The validity of serologic tests for *Trypanosoma cruzi* and the effectiveness of transfusional screening strategies in a hyperendemic region

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BACKGROUND: This study aims at obtaining unbiased estimates of the sensitivity and specificity of existing screening tests for *Trypanosoma cruzi* and at simulating the effectiveness of alternative screening strategies at different prevalence rates.

STUDY DESIGN AND METHODS: A systematic random sample of 400 was taken from 1200 banked serum samples of donors screened between August 1998 and January 1999 in Santa Cruz, Bolivia. Samples were tested with indirect hemagglutination test (IHA), indirect immunofluorescence assay (IFA), and four enzyme-linked immunosorbent assays (ELISAs). Sensitivity and specificity of tests were estimated through latent class analysis.

RESULTS: The sensitivity of individual tests ranged from 96.5 to 100 percent, and their specificity from 87.0 to 98.9 percent. Combinations of two tests used in parallel would, even at 40 percent prevalence, only miss approximately 1 infected unit per 10,000 screened. At 5 percent prevalence, however, they would yield 75 to 120 false-positive units per 1000 units screened. Parallel testing with IHA plus ELISA or with IHA plus IFA is marginally more cost-effective, compared to single IHA testing, than single ELISA or single IFA testing, regardless of the *T. cruzi* prevalence.

CONCLUSIONS: Routine blood donor screening for *T. cruzi* with a single test results in unacceptable numbers of false-negative samples in highly endemic areas or in at risk population groups. Adding a second test seems mandatory, but which one to choose depends on local cost components and feasibility.

The prevalence rates of *Trypanosoma cruzi* infection in blood donors in Latin America show a downward trend over the past decades, but there are still wide variations across the region and even within countries.[1] Santa Cruz, Bolivia, the main city in the lowlands, reported a seroprevalence of 51 percent whereas the capital La Paz found 5 percent of its blood donors infected.[2] The overall risk for acquiring a transfusion-transmitted infectious disease in Bolivia was estimated at 233 per 10,000 transfusions for 1993, with *T. cruzi* accounting for 94 percent of the infections.[3] This estimate resulted from the high overall prevalence of *T. cruzi* infection in the population (14.7%) and the low coverage of screening activities (29.4% of donors). Growing awareness of this problem led in 1996 to the adoption of a law that makes screening of blood donations for *T. cruzi* mandatory.

Serologic screening of blood donors is, indeed, the most important strategy to secure blood safety for Chagas' disease in a hyperendemic region, possibly combined

ABBREVIATIONS: IFA = indirect immunofluorescence assay; IHA = indirect hemagglutination test; LCA = latent class analysis; RBB = Regional Blood Bank.

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with chemical treatment of positive blood units when the availability of blood gets compromised.[4] Recently the US Red Cross also announced its plans to screen all donated blood for *T. cruzi* in spite of a low risk of infection.[5] The choice of a particular screening test or strategy, however, is far from straightforward. The conventional serologic tests for *T. cruzi* suffer from too low sensitivity and specificity, the latter being due to cross-reactions with other pathogens.[6] Higher specificities are possibly obtained with tests based on recombinant antigens.[7,8] The absence of a gold standard for assessing *T. cruzi* infection in asymptomatic subjects, however, hinders the rigorous evaluation of these new diagnostic tools. Parasitologic tests lack sensitivity and their use as a reference for validation of new tests leads to biased specificity estimates.[9,10] Additionally, between-laboratory reproducibility of serodiagnosis was found to be poor.[11]

A WHO expert committee recommended in 1991 the use of either a single indirect hemagglutination test (IHA; cutoff, 1:8 dilution) or a single latex agglutination test for donor screening.[4] In 1994, in line with several authors,[12,13] the Pan American Health Organization advocated the parallel use of at least two different serologic tests.[14] Various Latin American countries with low *T. cruzi* endemicity have now adopted this two-test screening strategy as their national policy.[15,16] The sensitivity of a strategy with complement fixation and IHA, however, has been reported to be variable, and it was suggested that the cost and benefits of the use of the enzyme-linked immunosorbent assay (ELISA) technique in conjunction with IHA or indirect immunofluorescence assay (IFA) be studied.[12] In Argentina, moreover, the cost-effectiveness of adding a second test with an estimated marginal cost of US$9811 for 1996 was questioned.[16] A recent WHO technical report recommends for blood-bank screening a single ELISA test, assumed to have approximately 99 percent sensitivity.[17] Through this study we aimed at obtaining unbiased estimates of the sensitivity and the specificity of IHA, IFA, and different ELISA tests and at assessing, with particular reference to hyperendemic areas, the effectiveness of *T. cruzi* screening strategies that use two different serologic tests.

MATERIALS AND METHODS

Setting

Santa Cruz de la Sierra is a city situated in the tropical lowland areas of Bolivia with approximately 1 million inhabitants. The population grows explosively owing to rural-urban migration, partly from *T. cruzi* endemic areas. Vector-borne transmission is no longer an issue in the town, but the prevalence of *T. cruzi* infection in blood donors was estimated at 62 percent (174/280) in 1982[18] and at 51 percent (105/206) in 1990.[2] The regional blood bank (RBB) opted, in line with WHO recommendations,[4] for a screening strategy based on a single IHA. Positive units were chemically treated with the dye crystal violet.[19] The National Center for Tropical Diseases (CENETROP) is responsible for the external quality control (QC) of the serology laboratory of the RBB. CENETROP participates in the Pan American Health Organization's blood safety program and is subject to external QC by Hemocentro, Sao Paulo, Brazil.

Patients

A systematic random sample of 400 was taken from a consecutive series of 1200 banked serum samples of blood donors screened by the RBB of Santa Cruz between August 1998 and January 1999. The IHA routine results obtained by the RBB and the variables age, sex, residence, and voluntary nature of donation were extracted from the records. The corresponding serum samples which had been stored at –20°C at the RBB, were unfrozen and divided into several aliquots and blindly retested with a panel of serologic tests described below. For four samples there was not enough serum to process the complete set of serologic tests. Anonymity and data confidentiality were assured throughout the study.

Serologic tests

The RBB routinely used a single IHA, based on the Averbach-Yanovsky technique and provided as a commercial kit by Polychaco, Buenos Aires, Argentina. The cutoff titer for a positive IHA was 1:8. IHA was repeated with the same method at the CENETROP laboratory.

For the IFA, antigen was prepared in CENETROP according to the method described by Alvarez and coworkers[20] from log-phase *T. cruzi* epimastigotes cultured in Lit medium. Fluorescent-labeled anti-human immunoglobulin conjugate (BioMérieux, Paris, France) was used. The cutoff titer for positivity was 1:32.

Four different indirect ELISAs were used, two with crude antigen and two with recombinant antigen. Crude ELISA 1 was performed according to the method described by Cura and colleagues[21] with lyophilized crude antigen produced at the National Institute for the Diagnosis and Investigation of Chagas Disease "Dr Mario Fatal Chaben" (INDIECH), Buenos Aires, Argentina. Flat-bottomed microplates (Evergreen Scientific, Los Angeles, CA) were sensitized with the antigen (10 µg/mL) and incubated with patient serum samples at 1:200 dilution. Stable immune complexes were detected by incubation with peroxidase-labeled goat anti-human immunoglobulin G. The enzyme reaction was carried out with ABTS and H_2O_2 as substrate (Kirkegaard-Perry Laboratories, Gaithersburg, MD). Crude ELISA 2 was a commercial kit obtained from Gull Laboratories Inc. (Salt Lake City, UT). Recombinant ELISA 1 was a third-generation ELISA

(Chagatest, Wiener Laboratories, Rosario, Argentina) and recombinant ELISA 2 was obtained from BIOSChile, Santiago, Chile (second-generation ELISA for Chagas disease).

For QC in this study we used a panel of well-characterized control serum samples. Positive controls consisted of 12 positive serum samples from Bolivian patients with positive xenodiagnosis for *T. cruzi* and three positive serum samples provided by the blood bank of Sao Paulo (Hemocentro, Sao Paulo, Brazil). The negative control sera were 12 negative serum samples from Belgian blood donors who had not traveled to endemic areas and three negative control samples provided by the same Sao Paulo blood bank. All tests classified all control samples correctly.

Statistical analysis

Sensitivity and specificity. We estimated the sensitivity and specificity of each single test with latent class analysis (LCA).[10,22] LCA is a mathematical modeling technique based on the assumption that a nonobserved (latent) variable is determining the associations between observed categorical variables. In this case, infection with *T. cruzi* is the latent variable in a two-latent-class model that assumes the sample of sera to be composed of two mutually exclusive and exhaustive groups: the donors infected and those not infected. This latent variable explains the observed response patterns for the serologic tests. Whereas the true prevalence of infection and the true sensitivity and the specificity of each single test cannot be directly observed, they can be estimated with LCA from the observed frequencies of the different test patterns. This approach has been successfully used to obtain unbiased estimates of the sensitivity and specificity of serologic tests for *Leishmania* sp. infections, leptospirose, pneumococcal pneumonia, and human herpesvirus 8 seroprevalence among blood donors.[23-27] In basic latent class models, the observed variables (test results) are assumed to be independent conditional on latent class (infection); that is, there are no associations between the observed variables within each category of the latent variable. More advanced models exist, where this condition is relaxed.[28,29] A series of latent class models were fitted with the LEM package (Vermunt, 1997, unpublished). They included the latent variable, the observed diagnostic test variables, and interaction terms to control for associations between test results. Models were compared by the difference in likelihood statistic (G^2)[30] and by Akaike's Information Criterion.[31] An approximate 95 percent confidence interval (CI) was computed for the LCA sensitivity and specificity estimates (the interval lying within $\pm 1.96 \times$ standard error of the estimate).

Reproducibility. We used Cohen's kappa to assess the reproducibility of IHA performed at the RBB and at CENETROP. Kappa results were interpreted as suggested by Landis and Koch.[32]

Effectiveness of strategies. With the Bayes theorem, we subsequently simulated the effectiveness of alternative donor blood screening strategies from the sensitivities and specificities of each individual serologic test. The strategies were based on isolated or parallel use of different assays (assuming independence of tests). In a particular screening strategy a blood unit was considered infected, for the purpose of assuring blood safety, if at least one serologic test used in the strategy was positive. As indicators for the effectiveness of a particular screening strategy, we computed the number of undetected infected blood units and the number of units falsely labeled positive per 1000 donations screened, for *T. cruzi* infection rates of 40, 20, and 5 percent.

Cost-effectiveness of strategies. Finally, we compared the selected strategies with regard to their effectiveness and reagent cost. We used US$0.20 for HAI reagents (just above the real cost for the RBB) and US$1 for IFA and ELISA reagents[16] to calculate the total reagent cost per 1000 donations screened and the (marginal) costs per (extra) infected unit detected.

RESULTS

A total of 173 donations of the 400 were positive for the presence of *T. cruzi* when screened in the RBB with IHA at dilution 1:8. Because there were no repeat donations, this corresponds to a *T. cruzi* prevalence rate in donors of 43.3 percent (95% CI, 38.4%-48.1%). The mean age of the donors was 31.1 years (standard deviation, 9.2 years). The donors were mainly recruited among relatives and only 4.3 percent were altruistic volunteers in the strict sense. The infection rate varied significantly with sex, residence, and age (Table 1).

Table 2 gives the frequencies of the observed serologic test patterns for the 396 donor serum samples on which the complete panel of serologic tests could be performed. Two-hundred seven (52.3%; 95% CI, 47.4%-57.2%) of them were positive for at least one test.

A series of LCA models with different conditional dependencies between tests was fitted to estimate the prevalence of infection and the validity characteristics of the serologic tests. The conditional independence model gave the best fit (deviance, 58.7; degrees of freedom, 110; $p = 1.00$) and produced the parameter estimates shown in Table 3. Whereas the observed frequency of a positive IHA serology result was 43.3 percent and of positivity to any test 52.3 percent, the LCA model estimated the true frequency of *T. cruzi* infection at 36.5 percent (95% CI, 31.8%-41.3%). IHA specificity was considerably lower under routine conditions than in the reference laboratory and the agreement between IHA in the RBB and IHA in CENETROP was far from optimal ($\kappa = 0.73$). The sensitivity

TABLE 1. Characteristics of blood donors and *T. cruzi* prevalence, RBB, Santa Cruz, Bolivia (1998-1999)

Characteristic	Number	Positive IHA	Prevalence rate (%)	Relative risk (95% CI)
Sex				
Female	134	68	50.7	1.29 (1.03-1.61)
Male	266	105	39.5	*
Residence				
Rural	53	30	56.6	1.37 (1.05-1.79)
Urban Santa Cruz	347	143	41.2	*
Age (years)				
≥30	191	100	52.4	1.48 (1.18-1.87)
<30	207	73	35.3	*
Motivation				
Relative	383	169	44.1	1.88 (0.79-4.45)
Altruistic	17	4	23.5	*

* Reference category.

TABLE 2. Frequencies of the different observed test patterns (n = 396)*

Response pattern†	Observed frequency	Percentage
0 0 0 0 0 0 0	189	47.7
0 0 0 0 0 0 1	4	1.0
0 0 0 0 1 0 0	5	1.3
0 0 0 1 0 0 0	3	0.8
0 0 1 0 0 0 0	3	0.8
0 1 0 0 0 0 0	9	2.3
1 0 0 0 0 0 0	23	5.8
0 0 0 1 0 0 1	1	0.3
0 0 1 0 0 0 1	1	0.3
1 0 0 0 0 1 0	1	0.3
0 0 1 1 0 0 0	1	0.3
1 0 0 1 0 0 0	1	0.3
0 1 1 0 0 0 0	1	0.3
1 1 0 0 0 0 0	2	0.5
1 0 0 0 0 1 1	1	0.3
1 0 0 0 1 0 1	2	0.5
0 1 1 0 0 0 1	1	0.3
1 0 1 1 0 0 0	1	0.3
0 1 0 0 1 1 1	1	0.3
1 1 1 0 0 0 1	1	0.3
1 0 1 1 1 0 1	1	0.3
1 1 1 1 1 1 0	1	0.3
1 1 1 1 1 0 1	1	0.3
1 1 1 1 0 1 1	2	0.5
1 0 1 1 1 1 1	3	0.8
0 1 1 1 1 1 1	5	1.3
1 1 1 1 1 1 1	132	33.3
Total	396	100.0

* Four samples were excluded for incompleteness of data.
† Negative results reported as 0, and positive results as 1. Order of results: IHA in RBB, IHA in CENETROP, IFA, crude ELISA 1, crude ELISA 2, recombinant ELISA 1, and recombinant ELISA 2.

of the different ELISAs ranged from 98.6 to 100 percent, and their specificity was from 95.3 to 98.9 percent. Crude ELISA 1 and IFA were the only tests with sensitivity estimates fixed at 100 percent by the estimation algorithm.

Table 4 shows the effectiveness of single IHA testing in the RBB and of selected alternative screening strategies, based on test characteristics as reported in Table 3. No single test in our study achieved perfect effectiveness. The IHA in the RBB, that is, under routine conditions, resulted—at a prevalence rate of 40 percent—in 14 nonidentified infected units per 1000 units screened and in 78 units falsely labeled positive. This corresponds to a positive predictive value of 83.2 percent and a negative predictive value of 97.4 percent.

Based on the test characteristics observed in this study, we subsequently hypothesized that for IHA, ELISA, and IFA sensitivities of 97, 99, and 99 percent, respectively, and specificities of 91, 96, and 96 percent, respectively, should be attainable under routine conditions and computed the corresponding effectiveness (Table 4, "hypothetical strategy" section). A small, but not negligible, number of false-negative samples seem unavoidable when using a single test, even with ELISA and IFA technology. Finally, we derived the potential effectiveness of combinations of two tests when used in parallel. Use of two different tests resulted in virtually 100 percent sensitivity and nearly perfect detection of infected blood units (only approx. 1 missed in every 10,000 screened at a prevalence rate of 40%). The "price" to pay was an increase in the number of units that were falsely labeled positive (from approx. 50 to approx. 100 in every 1000 units screened at low and high prevalences, respectively).

The total reagent cost of a strategy with a single IHA amounts to US$200 per 1000 blood units screened or US$0.5 per infected unit detected, at prevalence 40 percent (Table 5). A single ELISA or IFA detects 8 more infected units. The total additional cost is US$800 and the marginal cost is US$100 per extra infected unit detected. A strategy that combines IHA with ELISA or IFA (parallel testing) detects, at 40 percent prevalence, 11.88 infected units more than a single IHA for a marginal cost of US$84 per extra detected unit. Parallel testing with ELISA and IFA gives no meaningfully different yield but is far more expensive.

The above results carry over, qualitatively, to situations with a different prevalence of infected blood units. Strategies based on a combination of two tests perform better, regardless of the prevalence level of *T. cruzi* infection, but at lower prevalences the gain in detected infected units diminishes and the marginal cost rises steeply. Notwithstanding, the marginal cost per extra infected unit detected, when compared to single IHA testing, is always lower for parallel IHA plus ELISA or parallel IHA plus IFA testing than for a strategy based on a single ELISA or on IFA plus ELISA in parallel. This conclusion remains valid in a plausible range of sensitivity val-

ues for the different tests, in particular for IHA sensitivity of 0.965 (the one actually observed in the routine RBB procedure) and for a ELISA and IFA sensitivity up to 0.99995.

TABLE 3. Test characteristics of different serologic tests for *T. cruzi* estimated by a two-latent-class model

Serologic test	Sensitivity (95% CI)	Specificity (95% CI)
IHA in RBB*	0.965 (0.935-0.995)	0.870 (0.828-0.912)
IHA in CENETROP†	0.975 (0.948-1.000)	0.939 (0.910-0.969)
IFA	1‡	0.963 (0.940-0.987)
Crude ELISA 1	1‡	0.970 (0.948-0.992)
Crude ELISA 2	0.986 (0.967-1.000)	0.966 (0.943-0.989)
Recombinant ELISA 1	0.989 (0.969-1.000)	0.989 (0.976-1.000)
Recombinant ELISA 2	0.993 (0.980-1.000)	0.953 (0.926-0.979)

* Routine testing at donation.
† Retested by reference laboratory in this study.
‡ Parameter fixed at 1 by the estimation algorithm.

DISCUSSION

The use of LCA permitted us to obtain unbiased estimates of the validity characteristics of the serologic tests commonly used for detecting *T. cruzi* infection. Our estimates are fairly consistent with those of other authors, although figures are hard to compare owing to the widely varying method used in other studies that did not address the lack of golden standard problem. Reported sensitivity figures range between 75.2 and 99.02 percent for IHA,[33,34] 95.1 to 100 percent for IFA,[33,35] 97.7 to 100 percent for crude ELISAs,[35,36] and 82 to 100 percent for recombinant ELISAs.[7] Specificity ranges between 95 and 99.55 percent for IHA,[3,34] 96.7 to

TABLE 4. Observed test effectiveness and effectiveness of hypothetical strategies for transfusional *T. cruzi* screening

Strategy	Sensitivity	Specificity	Prevalence rate (%):‡	Number of false-negative units per 1000 screened* 40	20	5	Number of false-positive units per 1000 screened† 40	20	5
Observed in study									
IHA in RBB	0.965	0.870		14	7	1.75	78	104	123.5
IHA in CENETROP	0.975	0.939		10	5	1.25	36.6	48.8	58
Crude ELISA 1	1	0.970		0	0	0	18	24	28.5
Recombinant ELISA 1	0.989	0.989		4.4	2.2	0.55	6.6	8.8	10.5
IFA	1	0.963		0	0	0	22.2	29.6	35.2
Hypothetical strategy§									
IHA alone	0.970	0.910		12	6	1.5	54	72	85.5
ELISA alone	0.990	0.960		4	2	0.5	24	32	38
IFA alone	0.990	0.960		4	2	0.5	24	32	38
IHA plus ELISA	1.000‖	0.874		0.12	0.06	0.015	75.8	101.1	120.1
IHA plus IFA	1.000‖	0.874		0.12	0.06	0.015	75.8	101.1	120.1
ELISA plus IFA	1.000¶	0.922		0.04	0.02	0.005	47.0	62.7	74.5

* False-negative units per 1000 screened = prevalence of infection × (1 – sensitivity of the strategy) × 1000.
† False-positive units per 1000 screened = (1 – prevalence) × (1 – specificity of the strategy) × 1000.
‡ Hypothetical prevalence rate of *T. cruzi* infection in donors.
§ See text for explanation.
‖ Sensitivity 0.9997; rounded to 1.000 for presentation in table.
¶ Sensitivity 0.9999; rounded to 1.000 for presentation in table.

TABLE 5. Cost analysis of hypothetical screening strategies for transfusional *T. cruzi* screening

Hypothetical strategy§	Total cost per 1000 units screened*	Prevalence rate (%):‡	Number of infected units detected per 1000 units screened 40	20	5	Cost per infected unit detected* 40	20	5	Marginal cost per extra infected unit detected† 40	20	5
IHA alone	200		388.00	194.00	48.50	0.5	1.0	4.1	Baseline	Baseline	Baseline
ELISA alone	1000		396.00	198.00	49.50	2.5	5.1	20.2	100	200	800
IFA alone	1000		396.00	198.00	49.50	2.5	5.1	20.2	100	200	800
IHA plus ELISA	1200		399.88	199.94	49.98	3.0	6.0	24.0	84	168	673
IHA plus IFA	1200		399.88	199.94	49.98	3.0	6.0	24.0	84	168	673
ELISA plus IFA	2000		399.96	199.98	49.99	5.0	10.0	40.0	151	301	1204

* All costs are expressed in US$.
† Marginal cost compared to a strategy with a single hypothetical IHA.
‡ Prevalence rate of *T. cruzi* infection in donors.
§ With sensitivities and specificities as under "Hypothetical strategy" in Table 4.

99.65 percent for IFA,[34,35] 95 to 99.69 percent for crude ELISAs,[37,38] and 100 percent for recombinant ELISAs.[8] Furthermore, it should be noted that our test results, except for IHA RBB, were obtained under ideal laboratory (study) conditions. The chance-corrected agreement between the routinely obtained result and that in the reference laboratory was only moderate ($\kappa = 73\%$). Other authors have reported similar problems when evaluating the serology performance in blood bank settings.[15] The danger of relying exclusively on published efficacy estimates for serologic tests obtained under optimal conditions is also illustrated in a study in six blood banks reported by Andrade and associates:[12] sensitivity for complement fixation and IHA in field conditions dropped as low as 50 percent of the theoretical attainable one. Routine conditions for blood bank screening imply that quality of processing may be suboptimal or vary over time and that 100 percent effective sensitivity will never be attained. This adds further arguments for adopting a screening strategy that relies on two tests and supports the view of Salles and coworkers[39] that no single test is sufficiently sensitive to prevent *T. cruzi* transmission.

To decide on alternative strategies under routine conditions, we compared several scenarios. We assumed that appropriate procedures and staff, good quality reagents, and regular QC and training would lead to near optimal test execution and performance. Hence the 100 percent sensitive crude ELISA 1 was not included among the plausible scenarios and the 100 percent sensitivity estimate of IFA observed under study conditions was deemed to be overoptimistic in view of the subjective element in its reading[13] and reduced to a more realistic value (99.0%).

With 12 and 1.5 residual infected units per 1000 screened, at prevalence 40 and 5 percent, respectively, single IHA testing is inadequate to rule out *T. cruzi* infection. Single ELISA testing can improve the effectiveness of Chagas screening and reduces in a substantial way the number of undetected infected units to, respectively, 4 and 0.5 per 1000 screened. For contexts with a 100 percent screening coverage, as is the case now in the Santa Cruz RBB, the probability of receiving an infected unit depends on prevalence and sensitivity. For blood banks with a two-test screening, this risk is assumed to be zero,[16] but even parallel testing with nearly perfectly sensitive tests results in 4 false-negative units per 100,000 screened at a prevalence of 40 percent.

For the Santa Cruz RBB in particular, the presently adopted strategy (IHA alone) resulted in 14 false-negative units per 1000 units screened at the current prevalence rate of 40 percent. The actual number of iatrogenic infections, however, is lower, because the transmission risk for a person receiving an infected unit is estimated at 20 to 50 percent.[4,18] One can assume as well that 40 percent of the recipients are no longer susceptible, although little is known about the consequences of reinfection. A rough estimate of the maximum number of transmitted transfusional infections would thus be 14 infected units per 1000 screened $\times$ 50 percent transmission risk $\times$ 60 percent uninfected recipients $\times$ 1000, that is, 5 persons infected per 1000 screened units. For the Santa Cruz RBB the strategy based on a single IHA entails thus a low, but definite, risk for infection. Because the false-negative samples that go undetected in the screening procedure determine infection risk, test sensitivity is indeed the main concern for blood banks.[40]

Nevertheless, lack of specificity has a social and an economical cost: it causes unnecessary psychological suffering in false-positive donors who are (erroneously) informed about their status and induces unnecessary health-care expenses for further testing and possibly treatment of these false-positive donors. If positive units would be discarded instead of treated chemically, lack of test specificity will also become of direct concern for the blood bank[41] and even more so in situations of high prevalence and scarcity of donors. Again the observed IHA performed poorly, because it misclassified 78 and 123.5 units per 1000 screened as positive in case of a 40 and 5 percent prevalence, respectively. Single ELISA and single IFA led to better results than single IHA testing. Parallel IFA plus ELISA led to similar and parallel IHA plus ELISA or IHA plus IFA to worse results. The latter figure is determined by our specificity estimate of 87.4 percent for IHA plus ELISA or IHA plus IFA, which deviates from previously published figures that merely assumed 99 percent specificity.[16] Not withstanding, although a two-test strategy that trades off specificity to increase sensitivity may be critical in a hyperendemic country, specificity is a much greater issue in countries with low seroprevalence.

The cost of alternative screening strategies is another element to be considered. Our reagent cost estimates lie within the range of the reported reagent cost of several Latin American countries.[3,16] A formal cost-effectiveness analysis that takes all cost elements (e.g., investment, equipment, personnel, chemoprophylaxis) into account would be needed to fully explore this issue. In view of its complexity and huge local variations in, for example, salary costs, we simplified our analysis with, as described, estimated costs of test execution only. The hypothetical cost of detecting 1 infected unit for the alternative strategies and prevalence range (40-5%) varied between US$0.5 and US$40. Cost estimates between US$11 and US$209 were reported in 1993 for a variety of single tests in Latin American countries with prevalences between 0.2 and 14.7 percent.[3]

Parallel IHA and ELISA screening is barely more expensive than single ELISA testing but more effective and more cost-effective. The combination of ELISA and IFA does not really perform better over a realistic range of sensitivities and specificities: this strategy is slightly more effective in detecting infected units but its total and

marginal cost (compared to IHA alone) are nearly twice as high. Notwithstanding, sequential testing could substantially lower this cost in high prevalence conditions. The best strategy will ultimately depend on the local feasibility; for example, the preparation of reagents for crude ELISA 1 might not be possible in blood banks.

A rational use of indication for blood transfusion and selection of low-risk donors, screening of blood units, and subsequent chemical treatment of infected ones are the only building stones of strategies to limit transfusional Chagas disease transmission. This study indicates that blood screening with two tests is mandatory in settings with high to moderate prevalence of *T. cruzi* infection in donors.

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