1 **Serological diagnosis of chronic Chagas disease: Is it time for a change?**

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21 **Running title:** Changes in the serological diagnosis of chronic Chagas disease

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23 **Keywords:** chronic Chagas disease, serology, Architect Chagas

ABSTRACT

Chagas disease has spread to non-endemic areas with human migration. Since no single reference standard test is available, serological diagnosis of chronic Chagas disease requires at least two tests. New generation techniques have significantly improved the accuracy of Chagas disease diagnosis by the use of a large mixture of recombinant antigens with different detection systems, such as chemiluminescence. The aim of the present study was to assess the overall accuracy of a new generation kit, Architect Chagas (cut-off ≥ 1 S/CO, sample relative light units/cut-off value), as a single technique in the diagnosis of chronic Chagas disease. Architect Chagas showed a sensitivity of 100% (95% confidence interval, CI = 99.5-100) and a specificity of 97.6% (95% CI = 95.2-99.9). Five out of six false-positive sera were a consequence of cross-reactivity with *Leishmania* spp. and all of them achieved results < 5 S/CO. We propose Architect Chagas as a single technique for screening in blood banks and for routine diagnosis in clinical laboratories. Only grey zone and positive sera with a result ≤ 6 S/CO would need to be confirmed by a second serological assay, thus avoiding false-positive sera and the problem of cross-reactivity with *Leishmania* spp. The application of this proposal would result in important savings in the cost of Chagas disease diagnosis and therefore in the management and control of the disease.

INTRODUCTION

Chagas disease or American trypanosomiasis is a parasitic infection traditionally linked to rural areas of Latin America (1). Based on 2010 data, an estimated 5,742,167 people are infected in 21 Latin American countries (2). The epidemiology of Chagas disease has changed because of migratory trends and it is now an emerging public health problem in the United States and Europe (3, 4), notably in Spain, the European country with the largest number of immigrants from Latin America (3, 5).

The flagellated protozoan *Trypanosoma cruzi* is mainly transmitted in endemic areas through contact with the dejections of blood-feeding triatomine bugs (6, 7) and more rarely by oral transmission through contaminated food (8, 9). The infection may also occur in both endemic and non-endemic areas through blood transfusion (10), organ transplant (11), congenital transmission (12) and laboratory accidents (13), allowing the disease to spread to urbanized areas (14).

Chagas disease occurs in two stages: the acute phase, without symptoms or with nonspecific manifestations in the majority of cases, and the chronic phase, characterized by cardiac and/or gastrointestinal disorders. In the chronic indeterminate phase of the disease most patients remain asymptomatic all their lives (15, 16).

Due to the low and intermittent parasitemia, diagnosis during the chronic phase of Chagas disease is made by serological methods (10, 15, 16). There are two types of serological techniques for the detection of anti-*T. cruzi* antibodies: conventional tests using a whole parasite antigen, and non-conventional tests based on recombinant antigens (17, 18). Cross-reactivity, especially in conventional assays, is a particular problem for the serological diagnosis of Chagas disease in regions where Leishmaniasis also occurs (15, 19). Although numerous assays are available for diagnosing Chagas disease, no single test is considered the reference standard (19–21).

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To date, an individual is diagnosed as infected with *T. cruzi* in the chronic phase of the disease when the results of two serological tests are positive (17). When inconclusive or discordant results appear, a third technique (17) or additional samples are required (22), thereby increasing the cost of diagnosis. The plethora of serological tests used to identify *T. cruzi* infections often demonstrate discrepant results, which makes serum interpretation difficult (22, 23). Moreover, *T. cruzi* has great genetic diversity and is currently divided into six genotypes known as discrete typing units (DTUs TcI-TcVI) (24). Discordant results between assays are often attributed to antigenic differences among recombinant proteins or *T. cruzi* DTUs (23, 25).

New generation tests with potentially improved accuracy have been recently developed. The use of a large mixture of recombinant antigens and the incorporation of different detection systems, such as chemiluminescence, increase the sensitivity and specificity of the techniques. Other advantages of new generation tests are automation, rapidity and high-performance. Among them, Architect Chagas (Abbott Laboratories, Wiesbaden, Germany), a chemiluminescent microparticle immunoassay (CMIA), uses four recombinant proteins as the antigen (26–28).

The aim of the present study was to assess the overall accuracy of a new generation kit that combines a mixture of recombinant proteins with chemiluminescence (Architect Chagas). The application of this single technique in the diagnosis of chronic Chagas disease modifies the aforementioned diagnostic recommendations. Accordingly, it could lead to a reduction in the cost and time of diagnosis and be the first step to reach a consensus on a standard protocol.

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MATERIAL AND METHODS

Ethics statement. This study was approved by the Clinical Research Ethics Committee (CEIC) of the Hospital de la Santa Creu i Sant Pau in Barcelona (Project code: IIBSP-CHA-2013-33; CEIC number: 53/2013). All samples were anonymized before being evaluated and included in the study.

Study population and serum samples. A total of 315 sera of adults attended in the Hospital de la Santa Creu i Sant Pau of Barcelona (Spain) were used in this work. Clinical data were recorded by a retrospective review of patient files through the computer system Systems, Applications and Products for Data Processing (SAP). Serum samples (conserved at -40ºC) were collected during the period January 2009 to December 2012 and divided in four panels (I to IV):

Panel I (n = 107): samples of chronic chagasic seropositive patients from endemic countries for Chagas disease in Latin America diagnosed in Spain (96% from Bolivia, 2% from Argentina, and 2% from Paraguay).

Panel II (n = 125): samples of non-chagasic individuals from both endemic (n = 64) and non-endemic countries (n = 61) for Chagas disease.

For panels I and II, samples had concordant results for two enzyme-linked immunosorbent assays (ELISAs) using whole-parasite antigen (ELISAc) (29) and recombinant antigens (ELISAr) (BioELISA Chagas, Biokit, Lliçà d'Amunt, Spain). Clinical and epidemiological data were considered for the selection.

Panel III (n = 12): samples of individuals from endemic countries for Chagas disease with discrepant serological results diagnosed in Spain. These samples had discordant results for ELISAc and ELISAr and were also tested by a Western blot (WB) (19) in order to get the final interpretation (11 considered negative and 1 positive). Clinical and epidemiological data were also considered for the selection.

Panel IV (n = 71): samples of patients with other infectious diseases to evaluate cross-reactions (8 individuals with leishmaniasis, 7 with toxoplasmosis, 6 with amebic hepatic abscess, 3 with malaria, 6 with strongyloidiasis, 1 with visceral larva migrans [VLM], 3 with cytomegalovirus, 7 with human immunodeficiency virus [HIV], 4 with parvovirus B19, 5 with Epstein-Barr virus [EBV], 5 with hepatitis B virus [HBV], 2 with hepatitis C virus [HCV], 9 with syphilis, and 5 with Lyme borreliosis). All samples had serological and/or parasitological or molecular evidence of the infectious diseases studied.

Serological assays and interpretation of results. Since there is no single widely accepted reference standard test for the diagnosis of *T. cruzi* infections, 244 sera were pre-characterized using two serological tests, according to the WHO recommendations (17). The remaining 71 samples were taken from patients with other diagnoses (panel IV). For the sera pre-characterization, the techniques used were two ELISAs, one of them *in house* and using sonicated epimastigotes of *T. cruzi* (ELISAc) (cut-off ≥ 20 units) (29) and the second one with recombinant antigens (ELISAr) (results [sample ratio absorbance/cut-off value] < 0.9 were considered negative, ≥ 1 positive and the grey zone was from ≥ 0.9 to < 1). Samples with positive results for both assays were included in panel I and sera with negative results were included in panel II. Samples with discordant results by these techniques were included in panel III and they were tested by an *in house* WB based on lysate *T. cruzi* epimastigotes, as described elsewhere (19). The final interpretation of panel III samples was based on results coinciding in two out of the three techniques performed; thus, 11 were considered negative, and one positive. In order to rule out Chagas disease, samples of patients with other infectious diseases (panel IV) were also analyzed through WB.

All sera were tested for the presence of *T. cruzi* antibodies by the CMIA Architect Chagas assay. This fully automated assay is based on recombinant proteins FP3, FP6, FP10, and TcF. In aggregate, these four hybrid recombinant proteins represent 14 distinct antigenic regions (30, 31). Testing was performed according to the manufacturer's instructions. The chemiluminescent reaction is measured in relative light units (RLUs). Results are expressed as samples RLUs/cut-off value (S/CO). Ratios < 0.8 are considered negative, ≥ 1 are considered positive, and the grey zone was from ≥ 0.8 to < 1.

Data analysis. The following measures of diagnostic accuracy were calculated (TP: true positive, TN: true negative, FP: false positive, FN: false negative): sensitivity (calculated as TP/[TP+FN]), specificity (calculated as TN/[TN+FP]), validity index defined as the percentage of patients correctly classified (32) (calculated as [TP+TN]/[TP+TN+FP+FN]), positive and negative predictive values (PPV and NPV), which are the proportion of correctly diagnosed individuals with positive (PPV) or negative (NPV) results (33) (calculated as TP/[TP+FP] and TN/[TN+FN], respectively), positive and negative likelihood ratios (LR+, the highest value being the best result, and LR-, the lowest value being the best result), which express how many times more or less frequent the test result is obtained among individuals with the disease compared with those without the disease (34) (calculated as sensitivity/[1-specificity] and [1-sensitivity]/specificity, respectively), Youden index, which is a measure of the overall discriminative power of a diagnostic procedure (35) (calculated as [sensitivity+specificity]-1), and Cohen's kappa coefficient, which describes the level of concordance among tests relating the observed agreement (Ao) and the agreement expected by chance (Ae) (36) (calculated as [Ao-Ae]/[1-Ae]) (values > 0.8 indicate a high level of agreement) (37). Calculations were performed with the software EPIDAT

3.1, which is available online at http://www.sergas.es.

Economic evaluation. An economic assessment of the annual cost of Chagas disease serology in the Hospital de la Santa Creu i Sant Pau in Barcelona was done. During the period from March 2014 to February 2015, a total of 718 sera were analyzed for the presence of *T. cruzi* antibodies in our hospital. Several calculations were done: (i) the annual cost of performing two assays (Architect Chagas and ELISAr) for all the 718 sera according to the WHO recommendations, (ii) the annual cost of performing Architect Chagas for all sera and confirming by ELISAr grey zone (2 sera) and all positive samples (98 sera), and (iii) the annual cost by having to confirm by the second test only grey zone (2 sera) and positive ≤ 6 S/CO samples (19 sera), strategy proposed in this study.

RESULTS

Sera were divided in four panels: panel I (samples of chronic chagasic patients), panel II (samples of non-chagasic patients), panel III (samples with discrepant serological results), and panel IV (samples of patients with other infectious diseases).

A coincident result of Architect Chagas with the pre-characterization was considered as true positive (TP) or true negative (TN) and a discordant result with the pre-characterization was considered as false positive (FP) or false negative (FN) (Table 1). In this study, no FN for Architect Chagas were observed.

Among the 244 sera pre-characterized as positive or negative for Chagas disease, 242 were concordant with Architect Chagas results. Only one serum of panel II tested positive and was considered as FP and one serum of panel III gave a result in the grey zone. Therefore, the concordance level between pre-characterized sera and the results obtained with Architect Chagas was 99.2%.

The overall serum value distribution of ELISAc, ELISAr and Architect Chagas is shown in Fig. 1.

In reference to TP serum values (n = 108), 94 samples (87.04%) achieved results > 6 S/CO. The remaining 14 sera (12.96%) obtained values ≤ 6 S/CO; 9 samples (8.33%) obtained values from 1 to 4.9 and 5 samples (4.63%) from 5 to 6.

When sera from patients with other infectious diseases were analyzed, 5 out of 71 samples were reactive by Architect Chagas. All of them came from *Leishmania*-infected patients with Chagas disease ruled out by a WB method (19). These FP sera for Architect Chagas also showed positive results for ELISAc (values between 53 and 84 units) and negative results for ELISAr except in one case in which the sample obtained a value in the grey zone.

The serum from panel III with a grey zone result for Architect Chagas was positive for ELISAc (FP), negative for ELISAr, and negative for WB. The serum from panel IV (*Leishmania* infection) with a grey zone result for ELISAr was positive for both ELISAc and Architect Chagas (FP), and negative for WB. These samples were not included in the calculations, resulting in a final panel of 313 sera.

Measures of diagnostic accuracy of the Architect Chagas assay are shown in Table 2. Sensitivity, calculated using panels I and III, was 100%. Specificity, calculated using panels II, III and IV, was 97.6%. FP sera obtained results between 1.8 and 4.6, and 5 out of 6 samples came from *Leishmania*-infected patients (Table 3). A high proportion of patients were correctly classified (validity index of 98.4%) and the test showed a high level of agreement with the two techniques used in the pre-characterization; Kappa index of 0.91 (95% confidence interval, CI = 0.86-0.95) with ELISAc and a value of 0.94 (95% CI = 0.90-0.98) with ELISAr.

ELISAc scored 17 FP, 8 in panel III and 9 in panel IV (7 sera with *Leishmania* infection and 2 with EBV). Therefore, the test showed 100% sensitivity (95% CI = 99.5-100), the specificity was 91.7% (95% CI = 87.7-95.7), and the validity index was 94.6% (95% CI = 91.9-97.2). ELISAr achieved 3 FP and 1 FN: 2 FP and the FN in panel III and 1 FP in panel IV (serum with EBV). Consequently, the sensitivity and specificity of the technique were 99.1% (95% CI = 96.8-100) and 98.5% (95% CI = 96.7-100), respectively, and the validity index was 98.7% (95% CI = 97.3-100).

The annual cost of performing to assays for Chagas disease diagnosis in our hospital in Barcelona is €6,864.08 or US$7,413.21. From the 718 samples analyzed from March 2014 to February 2015, 618 (86.1%) tested negative using Architect Chagas. Taking into account the 100% sensitivity of the test found in this study, it was possible to classify the sera as negative with only a single technique. The remaining 100 sera (13.9%) were analyzed by two tests (Architect Chagas and ELISAr), since Architect Chagas gave grey zone (2 sera, 0.3%) or positive results (98 sera, 13.6%). Positive samples with results > 6 S/CO (79 sera, 11%) were also analyzed with a second test (ELISAr), confirming that all of them were TP. This represents an annual cost of €3,156.08 or US$3,408.57. We propose that grey zone (2 sera, 0.3%) and positive ≤ 6 S/CO (19 sera, 2.6%) samples require further confirmation (TP 57.9%). If inconclusive results appear, a third technique or additional samples are required. Confirmation by a second test was only necessary in 21 sera, instead of the 100 positive and inconclusive samples. As a result, the annual cost by not having to confirm all positive samples would be €2,682.08 or US$2,896.65 in the hospital population which represents savings of €4,182 or US$4,516.56 per year.

DISCUSSION

Despite the absence of the vector, Chagas disease is now an emerging public health problem in Europe and the United States due to immigration from endemic areas (3, 4). Chronic forms of the disease have appeared in non-endemic countries (4, 38, 39) as well as acute forms, principally due to vertical transmission (40–42). In Europe, chronic forms are more abundant than congenital cases.

Chronic forms of Chagas disease are diagnosed serologically, requiring two tests for confirmation (17). According to the World Health Organization (17), an ideal serological test should be easy to perform in a single step, be fast, cheap, require no special equipment or refrigeration of reagents and have 100% sensitivity and specificity, but unfortunately, no such test exists for Chagas disease. The lack of a reference standard serological assay for the diagnosis of *T. cruzi* infection has prompted the development of new tests, which require further evaluation. Among them, Architect Chagas, a fully automated assay using four recombinant proteins as the antigen, has been scarcely studied to date (26–28).

Sera pre-characterization was performed by ELISAc, a conventional method using parasite lysate as the antigen (29), and ELISAr, based on *T. cruzi* TcF antigen, a recombinant fusion protein that comprises four serologically active peptides (PEP-II, TcD, TcE, and TcLo1.2) (43, 44). The assay evaluated here, Architect Chagas, incorporates three recombinant proteins (FP3, FP6, and FP10) in addition to the TcF of ELISAr (30, 31, 45, 46). These four proteins in aggregate represent 14 different antigenic regions present throughout the life cycle of *T. cruzi* (30, 45). Moreover, *T. cruzi* is currently divided into six DTUs with distinct genetic profiles (24). Architect Chagas is capable of detecting the genetic diversity of *T. cruzi* by the incorporation of

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highly conserved antigenic proteins with tandemly repeated amino acid domains (26, 45).

A well-known problem in the serological diagnosis of Chagas disease is cross-reaction with antibodies produced by other pathogens, especially *Leishmania* spp. (15, 19, 47). All FP sera for Architect Chagas except one (5 out of 6) came from patients with leishmaniasis (panel IV) (see Table 3). Although all patients were from Spain, these samples were analyzed by a WB using *T. cruzi* lysate epimastigotes as antigen (19) in order to check possible *Leishmania* spp.-*T. cruzi* co-infections. Chagas disease was ruled out in all five cases because of negative results. The remaining FP serum belonged to a pre-characterized negative patient (panel II) from an endemic area in which leishmaniasis was ruled out. No data of other possible pathologies of the patient were known.

In this report, the Architect Chagas recombinant test showed 100% sensitivity, while specificity was 97.6% due to cross-reactions in the leishmaniasis patients. The specificity achieved by the Architect Chagas assay excluding cross-reactions with *Leishmania* spp. would be 99.5%. Architect Chagas results were highly concordant with tests using crude antigens, such as ELISAc (Kappa index = 0.91), but with higher specificity (ELISAc sensitivity 100%; specificity 91.7%). While Architect Chagas gave positive results in 5 out of 8 sera from *Leishmania*-infected patients, indicating cross-reactions, ELISAc scored positive results in all the 8 sera with *Leishmania* spp. The technique evaluated here also showed a high level of agreement with ELISAr results (Kappa index = 0.94). Although specificity shown by ELISAr, and even the validity index, was higher than Architect Chagas, this technique did not detect all positive sera (ELISAr sensitivity 99.1%; specificity 98.5%; validity index 98.7%). Indeed, Architect Chagas is better able than ELISAc and ELISAr to discriminate between positive and

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negative sera (see Fig. 1). The higher sensitivity of Architect Chagas is probably due to the greater diversity of proteins used as antigens, representing the three morphological forms (trypomastigote, epimastigote and amastigote) and the genetic diversity of *T. cruzi* (26, 45). Among current tests in which the number of recombinant proteins is known, Architect Chagas uses the most. This higher number of recombinant antigens could also explain the high level of cross-reactions with *Leishmania* spp. infection. Consequently, this fact should be considered when studying the diagnosis of Chagas disease in visceral leishmaniasis endemic areas. Other authors have previously reported that mixtures of recombinant proteins are very useful as antigens for the immunodiagnosis of Chagas disease (48, 49).

New generation techniques such as Architect Chagas or Bio-Flash Chagas (Biokit, Lliçà d'Amunt, Spain) (50) have improved the diagnosis of Chagas disease with innovative new tools (large mixture of recombinant antigens and chemiluminescence as detection system). Previous studies have also proposed a chemiluminescent ELISA (CL-ELISA) with purified trypomastigote glycoproteins for the detection of lytic protective antibodies against *T. cruzi* in human sera (33, 51, 52). CL-ELISA achieved high diagnostic accuracy in both endemic (51, 52) and non-endemic areas (33). Detection systems such as chemiluminescence increase light amplification and signal duration in comparison with traditional ELISA assays.

Both characteristics, a larger number of recombinant antigens and signal amplification, lead to higher accuracy in the diagnosis of Chagas disease compared to conventional and recombinant techniques used in this study.

Other authors have evaluated Architect Chagas using different populations or sample conditions (26–28). Their overall results (26–28) suggest Architect Chagas is a highly suitable assay for the detection of chronic *T. cruzi* infection and its use as a

single technique for routine testing in high-prevalence areas has already been recommended (26). In contrast with what is proposed here, a reduction from 1 to 0.88 in the CO value has been recommended, but only when blood samples on filter paper are used (28).

According to the results in the present study, and preserving the manufacturer's criteria for the interpretation of results, we propose Architect Chagas, or other similar new generation tests, as a single technique for the diagnosis of chronic Chagas disease in blood banks and clinical laboratories in both endemic and non-endemic areas. Taking into account the positive and cross-reactivity results obtained and the overall distribution of serum values (see Fig. 1C), we suggest that only grey zone and positive sera with results ≤ 6 S/CO would need to be confirmed by a second serological assay, in agreement with WHO recommendations. Sera with these results represented less than 18% of positive samples and 6.3% of the total sera analyzed in this study. Further studies with other new generation techniques with similar characteristics (recombinant antigens and chemiluminescence) are necessary.

Several control measures exist for Chagas disease, according to the different transmission scenarios (7, 14, 53), some of which have been applied by health organizations or administrative governments (54–58). Previous studies on the cost-effectiveness of Chagas disease management have been undertaken (59–62), but the costs of different diagnostic methods have not been compared.

The adoption of a single high performance technique, like the one studied here, would entail a significant saving. Indeed, the savings would be €4,182 or US$4,516.56 per year in our hospital, if the comparison is with the cost of performing two assays for all sera, the WHO-recommended strategy used to date. Our proposal would allow the

optimization of screening procedures and cost according to the document of the Sixty-third World Health Assembly (63).

According to Sicuri et al. (59), 1.7 million migrants from Latin American countries endemic for Chagas disease live in Spain, where 42,173 adult immigrants are estimated to be infected with *T. cruzi* (64). By 2009, in Europe an estimated 68,000 to 122,000 Latin American immigrants were thought to be infected by *T. cruzi*, but only 4,290 of them were diagnosed (65). Although Chagas disease has become a real problem for countries hosting Latin American migrants, not all European countries screen for the infection (57, 66), a problem that may have been exacerbated by the recent economic crisis (57). Therefore, the management of Chagas disease in non-endemic countries is crucial to control infection. For an individual with chronic Chagas disease, the estimated average lifetime cost of health-care is US$27,684, with considerable variations between countries (60). Other authors have reported that, in the long term, it is cheaper to diagnose and treat individuals with Chagas disease than not (61). Accordingly, the high rate of underdiagnosis in non-endemic countries could be increasing the final cost of Chagas disease patients. The use of a single technique would reduce diagnosis costs and therefore allow the application of screening and control programs in countries where such systems have not yet been implemented.

In conclusion, Architect Chagas is a highly effective assay for the detection of Chagas disease, with 100% sensitivity, and it allows the correct diagnosis of the majority of samples when applied as a single technique. Architect Chagas can be used as a single assay in blood banks and clinical laboratories for routine diagnosis. Only grey zone and positive sera with a result ≤ 6 S/CO would need to be confirmed by a second serological assay to avoid both FP sera and cross-reactions with *Leishmania* spp.

363 The application of this proposal would result in important savings in the cost of Chagas
364 disease diagnosis, and therefore in the management and control of the disease.
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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

FUNDING INFORMATION

This work was partially supported by Departament d'Universitats, Recerca i Societat de la Informació de la Generalitat de Catalunya, Spain (2014SGR026). AA, MG and ST belong to the RICET, a Tropical Disease Cooperative Research Network in Spain (grant number RD12/0018/0010). CM belongs to the Spanish Network for Research in Infectious Diseases (REIPIRD12/0015), Instituto de Salud Carlos III, Madrid, Spain.

ACKNOWLEDGMENTS

The authors would like to thank Drs. Montserrat Portús, Joaquim Gascón, and Pere Coll for their support and helpful scientific discussions.

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609 **TABLES**

610 **Table 1. Overview of the results obtained with the Architect Chagas assay for the**

611 **four panels of sera studied.**

		Pre-characterized sera			Other infections	Total
		Panel I (n=107)	Panel II (n=125)	Panel III (n=12)	Panel IV (n=71)	(n=315)
CMIA	Positive	107	1	1	5	114
	Negative	0	124	10	66	200
	Grey zone	0	0	1	0	1
	Total	107	125	12	71	315

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627 **Table 2. Measures of diagnostic accuracy of the Architect Chagas assay results.**

Measure	Result (numerator/denominator)	95% CI
Sensitivity (%)	100 (108/108)	99.54-100
Specificity (%)	97.56 (200/205)	95.21-99.92
Validity index (%)	98.40 (308/313)	96.85-99.95
PPV (%)	95.58 (108/113)	91.34-99.81
NPV (%)	100 (200/200)	99.75-100
LR+	41.00	17.25-97.45
LR-	-	-
Youden index	0.98	0.95-1

628 95% CI, 95% confidence interval; PPV, positive predictive value; NPV, negative
629 predictive value; LR+, positive likelihood ratio; LR-, negative likelihood ratio.
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Table 3. False positive (FP) serum results of the Architect Chagas assay (n = 6).

FP sera	Architect Chagas (S/CO)	Other infections
1	2.22	Unknown
2	1.83	Leishmaniasis
3	4.57	Leishmaniasis
4	4.09	Leishmaniasis
5	3.21	Leishmaniasis
6	2.40	Leishmaniasis

S/CO, sample relative light units/cut-off value.

FIGURE LEGEND

Figure 1. Overall serum value distribution of ELISAc (A), ELISAr (B) and Architect Chagas (C). Sera from panel I (samples from chronic chagasic seropositive patients, n = 107), panel II (samples from non-chagasic patients, n = 125), panel III (samples with discrepant serological results, n = 12) and panel IV (samples from patients with other infections, n = 71) are represented. Full circles (●) indicate true positive and negative results, empty circles (O) indicate false positive and negative results, and crosses (✕) represent results in the grey zone. Dashed lines represent the cut-off value established for each test: 20 units for ELISAc (A), 1 absorbance/ cut-off value for ELISAr (B) and 1 relative light unit/ cut-off value for Architect Chagas (C). Dotted line in C indicates the point of 6 relative light units/ cut-off value in the Y-axis.