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Evaluation of Three Commercial Enzyme-Linked Immunosorbent Assays for Diagnosis of Chagas' Disease

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Chagas' disease is a common cause of morbidity in Latin American countries. In Brazil, naturally occurring transmission of its etiologic agent, *Trypanosoma cruzi*, has been almost completely abolished through effective control programs aimed at the triatomid insect vector. Thus, transfusion of blood from infected donors has become the major route for contracting Chagas' disease due to the socioeconomically motivated migration of residents from areas where the disease is endemic to the larger urban centers. Therefore, the employment of screening tests is mandatory for all blood banks throughout the country. We compared the diagnostic performances of three commercially available screening assays used in routine testing in Brazilian blood banks: the Abbott Chagas antibody enzyme immunoassay (Abbott Laboratórios do Brasil, São Paulo), the BIOELISA-CRUZI kit (Biolab-Mérieux, Rio de Janeiro, Brazil), and the BIOZIMA Chagas kit (Polychaco S.A.I.C., Buenos Aires, Argentina). The evaluation was performed with sera obtained from chagasic patients and healthy residents of four different areas in Brazil where Chagas' disease is either endemic or emergent and where clinical manifestations of the disease and circulating parasite strains vary. The results obtained with each kit were compared to matched in-house enzyme-linked immunosorbent assay and immunofluorescence assay data obtained for each sample. Depending on the area under investigation, the three commercial kits produced specificity values between 93.3 and 100.0%, sensitivity values between 97.7 and 100%, and accuracies ranging from 93.6 to 100.0%.

The protozoan parasite *Trypanosoma cruzi* is the etiologic agent of Chagas' disease, which is endemic throughout Latin America and which is a major cause of morbidity and death in the affected countries. According to World Health Organization estimates (31), 16 to 18 million people are infected by the parasite and about 50,000 chagasic patients die each year from the disease. In Brazil, the area in which the disease is endemic extends over 17 states in the northeastern, southeastern, southern, and central western regions (21), but successful vector control programs have abolished almost completely the natural transmission of *T. cruzi* by its reduviid insect vector. Recent studies reported few chagasic patients younger than 12 years in the state of Minas Gerais (8, 20). Apart from vectorial transmission, Chagas' disease can be contracted either orally (39), congenitally (23), or by transfusion of blood from an infected donor (38). Due to socioeconomic factors, the migration of infected people from the areas in which the disease is endemic to the urban centers is very frequent, and blood transfusion has become the principal way of infection, accounting for an estimated 20,000 new cases per year in Brazil, a country with five to six million blood transfusions per year (21). Therefore, efficient donor screening is very important in order to identify and discard contaminated blood without negatively affecting the country's blood supply.

T. cruzi infection is lifelong, and after a short and mostly asymptomatic acute phase, during which the parasites can be detected in blood smears, patients enter the indeterminate phase of the disease, which is marked by an extremely low parasitemia and no sequelae. This stage can last for 10 to 30 years, after which a significant percentage of patients develop the chronic manifestations of Chagas' disease (cardiopathy, megacolon, and/or megaesophagus). While traditional methods of parasite detection (hemoculture and xenodiagnosis) are time-consuming and of low sensitivity, PCR amplification of nuclear (32, 40) or kinetoplast (3, 43) DNA was shown to be very sensitive (10, 46). However, at present, PCR is not feasible for blood bank screening, and the best way of diagnosing an indeterminate or chronic *T. cruzi* infection is the serologic detection of antibodies directed against the parasite. Serologic assays include the indirect immunofluorescence assay (IFA), indirect hemagglutination, complement fixation, the radioimmunoprecipitation assay, the enzyme-linked immunosorbent assay (ELISA), and Western blots. Antigen preparations employed in these tests range from crude parasite extracts and subcellular fractions to cloned antigens and synthetic peptides (24, 27–30, 34–36, 41, 44, 45). Some of these tests are available commercially, while others are in-house assays being used only in research settings. In Brazilian blood banks today, the screening of donors for Chagas' disease by at least two tests based on different methodologies is obligatory. Although IFAs and hemagglutination often lead to false-positive or -negative test results due to subjective interpretation, both assays are still widely used in blood bank screening and epidemiological surveys, and the results are generally confirmed by an ELISA.

T. cruzi is polymorphic, and different parasite strains circulate in different areas (21). While to date no definite correlation between infecting strain and clinical manifestation has been demonstrated, survey studies in regions in which the

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TABLE 1. Consensus classifications of sera from four regions of Brazil by in-house ELISAs and IFAs

Source of sera	No. of sera collected	No. of sera classified as:			
		Positive by both assays	Negative by both assays	Positive or negative	Discrepant (assay results differed)
Minas Gerais	261	180	81	261	0
Paraíba	466	135	305	440	26
Piauí	253	202	44	246	7
Amazon	85	3	75	78	7
Total	1,065	520	505	1,025	40

disease is endemic show differences in antibody titers found in the patients and in the degree of the clinical manifestations in the chronic phase of the disease (21). Since the infected donor populations encountered in the large urban centers of Brazil migrated from many different regions of the country in which the disease is endemic, in this study, we compared the performances of three commercial enzyme immunoassays (EIAs) by using panels of sera obtained from patients and healthy residents of four Brazilian areas where Chagas' disease is either endemic or emergent.

MATERIALS AND METHODS

Study population and description of areas in which Chagas' disease is endemic. Sera were obtained from patients and healthy residents from the following areas: the state of Minas Gerais in the south-central region of Brazil (municipality of Virgem da Lapa, $n = 261$; 12.6% seroprevalence), where the cardiac and digestive forms of the disease are common (4, 5); the hinterlands of the northeastern states of Paraíba ($n = 466$; 9.5% seroprevalence) and Piauí ($n = 253$; 5.9% seroprevalence), where the indeterminate form of the disease is common (7, 16, 17); and the Amazon state in the north of Brazil (municipality of Barcellos, $n = 85$; 13.2% seroprevalence), where Chagas' disease is emergent (15, 18, 19).

The municipalities and regions situated in the states of Minas Gerais, Paraíba, and Piauí are part of the dry hinterlands with sparse vegetation and rainless periods lasting from 1 to 3 years. On the other hand, the study area located in the Amazon state is part of the rain forest. The chagasic patients in the areas under investigation became infected mainly by vectorial transmission, with the predominant vectors being the triatomid bugs *Panstrongylus megistus* and *Triatoma infestans* (Minas Gerais) (6), *Triatoma brasiliensis* (Paraíba and Piauí) (16), *Triatoma pseudomaculata* (Paraíba) (16), and *Rhodnius brethesi* (Amazon) (19). Professional occupations in these regions are agriculture and stock raising (Minas Gerais, Paraíba, and Piauí) and gathering of palm fibers (Amazon). Illiteracy reaches levels between 30 and 40%. Most members of the study population were less than 30 years or more than 50 years of age. The intermediate age group (30 to 50 years old) is underrepresented in these areas as a consequence of the migration to the cities of Manaus, Salvador, São Paulo, and Rio de Janeiro.

Indirect immunofluorescence. All sera were tested at a final dilution of 1/40 in in-house tests according to the method of Camargo (12) with *T. cruzi* Y epimastigotes as antigen and fluorescein isothiocyanate-conjugated goat anti-human immunoglobulin G (IgG) (Cappel Biomedical Inc., Malvern, Pa.).

In-house ELISA. The cytosolic fraction of *T. cruzi* Y epimastigotes was used as antigen. Briefly, Nunc microtiter plates were sensitized overnight at 4°C with 100 μl of antigen solution in 0.05 M sodium bicarbonate buffer (pH 9.6) at a concentration of 200 ng/ml. Sera were diluted 1/200 in phosphate-buffered saline–Tween 20 (PBST) (0.3%)–fetal calf serum (5.0%), and 100 μl of the mixture was added to the wells. After 30 min at 37°C, the plates were washed eight times with PBST, anti-human IgG-peroxidase conjugate (Cappel Biomedical Inc.) was added to the wells at a dilution of 1/10,000 in PBST-fetal calf serum, and the wells were incubated at 37°C for 30 min. After eight additional washes, the immune complexes were developed with tetramethylbenzidine-H_2O_2 (Sigma), and the absorbances were read at 450 nm. Cutoff values were calculated by dividing the difference of the average absorbances of two positive and three negative controls by three.

To compare the results of ELISAs performed on different days, the results were expressed as ratios by dividing the absorbance values of each plate by the cutoff value obtained for the same plate. A sample was considered positive if the ratio was equal to or greater than 1.1, negative if the ratio was equal to or smaller than 0.9, and indeterminate if the ratio was between 0.9 and 1.1. After the results of the in-house ELISA were compared to those previously obtained by IFA, the sera were classified as either positive, negative, or discrepant. Sera with repeatedly indeterminate ELISA results were a priori considered discrepant. The results are shown in Table 1.

Commercial EIAs. Three commercial EIAs were evaluated in this study: the Abbott Chagas antibody EIA (Abbott Laboratórios do Brasil, Sãn Paulo), the BIOELISACRUZI kit (Biolab-Mérieux, Rio de Janeiro, Brazil), and the BIOZIMA Chagas kit (Polychaco S.A.I.C., Buenos Aires, Argentina). Each EIA was carried out strictly according to the instructions provided by the manufacturer. Calculations of the cutoff values and evaluation of the test results were performed as described in the respective sections of each manual.

TABLE 2. Comparison of results for sera from four regions of Brazil obtained by IFAs and in-house assays with results obtained with three commercial assay kits

Source of sera and result	No. of serum samples:								
	Tested by IFAs and in-house assays	With Abbott Chagas antibody EIA kit result			With Biolab-Mérieux BIOELISACRUZI kit result			With BIOZIMA Chagas kit result		
		Positive	Negative	Indeterminate	Positive	Negative	Indeterminate	Positive	Negative	Indeterminate
Minas Gerais										
Positive	180	179	0	1	178	1	1	180	0	0
Negative	81	2	79	0	1	80	0	1	80	0
Paraíba										
Positive	135	133	2	0	126	3	6	135	0	0
Negative	305	12	287	6	0	305	0	20	285	0
Piauí										
Positive	202	199	3	0	196	3	3	202	0	0
Negative	44	2	41	1	0	43	1	1	43	0
Amazon										
Positive	3	3	0	0	3	0	0	3	0	0
Negative	75	0	74	1	0	75	0	5	70	0
Total for all sera										
Positive	520	514	5	1	503	7	10	520	0	0
Negative	505	16	481	8	1	503	1	27	478	0

TABLE 3. Diagnostic performance of three assay kits with sera from four regions of Brazil[a]

Source of sera	Performance (%) by assay type								
	Abbott Chagas antibody EIA			Biolab-Mérieux BIOELISACRUZI kit			BIOZIMA Chagas kit		
	Relative sensitivity	Relative specificity	Accuracy	Relative sensitivity	Relative specificity	Accuracy	Relative sensitivity	Relative specificity	Accuracy
Minas Gerais	100.0	97.5	99.2	99.4	98.8	99.2	100.0	98.8	99.6
Paraíba	98.5	96.0	96.8	97.7	100.0	99.3	100.0	93.4	95.5
Piauí	98.5	95.3	98.0	98.5	100.0	98.8	100.0	97.7	99.6
Amazon	100.0	100.0	100.0	100.0	100.0	100.0	100.0	93.3	93.6
Total for all sera	99.0	96.8	97.9	98.6	99.8	99.2	100.0	94.7	97.4

[a] Indeterminate results have been omitted.

RESULTS

Evaluation of the EIAs. The results of the evaluation of the three kits are presented in Tables 2 and 3 for each study area and for the study population as a whole. Due to the lack of a serologic "gold standard" for the indeterminate and chronic phases of Chagas' disease (see also Discussion), the sera employed in the evaluation were characterized by matched IFA and in-house ELISA results. Of a total of 1,025 sera, 520 were consensus positive and 505 were consensus negative. Performances of the commercial tests were expressed as relative sensitivity, relative specificity, and accuracy (11).

Looking at the results obtained for each study area and for the population as a whole, all three kits performed comparably (Table 3). Considering the entire study population, the BIOELISACRUZI kit had the highest relative specificity and was the most accurate test, whereas the BIOZIMA Chagas kit showed the highest relative sensitivity.

With respect to the IFA and in-house ELISA consensus classifications, 63 sera of a total of 1,025 (6.1%) gave either a discrepant or indeterminate result with at least one of the kits evaluated in this study (some of the 76 discordant results shown in Table 2 appeared in the same sample). Due to the lack of a gray-zone definition for the BIOZIMA Chagas kit, indeterminate results were observed only for the Abbott Chagas antibody EIA (9 of 1,025; 0.9%) and the Biolab-Mérieux BIOELISACRUZI kit (11 of 1,025; 1.1%).

One of the four sera that tested indeterminate with the BIOELISACRUZI kit was a consensus-negative serum from Piauí that tested positive with the two other kits. The remaining 10 sera (1 from Minas Gerais, 6 from Paraíba, and 3 from Piauí) were consensus positive and were classified as such by both the Abbott Chagas antibody EIA and BIOZIMA Chagas kit.

On the other hand, the BIOELISACRUZI kit diagnoses were in agreement with the consensus on all nine sera which gave indeterminate results with the Abbott Chagas antibody EIA (six sera from Paraíba and one each from Minas Gerais, Piauí, and Amazon), whereas the BIOZIMA Chagas kit classified as positive three consensus-negative sera of the six from Paraíba.

The BIOELISACRUZI kit showed the highest relative specificity, with only 1 of 505 (0.02%; from the panel of Minas Gerais sera) consensus-negative sera diagnosed as positive. This serum was classified as negative by the two other kits. The Abbott Chagas antibody EIA and the BIOZIMA Chagas kit showed much lower relative specificities, with 16 (3.2%) and 27 (5.3%) positive results for consensus-negative sera, respectively. From the 16 consensus-negative sera that were positive in the Abbott test, 12 (75%) were from the Paraíba panel and 2 each were from Minas Gerais and Piauí. Six of these Paraíba sera and one of the Piauí sera also tested positive with the BIOZIMA Chagas kit. Additionally, the latter test gave positive results with another group of 20 consensus-negative sera (14 from Paraíba, 5 from the Amazon, and 1 from Minas Gerais), which were all diagnosed as negative by the Abbott and Biolab-Mérieux EIAs.

The BIOZIMA Chagas EIA did not yield any negative result for consensus-positive sera and, therefore, was the most sensitive test in this study. On the other hand, the Abbott Chagas antibody EIA gave negative results for five (1.0%) consensus-positive sera, three of which were from Piauí and two of which were from Paraíba. The BIOELISACRUZI kit yielded negative results for seven (1.3%) consensus-positive sera. Of these, three sera were from Piauí, three sera were from Paraíba, and one serum was from Minas Gerais. Two of the three sera from Piauí also tested negative in the Abbott Chagas antibody EIA. However, the remaining five sera were classified as positive by the other two tests. Taken together, these results clearly indicate that sera from patients residing in the states of Paraíba and Piauí have to be considered problematic for routine serology testing (see also Discussion).

DISCUSSION

In the present study, we compared the performances of the Abbott Chagas antibody EIA, the Biolab-Mérieux BIOELISACRUZI kit, and the BIOZIMA Chagas kit, which are routinely employed in Brazilian blood banks for the detection of antibodies against *T. cruzi*.

The BIOZIMA Chagas and BIOELISACRUZI kits are 96-well ELISAs, while the Abbott Chagas antibody EIA employs coated beads as the solid matrix. The Brazilian prices (in U.S. dollars) for a single test are $2.17, $1.59, and $3.51, respectively. The total test incubation times varied from 50 to 120 min, with the BIOZIMA Chagas kit being the fastest, providing a result by visual reading after a little over 1 h. In addition, it was the most easily performed, with controls, conjugate, and substrate supplied in dropper bottles as ready-to-use solutions.

All three assays gave satisfactory results with sera which were obtained in four Brazilian areas and classified as either consensus positive or negative by matched in-house IFA and in-house ELISA results. Relative assay sensitivities and specificities varied depending on the area under investigation (Table 3) and ranged for the total population from 98.6 to 100% and 94.7 to 99.8%, respectively. The observed area-dependent differences may in part be attributed to the disproportional fractions of positive and negative sera obtained in each area (e.g., 3 positive versus 75 negative sera from the Amazon and 202 positive versus 44 negative sera from Piauí [Table 2]).

However, for the total population, we employed 520 (50.7%) consensus-positive and 505 (49.3%) consensus-negative sera. Consequently, assay performances calculated for the four panels as a whole should reflect interassay differences more precisely.

The ELISAs yielded conflicting results for a number of sera, but the same sera were not necessarily problematic for each of the three kits evaluated in this study. Thus, for 21 of 520 (4.0%) positive and 42 of 505 (8.3%) negative sera, the results obtained with at least one of the three kits were not in agreement with the consensus. These findings corroborate the results obtained by others (1, 26). A chemiluminescent ELISA for the diagnosis of active infection by *T. cruzi* (1) was evaluated with sera which yielded inconclusive results in eight conventional serologic tests. Depending on the combination of test results, the percentage of inconclusive results varied between 18 and 78%. In another study (26), the Abbott Chagas antibody EIA, the Biolab-Mérieux BIOELISACRUZI kit, and the Chagas IgG ELISA (Gull Laboratories, Salt Lake City, Utah) were evaluated with 60 sera obtained at a blood bank. The authors defined a combined assay performance in which a serum was considered positive if at least two of the three ELISAs to be evaluated plus a confirmatory IFA were positive. Using the combined assay performance results as the gold standard, ELISA sensitivities were reported to be 100% and specificities varied from 87 to 97%. Carvalho et al. (13) compared the performances of an in-house recombinant-antigen ELISA and four commercial ELISAs (Abbott, Biolab-Mérieux, Gull Laboratories, and Ortho Diagnostic, Buenos Aires, Argentina) with sera obtained in Virgem da Lapa, Minas Gerais, and at the state blood bank of São Paulo. The authors report for the commercial tests specificities ranging from 95.0 to 98.0% and sensitivities from 99.0 to 100.0%.

The observed variation of sera that were problematic for a given assay is not surprising since the antigen preparations employed in each of the evaluated kits are obtained by different procedures. Furthermore, the *T. cruzi* Y epimastigotes are cultivated according to different protocols in various culture media. As previously reported (37), extraction procedures influence drastically the epitopes retained on antigenic molecules. Furthermore, binding of these molecules to solid surfaces hides or exposes epitopes that have different affinities for both specific and nonspecific antibodies present in the sera, thus accounting for conflicting results.

The Abbott Chagas antibody EIA was also evaluated in two studies published earlier (9, 33). Pan et al. (33) reported a sensitivity of 93.48% and a specificity of 99.48% with 1,392 sera from Brazil and Argentina which had been previously characterized by a commercial indirect hemagglutination assay.

Brashear et al. (9) used the Abbott Chagas antibody EIA to screen 13,309 sera from a potentially high-risk U.S. donor population and calculated a specificity of 99.98% and positive and negative predictive values of 81.25 and 99.99%, respectively.

The sera employed in our study were obtained in Minas Gerais, Paraíba, Piauí, and the Amazon, regions where disease manifestation, circulating parasite strains, and parasitemia vary (6, 7, 16, 18, 19). As a consequence of the sampling technique, in which houses and dwellings were first investigated for the presence of triatomid bugs and then, in a second step, blood samples were drawn from the residents and their neighbors (Minas Gerais, Piauí, and Paraíba), the panels we used did not reflect the overall prevalences of *T. cruzi* infection described in serologic surveys for the populations in the study areas. However, in the Amazon region, triatomid bugs are not found in houses, and people get infected while working in the rain forest. The Amazon panel utilized in this study consists of a small part of the samples obtained during the serologic survey (19).

In the particular case of Chagas' disease, no serologic gold standard for the definition of the disease status exists, since detection of *T. cruzi*-specific antibodies depends on the patient's immune status and since cross-reactivity of *T. cruzi* antigens with antibodies raised against other coendemic parasites (*Leishmania* and *Trypanosoma rangeli*) is frequent (2, 25, 42). Nevertheless, despite its drawbacks, IFA is the most commonly used serologic test for Chagas' disease and, as a result, is widely accepted as the gold standard (22). Therefore, as a first step we determined the status of the sera according to the results obtained in an in-house IFA and an in-house ELISA (Table 1). The sera were considered either positive or negative if IFA and ELISA results were concordant and indeterminate if the two results were discrepant. However, while no indeterminate serum was found in the panel from Minas Gerais, 26 (5.6%) of the 466 sera from Paraíba were found to be indeterminate, as were 7 (2.8%) of the 253 sera from Piauí and 7 (8.2%) of the 85 sera from the Amazon. These findings can be explained by the epidemiological characteristics and circulating parasite strains in the different areas. In Virgem da Lapa, Minas Gerais, the cardiac and digestive forms of Chagas' disease are frequent, and the circulating *T. cruzi* strains generally cause a high-titer immune response in the patients (6). Furthermore, this area is not one in which *Leishmania* spp. (5), which can cause false-positive results in Chagas' disease serology (14), is endemic. On the other hand, in the states of Paraíba and Piauí, the indeterminate form of the disease predominates, and patients show mostly moderate or weak immune responses to the infection (7). Also, in these areas leishmaniasis is frequent. As far as the Amazon is concerned, cross-reactions with *Leishmania* spp. may account for the high seroprevalence reported for this region (15, 19), and infections with the nonpathogenic parasite *T. rangeli* have been demonstrated (18). Taken together, these facts are likely to account for the indeterminate classification of some sera by our in-house tests. In addition, we cannot rule out the possibility that some of the discrepancies observed between the in-house consensus results and those obtained with the three kits were due to a misclassification of the sera by our in-house assays. However, the use of in-house tests for the characterization of serum panels and subsequent evaluation of a commercial kit has been reported by others (35).

This study shows that the Abbott Chagas antibody EIA, the Biolab-Mérieux BIOELISACRUZI kit, and the BIOZIMA Chagas test are well suited for the detection of IgG antibodies against *T. cruzi*. Nevertheless, when used for routine diagnoses and blood bank screening, problems can occur if the patients or donors come from areas in which the epidemiology of Chagas' disease is complex. Therefore, confirmatory tests with higher specificities need to be developed, and good candidates for such tests are those that include a combination of *T. cruzi*-specific cloned antigens and/or synthetic peptides (13, 28, 35, 36).

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