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Serologic Testing for *Trypanosoma cruzi*: Comparison of Radioimmunoprecipitation Assay with Commercially Available Indirect Immunofluorescence Assay, Indirect Hemagglutination Assay, and Enzyme-Linked Immunosorbent Assay Kits

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The radioimmunoprecipitation assay (RIPA) has been used as a confirmatory test in several ongoing and published studies of *Trypanosoma cruzi* in blood donors in the United States. Despite its use as a confirmatory test, few studies are available comparing RIPA to commercially available serologic test methods. Thus, we compared RIPA with two indirect hemagglutination assays (Biolab Diagnostica SA, São Paulo, Brazil; Hemagen Diagnostics, Inc., Waltham, Mass.) and four different enzyme-linked immunosorbent assays (Abbott Laboratories, Abbott Park, Ill.; Embrabio, São Paulo, Brazil; Organon Teknika, São Paulo, Brazil; and Gull Laboratories, Salt Lake City, Utah) using a panel of 220 serum specimens from Brazilian blood donors with a range of *T. cruzi* antibody titers as determined by indirect immunofluorescence assay (IFA). A titer of 1:20 was used as the baseline for seropositivity. All IFA-negative serum specimens ($n = 19$) were nonreactive on all tests. At a titer of 1:20 ($n = 9$), reactivity rates varied considerably among the tests, with only the RIPA and the Organon and Gull assays identifying reactive specimens. For specimens at a 1:40 titer ($n = 35$), most assays identified at least 32 of 35 (91%) specimens as reactive, but the Biolab assay only identified 24 (69%). At higher titers (1:80, $n = 56$; 1:160, $n = 101$) the assays were comparable, with the exception of the Biolab assay, demonstrating rates of agreement with IFA of ≥98%. Overall, when compared with several other test formats, RIPA demonstrated equivalent or superior rates of agreement with IFA-positive specimens across all titers examined. In particular, at titers of >1:40, the RIPA compared favorably with other test methods currently in use, supporting its application as a confirmatory test, particularly in a research setting.

In many areas of Latin America, Chagas' disease remains a public health concern despite efforts to reduce vectorial transmission of the etiologic agent, *Trypanosoma cruzi*. Government and World Health Organization efforts to eliminate domiciliary vectors via the Southern Cone Initiative have resulted in a dramatic reduction of newly acquired *T. cruzi* infections, particularly among children (3, 9, 17). As vectorial transmission has been reduced, residual transmission of *T. cruzi* by blood transfusion has received increased attention. Indeed, in some areas with intensive vectorial control in which the disease is endemic or in areas in which vectorial transmission is rarely (the United States) or never (Canada, Europe) observed, transfusion is the primary route of *T. cruzi* transmission (10, 19, 22). Because established infections with *T. cruzi* are chronic and untreatable, infected people can serve as reservoirs for transmission by transfusion throughout their lifetimes. Thus, concerns have been raised in the United States that blood donors who have emigrated there from countries where infection with *T. cruzi* is endemic may transmit infection via blood transfusion. Several recent studies, which have identified *T. cruzi*-positive blood donors from different geographic locations within the United States, support this growing public health concern (4, 15, 16, 20).

Blood screening for antibodies to *T. cruzi* has been implemented in many portions of Latin America to enhance blood safety. *T. cruzi*-infected individuals maintain a lifelong detectable antibody response; potentially infectious blood may be identified by serological screening and *T. cruzi*-positive blood may be withdrawn from use. No one test has been found to be sufficiently sensitive and specific to be designated the sole screening assay. The Pan American Health Organization and others have suggested that blood donors be tested by at least two different methods to increase the sensitivity of detecting true seroreactive donors (6, 11). South American blood banks, for example, often perform three serologic tests for *T. cruzi*, resulting in an algorithm that considers a donor positive if the sample is reactive in two or three out of three tests. Currently, the tests most frequently used are the indirect immunofluorescence assay (IFA), the indirect hemagglutination assay (IHA), and the enzyme immunoassay (EIA). In contrast, blood screening has not been implemented in the United States, in part because no test for blood bank screening has been licensed by the U.S. Food and Drug Administration (FDA). Several seroprevalence studies have been published indicating that there is a small percentage of *T. cruzi*-seropositive donors in the U.S. donor pool (1, 2, 4, 12, 15, 16, 20). Most of these studies have used algorithms that generally conform to FDA guidelines; repeat reactive samples are identified by screening and confirmed as seropositive by a more specific and sensitive test method. While studies in the United States have used a variety of screening tests (mostly EIA), almost all studies have used the radioimmunoprecipitation assay (RIPA) for confirmation (14).

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TABLE 1. Comparison of RIPA, IHA, and ELISA reactivity results with positive (titer of ≥1:20) IFA results[a]

IFA titer	n	No. of reactive specimens with assay						
		RIPA	IHA		ELISA			
			Hemagen	Biolab	Abbott	Embrabio	Organon	Gull
1:20	9	1	0	0	0	0	4	3
1:40	35	32	34	24	32	32	35	35
1:80	56	56	56	50	56	56	56	56
1:160	101	101	101	99	99	100	101	101
Total no. positive	201	190	191	173	187	188	196	195
% Agreement	NA[b]	95	95	86	93	94	98	97

[a] Data are grouped by the number of reactive specimens identified by each test at various IFA-positive (values of ≥1:20) titers. The percentage of agreement was defined as the total number of positive specimens for each test divided by 201 (total number IFA positive) and multiplied by 100.
[b] NA, not applicable.

The use of RIPA as a confirmatory test, however, has remained controversial despite reports indicating that it is extremely specific and sensitive (4, 14, 24). In part, this may be due to its laborious procedure, relatively high cost compared to other tests, and general lack of implementation outside of the United States. Further, and perhaps more importantly, few studies are available comparing the RIPA with other tests, particularly studies involving samples from blood donors. Thus, the present study was designed to compare the performance of RIPA with a variety of commercially available test kits using a panel of specimens from Brazil.

MATERIALS AND METHODS

Sera. A panel of 220 serum specimens from blood donors presenting at the Hospital Sírio Libanês Blood Bank (São Paulo, Brazil) with IFA-positive (n = 201) or -negative (n = 19) test results for *T. cruzi* antibodies was used to compare the performance of RIPA to a variety of commercially available tests for *T. cruzi*. All testing of specimens, with the exception of the RIPA, was performed at the Hospital Sírio Libanês.

IFA. IFA was assayed using fixed epimastigotes and anti-human immunoglobulin G-fluorescein conjugate (Biolab Diagnostica SA, São Paulo, Brazil). Specimens were considered reactive when fluorescence was observed at a 1:20 or higher dilution.

IHA. IHA was conducted using two commercially available kits (Biolab Diagnostica; Hemagen Diagnostics, Inc., Waltham, Mass.). For the former test kit, IHA was performed with specimens treated with 2-mercaptoethanol (2-ME) at a dilution of 1:40 according to the manufacturer's instructions. The latter test was performed according to the manufacturer's instructions, including the absence of 2-ME treatment. Both assays were read and interpreted manually.

ELISA. Four commercially available enzyme-linked immunosorbent assay (ELISA) kits (Abbott Laboratories, Abbott Park, Ill.; Embrabio, São Paulo, Brazil; Organon Teknika, São Paulo, Brazil; Gull Laboratories, Salt Lake City, Utah) for detection of antibodies to *T. cruzi* were used according to the manufacturers' instructions.

RIPA. RIPA testing was conducted at the American Red Cross's Holland Laboratory (Rockville, Md.) using procedures described previously (14, 15). All specimens were assayed in parallel with three negative- and three positive-control specimens, the latter obtained from parasitologically confirmed cases of Chagas' disease. Diagnostic confirmation of reactivity by RIPA was defined as the presence of bands in autoradiographs indicative of antibodies specific for the 72- and 90-kDa glycoproteins of *T. cruzi*.

Data analysis. IFA is widely recognized throughout Latin America and is commonly used as the laboratory standard for the measurement of *T. cruzi* antibodies (5, 18, 23, 25, 26). To facilitate the comparison of test results among the various assays examined, we grouped the data by IFA titer, using a titer of ≥1:20 as a baseline for positivity. The percentage of agreement was calculated by determining the total number of positive specimens identified by each test, dividing that number by the total number of IFA-positive (values of ≥1:20) specimens, and multiplying by 100.

RESULTS

All IFA-negative specimens (n = 19) were nonreactive on all assays examined. For the baseline positive group (titers of 1:20), all samples were identified as nonreactive except for one, three, and four samples identified as reactive by the RIPA, Gull assay, and Organon assay, respectively (Table 1). At a midlevel IFA titer of 1:40 (Table 1), most assays identified at least 32 of the 35 (≥91%) tested specimens as reactive. The Biolab IHA, however, only detected 24 of 35 (69%) specimens as reactive. When high-titer (1:80 and 1:160) IFA-positive sera were assayed by the various tests (Table 1), the results were comparable in most instances. At a titer of 1:80, all samples (n = 56) were reactive by all tests, except for six (11%) samples that were nonreactive by the Biolab IHA. At a titer of 1:160, all assays detected at least 99 of 101 (98%) IFA-positive samples; only the Biolab IHA, Abbott ELISA, and Embrabio ELISA failed to identify all positive specimens. Overall, the RIPA and the other assays generally demonstrated comparable rates of agreement with the IFA (between 93 and 98%). The lone exception was the Biolab IHA, which demonstrated an 86% agreement rate with the IFA (Table 1).

DISCUSSION

A wide variety of serologic tests are used in algorithms designed to identify blood donors with antibodies to *T. cruzi*. These tests, as indicated by the current study, demonstrate a range of specificities and sensitivities that can lead to false-positive test results or, perhaps more importantly, to an inability to detect true positives. The type of specimen investigated also influences test results and their interpretation. Indeed, the majority of problems and pitfalls associated with serologic testing for *T. cruzi* involves specimens from donors with unproven infections. In the present study, many of these problems could have been avoided by using specimens from donors with demonstrable parasitemia, but most tests readily detect such specimens. Thus, we sought to examine a panel of donor specimens that more closely reflected those encountered during routine testing in blood banks.

The IFA-negative sera in the present study were negative on all tests, but the results at a titer of 1:20 exemplify the difficulty in interpreting serologic test results. The RIPA, Gull assay, and Organon assay identified one, three, and four specimens, respectively, at a titer of 1:20 as reactive, while the remaining assays detected no reactive specimens at this titer. One could argue that the RIPA and Gull and Organon assays demonstrate lower specificity; however, one could alternatively argue that these tests are more sensitive and capable of identifying samples missed by other tests. Any discussion of sensitivity must be tempered, because no specimens from parasitologi-

cally confirmed cases of *T. cruzi* were included in this study, thereby precluding true sensitivity determinations. However, for this study, percent agreement between the IFA result and each test result was calculated as a means of comparison. At titers of 1:40 and higher, the agreement rates were generally greater than 91% with the exception of the Biolab IHA. Similarly, at a titer of 1:80 ($n = 56$), the Biolabs IHA demonstrated an agreement rate with IFA of 89%, while all other assays demonstrated 100% agreement. RIPA performed particularly well at titers of >1:40 where it, like the Organon and Gull assays, identified all samples tested ($n = 157$) as reactive. Thus, the RIPA appears to demonstrate equivalent or superior specificity and sensitivity when compared to other tests examined, supporting its use as a confirmatory test, at least in a research setting. As a caveat, specificity determinations were based on only 19 specimens determined to be IFA negative; consequently, additional specimens need be tested to further validate the specificity claim.

The present study grouped serum samples by observed IFA titers, using a titer of ≥1:20 as an indicator of reactivity for *T. cruzi*. However, at a titer of 1:20 there was limited consensus among the tests regarding reactivity for *T. cruzi*. While the number of available serum samples in this group is relatively small ($n = 9$), a point that needs to be addressed in future studies, the results suggest that a baseline IFA titer of greater specificity may be obtained using a value of 1:40. RIPA performed particularly well for samples at or above an IFA titer of 1:40, identifying 189 of 192 (98%) samples as reactive. For most studies of this nature, the greatest difficulty occurs when one attempts to establish a baseline value for reactivity. Indeed, for studies involving *T. cruzi*, the selection of one test or a multiple-test algorithm as the "gold standard" has proven problematic and elusive.

Perhaps the only assays that could presently be considered gold standards are xenodiagnosis and hemoculture. In both instances, the end point of the assay is visualization of the parasite, thereby providing indisputable evidence of infection. However, the sensitivity of these assays is only 30 to 55%, values that are often obtained only after repeat testing due to the intermittent nature of parasitemia (7). Additionally, these assays can take weeks or months to complete. Xenodiagnosis has the added drawback that live, hematophagous insects are allowed to feed on the individual for several hours, a process that can prove quite unpleasant (8). For these reasons, these tests are impractical for use in blood banks which require rapid results, but these tests remain useful in research settings, particularly for confirming active parasitemia.

Serologic testing has remained the method of choice in large part because of demonstrable antibody titers in people infected with *T. cruzi*, even decades after primary infection (13, 21). Blood banks routinely obtain serum samples from blood donors for testing, and most testing or reference laboratories are well equipped to handle routine serologic assays including IHA and EIA or ELISA. However, as indicated earlier, many serologic tests for *T. cruzi* suffer from problems with specificity and sensitivity. In particular, many of these tests have cross-reactivity problems with several other diseases, especially leishmaniasis (5, 24). Our experience and that of others, however, suggests that RIPA does not cross-react with sera from cases of leishmaniasis (cutaneous or visceral), falciparum malaria, toxoplasmosis, syphilis, schistosomiasis, or *Trypanosoma rangeli* (14, 24). Finally, RIPA test results are easily interpreted as positive or negative (described earlier), while indeterminate test results (i.e., only one band present) have been extremely rare, occurring only once among over 1,000 samples we have tested to date.

As for other tests, the RIPA has several drawbacks that make it less than attractive in certain testing situations. First, the RIPA is labor intensive, requiring access to live parasites and radioactive iodine (^{125}I). Second, the reagents required for RIPA, particularly the iodine, protein A-Sepharose, and polyacrylamide gel electrophoresis supplies, are expensive compared to those required for other serologic tests. Thus, while RIPA may be highly specific and sensitive, with corresponding low cross-reactivity, it remains a test amenable to only the research laboratory and not as part of a blood-screening algorithm.

In the future, a potential scenario for *T. cruzi* blood screening may involve several of the methods described above; however, from the United States' perspective, this discussion remains speculative in the absence of an FDA-approved test for blood screening. Initial identification of *T. cruzi*-positive donors will probably depend upon a serologic test in an EIA or ELISA format, or perhaps, less likely, a hemagglutinin format; both formats would fit easily into the present testing environment. It is less clear, however, what test would be used as a supplemental or confirmatory test. In addition to the tests described in the present study, several other tests are available, including PCR and those using a Western blot format, but specificity and sensitivity data are not readily available for these tests. Further, as for the blood-screening assay, the supplemental and/or confirmatory test will also likely require FDA approval or at least be submitted in conjunction with the blood-screening application. Thus, potential testing algorithms remain problematic and in a state of flux, particularly in the United States, where it is not yet clear if blood screening for *T. cruzi* will be implemented or is needed.

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