Serologic Screening for *Trypanosoma cruzi* among Blood Donors in Central Brazil[1]

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The study reported here compares results obtained by blood banks screening sera for chagasic (Trypanosoma cruzi) *infection with results obtained by the Chagas' Disease Reference Laboratory of the Federal University of Goiás in Goiânia, Brazil. It also evaluates results obtained using the ELISA technique to screen the study sera. The survey used data from six of eight blood banks serving the city of Goiânia, an urban region of Central Brazil where Chagas' disease is highly endemic. The survey population consisted of 1,513 voluntary first-time blood donors whose donations occurred between October 1988 and April 1989. This group included 50% of all the first-time blood donors in that period.*

The six participating blood banks, which accounted for about 90% of all blood donations in Goiânia during the study period, routinely used indirect hemagglutination (IHA) and complement fixation (CF) tests to screen sera for antibodies to T. cruzi. *Comparison of the results provided by the blood banks with the reference laboratory's results indicated a relative sensitivity of 77%, which ranged from 50% to 100% depending on the blood bank studied. The comparison, which found 12 false negative results, indicated that transfusions of infected blood might have occurred despite the serologic screening performed by the blood banks.*

Relative to the standard of positivity established for the study, the enzyme-linked immunosorbent assay (ELISA) technique was found to have a sensitivity of 96.3%. Considering as positive only those sera yielding positive IHA and indirect immunofluorescence (IIF) test results, the ELISA technique yielded 2 false negative and 41 false positive responses. Among the 41 were 34 sera found positive by the ELISA technique alone. These results point to a need for assessing the costs and benefits of introducing the ELISA technique in parallel with blood banks' serologic testing and underscore the desirability of establishing a coherent overall system for monitoring the screening of donated blood for T. cruzi *infection.*

To prevent Chagas' disease, improvements are required in socioeconomic conditions, vector control activities, and the methods used for serologic screening of blood donors in areas where the disease is endemic. It has been shown that actions against the vector and better housing conditions can interrupt Chagas' disease transmission in rural areas of Minas Gerais State (*1*); but, on the other hand, more recent studies have shown that migratory population flows from rural to urban areas have led to considerable levels of *T. cruzi* infection in the cities (2). This has made transmission of Chagas' disease via transfusions an important public health problem, and so ensuring the quality of blood or blood

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derivatives has become a priority goal for control of the disease (3–5).

Serologic screening to detect infection by *T. cruzi* in blood donors was introduced in Brazil as a health policy measure in 1969, but its coverage is still inadequate. Furthermore, hemotherapeutic services are performed by a variety of public, private, and philanthropic institutions whose laboratory tests are carried out independently.

The emergence of HIV infection in Brazil led to a review of the policy adopted for blood and blood derivatives, and the Ministry of Health (regulation 721 of 9 August 1989) (6) recommended that blood from donors be screened for *T. cruzi* antibodies using at least two serologic techniques. The blood banks of the city of Goiânia in Central Brazil use both indirect hemagglutination (IHA) and complement fixation, employing indirect immunofluorescence (IIF) in case of discordant results.

In population surveys, the enzyme-linked immunosorbent assay (ELISA) technique has shown greater sensitivity than either hemagglutination or complement fixation. Among its advantages are reagent stability, standardization, and easy execution, all of which increases the viability of large-scale use (2, 7). Hence, utilization of the ELISA technique, alone or in association with other methods, needs to be evaluated through checks on blood banks, principally in areas where Chagas' disease is endemic.

The study reported here is part of a more comprehensive research project designed to improve blood banks' sero-epidemiologic screening for infectious diseases (8). It compares the results of serologic screening performed by blood banks in an urban area endemic for *T. cruzi* infection with the results obtained by the Chagas' disease reference laboratory (9) for the area and assesses the desirability of having blood banks use the ELISA technique to screen for *T. cruzi* infection.

MATERIAL AND METHODS

Study Population

The survey was carried out in the city of Goiânia, 250 km southwest of Brasília. In recent decades this city has experienced about the highest rate of demographic growth in Brazil, principally as a result of migration from rural areas. The survey population consisted of voluntary, first-time blood donors. In order not to influence observed seroprevalence levels, several groups were excluded. These included individuals who reported giving blood previously, donors who appeared in groups (coworkers in the same enterprise), and members of the military (because of difficulty determining whether their donations were entirely voluntary).

The first-time donors included in the survey were processed during the morning hours between October 1988 and April 1989 at six of the eight blood banks (three private, two public, and one philanthropic) which accounted for 90% of blood donations in the city. Overall, the survey period included an average of 106 working days per blood bank. The number of first-time donor subjects (see Table 1) was 1,513 (50% of all the first-time donors), a sufficiently large sample to gauge the anticipated *T. cruzi* infection prevalence of 3% with a margin of error of 1%. The donor subjects' average age was 27.5 years (standard deviation ±8.9 years); 87% were males; 94% lived in the metropolitan area, and among these approximately 26% were born in rural zones.

Serologic Tests

Independently of the routine serologic screening carried out in each of the blood banks, a 10 ml blood sample was ob-

Table 1. Data on the Goiânia blood banks participating in the survey—including institutional status, the year blood collection commenced, average monthly number of first-time donors, and number of first-time donors included in the survey.

Blood bank (BB)	Institutional status	Start of operations (year)	First-time donors per month (no.)	First-time donors surveyed (no.)
BB1	Public	1962	61	177
BB2	Philanthropic	1967	65	354
BB3	Private	1975	150	318
BB4	Private	1964	115	331
BB5	Private	1964	200	248
BB6	Public	1962	88	85

tained from each study subject for testing and analysis by the WHO Reference Laboratory for Chagas' Disease, at the Federal University of Goiás' Institute of Tropical Diseases and Public Health (IPTESP) in Goiânia (*9*). At the end of each morning the sera were taken to the reference laboratory in insulated, ice-packed boxes, and after being distributed into aliquots were stored in a freezer at −20°C. The samples were then stored for approximately a month until being subjected to the following IHA, IIF, and ELISA test procedures.

• **Indirect hemagglutination (IHA).** The IHA test was carried out according to the method described by Camargo et al. in 1973 (*10*), using commercial kits supplied by Imunoserum®, São Paulo. The samples were first tested at a 1:2 dilution and then progressively up to a dilution of 1:64, those samples reacting at a titer of 1:16 or greater being considered positive.

• **Indirect immunofluorescence (IIF).** The IIF test was performed using the method described by Camargo in 1966 (*11*). The reagent was antihuman IgG antibodies conjugated with fluorescein isothiocyanate obtained commercially (Imunoserum®, São Paulo). The antigen was prepared from cultured epimastigotes of *T. cruzi*, strain Y, being maintained in vitro on a liquid LIT (liver infusion tryptose) culture at IPTESP. The samples were initially screened at a 1:40 dilution, and those yielding positive results were retested at progressively higher dilutions up to 1:1,280. Samples yielding a response at titers of 1:40 or more were considered positive.

• **Enzyme-linked immunosorbent assay (ELISA).** This test was performed according to the method described by Voller et al. in 1975 (*12*), using polyvinyl U plates (Hemobag®, Produtos Cirúrgicos Ltda., São Paulo) sensitized with antigen obtained from cultured epimastigotes of *T. cruzi* strain Y maintained in vitro on a liquid LIT culture. Human anti-IgG conjugate marked with peroxidase was obtained through Biolab®, São Paulo. The test results were read using a Cambridge Life Sciences (CLS) microplate spectrophotometer and 492 nm filter. To determine a cutoff point for each plate a curve was developed using a positive control sample of low reactivity, another of minimum reactivity (very low positive control), a negative control sample with relatively high absorbance (high negative control), and an ordinary negative control—together with wells without samples that were used to establish an absorbance baseline. Two duplicate samples were assayed on all plates. The cutoff point for positivity was defined as the arithmetic mean of the reading (absorb-

ance) of the two negative control samples and the lowest of the positive controls. Samples with a value of 1.0 relative to the cutoff point were considered positive.

The results of the IHA, IIF, and ELISA tests were compared with results of the screening tests performed by the blood banks. All the blood banks performed the IHA test using Camargo's method (*10*), and four of them also performed the complement fixation test. For the IHA test, Biochagas® (Goiânia) commercial kits were used, and samples with titers equalling or exceeding 1:16 were considered positive. For the complement fixation test, Biochagas® reagents were employed, using Almeida's method (*13*) and considering the test result to be reactive or unreactive.

Analysis

The seroprevalence of *T. cruzi* antibodies and corresponding 95% confidence limits were calculated for each blood bank. The statistical significance (at the 5% confidence level) of differences in the frequency distributions of the serologic test results was determined by chi-square test.

For the purpose of comparing reference laboratory and blood bank results, a sample was considered positive when the results of both the IHA and IIF tests performed by the reference laboratory were positive. The relative sensitivity, or copositivity, of the tests performed at each blood bank was taken to be the ratio between the blood bank's positive results and the total number of positives determined by the foregoing criterion (*14*). False negative results were defined as those found positive by this reference laboratory criterion but negative by the blood banks (*15*). The relative sensitivity of the ELISA test was assessed by finding the ratio between the number of positive ELISA results and the total number of positives defined by the above (IHA plus IIF) criterion.

The Kappa index (*16*) was used to verify agreement between the positive and negative IHA test results obtained by the blood banks and those obtained by the reference laboratory.

RESULTS

The blood bank test results indicated an average seroprevalence of 3.5% (95% confidence interval = 2.6%–4.4%) for *T. cruzi* infection, this value ranging from 2.0% to 7.1% at different blood banks (Table 2). A comparison of the blood bank results with the reference laboratory results (using the above-cited criterion of positivity) indicated a sensitivity of 77% (40/52), this apparent sensitivity ranging from 50% to 100% at different blood banks. In all, 12 false positives and 12 false negatives were detected. The sensitivity and the number of false negatives, by blood bank, are shown in Table 2.

The reference laboratory's IHA, IIF, and ELISA tests produced apparent seroprevalences of 4.2%, 4.6%, and 6.4%, respectively (Table 3). Taking as the definition of a positive sample one that yielded positive results on both the IHA and IIF tests, the apparent prevalence of infection was 3.7%, a figure significantly lower than that indicated by the ELISA results alone ($p < 0.001$). As Table 4 shows, most of these doubly (IHA and IIF) positive sera (52 out of 54) were detected by ELISA, indicating an apparent ELISA sensitivity by this standard of 96.3%. According to this same standard, in addition to the two false negatives, the ELISA results yielded 41 false positive findings (5 positive by IHA and ELISA, 2 positive by IIF and ELISA, and 34 positive only by ELISA).

Comparison of the blood bank and reference laboratory IHA test results yielded

Table 2. Study sera found positive for *T. cruzi* antibodies at the blood banks, showing the sensitivities and numbers of false negatives indicated by the reference laboratory tests, by blood bank.

Blood bank (BB)	No. positive (BB tests)[a]/ total no. tested	% pos.	Sensitivity (BB pos./ ref lab pos.)[b]	Sensitivity (%)	False negatives (no.)
BB1	5/173	(2.9)	2/2	(100)	—
BB2	7/354	(2.0)	6/12	(50)	6
BB3	18/315	(5.7)	15/15	(100)	—
BB4	10/329	(3.0)	6/7	(86)	1
BB5	7/248	(2.8)	6/7	(86)	1
BB6	6/85	(7.1)	5/9	(56)	4
Total	53/1,504[c]	(3.5)	40/52[d]	(77)	12

[a]Positive according to the indirect hemagglutination and/or complement fixation tests performed by the blood banks.
[b]Sensitivity was assessed by taking the sera positive by the reference laboratory standard (positive by both IHA and IIF) and determining how many were found positive by the blood banks' indirect hemagglutination and/or complement fixation tests.
[c]Nine serum samples were not analyzed (4 from BB1, 3 from BB3, and 2 from BB4).
[d]There were no blood bank serologic results for two of the 54 positive samples found by the reference laboratory, leaving a set of 52 paired results suitable for comparison.

Table 3. Seroprevalence of *T. cruzi* infection in first-time blood donors, as indicated by the IHA, IIF, and ELISA tests performed at the reference laboratory. Goiânia, 1988–1989.

Type of test	No. pos./ no. tested	Prevalence
IHA	61/1,457	4.2%
IIF	67/1,470	4.6%
ELISA	93/1,457	6.4%[b]
IHA + IIF[a]	54/1,451	3.7%[b]

[a]Definition of positive: IHA and IIF both positive.
[b]The difference between the seroprevalence indicated by ELISA and the seroprevalence indicated by IHA + IIF is statistically significant ($p < 0.001$).

a Kappa agreement index of 0.75, the index of agreement for different blood banks ranging from 0.59 to 0.90 (Table 5).

DISCUSSION

The scope of chagasic infection in urban areas of Central Brazil was recently assessed in a demographic survey that tested 6,222 urban workers in the city of Goiânia and detected a seroprevalence of 13.1% (2)—a figure greatly exceeding the 7.4% obtained by the National Survey on

Table 4. Comparison between results of the reference laboratory's ELISA, IHA, and IIF tests for detecting antibody against *T. cruzi* in the sera of first-time blood donors (Goiânia, 1988–1989). The indicated sensitivity of the ELISA technique was 52/54 or 96.3%.

		IHA				
		Positive		Negative		
		IIF		IIF		
		Pos.	Neg.	Pos.	Neg.	Total
ELISA	Pos.	52	5	2	34	93
	Neg.	2	1	10	1,345	1,358
Total		54	6	12	1,379	1,451[a]

[a]Sixty-two of the 1,513 survey samples were excluded from the analysis because they were not tested by all three methods.

Table 5. Kappa index of agreement between results of the IHA tests performed by the blood banks and those performed by the reference laboratory, by blood bank.

Blood bank	Kappa index[a]
BB1	0.74
BB2	0.59
BB3	0.90
BB4	0.73
BB5	0.79
BB6	0.63
Total	0.75

[a]Kappa index = Po − Pc/1 − Pc where Po = proportion of agreement observed and Pc = proportion of agreement statistically expected.

Chagas' Disease carried out almost two decades ago in municipalities in the interior of the state of Goiás (*17*). This concentration in urban areas of individuals infected with *T. cruzi* (as a result of migration from rural to urban areas) confirms the progressive urbanization of the disease, a situation that increases the danger of its transmission through blood transfusions.

Various methods have been used to evaluate serologic screening techniques for *T. cruzi*. The methods most commonly employed to compare the results of serologic tests have used panels of samples that are clearly positive or negative (*18, 19*) and, less often, samples from the general population—which sometimes yield results that are clearly positive or marginally positive (*7*). The traditional way of evaluating the accuracy of new serologic techniques is by means of validity indicators that measure the sensitivity and specificity of the test as compared with a true diagnosis. When no such diagnosis is available, the comparison for epidemiologic survey purposes is made using a reference diagnosis, to which the designations reference sensitivity or copositivity, and reference specificity or conegativity, are applied (*14*).

In the study reported here, copositivity (relative sensitivity) was used to compare the results of the indirect hemagglutination (IHA) test performed by the blood banks with a positive group of sera (yielding positive results in both the IHA and IIF tests) established by the reference laboratory. Depending upon the blood bank studied, the degree of copositivity observed ranged from 50% to 100%. This comparison revealed 12 false negatives (positive sera identified as negative by the blood banks). Since the 1,513 samples studied were provided by only 50% of the first-time donors, there could easily have been as many as 24 false negatives in all, which means that there could have been transfusions of infected blood over the 106-day survey period. It should be noted that most of the false negatives came from those two of the six blood banks that used only the IHA technique in their tests.

The Kappa index of agreement between the blood bank and reference laboratory IHA test results ranged from 59% to 90%, depending on the blood bank in question. These data indicate that the blood banks operate in different ways and point up the need to have a system for systematically evaluating the quality of serologic screening by these institutions in order to ensure good quality control of transfused blood.

Given the importance of the blood banks' serologic screening with regard to breaking chagasic infection's transmission chain in urban areas, it is recommended that the use of new laboratory methods such as the ELISA technique be evaluated.

Demographic surveys of *T. cruzi* infection using the ELISA technique have shown that technique to be more sensitive than those already on the market (*2, 7*). Similarly, the study reported here found the ELISA technique's relative sensitivity to be 96.3%, with only two false negative results, when the control standard for positive was a positive result on

both the IHA and IIF tests conducted at the reference laboratory. Thirty-four sera yielded positive results only by ELISA; in comparison (see Table 4) there were only 13 sera yielding negative ELISA results that were positive by IHA, IIF, or both.

A recent comparison of ELISA, immunofluorescence, and hemagglutination results obtained with sera from São Paulo blood donors produced indexes of agreement higher than 99% (*20*). Like our results, these findings suggest that use of the ELISA technique, in conjunction with methods routinely used, would permit greater sensitivity in detecting *T. cruzi* infection; further studies are required to calculate the costs and benefits of this procedure.

The only statistical indicator of Chagas' disease morbidity that is routinely available at present is the prevalence of antibodies against *T. cruzi* in donated sera (*21*); and so the uniformity and reliability of this indicator depends on the quality of the laboratory tests performed (*8*). In this regard, one strategy that could prove worthwhile in controlling the transmission of Chagas' disease via transfusions would be random selection of serum samples from blood banks for blind retesting in a reference laboratory. An epidemiologic surveillance system of this sort would make it possible to establish a quality criterion for serologic screening by blood banks, while the uniformity of the results would permit reliable determination of secular trends in *T. cruzi* infection.

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Disaster Mitigation Meeting in Caribbean

One of the aims of the current United Nations International Decade for Natural Disaster Reduction (IDNDR) is to focus attention on the need to lessen the impact of disasters as well as to increase preparedness and response capacity. After a successful meeting of 19 Latin American countries on the IDNDR in September 1991, PAHO organized a similar meeting for Caribbean countries in Jamaica from 26–29 May 1992, cosponsored by CARICOM, the OAS, the Caribbean Development Bank, UNDP, UNDRO, and the IDNDR Secretariat. The meeting's purpose was to increase awareness among decision makers and planners about disaster vulnerability and the need for disaster prevention and mitigation in the private and public sectors. Participants were expected to develop an IDNDR Caribbean Policy to orient disaster management at all levels.

Source: Pan American Health Organization, *Disasters: Preparedness and Mitigation in the Americas,* Issue No. 50, April 1992, p. 4.