Evaluation of a new *Trypanosoma cruzi* antibody assay for blood donor screening


**BACKGROUND:** This multicenter prospective study was designed to evaluate the performance characteristics of a new commercially available enzyme-linked immunosorbent assay (ELISA) for the detection of antibodies to *Trypanosoma cruzi* in blood donors, the ORTHO *T. cruzi* ELISA Test System (Ortho-Clinical Diagnostics).

**STUDY DESIGN AND METHODS:** Assay specificity was evaluated among 40,665 serum and ethylenediaminetetraacetate (EDTA) plasma specimens from volunteer blood donors and 481 *T. cruzi* antibody-negative specimens from a high-risk population. Sensitivity was evaluated among 106 *T. cruzi*-infected subjects identified by parasite detection, among 93 radioimmunoprecipitation assay (RIPA)-positive specimens from high-risk subjects, and 662 specimens presumed positive for the presence of *T. cruzi* antibodies by serologic methods. Also assessed were the equivalence of serum and plasma as specimen sources, performance equivalence of automated and semiautomated processing methods, nonspecific reactivity in specimens from other disease states or clinical conditions, and assay precision.

**RESULTS:** Assay specificity was 99.998 percent in volunteer blood donors and 99.4 percent among high-risk subjects. Sensitivity was 100 percent among specimens positive by parasite detection, or by serologic methods, and 98.9 percent among RIPA-positive specimens from high-risk subjects. No differences were demonstrated between serum and plasma or between semiautomated and automated processing methods. Cross-reactivity was observed with known positive leishmaniasis specimens. Total inter- and intraassay variability was less than 10 percent with both the automated and the semiautomated methods.

**CONCLUSION:** The ORTHO *T. cruzi* ELISA Test System is an effective, qualitative assay for screening blood donors for immunoglobulin G antibodies to *T. cruzi*. The assay was licensed for donor screening by the FDA in December 2006.

**ABBREVIATIONS:** IFA = indirect immunofluorescence assay; IR = initially reactive; NR = nonreactive; RIPA = radioimmunoprecipitation assay; RR = repeatedly reactive; S/CO = signal-to-cutoff ratio.

*Trypanosoma cruzi*, the organism that causes Chagas’ disease, is a protozoan parasite endemic to Southern Mexico and Central and South America. Infection, via the vector the triatomine or reduviid bug, is usually chronic, asymptomatic, and generally untreated and may result in fatal cardiac or intestinal muscular failure. Chagas’ disease has long been known to be a transfusion risk in endemic areas and more recently outside the endemic areas.
endemic areas. A recent study documented an almost 1 percent prevalence of antibodies to *T. cruzi* among approximately 7,300 blood donors at five Mexican blood banks. Four of nine surviving recipients of seropositive blood components were infected, confirming the infectivity of such donations. With increased immigration to the United States from endemic countries, the risk of *T. cruzi* entering the US blood supply has also increased. A study by Leiby and coworkers documented that Chagas' disease may be an undiagnosed source of some cardiac failure in the United States. Seroprevalence has been estimated to be between 1 in 5,000 and 1 in 7,700 in areas of the Southern United States and approximately 1 in 25,000 nationwide.5,6

Eradication efforts to reduce the risk of acquisition of *T. cruzi* appear to have decreased the prevalence of antibodies to *T. cruzi* among first time donors in Brazil.7 Similar improvements were observed in Uruguay and Chile.8 Most Hispanic immigrants to the United States, however, come from Central America and Mexico, which continue to have a significant prevalence of seropositivity. A recent review elegantly maps the historically highest prevalence within Mexico to the states of Queretaro, Oaxaca, and Chiapas.9 Most recently, a prevalence of 0.8 percent has been noted in blood donors in Chiapas.3

Because of the relatively low sensitivity and specificity of currently available assays, institutions screening blood donors for *T. cruzi* antibodies in South America have historically used two or more assays to prevent excessive loss of noninfected donors. The combination of tests has often facilitated deferral and counseling by more accurately identifying truly infected prospective donors.10 Recently, confirmatory testing with the radioimmunoprecipitation assay (RIPA) has been shown to demonstrate “equivalent to superior” rates of agreement with indirect immunofluorescence assay (IFA)-positive specimens in comparison to enzyme immunoassay (EIA) screening assays.11 For this reason, the RIPA was chosen as the confirmatory method in the current study.

The purpose of this multicenter study was to establish the performance characteristics of a new blood screening enzyme-linked immunosorbent assay (ELISA), the ORTHO *T. cruzi* ELISA Test System (ORTHO-Clinical Diagnostics, Raritan, NJ) by assessing:

- Specificity among volunteer blood donors;
- Sensitivity in known *T. cruzi* antibody–positive specimens;
- Sensitivity and specificity among subjects at high risk for *T. cruzi* infection;
- Performance equivalence of semiautomated and automated processing methods;
- Equivalence of serum and plasma as specimen sources;
- Rates of nonspecific reactivity in other disease states or clinical conditions;
- Precision.

**MATERIALS AND METHODS**

**Assay procedure and instrumentation**

The ORTHO *T. cruzi* ELISA is a qualitative assay for immunoglobulin G (IgG) antibodies to *T. cruzi* in human serum and plasma. The assay has been described previously.12

The ORTHO *T. cruzi* ELISA is run on a fully automated system (the ORTHO Summit system, consisting of the ORTHO Summit sample handling system, the ORTHO Summit processor, and ORTHO assay software). In several instances, a semiautomated system consisting of the ORTHO Summit sample handling system, AutoReader IV, Autowash 96, Model 120 Incubator and ORTHO assay software was used. An ORTHO assay protocol disk for *T. cruzi* was also used in the testing of samples by both methods. A direct comparison between the two processing methods was conducted during the study.

Table 1 lists the subject-specimen populations from which the 46,975 unique specimens used in the study were drawn. The populations are described in further detail below.

**Volunteer blood donors**

A total of 40,665 specimens were collected from healthy, linked, nonautologous volunteer blood donors at four geographically dispersed blood centers in Minnesota, Oklahoma, Texas, and northern California, 13,742 as serum and 26,923 as ethylenediaminetetraacetic acid (EDTA)-anticoagulated plasma. All subjects signed a study-specific informed consent before donation. Enrollment in the trial was not required for blood donation. Despite the longer consent process and voluntary entry into the study, the majority of eligible donors enrolled. For the three participating centers where donor refusal was documented and quantified, the refusal rates averaged

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**TABLE 1. Populations used in the ORTHO *T. cruzi* ELISA Test System clinical evaluation**

<table>
<thead>
<tr>
<th>Subject/specimen description</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linked, nonautologous volunteer blood donors</td>
<td>40,665</td>
</tr>
<tr>
<td>Unlinked, matched serum-EDTA plasma paired</td>
<td>2,083</td>
</tr>
<tr>
<td>blood donor specimens</td>
<td></td>
</tr>
<tr>
<td>Unlinked serum or plasma blood donor specimens</td>
<td>2,121</td>
</tr>
<tr>
<td>Subjects presumed positive for <em>T. cruzi</em> by parasite detection methods</td>
<td>106</td>
</tr>
<tr>
<td>Subjects presumed positive for <em>T. cruzi</em> antibody by serologic methods</td>
<td>810</td>
</tr>
<tr>
<td>Subjects at high risk for <em>T. cruzi</em> infection</td>
<td>574</td>
</tr>
<tr>
<td>Subjects with potentially cross-reacting infections or clinical conditions</td>
<td>616</td>
</tr>
</tbody>
</table>
19.1 percent (individual rates were 19.6, 19.8, and 17.9%). In addition, 2,083 unlinked specimens were selected as matched serum-EDTA plasma pairs for the serum-plasma comparison and 2,121 unlinked serum or plasma specimens (depending on the collection site) were selected for the comparison of the automated and semiautomated processing methods.

**Presumed positive specimens**

Presumed positive specimens included either prospectively obtained linked specimens or linked or unlinked specimens obtained from researcher or clinician repositories, positive by definitive diagnostic methods delineated below. Documentation of a diagnosis of chronic Chagas’ disease at least 6 months before specimen collection was required. Two subgroups were identified:

**Positive specimens identified by parasite detection methods**

Of the 106 subjects in this group, *T. cruzi* parasites were identified by blood smear (Giemsa) in 4 (3.8%), by xenodiagnosis in 72 (67.9%), and by hemoculture in 30 (28.3%). Twenty-nine specimens (27.3%) originated in Bolivia, 43 (40.6%) in Chile, 30 (28.3%) in Colombia, and 4 (3.8%) in Nicaragua.

**Positive specimens identified by serologic methods**

This group contained 810 specimens with a positive *T. cruzi* antibody result in two or more of the following methods: RIPA, ELISA, IFA, indirect hemagglutination assay, or complement fixation. A total of 144 specimens (17.8%) originated in Bolivia, 200 (24.7%) in Brazil, 86 (10.6%) in Chile, 18 (2.2%) in Guatemala, 263 (32.5%) in Mexico, and 99 (12.2%) in Nicaragua.

**High-risk population**

A total of 574 specimens were obtained from subjects at high risk for *T. cruzi* infection. Specific inclusion criteria required that the subject be a current or prior resident of a Latin American country and had lived in a rural (nonurban) country setting for 1 year or more in an endemic country. A total of 166 specimens (28.9%) originated in Bolivia, 75 (13.1%) in Colombia, 134 (23.3%) in Guatemala, 50 (8.7%) in Mexico, and 149 (26.0%) in Nicaragua.

**Potentially cross-reacting specimens**

The specificity of the ORTHO *T. cruzi* ELISA was further assessed with 616 samples from individuals with infections or clinical conditions that might potentially exhibit cross-reactivity when tested with the assay. Samples from the following populations were included in the testing: leishmaniasis; malaria; schistosomiasis; syphilis; influenza vaccine; paraproteins, autoantibodies, and alloantibodies; and virally infected and other disease states.

**Precision**

The precision of the ORTHO *T. cruzi* ELISA was evaluated incorporating the intraassay (within plate) and interassay (between plate) variation for each processing method (semiautomated and automated). The precision panel consisted of three strongly reactive specimens (>3.000 signal-to-cutoff ratio [S/CO]), three reactive specimens near the cutoff value (1.000-2.000 S/CO), and two nonreactive (NR) specimens (<0.750 S/CO). The study was designed to run 10 replicates of each panel member with each of three kit lots by both semiautomated and automated processing methods. Two operators tested each of the three kit lots over 3 days by both processing methods at each of three volunteer blood donor testing sites. Therefore, by design, 180 observations were collected for each panel member at each of the three sites (total of 540 observations per panel member, 4320 observations among the eight panel members) for both the semiautomated and the automated processing methods.

**Volunteer blood donor testing and confirmatory strategy**

Each volunteer donor specimen in the study was tested with the ORTHO *T. cruzi* ELISA before release of the unit. Specimens with results at or above the cutoff for the assay were considered initially reactive (IR). For purposes of this clinical study, all specimens with S/CO ratios of at least 0.750 were retested in duplicate. The test results in the at least 0.750 to less than 1.500 range were used in the analysis of the cutoff constant.

If the initial screening showed a less than 0.750 S/CO ratio, the specimen was considered NR, the components were released, and the donor was not deferred. In the event that the initial test result was at least 0.750 S/CO, the components were quarantined and the specimen was retested in duplicate. If both repeat tests were NR (<0.750 S/CO), the donor was temporarily deferred pending supplemental RIPA testing. When the supplemental RIPA testing was negative, the donor was reinstated. If either or both of the repeat tests were reactive, the quarantined components were unacceptable for transfusion, the donor was deferred, and a specimen was sent for RIPA testing for counseling purposes. In addition, any repeatedly reactive (RR) specimens that were negative or indeterminate by RIPA were also evaluated for malaria or leishmaniasis by IFA, as described below.
Testing algorithm for presumed positive specimens
All presumed T. cruzi-positive specimens diagnosed by parasite detection methods were tested with the ORTHO T. cruzi ELISA only. For purposes of this clinical study, all specimens with S/CO ratios of at least 0.750 were retested in duplicate. All parasite detection method positive specimens were presumed to have a status of T. cruzi-positive.

All T. cruzi presumed-positive specimens diagnosed by serologic methods were tested with the ORTHO T. cruzi ELISA. All specimens with S/CO ratios of at least 0.750 were retested in duplicate. Any specimen that had an initial result of less than 0.750 S/CO or had two or more results of less than 1.500 S/CO after repeat testing with the ORTHO T. cruzi ELISA was tested by RIPA.

Testing algorithm for high-risk specimens
High-risk specimens were tested with the ORTHO T. cruzi ELISA and with a validated T. cruzi IFA as a comparator method in the absence of a licensed T. cruzi antibody donor screening test. All specimens with S/CO ratios of at least 0.750 were retested in duplicate. Any specimen that was IR (≥0.750 S/CO) with the ORTHO T. cruzi ELISA or was positive with the T. cruzi IFA was tested with RIPA. A specimen that demonstrated discrepant results between the ORTHO T. cruzi ELISA and the T. cruzi IFA or had two or more results of at least 0.750 S/CO with the ORTHO T. cruzi ELISA but was indeterminate or negative with RIPA was tested with a Leishmania IFA and a Plasmodium IFA.

Supplemental testing methods
In the absence of a licensed donor screening comparator T. cruzi assay, a validated RIPA method, performed in the laboratory of L.V. Kirchhoff, was used for supplemental testing.13

IFAs for antibodies to T. cruzi, Leishmania sp., and Plasmodium sp. were performed by Focus Diagnostics (Cypress, CA). The T. cruzi assay used epimastigotes as the solid phase to detect IgG antibodies to T. cruzi. The Leishmania assay used Leishmania organisms as the solid phase to distinguish antibodies to Leishmania donovani, L. braziliensis, L. mexicana, and L. tropica. The Plasmodium IFA used Plasmodium organisms as the solid phase to distinguish antibodies to Plasmodium falciparum, P. vivax, P. malariae, and P. ovale.

RESULTS
Specificity among volunteer blood donors
Assay specificity was estimated in a low-risk, presumably negative-target population of 40,665 serum and EDTA plasma samples from volunteer blood donors. The ORTHO T. cruzi ELISA results for volunteer blood donor specimens are presented in Table 2. There were no IR or RR serum specimens at Sites 1 and 2, yielding a NR rate of 100 percent (13,742/13,742) in serum.

A single EDTA plasma specimen tested at Site 3 was IR and RR with the ORTHO T. cruzi ELISA. A single EDTA plasma specimen tested at Site 4 was IR but not RR. The NR rate for EDTA plasma specimens was 99.993 percent (26,921/26,923), the IR rate was 0.007 percent (2/26,923), and the RR rate was 0.004 percent (1/26,923). Among the 40,665 volunteer blood donor specimens tested, the NR rate was 40,663 of 40,665 or 99.995 percent, the IR rate was 2 of 40,665 or 0.005 percent, and the RR rate was 1 of 40,665 or 0.002 percent.

The only RR specimen gave reactivity just above the cutoff with two of three replicates and reactivity just below the cutoff with one replicate. The specimen was negative by RIPA and was negative for antibodies to Leishmania and Plasmodium spp. with IFA tests. Although questioned extensively, the donor appeared to have no risk factors for T. cruzi infection. The overall specificity of the ORTHO T. cruzi ELISA among volunteer blood donors was 99.998 percent (40,664/40,665; 95% confidence interval [95% CI], 99.986%-100%).

Sensitivity among specimens presumed positive by parasite detection
All 106 specimens presumed positive by parasite detection were IR and RR with the ORTHO T. cruzi ELISA. The overall

<table>
<thead>
<tr>
<th>Test site</th>
<th>Number</th>
<th>Matrix</th>
<th>NR</th>
<th>IR</th>
<th>RR</th>
<th>Ratio IR/RR</th>
<th>Confirmed positive with RIPA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site 1</td>
<td>4,523</td>
<td>Serum</td>
<td>4,523</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>NA†</td>
<td>NA (NA)</td>
</tr>
<tr>
<td>Site 2</td>
<td>9,219</td>
<td>Serum</td>
<td>9,219</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>NA</td>
<td>NA (NA)</td>
</tr>
<tr>
<td>Subtotal serum (n = 13,742)</td>
<td>13,742</td>
<td>(100.0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>NA</td>
<td>NA (NA)</td>
<td></td>
</tr>
<tr>
<td>Site 3</td>
<td>12,118</td>
<td>EDTA plasma</td>
<td>12,117</td>
<td>1 (0.008)</td>
<td>1 (0.008)</td>
<td>1/1</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Site 4</td>
<td>14,805</td>
<td>EDTA plasma</td>
<td>14,804</td>
<td>1 (0.007)</td>
<td>0 (0)</td>
<td>1/0</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Subtotal EDTA plasma (n = 26,923)</td>
<td>26,921</td>
<td>(99.993)</td>
<td>2 (0.007)</td>
<td>1 (0.004)</td>
<td>2/1</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>Total (n = 40,665)</td>
<td>40,663</td>
<td>(99.995)</td>
<td>2 (0.005)</td>
<td>1 (0.002)</td>
<td>2/1</td>
<td>0 (0.0)</td>
<td></td>
</tr>
</tbody>
</table>

* Data are reported as number (%).
† NA = not applicable.
observed sensitivity in this group was 100 percent (106/106; 95% CI, 96.6%-100%).

Reactivity among specimens presumed positive by serologic methods

The 810 specimens presumed positive for *T. cruzi* antibodies by serologic methods were obtained from subjects from endemic countries. Of the 810 specimens, 664 were IR and RR and 146 were NR. This resulted in IR and RR rates of 82.0 percent and a NR rate of 18.0 percent. All 146 NR specimens tested negative with the RIPA assay. In addition, two specimens that were RR with the ORTHO *T. cruzi* ELISA just above the cutoff gave negative results with the RIPA assay.

Of the 148 serologic positive specimens that were RIPA negative, 144 had initial Ortho *T. cruzi* ELISA results in the range of 0.018 S/CO to 0.492 S/CO. These 144 specimens do not appear to belong to the positive population. Of the remaining four specimens, two reacted just below the cutoff and two just above the cutoff (1.000). All 148 RIPA-negative specimens were prospectively collected serum specimens, and 83 had matched EDTA plasma specimens that were randomly included in the study for comparison of serum versus plasma for presumed positive specimens. All 83 matched paired EDTA plasma specimens were also NR with the ORTHO *T. cruzi* ELISA.

The results of the ORTHO *T. cruzi* ELISA and the most probable *T. cruzi* antibody status for the specimens presumed positive by serologic methods are compared in Table 3. The 148 RIPA-negative specimens were considered to have a most probable *T. cruzi* antibody status of negative.

There was no statistical evidence of disagreement between the ORTHO *T. cruzi* ELISA results and *T. cruzi* antibody status for these specimens as assessed by McNemar’s test (p = 0.500). The agreement between the ORTHO *T. cruzi* ELISA and most probable *T. cruzi* antibody status was 100 percent (662/662) for positive samples and 99.8 percent (808/810) overall.

Sensitivity and specificity among specimens in a high-risk population

Sensitivity and specificity were evaluated among 574 specimens from subjects who reside in countries endemic for *T. cruzi* infection. The specimens were tested with both the ORTHO *T. cruzi* ELISA and a *T. cruzi* IFA because no licensed donor screening assay for *T. cruzi* was available during the study, and another assay was required to detect potential *T. cruzi* antibody–positive specimens that had NR ORTHO *T. cruzi* ELISA results. Supplemental RIPA testing was performed either if the ORTHO *T. cruzi* ELISA result was at least 0.750 S/CO or if the *T. cruzi* IFA was positive. Only three specimens of 104 required RIPA testing due to reactivity in the *T. cruzi* IFA alone. All three specimens were negative with RIPA. Thus, the *T. cruzi* IFA did not detect additional *T. cruzi* antibody–positive specimens that the ORTHO *T. cruzi* ELISA did not.

Of the 574 specimens, 97 were IR, 95 were RR, and 477 were NR. This resulted in IR and RR rates of 16.9 and 16.6 percent, respectively, and an NR rate of 83.1 percent. There were 93 specimens testing positive by RIPA among the 574 high-risk specimens, which represents an observed prevalence of 16.2 percent.

The final ORTHO *T. cruzi* ELISA results (NR or RR) and most probable *T. cruzi* antibody status for the 574 high-risk specimens are compared in Table 4. There was no statistical evidence of disagreement between the ORTHO *T. cruzi* ELISA results and *T. cruzi* antibody status as assessed by McNemar’s test (p = 0.625).

Three specimens in the high-risk population gave RR results with the ORTHO *T. cruzi* ELISA and negative results with RIPA. These specimens were classified as *T. cruzi* antibody negative based on the negative RIPA results. A fourth specimen was NR with the ORTHO *T. cruzi* ELISA but had initial reactivity just below the cutoff. The RIPA result for this specimen was positive and the specimen was classified as *T. cruzi* antibody positive based on the RIPA results. The specimens with discordant results are presented in Table 5.

The overall sensitivity of the ORTHO *T. cruzi* ELISA within the high-risk population was 98.9 percent (92/93; 95% CI, 94.2%-100%). The overall specificity within the

<table>
<thead>
<tr>
<th>TABLE 3. ORTHO <em>T. cruzi</em> ELISA results and most probable <em>T. cruzi</em> antibody status in specimens presumed positive by serologic methods</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Observed results</strong></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>RR</td>
</tr>
<tr>
<td>NR</td>
</tr>
<tr>
<td>Total</td>
</tr>
</tbody>
</table>

* *T. cruzi* antibody status was determined by RIPA for specimens with a S/CO of less than 1.500.

<table>
<thead>
<tr>
<th>TABLE 4. ORTHO <em>T. cruzi</em> ELISA results and most probable <em>T. cruzi</em> antibody status in high-risk specimens</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Observed results</strong></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>RR</td>
</tr>
<tr>
<td>NR</td>
</tr>
<tr>
<td>Total</td>
</tr>
</tbody>
</table>
high-risk population was 99.4 percent (478/481; 95% CI, 98.2%-99.9%).

**Matched serum-plasma evaluation**

**Volunteer blood donor specimens**
The performance of the ORTHO T. cruzi ELISA was also compared among 2083 matched serum-EDTA plasma–paired specimens, unlinked to the donors’ identities, at three volunteer blood donor sites (Sites 1, 2, and 3 from Table 2). Testing was performed with the Ortho Summit system processing method (automated testing). All 2083 serum-plasma pairs were NR in the ORTHO T. cruzi ELISA. Clinical equivalence between the S/CO results of the serum and EDTA plasma–paired specimens was established.

**Specimens presumed positive by serologic methods**
The performance of the ORTHO T. cruzi ELISA was compared among a subset of 251 matched serum-EDTA plasma–paired specimens randomly selected from the 810 specimens in this group. Testing was performed at two diagnostic testing sites with the semiautomated processing method and involved initial testing only. The 83 matched pairs with NR ORTHO T. cruzi ELISA results and negative RIPA results were removed, leaving 168 pairs in the study. All 168 matched serum-EDTA plasma–paired specimens were IR with the ORTHO T. cruzi ELISA. Clinical equivalence between the S/CO results of the serum and EDTA plasma paired specimens was established.

**Semiautomated versus automated testing**

**Volunteer blood donor specimens**
Serum or EDTA plasma specimens (depending on the testing site) from 2121 volunteer blood donors, unlinked to the donors’ identities, were tested with both the semiautomated and automated (Ortho Summit system) processing methods at three donor sites (Sites 1, 2, and 3 from Table 2). All 2121 specimens were NR in the ORTHO T. cruzi ELISA with both processing methods. Clinical equivalence between the S/CO results of both processing methods was established.

**Specimens presumed positive by parasite detection**
The performance of the ORTHO T. cruzi ELISA was compared among the 106 parasite detection–positive specimens tested (initial testing only) by both the semiautomated and the automated processing methods at one donor site. All 106 specimens were IR in the ORTHO T. cruzi ELISA with both processing methods. Clinical equivalence between the S/CO results of both processing methods was established.

**Specificity in other disease states or clinical conditions**
A summary of the results of testing 616 specimens from subjects with potentially cross-reacting conditions is presented in Table 6. Among the 100 subjects with *Leishmania* infection, 24 (24.0%) were NR, 76 (76.0%) were IR, and 74 (74.0%) were RR. Although 21 (21.0%) of the specimens were positive by RIPA, the specimens were obtained in India where *T. cruzi* is not endemic and therefore the most probable *T. cruzi* antibody status of the 100 *Leishmania* specimens is negative. The ORTHO T. cruzi ELISA may yield falsely reactive results among test subjects with *Leishmania* infection. These data are consistent with previously reported cross-reactivity between methods testing for antibodies against *Leishmania* species and *T. cruzi*.14-19

Of the 516 non-*Leishmania* specimens, 511 (99.0%) were NR, 5 (1.0%) were IR, and 4 (0.8%) were RR. Two of the 4 RR specimens (one syphilis and one malaria, *P. falciparum*) were RIPA negative. The remaining two of the four RR specimens were obtained from among five test subjects with *Paracoccidioides brasiliensis* infection. These two specimens were RIPA positive and were obtained from a *T. cruzi*-endemic area. Whether these represent false positive for *T. cruzi* infection due to cross-reactivity in both ELISA and RIPA or coinfection with *P. brasiliensis* and *T. cruzi* is not known.

**Precision**
Although 4320 observations were made among the eight panel members for each of the processing methods, there was one observation for panel member R7 that was

<table>
<thead>
<tr>
<th>Specimen ID</th>
<th>Country of origin</th>
<th>T. cruzi ELISA results (S/CO)</th>
<th>RIPA result</th>
<th>Leishmania IFA</th>
<th>Malaria IFA</th>
</tr>
</thead>
<tbody>
<tr>
<td>7312003</td>
<td>Bolivia</td>
<td>2.687</td>
<td>2.674</td>
<td>2.559</td>
<td>Negative</td>
</tr>
<tr>
<td>7312012</td>
<td>Bolivia</td>
<td>2.959</td>
<td>2.946</td>
<td>2.827</td>
<td>Negative</td>
</tr>
<tr>
<td>7319117</td>
<td>Guatemala</td>
<td>5.999</td>
<td>7.177</td>
<td>7.298</td>
<td>Negative†</td>
</tr>
<tr>
<td>7319161</td>
<td>Bolivia</td>
<td>0.870</td>
<td>0.815</td>
<td>0.787</td>
<td>Positive</td>
</tr>
</tbody>
</table>

* Positive for the presence of *P. falciparum*, *P. vivax*, and *P. ovale*.
† Positive for the presence of *L. braziliensis*.
‡ Positive for the presence of *P. falciparum* and *P. vivax*.

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determined to be an outlier. Therefore, a total of 4319 observations per processing method (semiautomated and automated) were included in the analysis.

Mean S/CO, standard deviation (SD), and coefficient of variation (CV%) results are shown in Tables 7 and 8 for the semiautomated and automated (ORTHO Summit processor) methods, respectively. The intraassay (within plate), interassay (between plate), and total precision estimates (CV%) were derived from a variance component analysis with intraassay = MS (plate) and interassay = MS (error), where MS is mean square or variance. The data shown in the tables were rounded after all calculations.

The total CV (intra- and interassay) is the observed precision for a single test on each of two different plates.

For both the semiautomated and automated methods, the S/CO ratios (target S/CO less than 0.750) of the NR panel members (R7 and R8) were NR 100 percent of the time. The S/CO ratios of the strongly reactive panel members R1, R2, and R3 (target S/CO >3.000) and those of the reactive, clinically most important panel members R4, R5, and R6 (target S/CO 1.000-2.000) were reactive 100 percent of the time.

The total within-plate and between-plate precision estimates for the strongly reactive panel members (R1, R2, and R3) ranged between 7.8 and 8.3 percent for the semiautomated method and 7.1 and 7.2 percent for the automated method. The total within-plate and between-plate precision estimates for the reactive panel members (R4, R5, and R6) ranged between 9.4 and 9.7 percent for the semiautomated method and 7.7 and 9.3 percent for the automated method.

**DISCUSSION**

Increasing demands for blood products in the United States combined with ever-stricter donor eligibility...
requirements have resulted in blood donor recruitment strategies that reach out to a more diverse donor pool, including donors who have immigrated from Latin-American countries. Consequently, the risk of transfusion-transmitted T. cruzi infection has also increased. To minimize risk, donors may be deferred for exposure to Chagas' disease or upon identification of risk factors commonly associated with T. cruzi infection.

Questioning of blood donors before donation, however, has been shown to be an imperfect method of reducing risk. In a study with a two-tiered approach with IFAs, indirect hemagglutination assays, and ELISAs have also been used as antigens and synthetic peptides have also been used as antigens consisting of whole-parasite lysates or antigen preparations from cultured epimastigotes and that have shown increasing refinement in sensitivity and specificity. Although recombinant proteins and synthetic peptides have also been used as antigens and may be able to resolve some cases discrepant by other means, sensitivity may be reduced with some applications. IFAs, indirect hemagglutination assays, and ELISAs have been used in diagnostic and blood screening work, although ELISAs are most readily adapted to the high-throughput and short-turnaround-time requirements of blood donor screening. Because the sensitivity and specificity of tests for T. cruzi antibody are not adequate in areas of high prevalence, blood banks in these areas often use a parallel testing strategy with two or even three tests for each donor, an approach clearly not desirable in the high-volume screening environment.

With a T. cruzi seroprevalence rate in the United States believed to be in the range of 1 in 10,000 to 1 in 25,000, assay specificity of 99.99 percent or better is required to ensure that the false-positive rate is no greater than the expected seroprevalence. To adequately assess specificity at that level with any confidence, an unprecedented number of donor samples would need to be tested. In this study, more than 40,000 donors were tested with the ORTHO T. cruzi ELISA with an observed specificity of 99.998 percent, which represents a new standard for donor screening assays in both performance and number of specimens tested. Although up to 20 percent of prospective donors (at the three sites that tracked this data) refused to participate in this study, anecdotally the most common reasons cited were fear of being permanently deferred due to potentially false-positive results with the new assay (i.e., poor specificity) or the time it would take to complete the study-related materials. Because the deferral rates were similar at the three sites, there is no reason to suspect that either donor demographics or selective enforcement of the study biased study results. The high rate of refusal provides insight into donor motivation and the desire to avoid deferral.

Although the specificity of the ORTHO T. cruzi ELISA among volunteer blood donors was extremely high, no confirmed-positive donors were identified in this study, so it was not possible to correlate test results with clinical evaluation of donors or to perform lookback studies. Wider application of this assay will allow these analyses to be performed.

The ORTHO T. cruzi ELISA compares favorably to other T. cruzi assays used in endemic areas and published in the literature. Malan and coworkers reported a comparison of three commercially available assays, finding from 94 to 100 percent agreement across assays and specificities from 93 to 100 percent. A rapid test is available in South America with reported 99.6 percent sensitivity and

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**TABLE 8. Precision with automated (ORTHO Summit processor) processing**

<table>
<thead>
<tr>
<th>Panel member</th>
<th>Number tested</th>
<th>Mean Ortho T. cruzi ELISA S/CO (ratio)</th>
<th>Interassay*</th>
<th>Intraassay†</th>
<th>Total‡</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>SD CV (%)</td>
<td>SD CV (%)</td>
<td>SD CV (%)</td>
<td></td>
</tr>
<tr>
<td>R1</td>
<td>540</td>
<td>4.803</td>
<td>0.118</td>
<td>2.5</td>
<td>0.284</td>
</tr>
<tr>
<td>R2</td>
<td>540</td>
<td>5.104</td>
<td>0.128</td>
<td>2.5</td>
<td>0.321</td>
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<tr>
<td>R3</td>
<td>540</td>
<td>5.284</td>
<td>0.153</td>
<td>2.9</td>
<td>0.305</td>
</tr>
<tr>
<td>R4</td>
<td>540</td>
<td>1.681</td>
<td>0.052</td>
<td>3.1</td>
<td>0.134</td>
</tr>
<tr>
<td>R5</td>
<td>540</td>
<td>1.641</td>
<td>0.060</td>
<td>3.6</td>
<td>0.112</td>
</tr>
<tr>
<td>R6</td>
<td>540</td>
<td>1.871</td>
<td>0.070</td>
<td>3.7</td>
<td>0.110</td>
</tr>
<tr>
<td>R7</td>
<td>539</td>
<td>0.050</td>
<td>0.007</td>
<td>NA§</td>
<td>0.010</td>
</tr>
<tr>
<td>R8</td>
<td>540</td>
<td>0.057</td>
<td>0.007</td>
<td>NA§</td>
<td>0.010</td>
</tr>
</tbody>
</table>

* Between plate (between run [lot x site x technologist]): variability of the assay performance from plate to plate.
† Within plate (between replicate): variability of assay performance from replicate to replicate.
‡ Total: interassay and intraassay variability.
§ % CV are not meaningful when S/CO approaches zero.
99.9 percent specificity, although the study tested many high-risk specimens. In highly endemic regions, however, a single assay may not suffice. Hence, it will be important to evaluate the ORTHO *T. cruzi* ELISA and other newly developed assays to assess whether duplicate assays would no longer be required in the endemic regions.

This study utilized the RIPA, which has been documented to have high sensitivity and specificity in previous studies, for confirmation of *T. cruzi* antibody. In this study, RIPA-negative, RR donor samples were considered to be *T. cruzi* antibody negative and thus not to represent true infection given the low prevalence of highest-risk donors in the areas studied. There are no current plans for licensing the RIPA, which will be available through commercial laboratories.

The implications of a lack of a licensed confirmatory test are twofold. First, and perhaps foremost, the FDA has historically precluded any form of donor reentry or reinstatement pathway in the absence of a licensed confirmatory test. At this juncture, we presume that the FDA will require that the RIPA be used solely for counseling purposes and not for reentry or reinstatement. The fortunate result from this trial is that the observed rate of false-RR results is relatively low when compared to other EIA blood donor screening assays.6

Second, the FDA has allowed the use of an alternate licensed EIA strategy for confirmation, as in the case of human T-lymphotropic virus (HTLV) testing for which no licensed confirmatory assay is available. The donor is only deferred after two RR assay results, although the products from the first RR donation may not be released. Many donor centers choose to reflex RR specimens to repeat testing with an alternative licensed HTLV EIA test on the original positive sample. This allows rapid donor deferral and counseling, instead of requiring a second donation. Although the ORTHO *T. cruzi* ELISA is currently the sole blood donor screening assay for *T. cruzi* antibodies licensed in the United States, there are prospects of an alternative assay being licensed in the future. Hence, an alternative to the costly and longer turnaround time for confirmation via RIPA might be the use of assays from two different manufacturers when a second test is licensed. Although seven donors in the current study (of approximately 40,000 tested) were deferred after an IR assay result, only one with an RR assay result would be indefinitely deferred with the licensed assay criteria in the absence of other licensed *T. cruzi* screening or confirmatory assays. For plasma or platelet donations, the anticipated turnaround time for the RIPA may not support rapid donor notification required to prevent a subsequent donation, and specific donor notification strategies for such donors will need to be developed by each center.

The observation that even the RIPA is not entirely specific and that there may be cross-reactivity of the ORTHO *T. cruzi* ELISA with *Leishmania, malaria, and possibly syphilis add to the challenge of donor counseling. Because donor centers already screen for syphilis, there is less opportunity for confusion. Cross-reactivity for *Leishmania* and malaria, however, may pose greater challenges. Many US soldiers have potential exposure to *Leishmania*, especially Old World cutaneous leishmaniasis. The current practice is to defer blood donors who have traveled to Iraq for 12 months to address the potential for *Leishmania* exposure, so that a deferral for a true exposure might be beneficial. Having access to additional discriminatory testing via the CDC may be of great assistance to blood centers faced with counseling an RR, RIPA-positive blood donor.

Data generated from implementation of the licensed ORTHO *T. cruzi* ELISA Test System will begin to answer issues that remain, such as seroprevalence in the United States and the viability of various screening strategies. These could include testing every donation, testing only donors that answer affirmatively to a screening question, or testing all donors once then retesting donors with affirmative answers to screening questions.

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**REFERENCES**