

## Short Report: Use of a Rapid Test on Umbilical Cord Blood to Screen for *Trypanosoma cruzi* Infection in Pregnant Women in Argentina, Bolivia, Honduras, and México

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**Abstract.** We conducted a cross-sectional study of Chagas disease in five endemic areas in Argentina, Bolivia, Honduras, and México to estimate the prevalence of *Trypanosoma cruzi*-specific antibodies in pregnant women, and to assess the use of a rapid test (Chagas Stat-Pak) to screen for *T. cruzi* infection at the time of delivery. The prevalence of antibodies to *T. cruzi* measured by enzyme-linked immunosorbent assay (ELISA) in maternal blood was 5.5% (a range of 0.8–28.8% among the countries) in 2,495 women enrolled. Compared with ELISA in maternal blood samples, the Chagas Stat-Pak rapid test sensitivity and specificity in umbilical cord blood were 94.6% and 99.0%, respectively. These results show the ability for a rapid determination of the presence of *T. cruzi*-specific antibodies in umbilical cord blood as a pragmatic strategy to screen for infection in pregnant women.

Chagas disease, or American Trypanosomiasis, is caused by the protozoan parasite *Trypanosoma cruzi*. It is a major cause of morbidity and mortality in Latin America. Infection is transmitted mainly by vectors, but also by transfusion of infected blood or congenital transmission from a mother to her fetus.<sup>1</sup> A strategy to identify maternal infection at delivery will be an asset to select children needing care. Umbilical cord blood is readily available for testing among women delivering in health facilities, and avoids additional venipuncture of the mother. However, the placental transfer of maternal antibodies is not straightforward and can be reduced by infections as described for malaria.<sup>2</sup> Meanwhile, others showed an opposite phenomena during *T. cruzi* infection.<sup>3,4</sup> Furthermore, the mechanisms of maternal antibody transfer are still not fully understood in the case of Chagas disease,<sup>5</sup> and it would be important to evaluate and confirm the usefulness of detecting maternal *T. cruzi* antibodies in cord blood. We conducted a study in selected Latin American countries with different *T. cruzi* endemic situations. Our aims were: 1) to estimate the prevalence of antibodies to *T. cruzi* in pregnant women; and 2) to assess the accuracy of a rapid test (Chagas Stat-Pak, Chembio Diagnostic Systems, Medford, NY) for screening *T. cruzi* infection in pregnant women using umbilical cord blood.

We performed a cross-sectional descriptive study in five hospitals in endemic areas of *T. cruzi* infection in four countries: Instituto Maternidad Provincial “Nuestra Señora de las Mercedes” in Tucumán, Argentina; Instituto Maternológico Percy Boland in Santa Cruz, Bolivia; Hospital Enrique Aguilar Cerrato in Intibucá, Honduras; Hospital Materno-Infantil

in Mérida, Yucatán; and Hospital General in Celaya, Guanajuato in México. Study participants were women presenting for delivery between September 2006 and February 2007 and their newborns. Women  $\geq 18$  years of age at the time of delivery, having single live births, were invited to participate. Maternal and umbilical cord blood samples were obtained from each woman who accepted to participate in the study and signed an informed consent.

Enzyme-linked immunosorbent assay (ELISA) on both maternal and umbilical cord blood samples was performed according to the manufacturer’s instructions for the determination of antibody levels in plasma (Chagatest ELISA recombinant, version 3.0; Wiener Laboratories, Rosario, Argentina).<sup>6</sup> Whole blood of maternal and umbilical cord blood samples were also tested by the Chagas Stat-Pak, which is a rapid immunochromatographic screening test for detection of anti-*T. cruzi* antibodies in whole blood and serum.<sup>7,8</sup> External quality control of ELISA was performed by a central reference laboratory (Wiener Laboratory, Buenos Aires, Argentina) on all positive samples and 10% of the negative samples from Tucumán, Intibucá, Celaya, and Mérida.

Clinical and socio-demographic data were obtained by clinical records or by interviewing the mothers during their hospital stays. All data, including test results, digital photographs of the Chagas Stat-Pak cassette (at 15 minutes of reaction time), and ELISA optical density readouts, were sent by encrypted e-mail to the data center (Unidad de Investigación Clínica y Epidemiológica, Montevideo, Uruguay) for storage and double data entry into a secure data management system.

The maternal prevalence of *T. cruzi*-specific antibodies by site was reported as the prevalence of ELISA sero-reactive women with 95% confidence intervals (95% CI). We also calculated the sensitivity (Se), specificity (Sp), accuracy (Ac), and positive and negative predictive values (PPV and NPV) of Chagas Stat-Pak in umbilical cord samples in comparison

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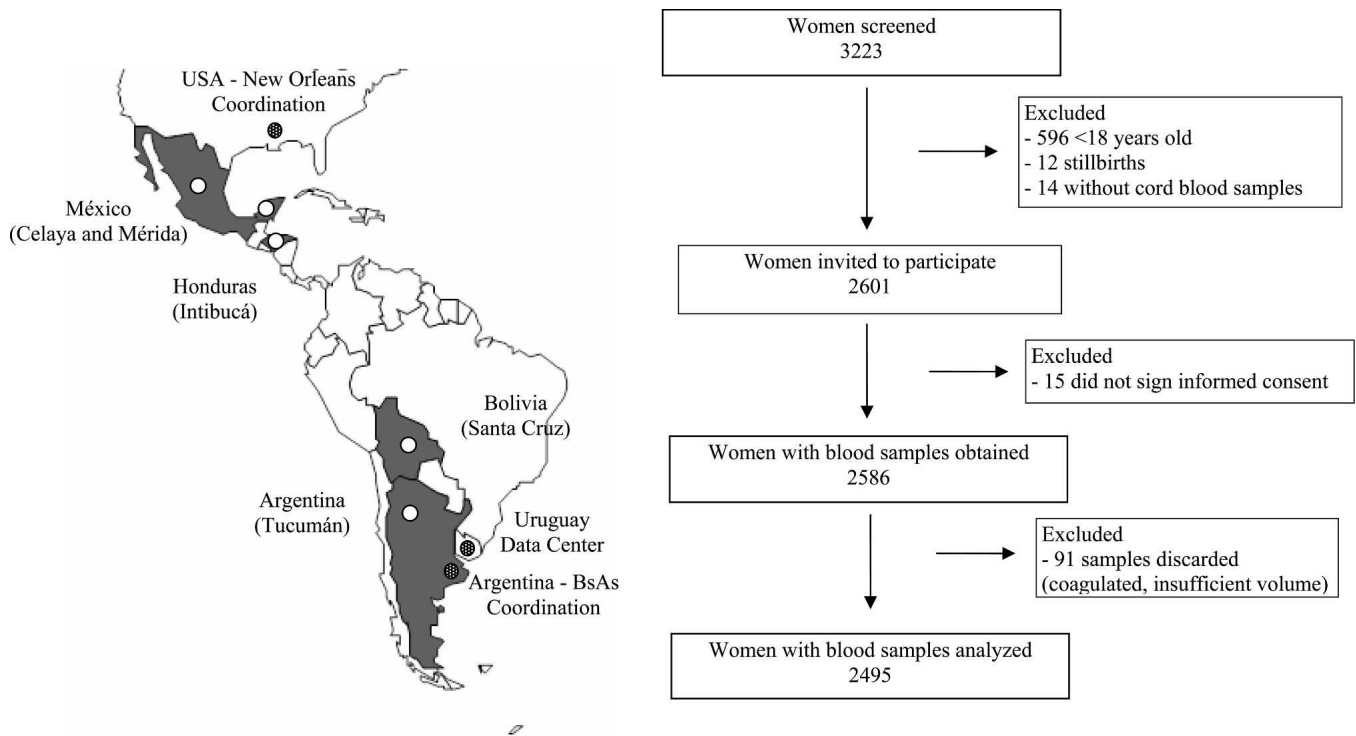


FIGURE 1. Study sites and flowchart of recruitment. Argentina, Bolivia, Honduras, and México, 2006–2007.

with maternal blood using ELISA as the gold standard, with their respective exact binomial 95% CI. A sample size of 500 women per site provided a 95% precision rate of  $\pm 2\%$  if the prevalence was 6%. All tests and procedures were standardized in all sites. The analyses were done using SPSS version 14.0 (SPSS, Chicago, IL) and Epi Info version 3.4 (CDC).

The study was approved by the Institutional Review Board of Tulane University and the ethics committees of the respective participating institutions. Newborns from mothers confirmed positive for *T. cruzi* infection were referred for appropriate clinical follow-up and treatment if needed, according to the established local practices at each site.

Among 2,495 female participants (Figure 1), the distribution of population by site was 518 women in Tucumán, 488 in Santa Cruz, 500 in Intibucá, and 988 in Celaya and Mérida. The mean age was  $25.3 \pm 6.0$  years. Among total women enrolled in the study, 205 volunteers were positive by ELISA for antibodies to *T. cruzi* infection, yielding a median prevalence of 5.5%. The seroprevalence rate was heterogeneous between countries; the highest rate was observed in Santa Cruz (Bolivia) (28.8%), followed by Tucumán (Argentina) (6.6%),

Intibucá (Honduras) (4.4%), and Celaya and Mérida (México) (0.8%) (Table 1). The absorbance of *T. cruzi*-specific antibodies in maternal samples assayed by ELISA showed a strong correlation with that of umbilical cord samples ( $r = 0.986$ ). Concordance among ELISA and Chagas Stat-Pak for maternal and umbilical cord samples were 98% (CI = 97.5–96.6) and 98.6% (CI = 98.1–99.1), respectively. At most sites, the prevalence from samples tested by Chagas Stat-Pak in cord blood were in close agreement with the rates obtained with the gold standard (ELISA in maternal blood). Only results from Intibucá (Honduras) showed a significant difference between maternal rates using ELISA and umbilical cord samples using Stat-Pak ( $P < 0.01$ ), (Table 1). Overall, the performance of the Chagas Stat-Pak for detection of anti-*T. cruzi* antibodies in umbilical cord samples in comparison with the gold standard showed values of 94.6% Se; 99% Sp; 98.6% Ac; 89% PPV; and 99.5% PNV. Test performance varied among countries (Table 2).

All sites used a standardized methodology to collect data and samples, which allowed us to reduce internal bias. However, the results presented in this study have some limitations,

TABLE 1

Prevalence of antibodies against *Trypanosoma cruzi* detected among 2,495 pregnant women and their newborns tested by ELISA and Chagas Stat-Pak, from Argentina, Bolivia, Honduras, and México, 2006–2007

	Tucumán (Argentina) (N = 518)		Santa Cruz (Bolivia) (N = 488)		Intibucá (Honduras) (N = 500)		Celaya and Mérida (México) (N = 988)*		Total (N = 2,495)	
	n	% (95% CI)	n	% (95% CI)	n	% (95% CI)	n	% (95% CI)	n	% (95% CI)
Reactive ELISA in maternal blood samples	34	6.6 (4.7–9.1)	141	28.8 (24.9–33.1)	22	4.4 (2.8–6.7)	8	0.8 (0.4–1.7)	205	8.2 (7.2–9.4)
Reactive Stat-Pak in cord blood samples	32	6.2 (4.3–8.7)	144	29.4 (25.5–33.7)	33	6.6 (4.7–9.2)	9	0.9 (0.4–1.8)	218	8.7 (7.7–9.9)

\* Total population under study in México N = 988 (Celaya N = 488 and Mérida N = 500).

TABLE 2

Detection of *Trypanosoma cruzi*-specific antibodies in cord blood samples tested by a Chagas Stat-Pak rapid test in comparison with the maternal blood samples tested by ELISA, among 2,495 pregnant women from Argentina, Bolivia, Honduras, and México, 2006–2007

Umbilical cord samples tested by Stat-Pak in comparison with maternal blood samples tested by ELISA	Tucumán (Argentina) (N = 518)		Santa Cruz (Bolivia) (N = 488)		Intibucá (Honduras) (N = 500)		Celaya and Mérida (México) (N = 988)		Total (N = 2,495)	
	%	[95% CI]	%	[95% CI]	%	[95% CI]	%	[95% CI]	%	[95% CI]
Sensitivity	85.3	[71.9; 98.7]	98.6	[96.3; 100.0]	95.4	[84.5; 100.0]	62.5	[22.7; 100.0]	94.6	[91.3; 98.0]
Specificity	99.4	[98.6; 100.0]	98.6	[97.2; 100.0]	97.5	[96.0; 99.0]	99.6	[99.2; 100.0]	99.0	[98.5; 99.4]
Accuracy	98.5	[97.3; 99.6]	98.6	[97.4; 99.7]	97.4	[95.9; 98.9]	99.3	[98.7; 99.9]	98.6	[98.1; 99.1]
Positive predictive value	90.6	[79.0; 100.0]	96.5	[93.2; 99.9]	63.6	[45.7; 81.6]	55.6	[17.5; 99.5]	89.0	[84.6; 93.4]
Negative Predictive value	99.0	[98.0; 100.0]	99.4	[98.5; 100.0]	99.8	[99.3; 100.0]	99.7	[99.3; 100.0]	99.5	[99.2; 99.8]

such as the pregnant women who participated may not be representative of pregnant women among the general population. Furthermore, participating hospitals were not randomly selected from among other hospitals in the country or study area, and only women older than 17 years of age were enrolled. For this reason, our results are restricted to women older than 17 years of age that sought care in the study sites selected for convenience. We also lost precision for the estimation of prevalence in Mexico and Honduras, because the measured prevalence was unexpectedly lower than that used for the sample size calculation, which was based on previous estimates of seroprevalence.<sup>9</sup> The external quality control of ELISA in maternal blood samples showed Kappa indexes of 0.83, 0.97, and 0.93 in Tucumán, Intibucá, and Celaya and Mérida, respectively ( $P < 0.05$ ).

Our study recruited women from five sites with some differences in socio-demographic characteristics, such as age, formal education level, and residence in urban or rural areas (data not shown). Differences in the characteristics of the study area,<sup>10–12</sup> the dynamics of vectorial transmission,<sup>13,14</sup> the history of programs for the control and prevention of vectorial and non-vectorial transmission in each country,<sup>15–23</sup> and inter-strain variability of *T. cruzi* may be additional factors to consider when interpreting differences in prevalence.

The Chagas Stat-Pak has been found to be appropriate for screening asymptomatic *T. cruzi* infection in a rapid assessment of schoolchildren.<sup>7,8</sup> However, a field evaluation in children and adolescents from Bolivia has shown lower sensitivity.<sup>24</sup> Our results have shown that the Chagas Stat-Pak was able to detect maternal antibodies in umbilical blood samples. However, its sensitivity was heterogeneous among countries.

Some of the potential explanations for this finding include: 1) Differences in levels of *T. cruzi*-specific antibodies in maternal samples in comparison with umbilical samples. The correlation of the absorbance of specific antibodies in maternal and umbilical cord blood samples were 0.98, 0.54, and 0.05 for concordant samples, ELISA reactive and Stat-Pak not reactive, and ELISA not reactive and Stat-Pak reactive, respectively. These results suggest that in some cases, differences in antibody concentrations in umbilical cord can affect the ability of Stat-Pak to detect it. 2) A different type of antigen used in the assays;<sup>6,7</sup> however, our data do not permit us to clarify this issue. The lower sensitivity of the rapid test in Mexico could be the result of poor detection by the antigens used in the test to the specific immune response induced for the autochthonous strain of parasites. 3) Technical errors while performing or reading the tests. Although the Chagas Stat-Pak test is easy to perform, it uses a visual inspection of bands, which may depend on the subjectivity of the operator.<sup>7,8</sup> The results in the external quality control show that the

validation of the Stat-Pak assay can be affected by the variable performance of the ELISA gold standard at the different study sites.

We have shown in this pilot study a pragmatic strategy using umbilical cord samples and a rapid test to screen *T. cruzi* infection in pregnant women, which permits assessment of results in minutes and would be convenient in primary health care settings. Other large-scale studies would be necessary to confirm the viability of the strategy and to assess cost-effectiveness and acceptability. The potential use of this strategy is based on the fact that the collection of cord blood samples is done routinely in several countries for perinatal screening, including all participating sites, as part of perinatal care, making this proposed strategy even more viable.

Research about the risk of congenital *T. cruzi* infection and the effective detection of infected newborns is essential, because it is recognized as a major cause of transmission in non-endemic countries and endemic countries with relatively successful vector control and blood screening programs.<sup>15,25–28</sup> The elimination of congenital *T. cruzi* infection will be a critical final step toward the elimination of Chagas disease, after elimination of transmission by vectors and blood transfusion.

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