EVALUATION OF THE WESTERN BLOT IN THE CONFIRMATORY SEROLOGIC DIAGNOSIS OF CHAGAS’ DISEASE

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Abstract. The Western blot method, using antigens from epimastigote forms of the Trypanosoma cruzi Y strain, was evaluated for the confirmatory diagnosis of Chagas’ disease. Serum samples were obtained from 136 chagasic patients (Group I), 23 patients with inconclusive serologic results for Chagas’ disease (Group II), 53 patients with other diseases (Group III), and 50 healthy individuals (Group IV). The Western blot results for Group I gave a confirmatory diagnosis of Chagas’ diseases in 118 (86.80%), an indeterminate pattern of reactivity in 16 (11.76%), and a negative pattern in only two (1.47%). Of the samples from Groups II, III and IV, none had a positive result in the Western blot; 60.86%, 52.83%, and 16.0%, respectively, showed indeterminate results; and 39.13%, 47.16% and 84.0%, respectively, showed negative results. The Western blot method showed a sensitivity of 86.60%, a specificity of 100.0%, a positive predictive value of 100.0%, and a negative predictive value of 73.50%, and the concordance coefficient kappa was high (0.7789). The results suggest that the previous serologic results for Chagas’ disease could be confirmed by Western blot for the detection of specific antibodies to T. cruzi antigenic fractions, which may reduce the medical, legal, and social consequences of an inconclusive serologic result for Chagas’ disease and also underscore the need for additional studies for continued efforts in the development of an ideal standard confirmatory test for Chagas’ disease.

Chagas’ disease or American trypanosomiasis is an endemic chronic infection caused by the protozoan Trypanosoma (Schizotrypanum) cruzi. It is a major health problem in rural and, more recently, urban and suburban areas on the American continent, where 16–18 million people are infected from Mexico to Argentina, and more than 90 million persons are at risk of being infected with the parasite. Several methods have been used for diagnosis of this disease. The classic parasitologic diagnosis is based on detection of the whole parasite, using methods such as blood smears, microhematocrit, xenodiagnosis, and hemoculture. Some of these methods are laborious and limited by the delay in obtaining results, and although highly specific, they detect parasites with a single examination in no more than 50% of the patients with chronic disease. The presence of antibodies in the sera of chagasic patients is commonly demonstrated by various serologic methods: the complement fixation test (CF), direct agglutination (DA), indirect hemagglutination (IHA), indirect immunofluorescence assay (IFA), and ELISA. None of the methods for diagnosis of Chagas’ disease can be regarded as 100% safe in blood bank screenings. Furthermore, with the low prevalence of infected donors, now less than 2%, one has to expect low positive predictive values, leading to a high false-positive rate of results that must be confirmed by other methods. Contradictory results have been obtained by different methods and laboratories, probably due to the use of different strains of the T. cruzi and different antigenic fractions and procedures, causing variations in sensitivity and specificity.

The main problem for blood bank screenings for chagasic patients is the variable number of cases reacting at about the cut-off value for positivity in the presently used serologic tests. In addition, for screening blood donors, the lowest possible cut-off value should be selected to ensure the highest sensitivity of the test. This fact should increase the risk of false-positive results.

Several investigators have studied the usefulness of the protein blotting technique (Western blot) in the diagnosis of Chagas’ diseases to confirm the serologic results obtained in the conventionally used tests, and have demonstrated high sensitivity and specificity.

The rates of inconclusive serologic results for diagnosis of Chagas’ disease obtained in routine procedures among the blood donors (2.46%) and patients (1.85%) seen at the Hospital Universitário Regional Norte do Paraná (HURNP) prompted us to evaluate the Western blot method to detect specific antibodies against T. cruzi antigenic polypeptides as an alternative method to confirm the laboratory diagnosis of Chagas’ disease.

MATERIALS AND METHODS

This study was reviewed and approved by the Internal Scientific Commission and the Bioethics in Research Committee of the Universidade Estadual de Londrina.

Human sera. The serum samples used were divided into four groups.

Group I. This group was composed of 91 serum samples obtained from patients seen at HURNP and the Ambulatório do Hospital de Clínicas (AHC) and 45 serum samples from blood donors of the Hemocentro of HURNP affiliated with the Universidade Estadual de Londrina. All 136 samples were reactive in at least two conventional serologic tests for Chagas’ disease (IFA, ELISA, or IHA).

Group II. This group was composed of 17 serum samples from patients seen at HURNP and AHC and six serum samples from blood donors of the Hemocentro; all 23 samples were reactive in only one conventional serologic test for Chagas’ disease.

Group III. This group was composed of 53 serum samples from patients seen at HURNP with a laboratory diagnosis of other infectious or autoimmune diseases or conditions;
cutaneous leishmaniasis (12), toxoplasmosis (12), antinuclear antibodies (6), rheumatoid factor (4), infectious mononucleosis (3), syphilis (4), rubella (4), antinuclear and anti-dsDNA antibodies (2), paracoccidioidomycosis (2), salmonellosis (2), listeriosis (1), and cytomegalovirus (1). All samples were negative for Chagas’ disease serology.

Group IV. This group was composed of 50 serum samples from blood donors of the Hemocentro seronegative for Chagas’ diseases, antibody to hepatitis C virus, hepatitis B surface antigen, antibody to hepatitis B virus core antigen, antibody to human immunodeficiency virus (anti-HIV), and antibodies to human T cell lymphotrophic viruses types 1/2 (anti-HTLV-1/2), and with negative Veneral Disease Research Laboratory (VDRL) test results and normal levels of alanine aminotransferase. These were used as negative controls.

Serologic tests for Chagas’ disease. Indirect hemagglutination. Qualitative and quantitative tests were conducted using commercial reagents (EBRAM; Produtos Laboratoriais Ltda, São Paulo, Brazil) at an initial serum dilution of 1:32 according to the manufacturer’s instructions.

Indirect immunofluorescence assay. The titers of antibodies against T. cruzi were measured using fixed T. cruzi epimastigotes on commercially available slides (LIO SERUM; Indústria e Comércio de Equipamentos e Produtos para Laboratórios Ltda., Ribeirão Preto, Brazil and Cecon, São Paulo, Brazil) and fluorescein-conjugated goat anti-human IgG (LABORCLIN; Produtos para Laboratórios Ltda, Pinhais, Paraná, Brazil and The Binding Site, Ltd., Birmingham, United Kingdom) as a second antibody at an initial serum dilution of 1:40.15

Enzyme-linked immunosorbent assay. This was performed with commercially available reagents (Abbott Laboratorios do Brasil, Ltda., Sao Paulo, Brazil and Weiner Laboratorios, Rosário, Argentina), according to the manufacturer’s instructions. Both ELISA kits used specific, inactivated T. cruzi antigens. The presence or absence of antibody to T. cruzi was determined by relating the optical density (OD) of the specimen to the cut-off value. A ratio between the sample OD value and the cut-off OD value (sample OD/cutoff OD) was determined for each serum sample assayed. A case was considered positive for Chagas’ disease when the serum samples showed positive serologic results (IHA titer ≥ 1:32; IFA titer ≥ 1:40; and ELISA results repeatedly higher than the cut-off value) in at least two tests.16

Antigen preparation. Antigens for the Western blot test were obtained from epimastigote forms of the T. cruzi Y strain (kindly provided by Dr. M. J. M. Alves (Department of Biochemistry, Institute of Chemistry, University of São Paulo, São Paulo, Brazil), cultured in liver infusion tryptose medium at 28°C without shaking, and collected during the exponential growth phase.17 The antigen was prepared by previously published methods, with some modifications.8121019

The cells were pelleted by centrifugation at 3,200 × g for 10 min at 4°C, and washed three times with 150 mM phosphate-buffered saline (PBS), pH 7.2, by centrifugation at 3,200 × g for 10 min at 4°C. The sediment was resuspended in 20 mM Tris-HCl, pH 7.5, containing 1 mM phenylmethylsulfonyl fluoride, 1 mM o-phenanthroline, and 10 μg/ml of L-1-tosylamide 2,2-phenylethylchlooromethylketone. The parasites were disrupted by sonication in an ice bath using five pulses of 30 sec at 60 cycles (Sonifer Cell Disruptor; Universidade Estadual de Londrina, Londrina, Brazil). The lysate was observed by light microscopy (400 ×) to ensure the absence of whole parasites. The suspension was centrifuged at 3,200 × g for 30 min at 4°C, and the supernatant was divided into aliquots and stored at −20°C until the time for use. Protein concentration in the antigenic suspension was determined by the method of Lowry and others,20 using bovine serum albumin (100 μg/ml) as the standard.

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis. The samples were electrophoresed in 1-mm thick slab gels with a 7.5–15.0% polyacrylamide gradient in the running gel, and a stacking gel of 3.5% acrylamide, with the use a discontinuous SDS buffer system.21 Carbonic anhydrase, egg albumin, bovine serum albumin, and β-galactosidase (SDS-200 Kit; Sigma Chemical Co., St. Louis, MO) were used as molecular weight markers. The molecular weight markers and the sonicated T. cruzi antigen were diluted in sample buffer (0.125 M Tris-HCl, 20% glycerol, 2% β-mercaptoethanol, 4% SDS, 10% bromophenol blue, 0.224% EDTA, pH 6.8), boiled for 3 min, and applied to the gel. Running buffer was added (0.025 M Tris-base, 0.192 M glycine, and 0.1% SDS, pH 8.8) and the samples were electrophoresed at 40 volts (10 mA) until reaching the running gel. Electrophoresis was then run at 100 volts (50 mA) until the stain front reached the bottom of the gel. The gel was then stained with Coomassie Brilliant Blue R 250 for proteins at room temperature overnight.22 The gel was dried between two wet cellophane sheets and 10% gelatin (w/v) for 72 hr at room temperature and the molecular weight of the T. cruzi proteins fractions electrophoresed was determined23 or mounted in a protein transfer apparatus and subjected to the electroimmunotransfer blot.

Enzyme-linked immunoelectrotransfer blot (Western blot). The polypeptides in the gel were transferred to nitrocellulose sheets (Life Technologies, Inc., Gaithersburg, MD) at 50 volts (150 mA) at 4°C overnight24 (EC-600; EC Apparatus Corporation, St. Petersburg, FL). The nitrocellulose sheet was cut into vertical strips. The strips were treated with a solution of 5% defatted milk (Molico, Nestlé, São Paulo, Brazil), 0.1% Tween 20 in PBS, pH 7.2, for 1 hr at room temperature with constant shaking, rinsed with PBS, and treated for 1 hr at room temperature with constant shaking with serum samples diluted 1:100 in PBS containing 5% defatted milk and 0.1% Tween 20. After washing three times (10 min each) with PBS containing 0.1% Tween 20, the strips were treated for 1 hour at room temperature with a peroxidase-labeled affinity purified rabbit anti-human IgG conjugate (Sigma Chemical Co.) diluted 1:2,000 in PBS containing 5% defatted milk and 0.1% Tween 20. After three additional washes (10 min each), the substrate (30% H2O2, 0.3% 4-chloro-1-naphthol in methanol dissolved in PBS) was added. The color was allowed to develop for 30 min at room temperature in the dark, rinsed with distilled water, dried, and photographed. The results were compared visually with the positive and negative controls run in parallel. In some nitrocellulose strips the human serum was omitted to control for nonspecific binding of the conjugate to the antigens.

The criterion of reactivity used for the interpretation of
FIGURE 1. Western blot analysis of sodium dodecyl sulfate–polyacrylamide gel electrophoresis of epimastigote forms of the *Trypanosoma cruzi* Y strain recognized by serum samples from patients with Chagas’ disease (Group I, lanes a–k), patients with only one serologic test result positive for Chagas’ disease (Group II, lanes l–o), patients with other diseases (Group III, lanes p–s), and healthy individuals (Group IV, lanes t–w). MW = molecular weight markers; P = positive control serum; N = negative control serum. kD = kilodaltons.

The protein concentration of the *T. cruzi* Y strain antigenic preparation was 2,080 μg/ml, and when subjected to SDS-PAGE and stained for proteins, it showed bands with apparent molecular weight ranging from 11 to 160 kD. The protein bands with molecular weights of 18–20, 22, 25, 29, 33, 40, 43, 45, 47, 52, 62, 70, 86, 94, 100, and 110 kD showed the highest staining intensity.

The serum samples from Groups I, II, III and IV recognized several antigenic bands of the *T. cruzi* Y strain at different frequencies and intensities of reactivity (Figure 1 and Table 1). The samples from Group I recognized an average of 7.71 bands, whereas the samples from Groups II, III and IV recognized an average of 1.47, 0.98, and 0.28 bands, respectively.

The sensitivity was determined by relating the number of samples with Western blot–positive results/number of samples with ELISA- and IFA-reactive results × 100. The specificity was determined by relating the number of samples with Western blot–negative results/number of samples seronegative for Chagas’ disease (Groups III and IV) × 100.

**RESULTS**

The protein concentration of the *T. cruzi* Y strain antigenic preparation was 2,080 μg/ml, and when subjected to SDS-PAGE and stained for proteins, it showed bands with apparent molecular weight ranging from 11 to 160 kD. The protein bands with molecular weights of 18–20, 22, 25, 29, 33, 40, 43, 45, 47, 52, 62, 70, 86, 94, 100, and 110 kD showed the highest staining intensity.

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The 136 serum samples from Group I showed a similar pattern of reactivity and nearly all (98.56%) of the chagasic patient sera recognized mainly 25 protein bands with apparent molecular weights ranging from 11 to 145 kD (11, 16, 25, 30, 33, 37, 39, 45, 49, 52, 60, 64, 70, 75, 82, 88, 92, 97, 100, 105, 120, 125, 130, 135, and 145 kD). The frequency of recognition and the intensity of the reactions were variable. Among the bands, the major bands with apparent molecular weight of 25, 30, 33, 37, 39, 45, 70, and 75 kD were recognized in more than 50% of the samples analyzed, giving frequencies of 55.1%, 82.3%, 69.1%, 50.7%, 52.9%, 50%, 50%, and 59.55%, respectively. In the 23 serum samples from Group II, the Western blot detected antibodies that recognized bands with apparent molecular weights ranging from 11 to 75 kD, and the bands with molecular weights of 30, 39, and 45 kD were recognized by 21.7% of the samples.
The data in Table 3 show that of 136 serum samples from patients with Chagas’ disease, 118 (86.8%) had positive results in the Western blot, 16 (11.76%) had indeterminate results, and two (1.47%) had negative results. Of 53 serum samples from patients with other diseases (Group III), 28 (52.83%) had an indeterminate reactivity pattern in the Western blot and 25 (47.16%) had a negative pattern. Of 50 serum samples from healthy individuals (Group IV), 42 (84.0%) had negative results, only eight (16.0%) had an indeterminate reactivity pattern, and none had a positive result in the Western blot. The sensitivity (95% confidence interval) of the Western blot was 86.80% (79.69–91.89%) and the specificity was 100.0% (91.10–100.0%). Without considering the prevalence of Chagas’ disease in the population studied, the positive predictive value of the Western blot was 100.0% (96.10–100.0%), the negative predictive value was 73.50% (61.20–83.20%), and the concordance coefficient kappa was high (0.7789) (Statistical Z = 10.89, P < 0.0001).

The positive results in the Western blot occurred in the serum samples with the highest OD values in the ELISA and the highest titers of anti-T. cruzi antibodies detected in the IFA. The indeterminate and the negative results obtained in the Western blot occurred mainly in the serum samples with low titers of anti-T. cruzi antibodies (Table 4).

Table 5 shows the positivity of 53 serum samples from patients with a laboratory diagnosis of other infectious or autoimmune diseases (Group III) in the IFA, ELISA, and Western blot for Chagas’ disease.

### Table 2

<table>
<thead>
<tr>
<th>Proteins* (kD)</th>
<th>Group I² No.</th>
<th>%</th>
<th>Group II³ No.</th>
<th>%</th>
<th>Group III§ No.</th>
<th>%</th>
<th>Group IV¶ No.</th>
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</table>

* T. cruzi Y strain proteins bands recognized by at least one sample analyzed.
² 136 serum samples from Chagas’ disease patients.
³ 53 serum samples from patients with other diseases seronegative for Chagas’ disease.
¶ 50 serum samples from healthy individuals.

When the 53 serum samples from patients with other diseases (Group III) were tested by Western blot, no band was recognized by 25 samples. In the remaining 28 samples, bands with apparent molecular weights ranging from 16 to 160 kD were recognized at a frequency < 19.0% with faint intensities of reactivity. Samples from patients with cutaneous leishmaniasis showed reactivity with some bands (18, 30, 39, 45, 52, 64, 70, and 82 kD), but the number of bands recognized was not sufficient for the test result to be considered positive. Of 50 samples from Group IV that showed no reactivity in conventional serologic tests for Chagas’ disease, 42 (84.0%) were also negative in the Western blot and only eight (16.0%) recognized bands with apparent molecular weights ranging from 18 to 97 kD, with a frequency of up to 6.0% (Table 2).

### Table 3

<table>
<thead>
<tr>
<th>Group*</th>
<th>Number of serum samples</th>
<th>IFA² T. cruzi antibody titers range</th>
<th>ELISA³ Sample OD/cutoff ratio range</th>
<th>Western blot</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>136</td>
<td>1:40–1:10.240</td>
<td>1.264–11.617</td>
<td>P</td>
</tr>
<tr>
<td>II</td>
<td>23</td>
<td>&lt;1:40–1:160</td>
<td>0.613–2.414</td>
<td>I</td>
</tr>
<tr>
<td>III</td>
<td>53</td>
<td>&lt;1:40–1:180</td>
<td>0.148–1.497</td>
<td>N</td>
</tr>
<tr>
<td>IV</td>
<td>50</td>
<td>&lt;1:40</td>
<td>0.099–0.871</td>
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</tbody>
</table>

* Group I = samples from patients with Chagas’ disease; Group II = samples from patients with at least one serologic test result for Chagas’ disease; Group III = samples from patients with other diseases and seronegative for Chagas’ disease; Group IV = samples from healthy individuals.
² Indirect immunofluorescence assay.
³ Enzyme-linked immunosorbent assay: range of the sample optical density (OD)/cutoff OD ratio obtained at 492 nm.

**DISCUSSION**

One of the properties of Chagas’ disease is that very often there are no clinical manifestations or they are mild. For this...
The sera tested reacted with the 55-kD protein and our results bands from a group of 10 with the highest frequency: I
of 92 kD. Teixeira and others
patient sera with the 90-kD protein, our results demonstrated screening is expected. Cross-reactivity between
one considers the low prevalence of Chagas’ disease among antibodies with different speciﬁcities.

The criterion used in this study for interpretation of the Western blot results was based on the number of bands rec-
ognized by the serum samples from Groups I, II, III, and IV (Table 1), and on the frequency of reactivity of different
proteins in the antigenic suspension of the

Schechter and Nogueira28 reported that variations in the surface antigen profiles of T. cruzi could be induced by dif-
ferent methodologies. The variation of the antigenic composition of the parasite associated with the particular
immune response of each individual could result in the activation of different lymphocyte clones and in production of antibodies with different specificities.29 A large number of T. cruzi Y strain antigens with apparent molecular weights ranging from 11 to 145 kD was recognized by serum samples from chagasic patients. However, differences in the molecular weights of the proteins recognized by sera have been observed when results from different studies are compared, and this may be due to variations in the measurement of the band migration on the gel.31 Whereas Snary and Hudson30 and Schechter and others31 reported reactivity of chagasic patient sera with the 90-kD protein, our results demonstrated reactivity with a protein with an apparent molecular weight of 92 kD. Teixeira and others32 demonstrated that 74% of the sera tested reacted with the 55-kD protein and our results showed that 43.8% of the sera reacted with the 52-kD pro-
tein. The highest frequency of reactivity was obtained with the 30-kD protein, which was recognized by 82.35% of the serum samples from chagasic patients. This result agrees with data reported previously, indicating 100% reactivity with the same protein.12 Another T. cruzi protein, gp 25,27 was recognized by 55.14% of the serum samples from Group I, and was not recognized by serum samples from Groups III or IV, which confirms its usefulness for the immunodiagnosis of Chagas’ disease. Different results were obtained by Grijalva and others with ELISA-reactive samples from patients with Chagas’ disease.10 When retested by Western blot, they showed reactivity with a 205-kD doublet T. cruzi antigen.

The high complexity of the molecular constitution of T. cruzi goes beyond the limits of resolution and sensitivity of biochemical and immunochimical techniques used in this analysis. There is no clear-cut consensus about the deﬁnition of major T. cruzi antigens in the literature, mainly because of the highly complex T. cruzi antigenic composition associated with limitations in the analysis of results obtained with different techniques.32 In fact, most of the results are deﬁned as a complex serum reactivity pattern displaying antigens with molecular weights ranging from 15 to 205 kD.

$^{*}$ Western blot results: P = positive when the sample recognized at least five antigenic bands from a group of 10 with the highest frequency; I = indeterminate when the sample recognized up to four antigenic bands from a group of 10 with the highest frequency; N = negative when the sample showed no reactivity.

$^\ddagger$ Indirect immunoﬂuorescence: assay of the anti-T. cruzi antibody titers obtained.

$^\ddagger$ ELISA: range of the sample optical density (OD)/cut-off OD ratio obtained at 492 nm (average ± SD).

TABLE 4

<table>
<thead>
<tr>
<th>Western blot result*</th>
<th>Number†</th>
<th>IFAC Anti-T. cruzi antibody titers range</th>
<th>ELISA§ Sample OD cut-off OD range (X ± SD)</th>
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<tr>
<td>Positive</td>
<td>118</td>
<td>1:40–1:10,240</td>
<td>1.333–11.627 (4.971 ± 1.993)</td>
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<td>Indeterminate</td>
<td>16</td>
<td>1:40–1:320</td>
<td>1.440–4.605</td>
</tr>
<tr>
<td>Negative</td>
<td>2</td>
<td>1:40–1:80</td>
<td>2.605 ± 1.112 (1.776 ± 0.475)</td>
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<tr>
<td>Total</td>
<td>136</td>
<td>1:40–1:10,240</td>
<td>1.264–11.627 (1.440 ± 2.113)</td>
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</table>

TABLE 5

<table>
<thead>
<tr>
<th>Laboratory diagnosis</th>
<th>Number of serum samples</th>
<th>IFAC*</th>
<th>ELISA†</th>
<th>Western blot‡</th>
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<td>Positive</td>
<td>12 2 10 2 10 0 8 4</td>
<td>P</td>
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<td>Cutaneous leishmaniasia</td>
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<td>Antinuclear antibodies</td>
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<td>Infectious mononucleosis</td>
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<td>Paracoccidioidomycosis</td>
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<td>Salmonellosis</td>
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<td>Rubula</td>
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<td>Cytomegalovirus disease</td>
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<td>Listeriosis</td>
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<td>Rheumatoid factor</td>
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<tr>
<td>Antinuclear + anti-dsDNA§</td>
<td>2 1 1 0 2 0 2 0</td>
<td></td>
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</tr>
<tr>
<td>Total</td>
<td>53 4 9 2 51 0 28 25</td>
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</table>

$^*$ Indirect immunoﬂuorescence: + reactive; – nonreactive.

$^\ddagger$ Western blot results: P = positive when the sample recognized at least five antigenic bands from a group of 10 with the highest frequency; I = indeterminate when the sample recognized up to four antigenic bands from a group of 10 with the highest frequency; N = negative when the sample showed no reactivity.

§ dsDNA = double-strand deoxyribonucleic acid.
in the IFA. In contrast, when the titers of anti-*T. cruzi* antibodies were low, the Western blot showed indeterminate or negative results, as shown in Tables 3 and 4. These results indicate that the results for samples with low titers of anti-*T. cruzi* antibodies were not confirmed by the Western blot. Although 23 serum samples from Group II, which showed inconclusive results in the initial serologic analysis, had antibodies that recognized *T. cruzi* antigens with apparent molecular weights of 11 to 75 kD, the recognition occurred at lower frequency than that observed with the serum samples from chagasic patients. No samples showed the minimum criterion of positivity in the Western blot; therefore, none had the diagnosis of Chagas’ disease confirmed. However, the blood collected will not be used by the blood bank and the donor should have a laboratory and clinical follow-up with other tests to confirm the previous results. If the results do not confirm the presence of *T. cruzi* infection obtained by the Western blot, associated with the clinical and epidemiology, the diagnosis of Chagas’ diseases could be discarded and this would reduce the medical, social, and legal consequences of a false-positive result.

The specificity of 100% for Western blot was obtained with 53 serum samples from patients with other diseases (Group III) and 50 serum samples from healthy subjects (Group IV). Although 28 samples from Group III and eight samples from Group IV showed antibodies that recognized some *T. cruzi* antigens, the number of bands was not sufficient to consider them positive. Eight samples from patients with cutaneous leishmaniasis recognized *T. cruzi* bands with molecular weights of 18, 30, 39, 45, 52, 64, 70, and 82 kD. Of 12 samples from patients with cutaneous leishmaniasis tested, two showed reactivity in the IFA and two in the ELISA for Chagas’ disease. However, when these samples were analyzed by Western blot, more details of the humoral response to different *T. cruzi* proteins were obtained because it was possible to identify how many *T. cruzi* proteins were recognized by the samples. No sample showed a positive pattern of reactivity.

The reactivity of serum samples from patients with cutaneous leishmaniasis with *T. cruzi* polypeptides is considered to be a product of specific stimulation due to common epitopes between *T. cruzi* and *Leishmania*. The intermediate results obtained in the Western blot due to cross-reactivity between *T. cruzi* and *Leishmania* could be elucidated with serial dilutions. Chiller and others showed that when the serum dilution was increased, sera suspected of containing specific IgG to *T. cruzi* continued to recognize proteins at high dilutions, whereas the serum from patients with leishmaniasis quickly lost its reactivity as the serum was diluted. The sensitivity of the test for the diagnosis of Chagas’ disease can be increased significantly by the use of complementary recombinant antigens. The use of recombinant antigens in a single ELISA may cause many technical problems not yet solved, especially regarding the reproducibility of various test kits. However, numerous *T. cruzi* polypeptides could be used individually or in combination, especially as confirmatory tests of the results obtained in the initial screening.

To be routinely useful, tests used in the diagnosis of any disease, beyond having good sensitivity and specificity, must have other features related to cost, technical procedures, facility in obtaining the reagents and results, and stability of the reagents. With regard to the technical procedures, serologic tests for detection and confirmation of anti-HIV and anti-HTLV-1/2 antibodies have brought the Western blot test from research status to routine use in diagnostic procedures. In addition to having a sensitivity of 86.8% and a specificity of 100%, the Western blot proved to be fast and easily done since it can be completed within 4 hr without the need for special equipment, the data are read visually or by densitometry, and the strips are stable.

We do not suggest the Western blot as a substitute for conventional serology, but only as a complementary and confirmatory alternative test. It is not an ideal test to confirm the diagnosis of Chagas’ disease, but at present it is the most viable and practical alternative at the HURNP. Other methods for diagnosis derived from molecular biology have been recently developed, but are not yet available to routine laboratories. Assessment of the usefulness of these new tests will be our future goal.

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