WHO comparative evaluation of serologic assays for Chagas disease

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BACKGROUND: Evaluation of commercially available test kits for Chagas disease for use in blood bank screening is difficult due to a lack of large and well-characterized specimen panels. This study presents a collaborative effort of Latin American blood centers and the World Health Organization (WHO) to establish such a panel.

STUDY DESIGN: A total of 437 specimens, from 10 countries were collected and sent to the WHO Collaborating Center in São Paulo and used to evaluate 19 screening assays during 2001 through 2005. Specimens were assigned a positive or negative status based on concordant results in at least three of the four confirmatory assays (indirect immunofluorescence, Western blot, radioimmunoprecipitation assay, and recombinant immunoblot).

RESULTS: Of the 437 specimens, 168 (39%) were characterized as positive, 262 (61%) were characterized as negative, and 7 (2%) were judged inconclusive and excluded from the analysis. Sensitivity and specificity varied considerably: 88 to 100 and 60 to 100 percent, respectively. Overall, enzyme immunoassays (EIAs) performed better than the other screening assays. Four EIAs had both parameters higher than 99 percent. Of the four confirmatory assays, only the RIPA gave a 100 percent agreement with the final serologic status of the specimens.

CONCLUSION: The sensitivities and specificities of at least four of the commercially available EIAs for Chagas disease are probably high enough to justify their use for single-assay screening of blood donations. Our data suggest that the majority of commercially available indirect hemagglutination assays should not be used for blood donor screening and that the RIPA could be considered a gold standard for evaluating the performance of other assays.

Chagas disease is a parasitic infection caused by *Trypanosoma cruzi,* which is naturally transmitted by hematophagous triatomine insects. The parasite can also be transmitted vertically and by transfusion of blood products and organ transplantation.[1] Chagas disease is endemic in all Latin American countries outside the Caribbean, where widespread implementation of serologic screening of donated blood has eliminated to a large extent transfusion-related transmission.[2] In some nonendemic areas, such as Europe, the United States, and Canada, there is increasing concern about Chagas disease due to the large numbers of individuals immigrating from endemic areas. In 2006, the US Food and Drug Administration approved a serologic enzyme-linked immunosorbent assay (ELISA) for screening donated blood for Chagas disease (Ortho *T. cruzi*

ABBREVIATIONS: IB = immunoblot; IF = indirect immunofluorescence; IHA(s) = indirect hemagglutination assay(s); PA = particle agglutination; PAHO = Pan American Health Organization; RIPA = radioimmunoprecipitation assay; WB = Western blot.

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ELISA Test System, Ortho-Clinical Diagnostics, Raritan, NJ) and subsequently the American Red Cross, as well as other entities involved in US blood banking, began screening donated units for markers of Chagas disease.[3]

Laboratory diagnosis of chronic *T. cruzi* infection is challenging. The direct detection of parasites is difficult, even with molecular techniques such as polymerase chain reaction, due to the low parasitemia during the chronic phase of the infection. Hence, in blood donors as well as in clinical patients, the laboratory diagnosis of chronic infection is based on serologic assays.[4] Many of the commercially available assays use lysates of the epimastigote form of the parasite grown in liquid culture. More recently assays using recombinant antigens also have been developed.[5,6]

A report published in 1986 compared the performance of serologic assays for Chagas disease and concluded that most assays had low sensitivity. This prompted the Pan American Health Organization (PAHO) to recommend that all donated blood collected in endemic areas be screened with two assays performed in parallel.[7] The problem with this approach, in addition to its economic implications, is that inconclusive results due to nonspecific reactivity are common. Given the lack of a widely accepted and accessible gold standard for serologic diagnosis, it is difficult to assign a final status to such specimens, and as a consequence many blood units are discarded needlessly.[8] Moreover, it is also difficult to validate new serologic assays because there is no easy method for resolving the true status of inconclusive and weakly reactive specimens. Most assays are validated using high-antibody-titer, consensus-positive specimens and hence their performance in resolving low-antibody-titer and inconclusive specimens is not really addressed and because of the selection bias calculated sensitivities are usually overestimated.

Any review of testing strategies for markers of Chagas disease necessitates a comparison of the currently available serologic assays with an emphasis on the original PAHO/World Health Organization (WHO) recommendation. We undertook this study by assembling a panel of 437 plasma units from 10 blood centers in different Latin American countries. Each of the participating blood centers used its own testing algorithm for determining the positive or negative status of the specimens they sent. Since the local testing algorithms used were all different, we thus avoided potential bias toward one assay or another in our study.

The results described here represent an evaluation of 18 screening assays, one rapid test, and four confirmatory assays. The decision as to the final serologic status assigned to the specimens was made using four confirmatory assays (indirect immunofluorescence [IF], Western blot [WB], radioimmunoprecipitation assay [RIPA], and recombinant immunoblot [IB]). By taking this approach, we believe that we were able to distinguish true-positive specimens with low levels of *T. cruzi* antibodies from false-positive specimens in our panel, thus allowing more accurate evaluation of the performance of the assays in relation to clinical sensitivity.

STUDY DESIGN

Specimens

Ten Latin American blood center directors were asked to provide Chagas-positive and -negative plasma units, as defined by their local serologic criteria, to the WHO Collaborating Center for Quality Control of Serology in Blood Banks (Fundação Pró-Sangue, Hemocentro de São Paulo [BCFSP]). The panel is composed of a total of 437 specimens collected in 2000. A list of the participating blood centers is presented in Table 1, as are the local

TABLE 1. Description of plasma units and their test results as submitted by each institution

			Local testing results: number of samples			
Country	Institution	Assays used locally for testing	Pos	Inc	Neg	Total
Argentina	Hospital de Pediatría Prof. Dr Juan P. Garrahan	Chagatek ELISA + Serodia–Chagas	25	0	20	45
Bolivia	Hospital Clínico "Viedma"	Chagatek ELISA + Chagas AHI Imunoserum	20	2	18	40
Brazil	Fundação Pró-Sangue	Hemacruzi + Imunocruzi + HBK 401 Hemobio Chagas	59	0	87	146
Colombia	Instituto Nacional de Salud	In-house IF	13	0	11	24
Ecuador	Cruz Roja Ecuatoriana	Chagatek ELISA + CRE ELISA	6	0	20	26
El Salvador	Cruz Roja Salvadoreña	Chagatek ELISA + ICMRT ELISA	9	0	22	31
Honduras	Cruz Roja Hondureña	Chagatek ELISA	21	0	19	40
Mexico	Centro Nacional de Transfusión	Chagatek ELISA	5	0	5	10
Nicaragua	Cruz Roja Nicaragüense	ICMRT ELISA	12	0	19	31
Paraguay	Instituto de Investigación en Ciencias de la Salud	Chagas-Test IICS, ELISA + in-house IF	18	0	26	44
		Total	188	2	247	437

Pos = positive; Neg = negative; Inc = inconclusive.

designations of the serologic status and the numbers of the specimens provided by each group.

Plasma specimen treatment

The plasma units were converted to serum by the following defibrination process: 0.5 mL of a 0.2 mol per L $CaCl_2$ solution was mixed with 100 mL of plasma and incubated at 37°C for 2 hours and then at 4°C for 24 hours. The plasma was centrifuged at 6000 × *g* for 30 minutes to separate the serum from the fibrin clot. To remove $CaCl_2$, the serum was dialyzed using a cellulose membrane that retains proteins of MW 12,000 kDa or greater (Cat. No. D-9652, Sigma, Steinheim, Germany) and then filtered through a 5.0-μm pore size membrane (Sigma Cat. No. N-3771) to remove fibrin particles. 5-Bromo-5-nitro-1,3-dioxane (Bronidox L, Henkel Chemicals, Dusseldorf, Germany) was added to a final concentration of 0.05 percent. A total of 10 aliquots of 1.5 mL each were prepared. The remaining serum was stored at –20°C.

Characterization of panel specimens

The following four confirmatory assays were used in the characterization of the panel:

- IF Imunocruzi (Biolab-Mérieux S.A, Rio de Janeiro, Brazil): Epimastigotes and anti-human immunoglobulin G (IgG)-fluorescein conjugate are used as reagents in this assay. The cutoff for IF was a dilution of 1/20. Specimens that gave an uninterpretable result at this dilution were considered inconclusive.
- INNO-LIA Chagas assay (IB; Innogenetics, Ghent, Belgium): This assay uses seven recombinant and synthetic *T. cruzi* antigens (Tc24, Ag 39, TcD, SAPA, MAP, CRA, and FRA) coated as discrete lines onto a nylon membrane with plastic backing. The strips were incubated with the sera at a 1/100 dilution for 18 hours at 25°C, and after being washed, the immune complexes were detected by incubation with an anti-human IgG conjugate and subsequent color development. The results were determined by visually comparing the intensities of the antigen lines with those of the controls according to the kit instructions.
- WB (TESA blot, bioMérieux, Rio de Janeiro, Brazil): As previously described,[9] native trypomastigote antigens bound to plastic strips were incubated with serum specimens at 1:100 dilution for 2 hours at room temperature, and after being washed, immune complexes were detected by incubation with an anti-human IgG conjugate and subsequent color development. The results were interpreted by visual inspection for the presence of bands between 130 and 200 kDa compared to a positive control run in parallel.
- RIPA: This assay was performed at the University of Iowa as previously described.[10] Radiolabeled *T. cruzi* surface antigens precipitated by specific IgG in the serum specimens were separated electrophoretically. Detection of 72- and 90-kDa glycoproteins of *T. cruzi* by autoradiography constitutes the criterion for positivity in the RIPA.

The final serologic status of each specimen was defined as follows:

- *Positive:* specimen positive in at least three of the four confirmatory assays;
- *Negative:* specimen negative in at least three of the confirmatory assays;
- *Inconclusive:* specimen positive in only two of the confirmatory assays.

Assays

The panel of specimens characterized by the confirmatory assays was used to evaluate the performance of 18 screening assays (11 enzyme immunoassays [EIA], 5 indirect hemagglutination assays [IHAs], and 2 particle agglutination [PA] assays) and one rapid test between 2001 and 2005 at the WHO Collaborating Center for Quality Control of Serology in Blood Banks (Fundação Pró-Sangue, Hemocentro de São Paulo [BCFSP]). For each assay, the specimens were tested and interpreted as either reactive or nonreactive using the cutoff calculated as suggested by the manufacturer. Screening assays were tested with all 437 specimens of the panel. The rapid test was evaluated with only 371 specimens due to a lack of availability of assays in the laboratory at the time of the study. The results were read by two independent readers to minimize observer bias.

Statistical analysis

Sensitivities and specificities were calculated after excluding the data from the seven specimens judged to be inconclusive. The 95 percent confidence intervals (CIs) were calculated using a statistical software package (STATA, Version 7, StataCorp., College Station, TX).

RESULTS

A panel of 437 well-characterized specimens were analyzed using 18 screening assays. The rapid test (Chagas Stat-Pak) could only be evaluated with 371 of the specimens. Figure 1 summarizes the results of the 18 screening assays by presenting the number of specimens as a function of the number of tests in which they were reactive. As shown, 89 specimens were not reactive in any of the assays, 126 specimens were reactive in 1 assay, 35

specimens were reactive in 2 assays, 7 specimens were reactive in 3 assays, 1 to 4 specimens were reactive in between 4 and 16 assays, and 18 specimens were reactive in 17 assays. Finally, as shown by the right-most vertical bar, 138 specimens were reactive in all 18 assays.

To present the results obtained with the four confirmatory assays as a function of their reactivity in the screening assays, we grouped the specimens into the following categories: Group A, specimens reactive in 3 or fewer screening assays (a total of 257 specimens); Group B, specimens reactive in at least 4 tests but less than 15 (18 specimens); and Group C, specimens reactive in 15 or more tests (162 specimens). As shown in Table 2, all specimens in Group A were judged to be negative by the final algorithm; conversely, all Group C specimens were judged to be positive. Of the 18 specimens in Group B, 6 were deemed positive, 7 were inconclusive, and 5 were negative. WB gave 5 false-positive results in Group A, while IB gave 1 false-negative result in Group C. IF gave inconclusive results for 2 specimens in Group A and for 1 in Group C. The RIPA was the only confirmatory assay that gave results that fully agreed with the final serologic status of all specimens.

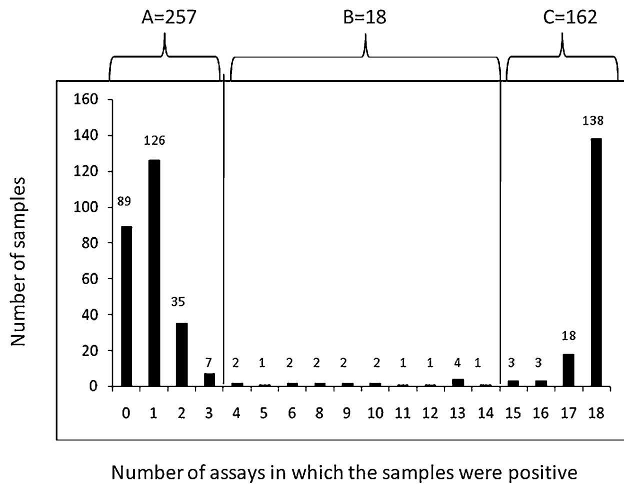

Fig. 1. The number of specimens shown as a function of the number of assays in which they were reactive.

To determine the sensitivity and specificity of each of the 18 assays under evaluation, we excluded seven specimens that were judged to be inconclusive in the confirmatory analysis. Details regarding these excluded specimens are shown in Table 3. Interestingly, all seven of these specimens were positive by WB, while only one was positive by RIPA. There were 30 challenging true-positive samples in this panel that were not reactive to all assays used: 6 from Group B and 24 from Group C (18, 3, and 3 samples reactive to 17, 16, and 15 assays, respectively).

The calculated sensitivities and specificities and 95 percent CI of the various screening assays assessed are shown in Table 4. In general, EIAs performed better than the assays based on other formats. Seven of the 11 EIAs evaluated had sensitivities and specificities greater than 98 percent, and 4 of these assays had both parameters

TABLE 2. Results obtained by testing 437 donor specimens in four confirmatory assays, grouped (A, B, and C) by the number of screening assays in which they were reactive*

	Number of results															
	IF			IB			WB			RIPA			Final status			
Group	Pos	Inc	Neg	Pos	Inc	Neg	Pos	Inc	Neg	Pos	Inc	Neg	Pos	Inc	Neg	Total
A	0	2	255	0	1	259	5	0	252	0	0	257	0	0	257	257
B	5	6	7	7	2	9	15	0	3	7	0	11	6	7	5	18
C	161	1	0	160	1	1	162	0	0	162	0	0	162	0	0	162

* Group A = specimens reactive in 1 to 3 of the 18 screening assays; Group B = specimens reactive in 4 to 14 of the screening assays; Group C = specimens reactive in 15 or more of the screening assays.
Pos = positive; Neg = negative; Inc = inconclusive.

TABLE 3. Results obtained in the four confirmatory assays with seven specimens that were excluded from the final analysis

Specimen ID	Country of collection	Number of assays with reactive results	IF (titer)	IB	WB	RIPA	Final interpretation
41	Bolivia	6	1/40	Neg	Pos	Neg	Inc
33	Argentina	6	Inc	Neg	Pos	Neg	Inc
40	Bolivia	9	Inc	Neg	Pos	Neg	Inc
31	Argentina	10	Neg	Pos	Pos	Neg	Inc
27	Argentina	12	Neg	Pos	Pos	Neg	Inc
36	Paraguay	11	Inc	Inc	Pos	Pos	Inc
34	Argentina	13	Inc	Inc	Pos	Neg	Inc

Pos = positive; Neg = negative; Inc = inconclusive.

greater than 99 percent. In contrast, only 2 of the 5 IHAs evaluated had sensitivities and specificities higher than 95 percent.

DISCUSSION

For a variety of reasons, the evaluation of serologic assays for identifying persons with Chagas disease is difficult. Chagas disease has a lifelong chronic phase during which most infected persons are asymptomatic, and thus clinical information is of little use in identifying infected individuals. Moreover, owing to the extremely low parasitemia during the chronic phase, parasitologic methods are insensitive and in a practical sense are not useful for assembling sizable panels of Chagas-positive specimens. In this context, the status of specimens to be used in assay evaluations must be defined using the available assays, which as noted often have shortcomings in terms of both sensitivity and specificity. As a result, the panels used often consist of high-antibody-titer "consensus-positive" specimens and the inherent selection bias can falsely increase the calculated sensitivities of the assays under evaluation.

A further difficulty in evaluating the performance of serologic assays for Chagas disease is that there is no widely accepted gold standard to identify true-positive status of specimens. Hence low-antibody-titer specimens giving inconclusive results by the various confirmatory methods are in general not included in panels for assay evaluation because of their ambiguous final serologic status. Yet these challenging low-antibody-titer specimens are critical for assessing the sensitivities of the assays under evaluation. The absence of large well-characterized panels of positive and negative specimens in which a broad range of *T. cruzi* antibody titers are represented was the impetus for the current study.

Many of the specimens ultimately judged to be positive were not reactive in all assays under evaluation. Using criteria based on the four confirmatory assays, we judged as positive 30 (18%) low-titer specimens that gave nonreactive results in some of the assays. This was key to better differentiate the performance of the various assays evaluated. In general, the EIAs showed better performance than the other assays and, as these tests can be automated, are in our view preferable for use as blood donation screening assays. The high sensitivities and specificities observed in this study by some EIAs suggest that such a single EIA test could be used by blood banks for screening of markers for Chagas disease with the goal of reducing costs for labor and reagents/consumables.

Overall, the sensitivities and/or specificities of the agglutination assays (IHA and PA) observed in this

TABLE 4. Sensitivity, specificity, and 95 percent CIs for each of the 19 assays under evaluation as compared to the final serologic status (n = 430 specimens)

Assays	Sensitivity (95% CI)	Specificity (95% CI)	Company	City/country
EIA assays				
HBK 401 Hemobio Chagas	100 (97.8-100)	99.62 (97.9-100)	Embrabio	Brazil
Chagas ELISA	97.62 (94.0-99.3)	97.71 (95.1-99.2)	Ebram	Brazil
Chagatek ELISA	99.40 (96.7-100)	99.24 (97.3-99.9)	Laboratório Lemos	Argentina
Premier Chagas IgG ELISA Test	94.04 (89.3-97.1)	100 (98.6-100)	Meridian Diagnostics	US
Test ELISA para Chagas	99.40 (91.2-98.1)	99.62 (97.9-100)	BIOSChile	Chile
Bioelisacruzi	98.21 (94.9-99.6)	99.24 (97.3-99.9)	Biolab-Mérieux	Brazil
Abbott Chagas Anticorpos EIA	99.40 (96.2-100)	98.09 (95.6-99.4)	Abbott Laboratories	US
Chagas test *IICS*, ELISA	97,02 (93.2-99.0)	99,24 (97.3-99.9)	IICS Univ de Asunción	Paraguay
Chagatest ELISA	98.81 (95.8-99.9)	99.62 (97.9-100)	Wiener lab	Argentina
Bioelisa Chagas	100 (97.8-100)	99.24 (97.3-99.9)	Biokit	Spain
Chagas Hemagen	100 (97.8-100)	96.56 (93.6-98.4)	Hemagen Diagnósticos	US
Hemagglutination assays				
Chagas HAI Imunoserum	97.62 (94.0-99.3)	78.62 (77.2-83.4)	Polichaco	Argentina
Teste Chagas–HAI	88.09 (82.2-92.6)	59.92 (53.7-65.9)	Ebram	Brazil
Imuno-HAI Chagas	100 (97.2-100)	95.80 (92.6-97.9)	WAMA	Brazil
Chagas Hemagen HA	92.26 (87.1-95.8)	89.31 (84.9-92.8)	Hemagen Diagnósticos	US
Hemacruzi	99.40 (96.7-100)	97.33 (94.6-98.9)	Biolab-Mérieux	Brazil
PA assays				
Serodia–Chagas	100 (97.2-100)	97.70 (95.1-99.2)	Fujirebio	Japan
ID-Chagas antibody test	97.02 (93.2-99.0)	99.62 (97.9-100)	DiaMed-ID	Switzerland
Rapid test				
Chagas Stat-Pak*	94.08 (89.1-97.3)	95.75 (92.1-98.0)	Chembio Diagnostic Systems	US
Confirmatory assays				
RIPA	100 (97.8-100)	100 (98.6-100)	University of Iowa	US
WB	100 (97.8-100)	97.3 (94.6-98.9)	bioMérieux	Brazil
IB	98.2 (94.9-99.6)	99.6 (97.9-100)	Innogenetics	Belgium
IF	98.2 (94.9-99.6)	98.0 (96.7-99.8)	bioMérieux	Brazil

* Could only be analyzed with 152 positive specimens and 212 negative specimens (n = 364 specimens). The 16 positive missing samples were from Group C. In the best scenario, if all missing samples were correctly assigned by the test, the sensitivity and specificity would increase to 94.60 (90.1-97.5) and 96.6 (93.6-98.4), respectively.

evaluation were relatively low with the exception of a few assays. An additional problem with agglutination assays is that interpretation is subject to reader bias. False-negative results by IHA have been described previously in external quality assessment schemes even for high-titer specimens (due to prozone effect).[11,12] Thus, we would not recommend their use for blood bank screening within large throughput facilities. The rapid test evaluated in our study did not have the sensitivity and specificity that would justify its use for screening donated blood based on our observations.

In this study, two IHA kits showed particularly low specificities (Imunoserum, 79%; IHA Ebran, 60%). If the results obtained with these two kits were excluded from the overall analysis, only 14 (3.2%) of the 262 negative specimens would be reactive to 2 or more of the 18 assays.

The results of this study support the notion that, at least in the endemic countries, it would be reasonable to use a single high-sensitivity and -specificity test for initial screening of donated blood for Chagas disease. It is imperative that the batch-to-batch variation in performance of a chosen assay be closely monitored.

With respect to a gold standard, the four confirmatory assays used are all good candidates. The RIPA was the only assay that gave results in all cases that agreed with our assigned final status (generated using the results of all four confirmatory assays). Thus, the RIPA is appropriate for use as a gold standard; this is consistent with its current use in the United States for confirmation of EIA-reactive specimens. Nonetheless, the complexity of the RIPA may limit its widespread use. In this context, the other confirmatory assays utilized in this assessment could also constitute reasonable alternatives. The low cost of IF might make it the preferred option, especially when financial resources are limited.

As described, seven specimens in our panel gave inconclusive results using our confirmatory algorithm and were excluded from the final analysis. Since their true serologic status cannot be determined, there is no way to conclude how they might have affected the calculated sensitivities and specificities of the assays under evaluation. It is well established that assays for Chagas disease, especially those based on native antigens, are more prone to false-positive results from individuals with leishmaniasis.[13] It is not known if this reactivity persists after treatment or in individuals with self-limited disease. Since the specimens in our panel were from blood donors, it is unlikely that any of the excluded seven specimens were from donors with symptomatic leishmaniasis. These persons are unlikely to have had prior treatment for Chagas disease but they might have had self-limited Chagas disease. Chagas disease is considered a lifelong infection by the scientific and clinical communities, although this point of view is not based on large studies, due to the logistical difficulties in carrying out long-term parasitologic and serologic surveillance. In at least three Chagas disease drug treatment trials, reversion to a negative serostatus was noted in a handful of untreated controls, but it is not clear if these observations resulted from actual parasitologic self-cures or the nonreproducibility of serologic testing for *T. cruzi* antibodies.[14-16]

In conclusion, we have established a well-characterized panel of specimens for assessing the performance of serologic assays for Chagas disease that includes a sizable number of low-titer positive specimens. We used this panel to conduct comparative evaluations of a sizable number of test kits available on the Latin America market in 2003 to 2005. Some of these kits showed excellent sensitivities and specificities and could be used as single assays for screening donated blood and blood products.

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CONFLICT OF INTEREST

Louis V. Kirchhoff performs the radioimmunoprecipitation assay (RIPA) on a fee-for-service basis in his laboratory at the University of Iowa. Also, he has licensed the RIPA technology to Quest Diagnostics in Chantilly, VA, where testing is available for donor specimens.

REFERENCES

1. Control of Chagas Disease. World Health Organ Tech Rep Ser 2002;905:i-vi, 1-109, back cover.
2. Tarleton RL, Reithinger R, Urbina JA, Kitron U, Gürtler RE. The challenges of Chagas disease—grim outlook or glimmer of hope. PLoS Med 2007;4:e332.
3. Blood donor screening for Chagas disease—United States, 2006-2007. MMWR Morb Mortal Wkly Rep 2007;56:141-3.
4. Schmunis GA, Cruz JR. Safety of the blood supply in Latin America. Clin Microbiol Rev 2005;18:12-29.

5. Chang CD, Cheng KY, Jiang LX, Salbilla VA, Haller AS, Yem AW, Bryant JD, Kirchhoff LV, Leiby DA, Schochetman G, Shah DO. Evaluation of a prototype Trypanosoma cruzi antibody assay with recombinant antigens on a fully automated chemiluminescence analyzer for blood donor screening. Transfusion 2006;46:1737-44.
6. Ferreira AW, Belem ZR, Lemos EA, Reed SG, Campos-Neto A. Enzyme-linked immunosorbent assay for serological diagnosis of Chagas' disease employing a Trypanosoma cruzi recombinant antigen that consists of four different peptides. J Clin Microbiol 2001;39:4390-5.
7. Camargo ME, Segura EL, Kagan IG, Souza JM, Carvalheiro Jda R, Yanovsky JF, Guimarães MC. Three years of collaboration on the standardization of Chagas' disease serodiagnosis in the Americas: an appraisal. Bull Pan Am Health Organ 1986;20:233-44.
8. Salles NA, Sabino EC, Cliquet MG, Eluf-Neto J, Mayer A, Almeida-Neto C, Mendonça MC, Dorliach-Llacer P, Chamone DF, Saéz-Alquézar A. Risk of exposure to Chagas' disease among seroreactive Brazilian blood donors. Transfusion 1996;36:969-73.
9. Umezawa ES, Nascimento MS, Kesper N Jr, Coura JR, Borges-Pereira J, Junqueira AC, Camargo ME. Immunoblot assay using excreted-secreted antigens of Trypanosoma cruzi in serodiagnosis of congenital, acute, and chronic Chagas' disease. J Clin Microbiol 1996;34:2143-7.
10. Kirchhoff LV, Gam AA, Gusmao RA, Goldsmith RS, Rezende JM, Rassi A. Increased specificity of serodiagnosis of Chagas' disease by detection of antibody to the 72- and 90-kilodalton glycoproteins of Trypanosoma cruzi. J Infect Dis 1987;155:561-4.
11. Oknaian S, Remesar M, Ferraro L, del Pozo AE. [External performance evaluation of screening in blood banks in Argentina: results and strategies for improvement]. Rev Panam Salud Publica 2003;13:149-53.
12. Saéz-Alquézar A, Otani MM, Sabino EC, Ribeiro-dos-Santos G, Salles N, Chamone DF. Evaluation of the performance of Brazilian blood banks in testing for Chagas' disease. Vox Sang 1998;74:228-31.
13. Caballero ZC, Sousa OE, Marques WP, Saez-Alquezar A, Umezawa ES. Evaluation of serological tests to identify Trypanosoma cruzi infection in humans and determine cross-reactivity with Trypanosoma rangeli and Leishmania spp. Clin Vaccine Immunol 2007;14:1045-9.
14. Andrade AL, Martelli CM, Oliveira RM, Silva SA, Aires AI, Soussumi LM, Covas DT, Silva LS, Andrade JG, Travassos LR, Almeida IC. Short report: benznidazole efficacy among Trypanosoma cruzi-infected adolescents after a six-year follow-up. Am J Trop Med Hyg 2004;71:594-7.
15. de Andrade AL, Zicker F, de Oliveira RM, Almeida Silva S, Luquetti A, Travassos LR, Almeida IC, de Andrade SS, de Andrade JG, Martelli CM. Randomised trial of efficacy of benznidazole in treatment of early Trypanosoma cruzi infection. Lancet 1996;348:1407-13.
16. Viotti R, Vigliano C, Lococo B, Bertocchi G, Petti M, Alvarez MG, Postan M, Armenti A. Long-term cardiac outcomes of treating chronic Chagas disease with benznidazole versus no treatment: a nonrandomized trial. Ann Intern Med 2006;144:724-34.