Evaluation of a prototype *Trypanosoma cruzi* antibody assay with recombinant antigens on a fully automated chemiluminescence analyzer for blood donor screening

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**BACKGROUND:** Chagas disease is caused by *Trypanosoma cruzi*, a protozoan parasite that can be transmitted by transfusion. The diagnosis of chronic *T. cruzi* infection is generally made by detecting specific antibodies that bind to parasite antigens. The aim of this study was to assess the sensitivity and specificity of a new serologic assay for antibodies to *T. cruzi* on a fully automated analyzer (PRISM, Abbott Laboratories).

**STUDY DESIGN AND METHODS:** A prototype chemiluminescent immunoassay based on chimeric recombinant antigens and run on the automated PRISM system was developed for detecting antibodies to *T. cruzi* in human serum and plasma. Assay specificity was evaluated by testing samples from random blood donors and from a diverse group of specimens from persons with diseases or conditions often associated with false-positive reactions in *T. cruzi* assays. Sensitivity was determined by testing 377 geographically diverse *T. cruzi* antibody–positive specimens.

**RESULTS:** Six of 7911 samples (0.08%) from random donors were repeatedly reactive in the prototype PRISM Chagas assay. One of these was reactive in three other tests, including the radioimmune precipitation assay and was presumed to be a true positive. Hence, the specificity was 99.94 percent (7905/7910) in the negative donor group studied. All 377 *T. cruzi* antibody–positive specimens were positive in the prototype assay and thus the sensitivity was 100 percent.

**CONCLUSION:** The results obtained to date, in terms of sensitivity as well as specificity, strongly suggest that the PRISM Chagas assay should function well as a tool for screening blood for serologic evidence of *T. cruzi* infection.

**ABBREVIATIONS:** IFA = indirect immunofluorescence assay; IHA = indirect hemagglutination assay; RIPA = radioimmune precipitation assay; RLU = relative light units; S/CO = sample-to-cutoff (ratio).

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A persistent problem with these serologic assays, however, has been the occurrence of indeterminate and false-positive results, most often with specimens from persons with other infectious diseases (e.g., leishmaniasis, malaria, and syphilis), as well as patients with autoimmune disorders. A lack of sensitivity with conventional assays has also been reported. Because of these problems, the Pan American Health Organization has recommended that specimens be tested with at least two assays in parallel before being accepted as positive, which obviously imposes an enormous logistical and economic burden for blood banks in endemic countries.

A number of highly conserved antigenic proteins of T. cruzi were described in the late 1980s, and many of these, in the form of synthetic peptides and recombinant proteins, have been used in serologic assays for diagnosing T. cruzi infection. The use of these reagents, in comparison to parasite lysates, offers clear advantages in terms of reagent quality control and reproducibility and also eliminates the biohazard associated with maintaining and processing live organisms from cultures of T. cruzi. In view of the long-recognized shortcomings of the conventional assays and the advantages of the use of antigens produced by genetic engineering techniques, we chose to develop a Chagas assay on an analyzer that is based on recombinant antigens (PRISM, Abbott Laboratories, Abbott Park, IL).

In this study we describe a PRISM-based serologic assay for antibodies to T. cruzi in which four chimeric recombinant proteins are used as antigens. The antigenic diversity of these proteins forms the basis of a highly sensitive assay that detects antibodies to T. cruzi in samples obtained from a geographically diverse group of Chagas disease patients. Specificity is determined from the testing of random donors, the vast majority of which we assumed are negative. Moreover, testing a broad range of samples from persons having diseases and conditions often associated with false-positive results in conventional assays indicates that the assay is highly specific.

MATERIALS AND METHODS

PRISM Chagas assay

The PRISM is a high-throughput, fully automated analyzer for screening plasma or serum for antibodies or antigens associated with transfusion-transmitted pathogens. The PRISM is currently used in 29 countries and 12 of them use this system to screen their entire national blood supply. The prototype Chagas PRISM assay uses a two-step testing protocol similar to that employed in the current PRISM hepatitis C virus (HCV) antibody assay. The assay format is illustrated in Fig. 1. In the first step, the instrument dispenses into the sample well of a reaction tray, 50 µL of sample, 50 µL of specimen diluent buffer, and 50 µL of microparticles coated with the four chimeric
recombinant *T. cruzi* proteins used in the assay. After a 20-minute incubation at 37°C, the tray is moved to a transfer station where the reaction mixture in each sample well is flushed by a transfer wash buffer onto a glass fiber matrix where the microparticles are captured on a filter. Excess fluid is absorbed by an underlying blotter. In the second step, 50 µL of a conjugate solution containing acridinium-labeled anti-human IgG monoclonal antibody (conjugate); SDB = specimen diluent buffer; *hv* = light. In Step 1, the rAg-coated microparticles capture hAnti-*T. cruzi*. After wash, the captured human antibodies on microparticles bind with the conjugate in Step 2. Subsequently, the bound conjugate is washed and emission of light is triggered with alkaline hydrogen peroxide.

**Volunteer blood donors**

A total of 7911 specimens (ethylenediaminetetraacetate plasma [n = 4911] and serum samples [n = 3000]) were obtained from the Gulf Coast Regional Blood Center (Houston, TX). These linked specimens were collected from random donors, and no samples were eliminated from this group because of positive results in any of the six routine tests performed on donated units. All specimens were tested on the PRISM Chagas assay within 5 days of collection, and the results obtained were used to determine a provisional cutoff for the assay. All specimens that were repeatedly reactive in the PRISM Chagas assay were tested in the ELISA-I and ELISA-II, as well as in the RIPA, except for one sample that could not be tested in the latter because of inadequate sample volume.

**Specimens with disease states or interfering substances**

The specificity of the PRISM Chagas assay also was assessed by testing 115 serum or plasma specimens from persons with conditions or diseases that might cause false-positive reactions in *T. cruzi* assays. Samples from persons who had positive serologic tests for autoimmune diseases (n = 10), human anti-mouse (n = 5), a history of
influenza vaccination (n = 5), hemolyzed (n = 8), lipemic (n = 10), monoclonal gammopathy (n = 5), multiple myeloma (n = 10), multiple sclerosis (n = 5), rheumatoid factor (n = 9), hemodialysis (n = 5), leishmaniasis (n = 10), cytomegalovirus (n = 5), Epstein-Barr virus (n = 5), herpes simplex virus (n = 5), syphilis (n = 5), and toxoplasmosis (n = 5) were purchased from various vendors including New York Biologicals (New York, NY), ProMedDx (Norton, MA), Boston Biomedica, Inc. (BBI, West Bridgewater, MA), and Teragenix Corp. (Ft. Lauderdale, FL).

**T. cruzi antibody–positive specimens**

A total of 377 serum or plasma specimens from persons seropositive for the presence of *T. cruzi* were obtained from the American Red Cross, Antibody Systems (Hurst, TX), BioClinical Partners (Franklin, MA), Biocollections Worldwide, Inc. (Miami, FL), BBI, Goldfinch Diagnostics Inc. (Iowa City, IA), and Teragenix. The requests sent to the suppliers were simply for *T. cruzi* antibody–positive specimens, and no guidance was provided in terms of high- or low-titer specimens. The specimens received represent a broad geographic range in South and Central America as well as in Mexico (Table 1). A total of 147 were tested by RIPA and were found to be positive; the other 230 specimens had given positive results in two or three immunoassays before our receiving them (IHA, IFA, or ELISA).

**RESULTS**

**Assay specificity**

A total of 7911 volunteer blood donor specimens were tested in the PRISM Chagas assay with the goal of establishing a provisional cutoff. The provisional cutoff established was 0.13 times the average mean RLU of the positive calibrator plus the average mean RLU of the negative calibrator. The positive calibrator was tested in duplicate, and the negative calibrator was tested in triplicate in both subchannels, A and B, at the front and at the back of each run. Samples with sample-to-cutoff (S/CO) values equal to or greater than 1.0 were considered initially reactive, and samples with S/CO values below 0.80 were considered negative. Samples with S/CO values between 0.80 and 0.99 were considered initial “gray zone” reactivies. All initial reactive and gray zone specimens were retested in duplicate. With the provisional cutoff for the assay, 6 of the 7911 specimens tested had S/CO values between 1.0 and 6.9 and thus were considered repeatedly reactive (Table 2). The repeat-reactive rate was 0.08 percent (6/7911) and thus the apparent specificity was 99.92 percent (7905/7911 × 100). A histogram depicting the S/CO values of a representative negative subgroup (n = 500) of the random donor specimens is shown in Fig. 2A.

As shown in Table 2, two of the six specimens that were repeatedly reactive in the PRISM Chagas assay (Samples 1661 and 3881) were negative in both reference ELISAs. Sample 3881 was also negative in the RIPA, and the other (Sample 1661) was not tested in the latter due to insufficient sample volume. Specimens 1497 and 1660 were negative in the lysate-based ELISA-I, positive in the recombinant ELISA-II, and negative by RIPA. One specimen (5060) had high S/CO values (>6.0) in the PRISM Chagas assay, was repeat-reactive in both reference ELISAs (S/CO values >2.5), and was positive by RIPA. The sixth repeatedly reactive specimen (5342) was just below the cutoff in repeat testing in ELISA-I (considered reactive per test instructions), reactive in ELISA-II, and negative in the RIPA. Hence, the non-confirming repeat-reactive rate was 0.06 percent (5/7910) and the true specificity was 99.94 percent (7905/7910 × 100). All 115 specimens from persons with conditions or diseases that might cause false-positive reactions in *T. cruzi* assays were nonreactive in the PRISM Chagas assay.

### Table 1. Countries of origin for 377 *T. cruzi* antibody–positive specimens

<table>
<thead>
<tr>
<th>Country of origin</th>
<th>Number of samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Argentina</td>
<td>107</td>
</tr>
<tr>
<td>Bolivia</td>
<td>53</td>
</tr>
<tr>
<td>Brazil</td>
<td>55</td>
</tr>
<tr>
<td>Chile</td>
<td>5</td>
</tr>
<tr>
<td>Colombia</td>
<td>1</td>
</tr>
<tr>
<td>Ecuador</td>
<td>1</td>
</tr>
<tr>
<td>El Salvador</td>
<td>2</td>
</tr>
<tr>
<td>Honduras</td>
<td>4</td>
</tr>
<tr>
<td>Mexico</td>
<td>53</td>
</tr>
<tr>
<td>Nicaragua</td>
<td>18</td>
</tr>
<tr>
<td>Surinam</td>
<td>1</td>
</tr>
<tr>
<td>United States</td>
<td>3</td>
</tr>
<tr>
<td>Venezuela</td>
<td>22</td>
</tr>
<tr>
<td>Unknown</td>
<td>42</td>
</tr>
</tbody>
</table>

### Table 2. Results (S/CO values in duplicates) of serological tests on six samples repeatedly reactive in the PRISM Chagas assay

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>PRISM Chagas</th>
<th>ELISA-I (lysate)</th>
<th>ELISA-II (Ag)</th>
<th>RIPA</th>
</tr>
</thead>
<tbody>
<tr>
<td>1497</td>
<td>2.23/3.60</td>
<td>0.28/0.31</td>
<td>2.20/2.27</td>
<td>Negative</td>
</tr>
<tr>
<td>1660</td>
<td>2.61/2.67</td>
<td>0.38/0.46</td>
<td>2.69/2.86</td>
<td>Negative</td>
</tr>
<tr>
<td>1661</td>
<td>1.23/2.10</td>
<td>0.26/0.27</td>
<td>0.12/0.13</td>
<td>NT*</td>
</tr>
<tr>
<td>3881</td>
<td>1.57/1.70</td>
<td>0.22/0.24</td>
<td>0.13/0.13</td>
<td>Negative</td>
</tr>
<tr>
<td>5060</td>
<td>6.65/6.90</td>
<td>2.56/2.72</td>
<td>6.44/8.10</td>
<td>Positive</td>
</tr>
<tr>
<td>5342</td>
<td>3.13/3.63</td>
<td>0.90/0.95†</td>
<td>1.91/2.32</td>
<td>Negative</td>
</tr>
</tbody>
</table>

* NT = not tested due to inadequate sample volume.
† Considered reactive if >0.9 S/CO in retests per test instruction.
Assay sensitivity

The results obtained in testing the 377 *T. cruzi* antibody-positive specimens are shown in Table 3. All the samples in this group were tested in the PRISM Chagas assay and the recombinant-based ELISA-II. Because of volume limitations, only 372 of the 377 specimens were tested in the lysate-based ELISA-I. All 377 specimens were reactive in both the PRISM Chagas assay and the ELISA-II, whereas only 360 of 372 were reactive in the ELISA-I. Hence, both the PRISM Chagas assay and the ELISA-II exhibited a 100 percent sensitivity (377/377), whereas the ELISA-I had a sensitivity of 96.77 percent (360/372). The 12 samples that tested negative in the ELISA-I had S/CO values between 0.55 and 0.89, which were considerably above...
background. A histogram depicting the S/CO values of the 377 *T. cruzi* antibody–positive specimens tested in the PRISM Chagas assay is shown in Fig. 2B.

**DISCUSSION**

In this study, we determined that the prototype PRISM Chagas assay has a sensitivity of 100 percent and a specificity of 99.94 percent when used to test a sizable and geographically diverse group of *T. cruzi* antibody–positive specimens and a large group of samples from nonendemic areas. To achieve this high level of sensitivity, we used as target antigens four chimeric recombinant *T. cruzi* proteins that in aggregate are composed of 14 distinct segments, 7 of which contain repetitive sequences. The data indicate that the diversity of these antigens performed well as shown by the fact that the assay detected as reactive all 377 specimens in the *T. cruzi* antibody–positive group (Table 1). The distribution of S/CO values for the 377 *T. cruzi* antibody–positive specimens shown in Fig. 2B indicates that even the least reactive specimens were clearly above the cutoff established in our initial experiments. This crisp segregation of the S/CO values of the *T. cruzi* antibody–positive specimens, ranging from 1.29 to 23.19, was not likely the result of selection of the specimens studied, because no selection criteria were communicated to the suppliers of the samples. Most of the low reactive specimens also gave relatively low S/CO values in both ELISAs. Achieving a sensitivity of 100 percent with the PRISM Chagas assay supports the use of a cocktail of recombinant proteins as target antigens and agrees with the conclusions reached by other assay investigators who have taken this approach.17,22,23

It is generally accepted that serologic screening is the most effective tool for reducing the risk of transfusion-associated transmission of *T. cruzi*.24 In Argentina, for example, during 1995 through 1997, when 100 percent of the blood supply was tested with two parallel assays as mandated by law (ELISA and IFA or IHA), the theoretical risk of transmission of *T. cruzi* was judged to be negligible.25 Moreover, accumulating data suggest that recombinant antigens can serve as the basis of assays that are more sensitive and specific than conventional tests. This perspective is supported by the results of a recent study done in the hyperendemic eastern lowlands of Bolivia (Santa Cruz de la Sierra), where the prevalence of *T. cruzi* infection among blood donors was 43 percent.26 In that study two assays were used as benchmarks, and five tests were evaluated in comparison to them. The two recombinant-based ELISAs in the latter group had the highest sensitivities, and one of these tests also had the highest specificity. In another study, conducted in Brazil,23 the results of which may be more broadly applicable because samples from six countries were assayed, similar conclusions were reached regarding the sensitivity and specificity of assays based on recombinant antigens. The study showed that recombinant-based tests had the highest sensitivities and that there were advantages in terms of specificity as well, in particular when samples from persons with leishmaniasis were assayed. Interestingly, some mixtures of recombinant antigens also detected anti-*T. cruzi* IgG in specimens from patients with acute Chagas disease. The results of these two studies and others22,27 clearly support the relatively greater utility of recombinant antigens as targets in serodiagnostic assays for *T. cruzi* infection, as does our finding (Table 3) that the sensitivities of the PRISM Chagas assay and the recombinant-based ELISA-II were greater than that of the lysate-based ELISA-I. Although both recombinant-based assays showed superior sensitivity, we found a lower specificity of 99.0 percent on testing of a small random population (n = 300, showed 3 nonconfirmed repeat reactives) with ELISA-II compared to the PRISM assay.

None of the 115 potentially problematic specimens with interfering substances or disease states, including 10 from patients with leishmaniasis, were reactive in the PRISM Chagas assay. Moreover, the specificity of the PRISM assay was found to be at least 99.94 percent in the random donor testing. These results indicate that the relative inputs of the four chimeric recombinant antigens were appropriate and suggest that the PRISM Chagas assay will perform well in terms of specificity when used to screen a low prevalence donor population such as that in the United States.

The six specimens in the group of 7911 random donors who were repeatedly reactive in the PRISM Chagas assay merit further comment (Table 2). The most straightforward is Specimen 5060, which was also positive in both ELISAs and in the RIPA. By all indications, this sample came from a *T. cruzi*-infected person and thus we can reasonably exclude it from the calculation of the specificity of the assay. Two specimens (1661 and 3881) were negative in both ELISAs; only the latter was tested in the RIPA, and it was negative. We view these two specimens as true negatives, and thus they were false-positives in the PRISM Chagas assay.

**TABLE 3. Comparison of three serologic tests on 377 *T. cruzi* antibody positive samples**

<table>
<thead>
<tr>
<th>Variable</th>
<th>PRISM Chagas assay (rAg)</th>
<th>ELISA-I (lysate)</th>
<th>ELISA-II (rAg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of chagasic specimens tested</td>
<td>377</td>
<td>372†</td>
<td>377</td>
</tr>
<tr>
<td>Reactive</td>
<td>377</td>
<td>360†</td>
<td>377</td>
</tr>
<tr>
<td>Sensitivity (%)</td>
<td>100</td>
<td>96.77</td>
<td>100</td>
</tr>
</tbody>
</table>

* Five fewer samples were tested due to volume limitation.
† Among 12 false-negatives: 8 were RIPA confirmed-positive and remaining 4 were positive by two to three other tests (IHA, IFA, and ELISA).
Interestingly, Specimens 1497 and 1660 gave discordant results, because they were repeat-reactive in the PRISM Chagas assay and the recombinant ELISA-II, but negative by ELISA-I and RIPA. The results obtained with the sixth specimen were also discordant in that it was reactive in both ELISAs, but negative by RIPA. The conflicting results obtained with these three specimens puts them in the indeterminate category and raise the question of what is actually known about the sensitivity of the RIPA.

As noted above, assays developed over the years for diagnosing T. cruzi infection had shortcomings in terms of both sensitivity and specificity. Unfortunately, no single test is considered the gold standard for unequivocally diagnosing T. cruzi infection. The RIPA was developed almost two decades ago by one of us (LVK) with the goal of filling this void, and the results presented in its original description, based on 89 T. cruzi antibody–positive samples and 52 diverse controls, including 11 with visceral leishmaniasis, suggested that it is both highly sensitive and specific.12 Since the publication of the latter report, more than 20 articles have appeared that describe projects in which the RIPA was used as a confirmatory assay.5,6,28 It is important to point out, however, that despite this widespread use of the RIPA as a confirmatory test, its sensitivity and specificity have never been systematically validated. Although there are no published reports of parasitologically positive specimens (by xenodiagnosis or hemoculture) being negative in the RIPA, nor of “consensus” positive specimens (in conventional serodiagnostic tests) giving negative RIPA results, it cannot be assumed that the RIPA is 100 percent sensitive in detecting as positive specimens with low levels of anti-T. cruzi antibodies. Our classification, then, of the three discordant specimens in the voluntary donor group (Specimens 1497, 1660, and 5342) as indeterminate is appropriate, and in this context the calculated specificity of the PRISM Chagas assay of 99.94 percent can be viewed as a minimal value. Moreover, it is possible that even a higher specificity may be achieved by modifying the blend of the four antigens used as targets and/or optimization of other technical aspects of the assay. In any event, the results obtained to date, in terms of sensitivity as well as specificity, strongly suggest that the PRISM Chagas assay should function well as a blood screening tool.

REFERENCES


