | 97.5 | 98.7 | 100   |
| Specificity | 200            | 100  | 100        | 99      | NA    | 100  | 100  | 100   |
| Specificity | 180            | 69.4 | 100        | 94.4    | NA    | 77.8 | 58.3 | 57.8  |
| Specificity | 380            | 85.5 | 100        | 97.4    | NA    | 89.5 | 80.3 | 80    |

a Specificity determined with seronegative human sera.
b Specificity determined with specificity control human sera.
c Specificity determined with specificity control human sera.
d NA, not applicable (did not render a positive value).

discussion

Serologic methods are widely employed for the diagnosis of Chagas’ disease, particularly in the indeterminate and chronic phases, when parasitemia is extremely low and very hard to detect. The World Health Organization has long emphasized the need to employ defined antigens as a way of improving the serodiagnosis of Chagas’ disease. In order to be useful, these antigens must meet several criteria: (i) they should be present in T. cruzi isolates from different areas of endemicity, (ii) they should be absent from other infectious disease agents, (iii) they should be highly immunogenic in populations with different genetic backgrounds, and (iv) they should be stable and easily amenable to quality control tests to guarantee reproducibility (19). Considering these guidelines, we herein evaluated the recombinant protein core of the TSSA antigen, derived from a mucin molecule expressed by the bloodstream forms of T. cruzi, in the development of a sensitive and specific CL-ELISA system for the diagnosis of Chagas’ disease. The choice was based on previous results in which the TSSA isoform from DTU Tc1 parasite stocks displayed a high reactivity with human infection sera from Southern Cone countries (15). Although the spectra of circulating parasite stocks, and therefore of their expressed TSSA isoforms, seem to vary in different areas of endemicity, a high frequency of TSSA VI recognition, as assessed by Western blotting, was also recently observed in...
patients with chronic Chagas’ disease from Colombia, Venezuela, and Mexico (29).

By using a much larger and more comprehensive serum panel, we estimated the overall sensitivity of GST-TSSA VI as 86.9% (Table 1). Even though the sensitivity of this antigen is in the range of other recombinant proteins proposed for Chagas’ disease immunodiagnosis (12, 25), its sensitivity among parasitologically positive samples was 98%. More importantly, TSSA VI showed minimal cross-reactivity with blood samples from *Leishmania*-infected individuals, a common source of false-positive results in conventional serodiagnosis tests for Chagas’ disease (Tables 1 and 2). In accordance, TSSA seems to be restricted to *T. cruzi*, since no homologous gene has been detected in the genome databases of other trypanosomatids (reference 17 and our unpublished results).

For comparison purposes, the same samples were evaluated in parallel using tGPI mucins, a putative gold standard for the diagnosis and follow-up of the treatment of Chagas’ disease (3, 5, 13), which resulted in 100% sensitivity and 100% specificity with the extensive panel of serum samples tested here. The basis for this sensitivity can be attributed in part to the abundance of tGPI mucins on the surface of a parasite (26) and to the high antigenicity of their nonreducing, terminal \( \alpha \)-Gal epitopes (4). Accordingly, treatment of tGPI mucins with \( \alpha \)-galactosidase abolished most (80 to 100%) of the reactivity of individual human Chagasic sera against these GPI-anchored glycoproteins (I. C. Almeida, unpublished data). In spite of its optimal specificity and sensitivity, some drawbacks could be envisaged for the routine implementation of tGPI mucins in serodiagnosis. First, the material is purified from cultured infective forms of the parasite, and this process is expensive and time-consuming. In addition, purification is a multistep process, requiring lyophilization of the parasites, extraction in organic solvents, and hydrophobic-interaction chromatography. These disadvantages are eased by the fact that very small amounts of tGPI mucins are required (~1 ng/well). Therefore, a single-batch preparation from \( 10^9 \) trypomastigotes should

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**TABLE 2. Specificities of CL-ELISA with distinct *T. cruzi* antigens and of conventional serologic methods to heterologous human sera**

| Disease or infection \( (n)^a \) | Test result\( b \) | No. of samples with each result with CL-ELISA and: | No. of samples with each result with: |
|---|---|---|---|
| | | EpEx | tGPI mucin | TSSA VI | IHA | IIF | ELISA |
| Leishmaniasis \( (60) \) | Positive | 36 | 0 | 0 | 35 | 46 | 40 |
| | Inconclusive | 6 | 0 | 1 | 3 | 5 | 0 |
| | Negative | 18 | 60 | 59 | 22 | 9 | 20 |
| | Specificity | 30 | 100 | 98.3 | 36.7 | 15 | 33.3 |
| Histoplasmosis \( (4) \) | Positive | 0 | 0 | 0 | 0 | 0 | 0 |
| | Inconclusive | 0 | 0 | 0 | 0 | 0 | 0 |
| | Negative | 4 | 4 | 4 | 4 | 4 | 4 |
| | Specificity | 100 | 100 | 100 | 100 | 100 | 100 |
| *Mycobacterium leprae* \( (9) \) | Positive | 0 | 0 | 0 | 0 | 3 | 0 |
| | Inconclusive | 0 | 0 | 0 | 0 | 2 | 0 |
| | Negative | 9 | 9 | 9 | 9 | 4 | 9 |
| | Specificity | 100 | 100 | 100 | 100 | 44.5 | 100 |
| Helminth and protozoan infections \( (28) \) | Positive | 2 | 0 | 0 | 0 | 0 | 5 |
| | Inconclusive | 2 | 0 | 1 | 0 | 0 | 2 |
| | Negative | 24 | 28 | 27 | 28 | 28 | 21 |
| | Specificity | 85.7 | 100 | 96.4 | 100 | 100 | 75 |
| Rheumatoid arthritis \( (53) \) | Positive | 4 | 0 | 3 | 0 | 4 | 20 |
| | Inconclusive | 1 | 0 | 4 | 0 | 5 | 0 |
| | Negative | 48 | 53 | 46 | 53 | 44 | 33 |
| | Specificity | 90.6 | 100 | 86.6 | 100 | 83.1 | 62.3 |
| Systemic lupus erythematosus \( (26) \) | Positive | 4 | 0 | 1 | 2 | 4 | 9 |
| | Inconclusive | 0 | 0 | 0 | 0 | 6 | 0 |
| | Negative | 22 | 26 | 25 | 24 | 16 | 17 |
| | Specificity | 84.6 | 100 | 96.2 | 92.3 | 61.5 | 65.4 |

\( ^a \) \( n \), number of samples from affected individuals.

\( ^b \) Specificity is expressed as a percentage.
render enough material to perform 20,000 assays. Different protocols to speed up and simplify the tGPI mucin purification processes should be evaluated.

It becomes clear that in order to improve the sensitivity of TSSA VI, it should be supplemented with other antigens, either by including it in existing serologic diagnostic kits or by developing a new mixture of antigens that might well include a synthetic terminal α-Gal-containing epitope(s) recognized by Chagas' anti-Gal antibodies in tGPI mucins. In this regard, it is worth noting that chemical synthesis of complex oligosaccharides attached to T. cruzi mucins has been recently achieved (14, 24). We speculate that the presence of both peptide and glycan epitopes in a single serodiagnostic reagent may render optimal results in terms of sensitivity and specificity. In summary, our results show that TSSA VI is a better alternative to the epimastigote extracts currently employed in T. cruzi serodiagnosis. It could be combined with other recombinant antigens to improve the sensitivities of current kits or used alone or in combination with other antigens as a confirmatory diagnostic test, as recommended by the World Health Organization (19). The CL-ELISA developed here also has a highly increased specificity, in particular with respect to Leishmania infections, compared to other, more cumbersome diagnostic techniques for T. cruzi detection, such as IHA and IIF.

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