Performance of six diagnostic tests to screen for Chagas disease in blood banks and prevalence of *Trypanosoma cruzi* infection among donors with inconclusive serology screening based on the analysis of epidemiological variables

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Objective: *The frequent occurrence of inconclusive serology in blood banks and the absence of a gold standard test for Chagas' disease led us to examine the efficacy of the blood culture test and five commercial tests (ELISA, IIF, HAI, c-ELISA, rec-ELISA) used in screening blood donors for Chagas disease, as well as to investigate the prevalence of Trypanosoma cruzi infection among donors with inconclusive serology screening in respect to some epidemiological variables.*

Methods: *To obtain estimates of interest we considered a Bayesian latent class model with inclusion of covariates from the logit link.*

Results: *A better performance was observed with some categories of epidemiological variables. In addition, all pairs of tests (excluding the blood culture test) presented as good alternatives for both screening (sensitivity > 99.96% in parallel testing) and for confirmation (specificity > 99.93% in serial testing) of Chagas disease. The prevalence of 13.30% observed in the stratum of donors with inconclusive serology, means that probably most of these are non-reactive serology. In addition, depending on the level of specific epidemiological variables, the absence of infection can be predicted with a probability of 100% in this group from the pairs of tests using parallel testing.*

Conclusion: *The epidemiological variables can lead to improved test results and thus assist in the clarification of inconclusive serology screening results. Moreover, all combinations of pairs using the five commercial tests are good alternatives to confirm results.*

Keywords: *Blood donors; Chagas disease; Sensitivity and specificity; Epidemiologic factors*

Introduction

Chagas disease, originally described by the Brazilian researcher, Carlos Justiniano Ribeiro Chagas, in 1909 is one of the most widely distributed infectious diseases on the American continent, especially in Latin America, with approximately 25 million people living in risk areas. An estimated 10 million individuals are infected worldwide mostly in Latin America[1]. The gradual control of natural transmission, mainly due to eradication of the vector in various countries where the disease is endemic, demonstrates the presence of secondary mechanisms of Chagas disease transmission, especially transfusional transmission[2-5].

Scientific-technical progress in the fight against Chagas disease intensified in the 1980s. Thus, the intensification and standardization of sanitary surveillance measures by public and private blood centers in endemic countries - that began in the late 1960s in some Latin American countries - markedly contributed to a reduction in the frequency of non-negative serology for *Trypanosoma cruzi* among blood donors. While in the 1980s the predominance of seropositive donors in Latin America was 6%, this index dropped to 1.28 % by 2006[2]. Concomitantly, transfusional transmission cases of Chagas disease have occurred in North America, Europe, Japan and Australia[1,4]. Thus Chagas disease, which until recently had been seen as a serious Latin-American public health problem, has become a global public health threat. Especially with respect to the quality control of serological screening for Chagas disease among blood donors, the occurrence of false-positive results may lead to unnecessary disposal of blood units and consequently compromise the blood supply at blood centers. In addition, there are psychological and social consequences for the ineligible donor who believes that he has a stigmatized chronic disease. On the other hand, the occurrence of false-negative results may lead to transfusional transmission of Chagas disease. The greatest challenge currently encountered by blood banks is the frequent occurrence of inconclusive reactions, most of which are observed among non-chagasic donors; this indicates failure in the specificity of screening tests[6-8].

In view of the lack of consensus in the international literature regarding the diagnostic test that correctly classifies a subject as seropositive or seronegative for Chagas disease (100% sensitivity and specificity), numerous researchers have proposed to improve the existing variations of the enzyme-linked immunosorbent assay (ELISA) - one technique recommended

Conflict-of-interest disclosure:
The authors declare no competing financial interest

Submitted: 9/16/2011
Accepted: 6/8/2012

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DOI: 10.5581/1516-8484.20120074

by ANVISA, the Brazilian National Heath Surveillance Agency for screening donors - for example, by replacing natural *T. cruzi* antigens with recombinant antigen mixtures[9-12].

Several investigators have also proposed the inclusion of a clinical-epidemiological chart to assist in the investigation and definition of the true serological profile of ineligible donors, especially those presenting inconclusive reactions[7,13-15]. This chart would include questions regarding the place and type of residence, a family history of Chagas disease, a history of contact with the vector, and a history of blood transfusions and surgery. This procedure has been shown to be effective in the differentiation between donors with or without *T. cruzi* infection when used simultaneously with serological and/or molecular biology tests[7,14], and will certainly be an important tool for the selection and exclusion of blood donors at blood centers in countries or geographic regions where Chagas disease is not endemic[15].

Thus, the goal of this work is to evaluate the efficacy of the blood culture test and five commercial tests (ELISA, IIF, HAI, c-ELISA, rec-ELISA) used in screening blood donors for Chagas disease, as well as to investigate the prevalence of *T. cruzi* infection among donors with inconclusive serology screening in respect to some epidemiological variables.

Methods

The target population of this study was stratified from a total of 95,990 donations based on a retrospective study of the database of the Uberaba Regional Blood Center (URBC) conducted between January 2000 and December 2005. From 269 non-negative donors for Chagas disease (0.28%), 60 participants were randomly selected: Stratum II: 30 donors repeatedly positive using two screening tests (ELISA and IIF) and Stratum III: 30 donors with inconclusive serology in the ELISA screening test or with discordant results in two tests. Additionally 30 donors with more than five negative donations at URBC over a six-year period (Stratum I) were included. This number was fixed due to the difficulty in contacting ineligible donors, especially those ineligible before 2003, which led to sample loss. The main reasons for this loss were changes of address and telephone number (30.4%), residing more than 120 km from Uberaba (26.4%), refusal to participate in the study (7%) and death (2%). All participants answered the following socio-epidemiological questionnaire: type of donor (first time, repeat), age (< 30: > 30 years), gender (male: female), living in an endemic region (yes: no), contact with the vector transmitting Chagas disease (yes: no) and a family history of Chagas disease (yes: no). A 30-mL sample of blood was drawn for each participant to perform five serological tests and a parasitological test.

In addition to the commercial ELISA test (ELISA cruzi® bioMérieux Diagnostica SA, Rio de Janeiro, Brazil), five other immunodiagnostic screening tests were performed in the ninety donors invited to participate in the study including: the commercial ELISA test (ELISA Chagatest® Wiener Lab, Rosario, Argentina); indirect immunofluorescence test (IIF - Imunocruzi®, bioMerieux Diagnostics SA, Rio de Janeiro, Brazil); indirect hemagglutination (IHA - Chagatest®, Wiener Lab, Rosario, Argentina); conventional ELISA (c-ELISA) which uses antigens derived from *T.* cruzi lysate and recombinant ELISA (rec-ELISA) using the recombinant antigens cytoplasmic repetitive antigen (CRA) and flagellar repetitive antigen (FRA) (EIE-Recombinant-Chagas Bio-Manguinhos®, Bio-Manguinhos Laboratory, Oswaldo Cruz Foundation / FIOCRUZ, Brazilian Ministry of Health) and the blood culture test (HEMO), the only test that is known to be 100% specific as it demonstrates the presence of the parasite.

Statistical Analysis

Due to the absence of a gold standard for the diagnosis of Chagas disease, a Bayesian latent class statistical model was considered in this particular case of six diagnostic tests, six covariates in the logit model[16,17] considering the assumption proposed by Hui & Walter[18], with different prevalence rates for chagasic infection but with a similar test performance among strata (SI: negative, SII: positive and SIII: inconclusive serology in the screening). The numerical Bayesian algorithm (Metropolis-Hastings algorithm) and the convergence evaluation were implemented in package R, which is freely available from www.r-project.org. The codes designed for the present study can be requested by e-mail from the authors.

Ethical Aspects

The study was approved by the Ethics Committee of Universidade Federal do Triângulo Mineiro (UFTM - protocol # 464) and all participants signed consent forms.

Results

Table 1 shows the results of the six tests under investigation for each of the three strata according to the result of serological screening at the time of donation. It was observed that 90% of donors with negative and 80% with inconclusive screening results were negative in all six tests investigated. Among the 30 donors with positive screening results, 96.6% had five or six positive results in the tests under investigation.

On analyzing the performance of tests without considering the covariates and the blood culture test, the general sensitivity between tests is very similar and higher than 97.63%. Two tests (IHA and IIF) were more specific with rates of 98.48% and 98.52%, respectively (Table 2).

There was an increase in these rates when the covariates were taken into account, for example, all tests had sensitivities equal to or greater than 99.05% for donors older than 30 years and with a history of Chagas disease in the family; the ELISA, IIF and IHA tests had specificity rates above 99.05% for these same categories (Table 2).

Despite the very similar nominal values, the sensitivity rates of all serological tests (ELISA, IIF, IHA, c-ELISA and rec-ELISA) were higher in over 30-year-old donors, females, who came from an endemic region, who had had contact with the vector and had a family history of Chagas disease. The IIF, IHA, c-ELISA and rec-ELISA tests were found to be more specific in repeat donors, over 30-year-old donors, females, who came from an endemic region, who had not had contact with the vector and had a family history of Chagas disease. In contrast, the ELISA test was more specific in first time donors who had had contact with the vector transmitting the disease (Table 2).

Table 1 - Results of the tests under investigation according to the result at the time of screening test

Tests under investigation						Screening results		
						Negative	Positive	Inconclusive
ELISA	IIF	IHA	HEMO	c-ELISA	rec-ELISA	n(%)	n(%)	n(%)
-	-	-	-	-	-	27(90.0)	0 (0)	24 (80.0)
-	-	-	-	+	-	2 (6.7)	0 (0)	5 (16.7)
+	-	-	-	-	-	1 (3.3)	0 (0)	1 (3.3)
+	+	-	-	+	-	0 (0)	1 (3.3)	0 (0)
+	+	+	-	+	+	0 (0)	17(56.7)	0 (0)
+	+	+	+	+	+	0 (0)	12 (40.0)	0 (0)
					Total	30 (100)	30 (100)	30 (100)

ELISA: enzyme-linked immunosorbent assay; IIF: indirect immunofluorescence; IHA: indirect hemagglutination; HEMO: blood culture; c-ELISA: conventional ELISA using the Bio-Manguinhos kit; rec-ELISA (recombinant ELISA using the Bio-Manguinhos-FIOCRUZ kit and CRA and FRA antigens)

Table 2 - Sensitivity (%) and specificity (%) of the six tests under investigation according to each level of the six covariates involved

Sensitivity (%)

Covariate	ELISA		IIF		IHA		HEMO		c-ELISA		rec-ELISA	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
General	97.76	0.88	97.64	0.93	97.70	0.85	58.07	0.80	97.63	0.93	97.76	0.90
First time donor	98.23	0.70	98.19	0.73	98.23	0.66	57.75	0.80	98.14	0.73	98.27	0.70
Repeat donor	97.17	1.11	96.94	1.20	97.01	1.11	58.38	0.79	96.98	1.17	97.11	1.15
Age < 30 years	94.53	2.13	94.17	2.23	94.29	2.08	55.71	0.84	94.17	2.21	94.41	2.21
Age > 30 years	99.10	0.37	99.06	0.39	99.09	0.36	60.38	0.82	99.05	0.39	99.12	0.37
Male	95.21	1.86	94.84	2.02	94.98	1.84	58.57	0.79	94.96	1.93	95.15	1.89
Female	98.96	0.43	98.94	0.44	98.96	0.40	59.57	0.80	98.90	0.45	98.98	0.43
Endemic region												
No	95.67	1.70	95.38	1.80	95.50	1.66	56.91	0.81	95.43	1.77	95.61	1.73
Yes	98.85	0.47	98.81	0.49	98.83	0.45	59.22	0.80	98.78	0.49	98.87	0.47
Contact with vector												
No	96.54	1.35	96.31	1.44	96.39	1.33	56.33	0.82	96.29	1.44	96.50	1.39
Yes	98.55	0.58	98.50	0.60	98.54	0.55	59.78	0.81	98.49	0.60	98.58	0.58
Family history												
No	94.44	2.16	94.03	2.31	94.20	2.13	56.01	0.83	94.21	2.23	94.30	2.26
Yes	99.11	0.37	99.09	0.38	99.10	0.35	60.09	0.81	99.05	0.39	99.14	0.36

Specificity (%)

Covariate	ELISA		IIF		IHA		HEMO		c-ELISA		rec-ELISA	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
General	97.25	0.99	98.52	0.66	98.48	0.67	100	0	97.71	0.89	97.77	0.83
First time donor	97.56	0.88	97.93	0.91	97.74	0.99	100	0	96.22	1.46	96.38	1.33
Repeat donor	96.90	1.11	98.95	0.47	98.98	0.45	100	0	98.62	0.55	98.63	0.51
Age < 30 years	92.18	2.84	95.51	2.00	95.48	2.02	100	0	95.53	1.73	95.63	1.61
Age > 30 years	99.06	0.36	99.52	0.22	99.50	0.23	100	0	98.83	0.47	98.87	0.43
Male	93.83	2.20	95.98	1.77	96.07	1.74	100	0	96.05	1.52	96.13	1.43
Female	98.79	0.46	99.46	0.25	99.42	0.27	100	0	98.68	0.53	98.72	0.49
Endemic region												
No	94.28	2.05	96.72	1.45	96.69	1.47	100	0	95.88	1.57	95.93	1.50
Yes	98.70	0.48	99.34	0.30	99.31	0.31	100	0	98.73	0.51	98.78	0.46
Contact with vector												
No	96.02	1.42	98.96	0.47	98.97	0.46	100	0	98.20	0.71	98.24	0.66
Yes	98.11	0.69	97.90	0.93	97.76	0.98	100	0	97.09	1.13	97.17	1.04
Family history												
No	91.66	2.98	95.11	2.18	95.10	2.13	100	0	93.63	2.43	93.70	2.37
Yes	99.12	0.35	99.56	0.21	99.54	0.22	100	0	99.19	0.34	99.22	0.30

SD: Standard Deviation; ELISA: enzyme-linked immunosorbent assay; IIF: indirect immunofluorescence; IHA: indirect hemagglutination; HEMO: blood culture; c-ELISA: conventional ELISA using the Bio-Manguinhos kit; rec-ELISA (recombinant ELISA using the Bio-Manguinhos-FIOCRUZ kit and CRA and FRA antigens)

With respect to the prevalence of Chagas disease, an overall estimate of 13.30% was observed among donors with inconclusive serology in the screening test at the time of blood donation but this rate varied considerably depending on the covariates (Table 3).

When we consider testing in series which is suitable for situations that require greater specificity, where the diagnostic result is positive if all tests have positive results, we find a specificity of over 99.93% for all pairs of tests (not including the blood culture test) and 100% when we consider the ELISA, IIF, IHA tests together. While, the parallel scheme, indicated for urgent cases or for quality control as in blood banks, in which the set of tests is considered to be positive when at least one of the tests is positive, we found a sensitivity of over 99.96% for all pairs of tests (not including the blood culture test) and 100% when we consider ELISA, IIF, IHA tests together (Table 4).

The predictive values (positive and negative) of all combinations of pairs of the five serological tests (ELISA, IIF, HAI, c-ELISA, rec-ELISA) confirm the presence of *T. cruzi* infection with a probability of 100% in the stratum of donors with positive serology screening when at least one of two tests has a positive result. In the stratum of donors with negative serology screening results, it is possible to affirm the absence of Chagas disease with a probability of 100% when at least one of two tests shows negative results. However, in the stratum of donors with inconclusive serology it is possible to confirm the absence of Chagas disease with a probability of 100% when the two tests have negative results depending on the epidemiological variables (Table 5).

Table 3 - Prevalence rates (%) of Chagas disease for donors with inconclusive serology (Stratum III), according to the six covariates

	Prevalence	
Covariate	**Mean**	**SD**
General	13.30	0.22
First time donor	15.22	0.25
Repeat donor	11.59	0.20
Age < 30 years	9.23	0.16
Age > 30 years	18.79	0.30
Male	9.90	0.17
Female	17.64	0.28
Endemic region		
No	13.73	0.00
Yes	15.71	0.00
Contact with vector		
No	11.98	0.00
Yes	19.38	0.00
Family history		
No	9.55	0.00
Yes	18.19	0.00

SD: Standard Deviation

Table 4 - Sensitivity (%) and specificity (%) of the five tests under investigation (not including the blood culture test) with parallel and serial testing schemes

	Parallel				Serial			
	Sensitivity		Specificity		Sensitivity		Specificity	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
ELISA + IIF	99.96	0.06	95.23	2.33	95.98	2.44	99.94	0.06
ELISA + IHA	99.96	0.07	95.13	2.38	95.94	2.50	99.94	0.07
ELISA + c-ELISA	99.96	0.06	94.61	2.48	95.97	2.41	99.93	0.07
ELISA + rec-ELISA	99.96	0.05	94.70	2.46	96.10	2.30	99.93	0.06
IIF + IHA	99.96	0.06	95.72	1.92	95.87	2.46	99.95	0.05
IIF + c-ELISA	99.96	0.07	95.20	2.06	95.91	2.40	99.94	0.05
IIF + rec-ELISA	99.96	0.05	95.29	2.05	96.04	2.32	99.94	0.05
IHA + c-ELISA	99.96	0.05	95.10	2.10	95.86	2.39	99.94	0.06
IHA + rec-ELISA	99.96	0.06	95.20	2.12	96.00	2.35	99.94	0.06
c-ELISA + rec-ELISA	99.96	0.05	94.68	2.25	96.03	2.27	99.93	0.07
ELISA + IIF + IHA	100	0.00	94.41	2.01	93.23	2.00	100	0.00

SD: Standard Deviation; ELISA: enzyme-linked immunosorbent assay; IIF: indirect immunofluorescence; IHA: indirect hemagglutination; HEMO: blood culture; c-ELISA: conventional ELISA using the Bio-Manguinhos kit; rec-ELISA (recombinant ELISA using the Bio-Manguinhos-FIOCRUZ kit and CRA and FRA antigens)

Table 5 - Negative predictive value (%) for three pairs of tests according to the parallel and serial testing schemes as well as epidemiological variables for donors with inconclusive serology screening

	Parallel						Serial					
	ELISA/c-ELISA		ELISA/rec-ELISA		c-ELISA/rec-ELISA		ELISA/c-ELISA		ELISA/rec-ELISA		c-ELISA/rec-ELISA	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
General	99.98	0.03	99.98	0.02	99.98	0.02	98.36	1.11	98.42	1.01	98.38	1.06
First time donor	99.99	0.01	99.99	0.01	99.99	0.02	99.41	0.55	99.42	0.56	99.40	0.58
Repeat donor	99.99	0.06	99.99	0.03	100	0.03	98.86	1.99	98.93	1.75	98.95	1.73
Age < 30 years	99.63	0.58	99.67	0.43	99.66	0.45	89.82	5.67	90.17	5.42	90.01	5.45
Age > 30 years	100	0.00	100	0.00	100	0.00	99.82	0.23	99.82	0.22	99.81	0.25
Male	99.96	0.09	99.96	0.05	99.96	0.05	96.93	2.24	97.07	2.00	97.04	2.08
Female	100	0.00	100	0.00	100	0.00	99.84	0.18	99.84	0.20	99.83	0.20
Endemic region												
No	99.93	0.10	99.94	0.08	99.94	0.08	97.11	1.70	97.19	1.65	97.20	1.50
Yes	99.99	0.03	99.99	0.02	99.99	0.02	98.67	1.16	98.72	1.04	98.67	1.14
Contact with vector												
No	99.96	0.09	99.96	0.06	99.96	0.06	96.86	2.54	96.95	2.32	96.88	2.43
Yes	100	0.00	100	0.00	100	0.00	99.82	0.19	99.83	0.18	99.82	0.19
Family history												
No	99.90	0.15	99.91	0.12	99.90	0.12	93.80	3.55	94.00	3.44	93.91	3.40
Yes	100	0.01	100	0.00	100	0.00	99.77	0.33	99.78	0.27	99.77	0.33

SD: Standard Deviation

Discussion

In the present study, the general sensitivity rates were 97.76%, 97.70% and 97.64% and the specificity was 97.25%, 98.48% and 98.52% for the ELISA, IHA and IIF tests, respectively. These results show performances similar to those found by Langui Junior et al.[19] but the sensitivity of IHA testing was higher in the present study. Studies on screening tests for Chagas disease reported sensitivity rates for the ELISA kit of bioMérieux ranging from 98.38% to 98.5% and specificity rates from 94.30% to 99.93%.[20,21] Sensitivity rates ranging from 52.75% to 96.5% and specificity from 87% to 100%[22-24] have been described for IIF. Sensitivity rates from 98.5% to 100% and specificity between 99.69% and 100% have been reported for IHA.[19,20] Regarding the Bio-Manguinhos-FIOCRUZ kits, Gadelha et al.[22] found 100% sensitivity and specificity for the c-ELISA kit and sensitivity of 98.2% for the rec-ELISA kit.

Hence, it is essential to continually assess the performance of commercial tests that are still not totally efficacious. Failures in Chagas disease screening, may occur due to cross reactions with other parasites, in particular those of the trypanosomatid genus such as Leishmania which may have many genetic similarities with *T. cruzi* antigens and the same epitopes to bind to antibodies present in the sera of infected patients[20,25].

The publication of government directives numbers 153 and 57 by ANVISA[26,27], made a single technique for the serological screening of blood donors for Chagas disease mandatory and that the blood bank must participate in external quality control programs. Thus it is important to seek screening methods by investigating specific *T. cruzi* antigens which minimize or exclude cross reactions.

Cross reactions in blood banks may be a cause of persistent serological ineligibility due to Chagas disease; this may correspond to up to 80% of inconclusive or false-positive serological results.[8,11,20,28,29] Many studies report that inconclusive samples repeatedly showed negative results on using other tests.[11,26]

As, in this study, the estimated prevalence of Chagas disease was 13.30% in the stratum of donors with inconclusive serology at screening, 86.70% of donors probably do not have Chagas infection. This prevalence rate is well below the rate reported by Furuchó et al.[10] who observed that 20.5% of donors in the inconclusive serology group were positive for Chagas disease. In addition, on considering epidemiological variables in this stratum, the absence of Chagas' infection can be affirmed with a probability of 100% when two tests give negative results in all pairs of serological tests.

Studies in epidemiology and statistics show that an association of two tests increases the quality of diagnosis, thereby reducing the number of false results. The simplest way of forming a set of tests is by using in series or in parallel design[30] as used in this study.

Although there is no individual screening test for Chagas disease that has a sensitivity of 100%, sensitivity levels of 99.96% were found for several sets of tests in the parallel analysis. Additionally all pairs of tests (excluding HEMO) had specificities in series testing greater than 99.93% and the set formed by the ELISA, IIF and IHA tests had specificities of 100%, suggesting that this is the best alternative to confirm procedures.

Studies report that the combination of epidemiological data with results of high performance testing allows a more accurate view of the serologic status of donors[10,11]. To confirm these data, we find estimates for the sensitivity and specificity close to 100% for certain epidemiological covariates suggesting that it is important to consider the inclusion of covariates in the structure of the model to evaluate the performance of the diagnostic tests.

In conclusion, commercial diagnostic tests (ELISA, IIF, HAI, c-ELISA, rec-ELISA) used to screen for Chagas disease, when used in pairs in serial testing schemes, proved to be an interesting alternative to confirm the procedure. In addition, epidemiological variables may contribute to improve the results of these tests and to clarify the true meaning of inconclusive serology screening. In search of a gold standard procedure and more reliable estimates, further studies are necessary with larger samples, clinical variables and further assume dependence between tests in statistical modeling.

Acknowledgements

We thank the Fundação Hemominas for providing the data and the Brazilian funding agencies Capes, ANVISA and CNPq for financial support. We thank the Laboratory Bio-Manguinhos, especially Antônio Gomes Pinto, Yara de Miranda Gomes, Edimilson Domingos da Silva and Marcelle Bral de Mello for providing the c-ELISA and rec-ELISA kits as well as processing of samples from the respective techniques. We also thank the anonymous reviewers for their comments and criticisms.

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