Field Evaluation of the InBios Chagas Detect Plus Rapid Test in Serum and Whole-Blood Specimens in Bolivia

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Trypanosoma cruzi causes Chagas disease, which affects an estimated 7 million to 8 million people. Chagas disease is endemic throughout Latin America, with the highest prevalence in Bolivia. Conventional diagnosis requires a well-equipped laboratory with experienced personnel. We evaluated the Chagas Detect Plus (CDP) (InBios, Seattle, WA), a rapid immunochromatographic assay for IgG antibodies to *T. cruzi*. CDP performance was compared to infection status based on results obtained by indirect hemagglutination assay, immunofluorescent-antibody test, and enzyme-linked immunosorbent assay. Confirmed infection required positive results by at least 2 conventional assays. We used specimens from adults of both sexes in a general hospital in the city of Santa Cruz and from pregnant women in a hospital and children in villages in the Bolivian Chaco, an area of hyperendemicity. CDP was performed in paired whole-blood and serum specimens from 385 individuals in the two hospital studies and in 200 serum specimens from the community study. CDP showed sensitivities/specificities of 96.2% (95% confidence interval, 92.7 to 98.4)/98.8% (95.9 to 99.9) in whole blood and 99.3% (97.5 to 99.9)/96.9% (94.2 to 98.6) in serum, with no differences by sex, age group, or study site. CDP showed excellent sensitivity and specificity in our study population, comparable to those of conventional serology. The test is reliable for field surveys, requires no laboratory equipment, and performed well in serum and whole blood. The CDP could also be used for accurate maternal screening to identify neonates at risk of congenital transmission. CDP performance data in diverse geographic areas are needed to strengthen the evidence base for its use.

An estimated 7 million to 8 million people are infected with *Trypanosoma cruzi*, the parasite that causes Chagas disease (1). Chagas disease is one of the most deadly of neglected tropical diseases, accounting for 670,000 disability-adjusted life years lost per annum in the Americas (2, 3). Bolivia has the highest infection prevalence in the world (4–6). *T. cruzi* is transmitted predominantly by triatomine vectors. Infection may also result from infected blood transfusion or organ transplant, ingestion of contaminated food or drink, or congenital transmission from mother to fetus (1). Acute infection lasts 4 to 8 weeks and usually causes only mild, nonspecific symptoms, such as fever and fatigue (1, 7). Without treatment, the infection then passes into the lifelong chronic phase. An estimated 30 to 40% of patients develop chronic manifestations of Chagas disease, most commonly cardiac or gastrointestinal abnormalities (1, 7).

In the chronic phase, parasite detection has low sensitivity and diagnosis relies on detection of anti-*T. cruzi* IgG antibodies. Because no single serological assay has sufficient sensitivity and specificity to be used alone, the World Health Organization and other expert committees recommend performing at least two tests based on different antigens and/or formats (7). Specimens with positive results by both assays are considered to represent confirmed infection (7, 8). For specimens with discordant results, the use of a third distinct assay is recommended as a tie breaker.

Current recommendations call for antitrypanosomal treatment of all infected children and for treatment to be offered to most infected adults (1, 7, 8). However, infection is nearly always asymptomatic in the first several decades of the chronic phase and is often asymptomatic throughout life. In the absence of population screening, the infection status of most residents of areas with endemic transmission is unknown. Current norms in areas of endemicity in Latin America also mandate screening all pregnant women for Chagas disease as the first step in detection of infants with congenital *T. cruzi* infection (9). For the purpose of widespread screenings of these types, highly sensitive rapid tests applicable in whole fingerstick blood are ideal (4, 9).

We evaluated the performance of a new rapid test, the InBios Chagas Detect Plus (CDP), in capillary whole blood and serum in hospital and local laboratory settings. The CDP utilizes the multiepitope fusion protein developed and evaluated by InBios for its earlier rapid test (10) with modifications of the test platform to improve sensitivity and allow use of whole-blood specimens. The intent of the rapid test is to serve as a screening test with later confirmation as recommended by the WHO and other organizations. We therefore compared the performance of the CDP with confirmed infection status based on conventional serological assays.

TABLE 1 Characteristics of the study populations and specimens used in this evaluation

Characteristic	Biomarkers study	Congenital Chagas disease study	Community study
Setting	Hospital in Santa Cruz	Hospital in the Chaco	Villages in the Chaco
Population	Adults with and without heart disease	Pregnant women presenting for delivery	Children up to 17 yr
Total no.	108	277	200
Mean age, yr (range)	56.0 (22–83)	24.3 (15–42)	9.5 (2–17)
% Female	50.9	100	47.0
Sample collection dates	April to May 2013	April 2013 to February 2014	September to December 2011
Specimen type(s)	Paired serum and whole blood	Paired serum and whole blood	Archived serum
Positive results (%) by conventional serology[a]	79/108 (73.1)	134/277 (48.4)	79/200 (39.5)

[a] Serological status based on indirect hemagglutination assay, immunofluorescent-antibody test, and enzyme-linked immunosorbent assay. Final status was defined as positive if results were positive by at least two of the three tests and negative if results were positive by none or one of the assays.

MATERIALS AND METHODS

Specimen sources and ethical approvals. Specimens used in the evaluation came from three studies in Santa Cruz Department, Bolivia. For the "biomarkers" study, inpatients, outpatients, and visitors were recruited in San Juan De Dios Hospital in the city of Santa Cruz. Recruitment was designed to yield a study population that included groups of patients without heart disease and with early and advanced heart disease. Following written informed consent, each participant provided two specimens, a fingerstick whole-blood specimen (volume, ~20 μl) and a venous-blood specimen (5 ml). A total of 108 paired sets of specimens were collected from consecutive patients during their hospital appointments for cardiac evaluation in April to May 2013. The biomarkers study protocol was approved by the ethics committees of Johns Hopkins Bloomberg School of Public Health, Asociacion Benefica PRISMA (Lima, Peru), and Universidad Catolica Boliviana (Santa Cruz, Bolivia).

The "congenital Chagas disease" study population comprised pregnant women presenting for delivery at the Camiri Municipal Hospital. Camiri is the capital of Cordillera province in the Bolivian Chaco, an area where the adult prevalence of Chagas disease is over 50% (5, 11). Following written informed consent, each participant provided two specimens, a fingerstick whole-blood specimen (volume, ~20 μl) and a venous-blood specimen (5 ml). A total of 277 sets of paired specimens were collected for the CDP evaluation from April 2013 to February 2014. The congenital Chagas disease protocol was approved by the ethics committees of Johns Hopkins Bloomberg School of Public Health, Asociacion Benefica PRISMA (Lima, Peru), and Universidad Catolica Boliviana (Santa Cruz, Bolivia).

The "community" study was conducted in 2011 to 2012 in villages of Gutierrez municipality, Cordillera province, where Chagas disease is highly endemic (5). At the time of the survey, written informed consent was obtained from the parent or guardian of each child and written assent from children age 7 to 17 years. A 5-ml venous-blood specimen was collected from each child. We evaluated the CDP with 200 archived serum specimens. The only selection criterion was age 2 to 17 years; specimens comprised all pediatric specimens collected from 22 September 2011 to 4 December 2011. The community protocol was approved by CDC, Asociacion Benefica PRISMA, and Universidad Catolica Boliviana.

Specimen handling and storage. In the two prospective evaluations, CDP was run immediately on fingerstick capillary blood. In all three studies, venous-blood specimens were centrifuged, divided into several aliquots, and stored at −20°C until testing in batches.

CDP. The Chagas Detect Plus (CDP) (InBios International Inc., Seattle, WA) is based on the ITC8.2, the same recombinant multiepitope fusion antigen used in an earlier InBios rapid test described previously (10). In contrast to the earlier test, the gold conjugate for the CDP is provided in a liquid form and kept in solution by a preservative, and the test is in a cassette format rather than a strip. As in the earlier assay, the test line is made up of the ITC8.2 fusion antigen, while the control line consists of chicken anti-protein A. All CDP tests were performed by technicians

blinded to the patient's infection and clinical status, following the manufacturer's instructions, as follows. Five microliters of serum or whole blood was applied to the sample pad area of the test cassette, followed by one drop of protein A gold solution. After 5 min, one drop of chase buffer was added to the sample pad area of the test cassette. After 15 min at room temperature, results were interpreted as directed by the product insert. Results were qualitative and were recorded as negative, weak positive, or positive.

Conventional serological testing. Confirmed infection status was based on the consensus results of three conventional assays for anti-*T. cruzi* IgG. All serum specimens were run in an indirect hemagglutination assay (IHA) (Chagas Polychaco kit; Lemos Laboratories, Buenos Aires, Argentina) and an immunofluorescent-antibody assay (IFA) following standard methods (12). The result by IHA was considered positive at dilutions of 1:16 or above. All specimens were also run in a commercial enzyme-linked immunosorbent assay (ELISA) (Wiener Recombinante v3.0 ELISA [reported sensitivity, 99.3%; specificity, 100%] or Wiener parasite lysate ELISA [reported sensitivity, 100%; specificity, 99.6%], both from Wiener Laboratories, Rosario, Argentina). ELISAs were performed following the manufacturer's instructions. The cutoff for both ELISA types was set at 0.300 optical density (OD) unit above the mean absorbance of two negative-control specimens. Confirmation of *T. cruzi* infection followed the recommendations of the WHO and CDC (7, 8). Infection was considered confirmed if the specimen had positive results by two or more conventional assays. Specimens with positive results by none or one of three assays were considered seronegative. If the results of one assay were considered equivocal, concordant results by at least two other assays were required to define the final status of a specimen. The WHO definition is one commonly used in evaluations of rapid tests (13, 14).

Statistical analysis. Sensitivity was defined as the proportion of patients correctly identified as infected by CDP compared to assignment of infection status based on conventional serological testing. Specificity was defined as the proportion of patients correctly identified as uninfected. Exact binomial confidence intervals (CIs) were calculated. McNemar's chi-square test was used to compare sensitivities and specificities between results of the CDP in serum and blood. The kappa statistic was calculated to assess agreement between tests (15). Comparison of the distribution of IFA titers between groups was performed using the two-sample Wilcoxon rank sum test. P values below 0.05 were considered significant.

RESULTS

In total, 385 paired sets of serum and whole-blood specimens were tested, 108 from biomarker study participants and 277 from women in the congenital Chagas disease study (Table 1). Two hundred archived serum specimens from children in the community study were tested. *T. cruzi* infection was confirmed by conventional serological testing in 79 (73.1%), 134 (48.4%), and 79 (39.5%) subjects from the biomarker, congenital Chagas disease,

TABLE 2 Results of individual serological assays and Chagas Detect Plus in serum and whole blood

Conventional assay results[a]			Final serological status[b]	CDP in serum		CDP in whole blood	
IHA	IFA	EIA		Total tested	No. (%) positive	Total tested	No. (%) positive
N	N	N	Negative	283	6 (2.1)	164	2 (1.2)
N	N	P	Negative	1	0	1	0
N	P	N	Negative	6	3 (50)	4	0
P	N	N	Negative	3	0	3	0
N	P	P	Positive	4	3 (75)	3	2 (66.7)
P	P	N	Positive	7	6 (85.7)	7	3 (42.9)
P	P	P	Positive	281	281 (100)	203	200 (98.5)

[a] Serological status based on indirect hemagglutination assay (IHA), immunofluorescent-antibody test (IFA), and enzyme-linked immunosorbent assay (EIA). N, negative; P, positive.
[b] Final status was defined as positive if results were positive by at least two of the three tests and negative if results were positive by none or one of the assays.

and community studies, respectively. Concordance among results of the 3 conventional serological tests was 96.4% (564/585; 283 negative on all 3 tests and 281 positive on all 3 tests) (Table 2). Ten (1.7%) specimens had positive results by 1 of 3 tests and were considered seronegative, whereas 11 (1.8%) specimens had positive results by 2 of 3 tests and were considered seropositive.

Compared to the results of conventional serology, the sensitivity and specificity of CDP were estimated to be 96.2% and 98.8% in whole blood and 99.3% and 96.9% in serum, respectively (Table 3). The test was slightly more sensitive and less specific in serum than in blood, but these differences failed to reach statistical significance (P values by McNemar's exact test = 0.07 and 0.11, respectively). There were no differences in performance by sex, age, or study population (Table 4). There was 95.3% (367/385) concordance between CDP results in serum and in whole blood (kappa = 0.905; P < 0.0001). Two samples tested by CDP in whole blood had weak positive readings; one of these was positive and the other negative by conventional testing. Of the 585 samples tested with CDP in serum, 20 had weak positive readings; 12 (60%) were confirmed positive, and 8 (40%) were confirmed negative. Confirmed seropositive specimens with weak positive or

false-negative CDP results had significantly lower antibody titers based on IFA than those with true-positive CDP results (median of 1:64 versus 1:512; P < 0.0001).

DISCUSSION

Diagnosis and antitrypanosomal treatment, especially of infected children, are crucial actions to control Chagas disease in Bolivia and other countries where it is endemic (7). Screening in the rural areas that harbor the highest Chagas disease burden will require accurate, sensitive, field-friendly rapid tests. In addition, rapid tests will be valuable as a first screen of pregnant women to identify infants at risk of congenital infection. Point-of-care tests do not require specialized equipment or training and give results in minutes rather than hours or days. We evaluated one such test, the new Chagas Detect Plus, in several study populations in Bolivia. This test shows high sensitivity in both serum (99.3%) and whole blood (96.2%), while maintaining high specificity.

A recent multicenter evaluation of 11 rapid tests in serum showed sensitivities ranging from 10.6 to 97.2% and specificities ranging from 70.7 to 97% (14). Several rapid tests have shown high sensitivity and specificity in their initial evaluations (16) but less favorable results when later tested in field trials or against a larger panel of specimens (14, 17). In initial evaluations in serum, sensitivity and specificity were reported to be 98.5% and 94.8% for the Chagas Stat-Pak (16) and 99.2% and 99.1% for the InBios *Trypanosoma*Detect (10). In our 2006 to 2007 screening of pregnant women in Santa Cruz, a study population comparable to those used in the current evaluation, sensitivity and specificity were 87.5% and 100% for Stat-Pak and 90.7% and 100% for *Trypanosoma*Detect (18). The improved results for the new CDP test likely reflect the sensitivity gained by the use of gold in solution. A study of the Stat-Pak rapid test showed 93% sensitivity in whole-blood specimens, compared to reported 98 to 99% sensitivities in studies of the test in serum (13). While we found a similar relative pattern, the sensitivity of CDP was excellent in both serum (99.3%) and whole blood (96.2%).

As in our earlier analysis of the *Trypanosoma*Detect (18), some positive CDP readings were noted to show a weak signal, and weak readings and false-negative readings were significantly more likely to occur when the antibody titers were low. Anti-*T. cruzi* antibody titers tend to be higher in infected populations in Bolivia than in some other tested regions (18), and this implies that evaluations that utilize specimens from Bolivia may overestimate the sensitivity that can be achieved in other populations. For example, we found low antibody titers and very high rates of false-negative

TABLE 3 Performance of Chagas Detect Plus in whole blood and serum compared to confirmed infection status defined by conventional serology

Parameter	CDP in serum		CDP in whole blood	
	No. negative	No. positive	No. negative	No. positive
Conventional serology[a]				
Negative	284	9	170	2
Positive	2	290	8	205
CDP performance				
Kappa statistic (95% CI)	0.962 (0.940, 0.984)		0.948 (0.916, 0.98)	
Sensitivity (95% CI)	99.3 (97.5, 99.9)		96.2 (92.7, 98.4)	
Specificity (95% CI)	96.9 (94.2, 98.6)		98.8 (95.9, 99.9)	
Positive predictive value (95% CI)	97.0 (94.4, 98.6)		99.0 (96.5, 99.9)	
Negative predictive value (95% CI)	99.3 (97.5, 99.9)		95.5 (91.3, 98.0)	

[a] Serological status based on indirect hemagglutination assay, immunofluorescent-antibody test, and enzyme-linked immunosorbent assay. Final status was defined as positive if results were positive by at least two of the three tests and negative if results were positive by none or one of the assays.

TABLE 4 Results of conventional serology and Chagas Detect Plus in serum and whole blood by source study

	No. of specimens									
	Cardiac biomarkers study				Congenital Chagas disease study				Community study	
	Serum (n = 108)		Whole blood (n = 108)		Serum (n = 277)		Whole blood (n = 277)		Serum (n = 200)	
Parameter	Negative	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative	Positive
Standard serology[a]	29	79	29	79	143	134	143	134	121	79
CDP										
Positive	2	79	0	77	6	132	2	128	1	79
Negative	27	0	29	2	137	2	141	6	120	0

[a] Serological status based on indirect hemagglutination assay, immunofluorescent-antibody test, and enzyme-linked immunosorbent assay. Final status was defined as positive if results were positive by at least two of the three tests and negative if results were positive by none or one of the assays.

Stat-Pak and *Trypanosoma*Detect results in Arequipa, Peru (18), and others have found lower sensitivity of the Stat-Pak rapid test in parts of Mexico compared to other locations in Latin America (17). Similar differences in the sensitivities of rapid tests and IgG antibody titers by conventional serology have been shown for visceral leishmaniasis in Sudan compared to India, and they appear to reflect genetic diversity between *Leishmania donovani* strains in the two regions (19, 20). To date, correlation of genetic analyses with antibody response have not been performed for *T. cruzi*. Before widespread implementation, rapid tests need to be evaluated in as many geographically diverse populations as possible. Systematic testing against specimens from patients with potentially cross-reacting diseases such as leishmaniasis is also needed to further establish specificity.

In summary, we found the new Chagas Detect Plus test to have high sensitivity and specificity in both serum and whole blood and to be a field-friendly screening tool to identify individuals to be further tested by confirmatory serological tests (7). The choice of the detection threshold for a fixed test always involves a trade-off between sensitivity and specificity, and the implications depend on the setting. For example, when used as the sole screening test in remote communities, a false-negative test signifies the loss of perhaps the only treatment opportunity for a child. In hospital screening of pregnant women, a blood specimen can easily be collected for later conventional testing. However, in this setting as well, a false-negative test may also mean the loss of that woman to follow-up; only 12% of women with false-negative rapid test results returned for infant follow-up when later informed of their true status based on conventional serology (4). In addition to more geographically diverse validation data, input from Chagas disease control programs and experienced clinicians will be required to inform decisions regarding acceptable false-negative and false-positive rates for the most important potential uses of these tests.

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