

## Serological Diagnosis of Chagas' Disease: a Potential Confirmatory Assay Using Preserved Protein Antigens of *Trypanosoma cruzi*

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**The diagnosis of Chagas' disease relies mostly on data provided by immunologic tests, but inconclusive results often require elucidation, especially in blood banks. When six different types of *Trypanosoma cruzi* epimastigote antigens were studied by an immunoblotting assay (IBA), a preserved protein antigen (Ag PP) was found to present the most interesting immunochemical features because of its high reactivity with anti-*T. cruzi* antibodies. Thus, the IBA with Ag PP (PP IBA) was assessed with panels of coded and noncoded serum samples prepared in different laboratories, including the Brazilian Reference Laboratory for Chagas' Disease. It was found that serum samples from patients proved (clinically, eletrocardiographically, serologically, and epidemiologically) to have Chagas' disease consistently recognized 12 bands (140, 100, 85, 78, 59, 57, 46, 35, 27, 23, 20, and 18 kDa) of Ag PP. In contrast, sera from nonchagasic patients, including patients with mucocutaneous leishmaniasis, were negative or reacted weakly, and one serum sample did not have more than five different bands. These bands were 78, 57, 46, 35, 27, 23, 20, or 18 kDa. A criterion was adopted to interpret the results obtained in the PP IBA. The criterion considered positive a serum sample recognizing all 12 bands and considered negative a serum sample that did not recognize any of the bands except the eight nonspecific bands mentioned above. The PP IBA indicated maximum sensitivity and specificity as well as high positive and negative predictive values. The data demonstrate that the PP IBA discriminates chagasic from nonchagasic infections and seems to be applicable as a confirmatory assay for elucidating inconclusive results obtained by standard serology.**

Chagas' disease is caused by *Trypanosoma cruzi* and constitutes a public health problem in South American countries. Economic and political instability has caused increased internal migration in these countries, and flows of immigrants have spread over the Americas and from the Americas to Europe. Among the people making these migratory movements are a number of individuals infected with *T. cruzi* (1). In the United States, for instance, 1.1% of blood donors in Los Angeles, Calif., were found to be seroreactive to *T. cruzi* (14), and so far, incidents of acute Chagas' disease were reported (11, 12, 25) in two patients who received blood transfusion and in a patient who had bone marrow transplant. A careful selection of blood or organ donors is one of the measures used to avoid the risk of *T. cruzi* transmission in countries to which those infected individuals migrated.

So the transmission of *T. cruzi* infection occurs nowadays not only through the insect vectors but also through blood transfusion and organ transplants (31). Under these circumstances, an accurate diagnosis of chagasic infection is a matter of priority. The acute phase of Chagas' disease can be diagnosed by parasitological methods, but during the chronic phase, in which indeterminate forms prevail, the parasites are seldom detected in the blood. During this phase of the disease, antibody-based immunoassays play a relevant role as diagnostic tools because of their high sensitivities (10). There is an array of highly sensitive assays, but in the routine diagnosis of Chagas' disease,

two or more assays are usually carried out in parallel and discrepant or inconclusive results corresponding to false-positive and false-negative results are observed (28). The inconclusive results constitute a limiting factor and have been considered to be mainly due to a lack of knowledge about the composition of the antigens used as well as the immunogenicity of *T. cruzi* in humans (34). Thus, the importance of developing reliable confirmatory assays with well-defined antigen preparations has been emphasized (1).

Several methods that use recombinant antigens and that show high sensitivities and specificities have recently been described (16, 18, 38), but they are not yet available for use in clinical laboratories or as confirmatory assays. Thus, the present study was undertaken to develop an appropriate antigen preparation to be used in an immunoblotting assay (IBA) suitable for use as a possible confirmatory assay for the diagnosis of Chagas' disease. Several investigators (30, 36) have studied *T. cruzi* epimastigotes using immunoblotting-based assays, with different procedures for antigen preparation. The major objective of the present study was to investigate by IBA the reactivities of the epimastigote antigens currently used in tests for the diagnosis of Chagas' disease.

### MATERIALS AND METHODS

**Antigens.** *T. cruzi* Y (33) epimastigotes were harvested from liver infusion and tryptose culture medium (6). Antigens for the complement fixation test (Ag CF) (20) and indirect hemagglutination test (Ag IHA) (13) were obtained as described previously. The antigen for the immunoenzymatic assay (9) (Ag IE), an enzyme-linked immunosorbent assay (ELISA), was kindly provided by A. W. Ferreira from the Tropical Medicine Institute of São Paulo, São Paulo, Brazil. The antigen for the intradermal test (Ag ID; delayed type) was supplied by Central de Medicamentos do Brasil (35). The preserved protein antigen (Ag PP) derived from formalin-treated epimastigotes was obtained as described previ-

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TABLE 1. Panels of serum samples studied

Panel no.	No. of serum samples	Diagnosis	Patients' data <sup>a</sup>				
			No. of patients	Clinical	EKG	Serology	Epidemiology
I	12	Chagas' disease	10	Indeterminate	Normal	Pos	Pos (Chagas' disease)
			2	Cardiac	Pos	Pos	Pos (Chagas' disease)
II	16	Nonchagasic	16	Healthy	Normal	Neg	Neg (Chagas' disease)
III	10	Leishmaniasis	10	Mucocutaneous	ND	Pos <sup>b</sup>	Pos (leishmaniasis)
IV	40 (coded) <sup>d</sup>	Chagas' disease (panel IVA)	11	Digestive <sup>c</sup>	Normal	Pos	Pos (Chagas' disease)
			3	Cardiac	Pos	Pos	Pos (Chagas' disease)
			5	Digestive & cardiac	Pos	Pos	Pos (Chagas' disease)
		1	Indeterminate	Normal	Pos	Pos (Chagas' disease)	
		20	Nonchagasic (panel IVB)	Healthy	Normal	Neg	Neg (Chagas' disease)

<sup>a</sup> EKG, electrocardiogram; Pos, positive; Neg, negative; ND, not done.

<sup>b</sup> Positive for *L. major* by ELISA and IFT.

<sup>c</sup> Esophagus or intestine X ray was also positive.

<sup>d</sup> Panels IVA and IVB were disclosed after double-blind tests.

ously for the antigen for the immunofluorescence test (13). Native whole epimastigote antigen (Ag WP) with no enzyme inhibitors was also included in the study.

**IBA.** The six types of epimastigote antigens described above, along with molecular mass markers (Sigma) and bromophenol blue, were submitted to sodium dodecyl sulfate-polyacrylamide gel electrophoresis as described previously (17) by using a 10% gel at 25 mA for 4 h. The protein bands were transblotted onto a nitrocellulose membrane (HATF; Millipore) by electrophoresis at 25 mA for 18 h. The membrane was cut into 4-mm strips and was blocked with 5% skim milk. The strips were incubated with serum samples (1:50) at 4°C for 18 h, and antibodies were detected with peroxidase-labeled conjugate for anti-human immunoglobulin G (gamma chain) (Biolab SA, São Paulo, São Paulo, Brazil) (1:800) at room temperature for 3 h and with chromogen solution (0.05% 4-chloro-1-naphthol, 0.03% H<sub>2</sub>O<sub>2</sub>). The numbers of antigenic bands and their intensities and molecular masses were recorded.

Also, the reactivities, expressed in terms of the numbers of stained bands, of chagasic sera with different types of antigens were correlated by the calculation of the Spearman correlation coefficient (32).

**Immunologic assays.** Serum samples from panels I and II (described below) were collected and tested at the Laboratory of Immunology, Federal University of Uberlândia by the indirect hemagglutination test (IHAT) (5), immunofluorescence test (IFT) (4), immunoenzymatic assay (ELISA) (9), and a dot ELISA with a recombinant antigen (8) for the diagnosis of Chagas' disease. Also, the individuals selected to participate in this study were subjected to the intradermal test (IDT) (35). The serum samples from panel III had been collected and tested by IFT and ELISA with *Leishmania major* 49 antigen and by ELISA with *T. cruzi* antigen at the Tropical Medicine Institute of São Paulo. The serum samples from panel IV were collected and tested by IHAT, IFT, ELISA, and the direct agglutination test (DAT) with 2-mercaptoethanol (2-ME) and were coded at the Brazilian Reference Laboratory for Chagas' Disease (Institute of Tropical Pathology and Public Health, Federal University of Goiás, Goiânia, Goiás, Brazil) (19, 23).

**Serum samples.** Four panels of serum samples (panels I, II, III, and IV) were studied (Table 1). Panel I consisted of serum samples collected from 12 chagasic patients and were positive by immunologic tests (IHAT, IFT, ELISA, dot ELISA, and IDT); however, one serum sample gave a false-negative result only by IDT. Panel II consisted of serum samples from 16 nonchagasic individuals which were negative by the same five tests; one sample, however, gave an inconclusive result by dot ELISA and another gave a false-positive result by IDT. A serum sample from a chagasic patient and a sample from a nonchagasic individual were taken as positive and negative controls, respectively, and were always included in the serologic tests. Panel III consisted of serum samples from 10 patients with mucocutaneous leishmaniasis and were all positive when tested by ELISA and IFT with the *L. major* antigen, but 4 of the serum samples presented false-negative results when they were tested by IFT. All except one of the serum samples showed cross-reactivity with *T. cruzi* antigen by ELISA; one sample had a negative result. Panel IV comprised 40 coded serum samples which, when decoded, were found to include 20 serum samples from chagasic patients (panel IVA). They were positive by IHAT, with antibody titers ranging from 16 to 4,096; IFT, with titers ranging from 80 to 2,560; and ELISA, with optical densities ranging from 1.7 to 4.0. In the DAT with 2-ME, these samples were all positive at a 1:64 dilution. For the remaining 20 serum samples from nonchagasic individuals (panel IVB), no antibodies to *T. cruzi* were detected by the four serological tests used.

**Two-step study.** This study was conducted in two steps. In the first step, six different types of *T. cruzi* epimastigote antigens were assayed by IBA against

panels of serum samples from chagasic (panel I) and nonchagasic (panel II) individuals, as well as from patients with mucocutaneous leishmaniasis (panel III). Four of these antigens are soluble epimastigote extracts used in conventional IDT, complement fixation test, IHAT, and ELISA, while the other two consisted of whole parasite antigens, one in its native form (Ag WP) and the other pretreated with formalin (Ag PP). In the second step, the Ag PP was further studied by IBA in order to confirm the data obtained in the previous step. A double-blind test was carried out with the coded serum samples in panel IV.

## RESULTS

The results obtained in the study of six different types of antigens against each serum sample from panel I are presented schematically in Fig. 1 and 2. The molecular masses of the bands recognized by these samples were determined (Table 2). Some antigenic bands were strongly reactive to chagasic sera, and most antigens had one or two major bands. The Ag ID had no major band and the Ag PP had 12 bands consistently detected by all chagasic serum samples. No strong antigenic bands or major bands were revealed by samples from panels II and III.

All except two serum samples (chagasic) from panel I reacted with different types of antigens, with the number of bands ranging from 1 to 12; serum samples 5 and 9 were negative when tested against Ag ID (Fig. 1 and Table 3). In contrast, serum samples (nonchagasic) from panel II reacted poorly with the same antigens. Serum samples from panel III (from patients with mucocutaneous leishmaniasis) showed reactivity to different types of antigens, but not to Ag ID or Ag WP.

In the second step of the study, 40 coded serum samples (panel IV) were numbered from 1 to 40 and were submitted to IBA with Ag PP (PP IBA), and 20 of these revealed a spectrum of 12 antigenic bands, with the bands of 78 and 27 kDa being broader and more strongly reactive than the other bands. These data were suggestive of chagasic infections, and these samples were later confirmed by the reference laboratory to belong to chagasic patients. The remaining 20 serum samples failed to show the pattern described above and were later identified as being from nonchagasic individuals. Of these 20 serum samples, 55% (11 serum samples) were completely negative and 45% (9 serum samples), the remaining ones, reacted weakly with a number of bands, but not more than five bands, as follows. Twenty-five percent (five serum samples) of the serum samples reacted with only one band, i.e., four with the 78-kDa band and one with the 27-kDa band; 10% (two serum

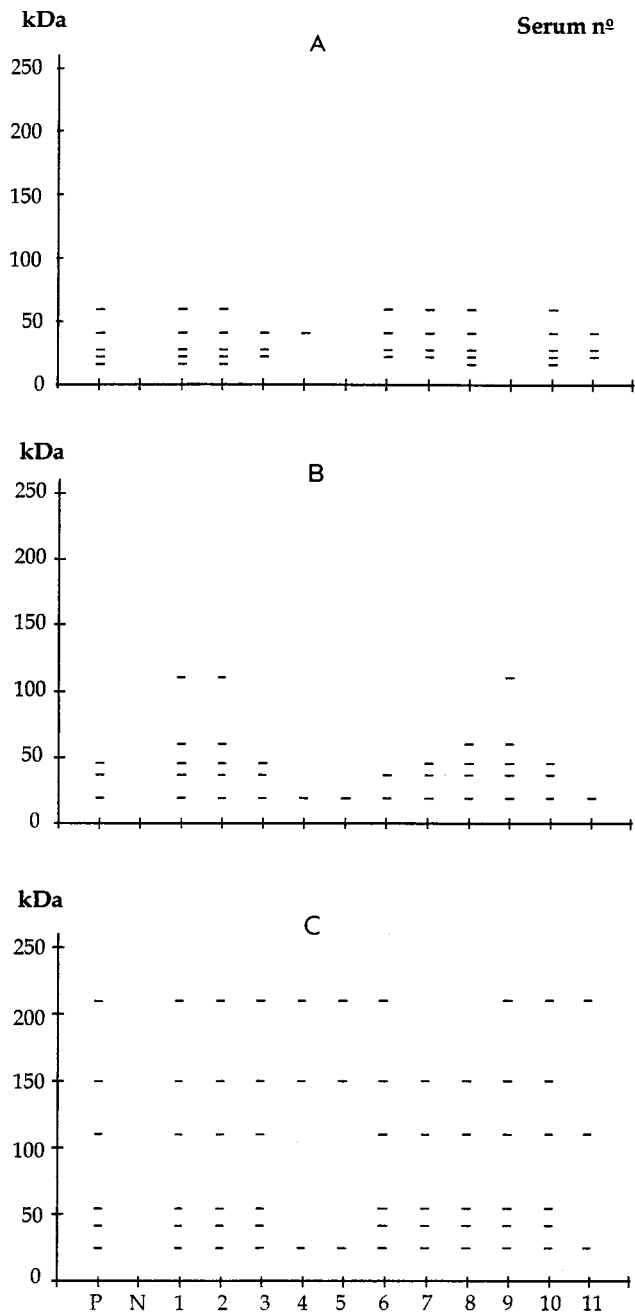


FIG. 1. Immunoblots of different *T. cruzi* epimastigote antigens. Ag ID (A), Ag CF (B), Ag IHA (C) were tested against serum samples (samples 1 to 11) from Chagas' disease patients. P, positive control serum; N, negative control serum.

samples) reacted with two bands, one with the 27- and 23-kDa bands and the other with the 78- and 27-kDa bands; 5% (one serum sample) reacted with 4 bands of 78, 27, 20, and 18 kDa; and the last 5% (one serum sample) reacted with five bands of 78, 57, 46, 27, and 18 kDa. The 78-kDa band was the most reactive band (40% of serum samples), followed by the 27-kDa band (20% of serum samples).

The nonspecific bands (panels II, III, and IVB) were 78, 57, 46, 35, 27, 23, 20, and 18 kDa.

The reactivities of chagasic sera with different types of antigens were correlated and the Spearman correlation coefficient ( $r_s$ ) that was obtained gives an idea of whether the features of sera with high, moderate, and low levels of reactivity were maintained when they were assayed against these antigens. The reactivities of the sera with Ag IE and Ag IHA showed a high correlation coefficient ( $r_s = 0.92$ ), followed by those with Ag ID and Ag WP ( $r_s = -0.89$ ), Ag IE and Ag

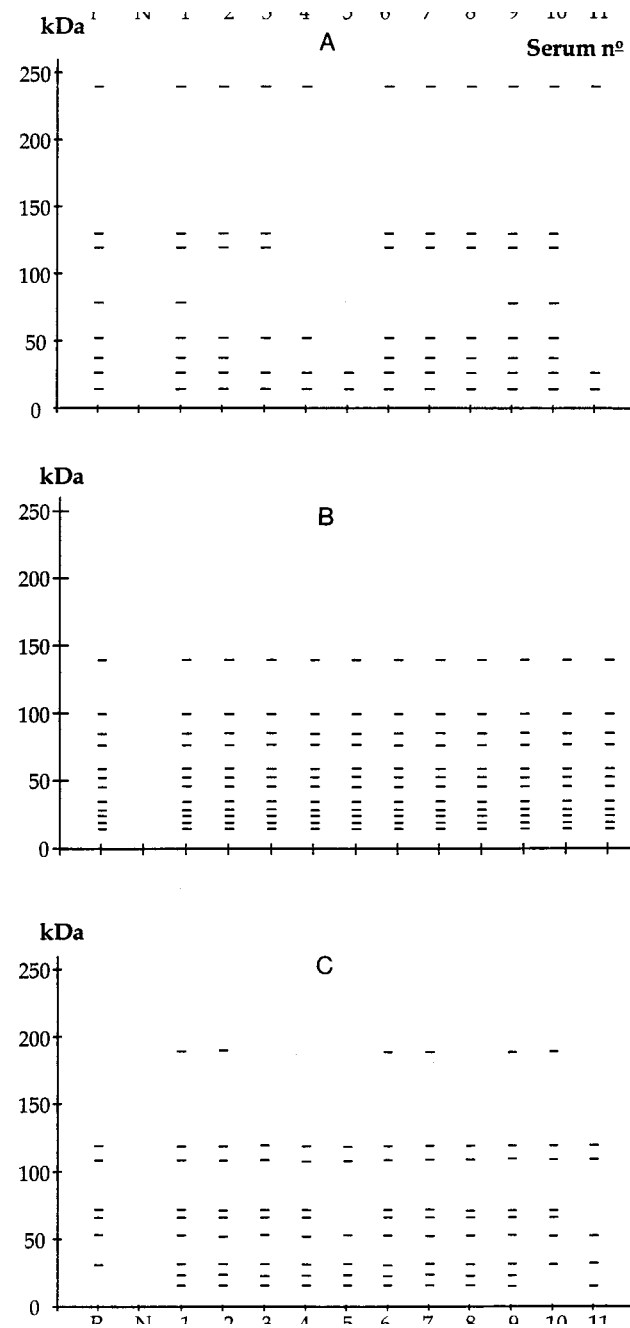


FIG. 2. Immunoblots of different *T. cruzi* epimastigote antigens. Ag IE (A), Ag PP (B), and Ag WP (C) were tested against serum samples (samples 1 to 11) from Chagas' disease patients. P, positive control serum; N, negative control serum.

TABLE 2. Bands from different types of *T. cruzi* antigens recognized by serum samples from panels I to III in the IBA

Type of antigen	Band(s) (kDa) <sup>a</sup>		
	Chagasic sera	Nonchagasic sera	Mucocutaneous leishmaniasis sera
Ag ID	60, <b>42</b> , 27, 23, 21	None	None
Ag CF	110, 60, <b>45</b> , <b>36</b> , 21	None	110, 60, 45, 36, 21
Ag IHA	210, 150, 110, <b>54</b> , 41, <u>25</u>	None	210, 85, 54, 41, 25
Ag IE	<b>240</b> , <b>130</b> , <b>120</b> , 79, 53, 38, <u>27</u> , <u>15</u>	None	240, 130, 120, 79, 53, 15
Ag PP	<u>140</u> , <u>100</u> , <u>85</u> , <b>78</b> , <u>59</u> , <u>57</u> , <u>46</u> , <u>35</u> , <u>27</u> , <u>23</u> , <u>20</u> , <u>18</u>	57, 35, 27	57, 35, 27
Ag WP	190, 120, 110, <b>72</b> , 68, 53, <u>31</u> , 24, 19	68	Not done

<sup>a</sup> Boldface numbers indicate strongly reactive band; underscores indicate major bands.

CF ( $r_s = 0.79$ ), Ag IHA and Ag CF ( $r_s = 0.77$ ), Ag Fc and Ag WP ( $r_s = 0.62$ ), Ag IE and Ag WP ( $r_s = 0.57$ ), Ag IHA and Ag ID ( $r_s = 0.57$ ), Ag IHA and Ag WP ( $r_s = 0.55$ ), Ag IE and Ag ID ( $r_s = 0.45$ ), and Ag FC and Ag ID ( $r_s = 0.44$ ). On the other hand, the reactivities of sera with Ag PP showed no correlation with the reactivity of the same sera with the five antigens studied.

A criterion was adopted to interpret the results obtained in the PP IBA. The criterion considered positive a serum sample capable of recognizing all 12 bands and considered negative a serum sample that did not recognize any of these bands, mainly those of 149, 100, 85, and 59 kDa. According to this criterion, the sensitivity and specificity, as well as the positive and negative predictive values, were estimated to be 1.00.

Three batches of Ag PP were successively prepared for this study and were assayed against 12 serum samples from panel I and 16 serum samples from panel II in 3 different months. A spectrum of 12 bands was always seen with serum samples from panel I and negative results were always observed with serum samples from panel II, according to the adopted criterion.

## DISCUSSION

Patients with Chagas' disease produce antibodies against many *T. cruzi* antigens, and these antibodies show qualitative and quantitative differences. A small part of these antibodies may be considered specific for a number of antigens from different developmental stages of *T. cruzi*. The rest of the antibodies usually show various degrees of cross-reactivity with other antigens present in some unrelated organisms or with ubiquitous antigens. Also, some antibodies cross-react with self-antigens as well as antibodies that elicit anti-idiotypic antibodies (7, 21, 22). In the immunodiagnosis of this disease, the type of *T. cruzi* antigen preparation to be used to obtain highly specific and sensitive tests is of crucial importance.

The data obtained here provide an interesting insight into different types of epimastigote antigens currently used in the immunodiagnosis of Chagas' disease. The immunochemical patterns developed by these antigens against three panels of sera from individuals with a well-defined diagnosis were found to depend on several factors related to the conditions used to process epimastigotes. Thus, the processes for obtaining a soluble parasite extract as well as the centrifugation conditions seem to have a strong influence on the features of the antigen, and these aspects corroborate the data observed by others (30).

The Ag ID, which is prepared after the epimastigotes have

been reduced to small particles and have been submitted to several ultracentrifugations (35), has fewer antigenic bands (one to five) than other types of antigens, and these bands have low molecular masses (60, 42, 27, 23, and 21 kDa). False-negative results (18%) were seen when the sera from chagasic patients (panel I) were tested against this antigen in the IBA. However, no false-positive results were observed against sera from nonchagasic (panel II) or mucocutaneous leishmaniasis (panel III) individuals. Ag ID is highly specific rather than sensitive. Low-molecular-weight antigens or haptens are also reported (27) to be appropriate for intradermal tests of delayed-type hypersensitivity in other infections such as visceral leishmaniasis.

The Ag CF is obtained under harsh conditions (20), since epimastigote fragments are treated with benzene, dried, homogenized in saline solution, and centrifuged to separate the soluble components. This antigen also had few bands (one to five) recognized by sera from panel I and no band recognized by sera from panel II, but 50% of the sera from panel III recognized the same bands, although weakly and in low numbers. Although this antigen appears to be nonspecific for chagasic infection by IBA, at the conventional serum dilutions used in the complement fixation test, because of the cutoff, it permits a reasonable discrimination of chagasic from nonchagasic infections.

Ag IHA and Ag IE are both prepared from the same alkaline-solubilized epimastigotes, except that they differ with respect to the *g* force to which they are submitted during the consecutive centrifugations. The former antigen undergoes much milder centrifugation (1,000 × *g*) than the latter (12,000 × *g*). All the sera from panel I reacted with several bands (three to six bands) of this antigen, as well as with bands of Ag IE (two to eight bands). Neither antigen showed bands reactive with sera from panel II. However, sera (40%) from panel III showed some cross-reactivity with five bands of Ag IHA; four of them were also recognized by chagasic sera and one band (85 kDa) was recognized only by sera from mucocutaneous leishmaniasis subjects. Thus, the bands of 150 and 110 kDa of Ag IHA appear to be specific for *T. cruzi* infection. As to Ag IE, six bands were recognized by sera (40%) from panel III. In contrast to the latter antigen, the bands of low molecular mass (38 and 27 kDa) seem to be specific for chagasic infection.

Ag WP was included here as a native control antigen. This antigen showed nine bands reactive with all sera from panel I and only one band of 68 kDa cross-reactive with sera from panel II. The scarce amount of sera from panel III did not permit a better assessment of this antigen. However, on the basis of previous findings (24, 36), it is expected that about five to six bands will be cross-reactive with sera from mucocutaneous leishmaniasis patients. In the IBA nonreducing conditions

TABLE 3. Numbers of bands from different types of *T. cruzi* antigens recognized by sera in panels I to III by IBA

Antigen type	Panel I		Panel II		Panel III	
	No. of bands	No. (%) of serum samples	No. of bands	No. (%) of serum samples	No. of bands	No. (%) of serum samples
Ag ID	1-5	9 (82)	0	0 (0)	0	0 (0)
Ag CF	1-5	11 (100)	0	0 (0)	1-3	5 (50)
Ag IHA	3-6	11 (100)	0	0 (0)	1-3	4 (40)
Ag IE	2-8	11 (100)	0	0 (0)	1-5	4 (40)
Ag PP	12	11 (100)	1-2	3 (20)	1-2	3 (30)
Ag WP	5-9	11 (100)	1	2 (15)	Not done	

were used to assess different types of *T. cruzi* antigens, and therefore, the data obtained with Ag WP can hardly be compared with those obtained by usual IBAs because 2-ME was used. In this work the nonreducing conditions yielded a relatively small number of antigenic bands compared with the number obtained under reducing conditions in other studies (24, 26, 36), in which 13 to 28 bands were revealed by chagasic sera, with difficulty in the interpretation of the data obtained. Also, under reducing conditions, 17 bands were seen to react with sera (67%) from visceral leishmaniasis patients (24), which were not included in the present study. Nevertheless, recent findings (36) have shown that this cross-reactivity with sera from visceral leishmaniasis patients is limited to only two bands.

Ag PP, in turn, showed a most unusual immunochemical feature since 12 bands were always recognized by chagasic sera, regardless of whether their antibody titers were low, moderate, or high by conventional serology. This antigen presented three bands of 57, 35, and 27 kDa recognized by 20% of the sera from panel II and 30% of the sera from panel III. The number of bands from Ag PP reactive with chagasic sera was comparatively higher than that of the other antigens studied here, but was lower in relation to the number of bands from antigens obtained under reducing conditions (18, 24, 26).

The previous fixation of epimastigotes with formaldehyde seems to preserve many epitopes which are labile under the conditions normally used to prepare antigens. The aldehyde treatment confers stability on the antigen and obviates the use of different enzyme inhibitors, which is required by most lysate antigen preparations (7, 15, 21, 24, 36). Other types of tissue fixatives should be investigated, but formaldehyde has long been used and is one of the best-known fixatives (3). It is also successfully used for several other purposes such as histopathology, immunohistochemistry, and immunoelectron microscopy, among others, and for the study of microorganism surface antigens (37). Also, in several proposed *T. cruzi* vaccine preparations (29, 39), this aldehyde is used not only to kill the parasites but also to enhance immunogenicity. The use of formaldehyde-treated epimastigotes in ELISA for the diagnosis of Chagas' disease has also been suggested (2).

Bands of high molecular mass have been reported (40) to be unstable, and those of less than 50 kDa have been reported to be stable. In this study the number of bands larger than 50 kDa increased from one to six, as shown in Table 1, in which the antigens are ranked according to the number of bands recognized by chagasic sera. Interestingly, the Ag PP showed half of the bands with a molecular mass higher than that value and half of the bands with a molecular mass lower than that value, implying that aldehyde preserves all the bands well, requiring no enzyme inhibitors, as in the case of native epimastigote antigens.

Figures 1 and 2 indicate that different types of antigens except Ag PP are recognized by chagasic sera, presenting parallel patterns; i.e., serum samples 5 and 11 recognized fewer bands than the other sera, and conversely, serum samples 1 and 8 recognized more bands than the other sera. This is explained by the high positive correlation ( $r_s > 0.77$ ) observed between the number of bands from different antigens recognized by chagasic sera. The pattern developed by Ag PP demonstrates that in Chagas' disease, antibodies are produced against a large number of *T. cruzi* antigen epitopes. However, in serologic tests, the universe of antigens is not well represented since many of them are missing. Possibly, they were denatured or lost during the preparation process.

The data obtained in the first step of the study with Ag PP could be confirmed in the second step by studying a panel

comprising 40 coded serum samples from the Brazilian Reference Laboratory for Chagas' Disease. Thus, in the PP IBA, 20 serum samples recognized 12 bands and were later identified as being from chagasic patients. The remaining 20 serum samples, identified as from nonchagasic individuals, included 11 serum samples which gave completely negative results and 9 serum samples which recognized one to five bands. So, the cross-reactive bands increased from the previous three bands to eight bands (78, 57, 46, 35, 27, 23, 20, and 18 kDa). The most false-reactive bands were 78 kDa (40%) and 27 kDa (20%). Thus, those two bands strongly reactive (Table 1) with chagasic sera were also found to react with sera from nonchagasic individuals. Bands with high molecular masses of 140, 100, 85 and 59 kDa from Ag PP appear to be specific for chagasic infections. All 12 bands were also well stained, and no doubtful result due to weak serum reactivity was observed.

Glycoproteins of 90 and 72 kDa from the epimastigote surface were reported (15) to be specific in the serodiagnosis of Chagas' disease. Moreover, a 25-kDa antigenic band from the epimastigote lysate was also shown to be specific (7). Although these antigens had been prepared in different fashions, the possibility that they were associated with Ag PP remains to be investigated.

A criterion was adopted for the easy evaluation of the diagnostic performance of PP IBA on the basis of the number of reactive bands rather than on the basis of the number of defined antigenic bands. If a serum sample recognized all 12 bands, it was considered positive, and if it did not recognize all of them, it was considered negative.

Taking together the data from the first and second steps of the work in the study of 76 serum samples, the estimated sensitivity, specificity, and positive and negative predictive values were 1.00. However, to validate the PP IBA as a confirmatory assay, further collaborative studies will be undertaken, including studies with different laboratories from other Latin American countries, where the prevalence of Chagas' disease varies considerably.

Thus, the PP IBA seems to be applicable as a confirmatory assay for the serodiagnosis of Chagas' disease, since it proved to be reproducible, with no doubtful results, and it required no complex equipment.

The antigen preparation and the criterion used to interpret the results obtained by the PP IBA may be useful in the diagnosis of other parasitic diseases.

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