Evaluation of the Elecsys® Chagas Assay for the Detection of Trypanosoma cruzi-Specific Antibodies in a Multicenter Study in Europe and Latin America

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ABSTRACT

Serology is the preferred method to confirm Chagas disease diagnosis and to screen blood donors. A battery of assays is often required due to the limited accuracy of single assays. The Elecsys® Chagas assay is a newly developed, double-antigen sandwich assay for use on the Elecsys® and cobas® e immunoassay analyzers, intended to identify individuals infected with Trypanosoma cruzi, for diagnosis and screening. The performance of the Elecsys® Chagas assay was evaluated in comparison with other widely used T. cruzi antibody assays, at multiple sites (Europe/Latin America). Relative sensitivity and specificity were assessed in samples from blood donors, pregnant women, and hospitalized patients, from endemic and non-endemic regions. The Elecsys® Chagas assay had an overall relative sensitivity of 100% (n = 674). Overall relative specificity was 99.90% (n = 14,681), 100% (n = 313) and 100% (n = 517) in samples from blood donors, pregnant women and hospitalized patients respectively. Analytical specificity was 99.83% (n = 594). The Elecsys® Chagas assay detected T. cruzi antibodies in two World Health Organization (WHO)-standard T. cruzi reference panels (09/188 and 09/186) at a 1:512 dilution, corresponding to a cut-off sensitivity of approximately 16 mIU/ml. The Elecsys® Chagas assay demonstrated a robust performance under routine conditions at multiple sites in Europe and Latin America. In contrast to other available Chagas assays, the Elecsys® assay uses a reduced number of recombinant T. cruzi antigens resulting in a significantly smaller number of cross-reactions having improved analytical specificity, while being highly sensitive.
INTRODUCTION

Chagas disease (American trypanosomiasis) is caused by the flagellate protozoan Trypanosoma cruzi and affects six to seven million people worldwide, mainly in Latin America (1, 2). Vector-borne transmission via insects of the subfamily Triatominae occurs in the Americas; however, the infection can also be transmitted congenitally and via blood transfusion, organ transplantation and by ingestion of food/beverages contaminated with parasites (3-6). Although mainly affecting individuals living in endemic regions, the disease has now spread to other regions and continents (7-10).

The infection is characterized by an acute, often asymptomatic stage, lasting 8 to 12 weeks, when active parasitemia is evident. During this stage, diagnosis can be performed by direct microscopy of the blood for circulating parasites, or via polymerase chain reaction (PCR). The infection subsequently enters a chronic phase (1, 11, 12) and the majority of individuals remain asymptomatic. However, up to 30% of individuals will progress to develop Chagas cardiomyopathy over decades, and up to 10% will develop gastrointestinal, neurological or oligosymptomatic alterations, requiring treatment (1, 11, 12); these pathologies typically develop over years to decades. Generally, in the chronic phase of infection, the management of specific symptoms/conditions is necessary (13-15). The availability of curative treatment is a controversial topic; the WHO recommends treatment of adults with anti-parasitic drugs to prevent disease progression and congenital transmission in pregnant women (1).

Importantly, chronically infected individuals represent a substantial population capable of transmitting the infection, particularly through blood or organ donation or from mother to child (4, 6). During the chronic phase, individuals exhibit low-level or no parasitemia and thus direct microscopy is inappropriate. PCR detects the parasite...
in 40–65% of patients with chronic disease (16, 17); consequently, diagnosis relies upon the detection of *T. cruzi* antibodies by serological methods (13, 17, 18).

The most commonly used serological methods are enzyme-linked immunosorbent assays (ELISA) and immunofluorescent (IFI) antibody tests (13, 17, 19); few automated systems based on chemiluminescence have been introduced (20, 21).

The diagnostic approach to Chagas disease is heterogeneous, with guidelines varying according to location (i.e. laboratory, region or country) and purpose (i.e. screening of blood/organ donors versus diagnosis of patients with symptomatic disease) (22-26). Conventional tests based on total antigens show cross-reactivity between *T. cruzi* and *Leishmania* spp. or *T. rangeli*; therefore, confirmation of the presence of *T. cruzi* antibodies commonly requires the use of at least two tests that are based on different methods/antigens. Furthermore, the resolution algorithms (i.e. sequence, type and number of tests used) also vary by region (22, 25, 27). However, with the availability of assays with improved sensitivity and specificity, it has been suggested that a single assay may now be adequate for screening and diagnosis (28).

The primary aim of this study was to assess the relative sensitivity and specificity of the fully automated Elecsys® Chagas assay in comparison with other state-of-the-art *T. cruzi* antibody assays. Secondary aims were the evaluation of analytical specificity and analytical sensitivity at the cut-off in dilution series against the WHO standards.
RESULTS

Relative sensitivity. A total of 674 pre-characterized positive frozen samples were tested (Table 1). Pre-characterization was performed via serology and PCR ($n = 158$), or via serology alone ($n = 516$) with and without clinical staging ($n = 135$ and $n = 381$, respectively) (Table 2).

All Chagas-positive samples were correctly identified by the Elecsys® Chagas assay (sensitivity: 100%; 95% confidence interval [CI], 99.45–100%). The Abbott ARCHITECT assay correctly identified all Chagas-positive samples (sensitivity: 100%; 95% CI, 97.90–100%; $n = 174$), while the ORTHO® T. cruzi assay detected positive samples with a sensitivity of 96% (95% CI, 93.89–97.54%; $n = 500$).

Relative specificity. A total of 14,681 samples from blood donors were tested (Fig. 1a); the relative specificities of the Elecsys® Chagas assay (and comparison methods) after resolution testing (Fig. 1b) are summarized in Table 3. The Elecsys® Chagas assay had an overall relative specificity of 99.88% (initially reactive [IR]; 95% CI, 99.81–99.93%) and 99.90% (repeatedly reactive [RR]; 95% CI, 99.83–99.94%). Relative specificity was 99.70% (IR; 95% CI, 99.51–99.83%) and 99.74% (RR; 95% CI, 99.56–99.86%) in the Latin American subgroup, and 99.98% (IR and RR; 95% CI, 99.93–100%) in the European subgroup. Overall, there were 26 qualitative discrepant results (Europe, $n = 10$; Latin America, $n = 16$) versus the comparator assays and 13 concordant reactive results (Latin America) were identified. Relative specificity (RR) was 100% with the ORTHO® T. cruzi ELISA (95% CI, 99.93–100%; $n = 5241$), 99.96% with the DiaSorin Liaison® assay (95% CI, 99.86–100%; $n = 5244$), 99.93% with the Abbott PRISM assay (95% CI, 99.80–99.99%; $n = 4391$), and 99.78% with the Abbott ARCHITECT assay (95% CI, 99.61–99.89%; $n = 5046$).
A total of 313 residual samples from pregnant women were tested. There were no discrepant or concordant reactive results. The Elecsys® Chagas assay had an overall relative specificity of 100% (IR and RR; 95% CI, 98.83–100%), which was generally consistent between the endemic region (Latin America) (IR and RR: 100% [95% CI, 95.14–100%]; n = 74 samples) and non-endemic region (European) subgroups (IR and RR: 100% [95% CI, 98.47–100%]; n = 239 samples). Comparable results were obtained with the DiaSorin Liaison® and Abbott ARCHITECT assays (data not shown).

A total of 517 residual samples from hospitalized patients were tested and there were no discrepant or concordant reactive results. The Elecsys® Chagas assay had an overall relative specificity of 100% (IR and RR; 95% CI, 99.29–100%), which was generally consistent between the Latin American (IR and RR: 100% [95% CI, 80.49–100%]; n = 17 samples) and European subgroups (IR and RR: 100% [95% CI, 99.26–100%]; n = 500 samples). Comparable results were obtained with the DiaSorin Liaison® XL MUREX Chagas and Abbott ARCHITECT assays (data not shown).

**Analytical specificity.** A total of 594 potentially cross-reactive samples were tested with the Elecsys® Chagas assay (Table 4) and overall analytical specificity was 99.83% (95% CI, 99.07–100%).

A subgroup of pre-characterized leishmaniasis-positive samples (n = 164) and malaria-positive samples (n = 100) from Spain were tested on both the Elecsys® Chagas assay and ORTHO® *T. cruzi* ELISA. In the leishmaniasis-positive cohort, all samples tested non-reactive for *T. cruzi* antibodies with the Elecsys® Chagas assay (analytical specificity: 100%; 95% CI, 97.78–100%) while 65 samples tested positive.
with the ORTHO® T. cruzi ELISA (analytical specificity: 60.37%; 95% CI, 52.44–
67.91%). In the malaria-positive cohort, one sample tested reactive with the Elecsys®
Chagas assay (low level cut-off index [COI] of 1.11; analytical specificity: 99.00%;
95% CI, 94.55–99.97%) and four samples tested positive with the ORTHO® T. cruzi
ELISA (analytical specificity: 96.00%; 95% CI, 90.07–98.90%). The co-infection
Plasmodium/T. cruzi was ruled out. These samples were from Spanish citizens and
immigrant residents who had traveled to South Asia and/or Africa.

Six additional samples (dengue, n = 5; leishmaniasis, n = 1) were excluded from the
analysis because a co-infection could not be ruled out. These samples originated
from a Chagas endemic region (Argentina) and were found reactive in the Elecsys®
Chagas assay (with COI values ranging from 13.3 to 206) as well as in at least one
additional Chagas antibody assay. Four out of five dengue samples thereof were
also found to be highly reactive in at least two comparator Chagas assays. Further
resolution testing was not possible due to lack of sample volume.

**Analytical sensitivity at the cut-off.** The analytical sensitivity at the cut-off of the
Elecsys® Chagas assay and comparator assays (n=3 automated assays and n=4
non-automated ELISA assays; reflecting local routine methods) was assessed using
two WHO-standard National Institute for Biological Standards and Control (NIBSC)
reference panels. The Elecsys® Chagas assay detected T. cruzi antibodies at a
1:512 dilution for both reference panels (Table 5), corresponding to a cut-off
sensitivity of approximately 1 mIU/ml. The earliest detection with a comparison assay
was 1:32 for panel 09/188 (T. cruzi I) and 1:16 for panel 09/186 (T. cruzi II),
corresponding to cut-off sensitivities of 15.6 mIU/ml and 31.3 mIU/ml respectively.
Typical distribution of values. Distribution of COI values for $n = 16,185$ samples (reactive and non-reactive samples from blood donors, pregnant women, hospitalized patients and confirmed Chagas positives) is displayed in Fig. 2. The Elecsys® Chagas assay revealed a good discrimination between reactive versus non-reactive samples. Only a minor number of the samples were found with low positive COI values in the Elecsys® Chagas assay ($n = 16185$; 12 samples with COI values ranging from 1 to 2, representing 0.07%; Fig. 2).

Lowest COI values observed for the pre-characterized Chagas-positive cohort ($n = 674$) were 1.79 and 1.80, thus representing just 0.3% of the total positive sample cohort. All other Chagas patient samples showed COI values ranging from 2.3 to $>300$.

Neutralization. A total of 10 Elecsys® assay-discrepant RR samples from the blood donor cohort underwent in-house neutralization testing to further assess the presence of antibodies to *T. cruzi* that might be undetectable on comparator methods with lower sensitivity. Five samples from endemic regions could successfully be neutralized (recovery of COI ≤25%) and one sample from an endemic region showed a borderline tendency for neutralization with the native antigen pre-treatment procedure (26% recovery). Four samples could not be neutralized and revealed COI recoveries of 28–98% (Table 6).
DISCUSSION

In the present study, the Elecsys® Chagas assay demonstrated excellent analytical performance in a multicenter study in Europe and Latin America when compared with established assays. Although the Elecsys® Chagas assay uses a new combination of just three different recombinant T. cruzi antigens, the performance observed in the present study supports its use as a diagnostic and screening test.

Since screening for blood products harboring T. cruzi is a critical component for blood safety, we also investigated the performance of the test in samples from blood donors from various endemic and non-endemic regions. Importantly, a substantial number of serum samples from patients known to have Chagas disease from different regions were included in the study. Furthermore the excellent analytical specificity was confirmed using a large panel of potential cross-reactive samples, or samples from individuals with other infectious diseases. Lastly, the performance of the Elecsys® Chagas assay was also verified in samples from pregnant women and hospitalized patients and the results were comparable, irrespective of whether the samples were sourced from endemic and non-endemic regions.

A number of studies have demonstrated various T. cruzi antigens (either native or recombinant; or as peptide or multi-epitope antigen) as potentially suitable for use as serodiagnostic tools (29-34). However, the number of samples used for evaluation varies and the statistical power of the results may be limited. Studies including a significant number of blood donor screening samples, potentially cross-reacting samples, and proven reactive samples are found less frequently (20, 35-38). WHO-driven comparison activities were conducted over a decade ago (39), with specificity and sensitivity varying considerably between the 18 screening assays evaluated. A definitive resolution of the discrepant findings was difficult since “consensus
positives” and “consensus negatives” always inherit a selection bias, while the true
serological status cannot be revealed (39). It may now be timely to conduct
comparative evaluations of the new assays that have become available since this
WHO study, to ascertain their relative efficacies.

The WHO seeks to promote identification of novel diagnostic tests for Chagas
disease (1). Although there are a variety of methods available to confirm the
presence of *T. cruzi* infection (ELISA-based, immunofluorescence-based,
immunoblots, PCR, microscopy), no gold standard currently exists and the testing of
samples with multiple assays is often necessary, creating a combined gold standard
as a surrogate (13, 17, 28). This leaves manufacturers of new, highly sensitive tests
with a dilemma.

In the present study, the surrogate ‘gold standard’ against which the Elecsys® assay
was compared included at least three serological assays or PCR (for the evaluation
of relative sensitivity), and resolution testing at two independent reference centers
using several commercial CE-labeled tests and well-evaluated in-house assays
representing state-of-the-art methods (for the evaluation of relative specificity).

Our study results support the notion that existing methods worldwide may not be
adequate to confirm samples with low-level antibody concentration, which may be
best confirmed according to epidemiological and clinical background. Equally,
samples with higher concentrations in this study could potentially be confirmed with
any test. Moreover, our findings add to the evidence suggesting that single assays
with improved sensitivity and specificity may be sufficient for screening and
diagnosis purposes, respectively (28).
Since the Elecsys® Chagas assay was developed without a gray zone, and a clear separation of reactive versus non-reactive samples was validated in this multicenter evaluation, the potential number of unclear and inconclusive or “low-titer” samples following the initial analysis is expected to be reduced significantly if compared with formerly described assays (28) or resolution algorithms (27). A highly sensitive automated method to screen for Chagas disease, such as the Elecsys® Chagas assay, could potentially increase throughput of samples and likely lead to improvements in diagnosis algorithms, and thus, in cost effectiveness (28). Ultimately, the availability of improved assays for the detection of *T. cruzi* would be expected to better safeguard patients who require blood and organ donation, and help to minimize misdiagnoses, which are a major factor in delaying the appropriate healthcare response (21).

Strengths of this study are the inclusion of a significant proportion of samples from Latin America to evaluate the Elecsys® Chagas assay under blood screening and diagnostic routine laboratory conditions in countries where the disease is endemic. This is important because geographical differences in the sensitivity of recombinant antigen-based rapid tests for *T. cruzi* infection have been demonstrated, possibly due to *T. cruzi* strain differences (40). Commercially available performance panels covering samples from an additional nine countries were all found reactive with the Elecsys® assay (Roche internal data, not shown), underlining the sensitivity for Chagas samples from South- and Central-America. The present study also included analysis of reactive samples stored frozen for a period of years, demonstrating the general stability of the analyte (IgG *per se*). Moreover, in samples that showed a loss of reactivity with competitor assays during the long-term storage, the Elecsys® COI values ranged from 1.8 to 70.9, supporting the high sensitivity of the assay. Finally,
the Elecsys® Chagas assay was compared with several existing assays to ensure relevance to local protocols and thus to current benchmarks for performance.

Evaluation with a commercially available seroconversion panel revealed identical seroconversion-sensitivity to competitors, thus reconfirming the sensitivity for samples derived from the early phase of the infection (Table 7).

Compared with the comparator tests, discrepant results were observed in 26 of 14,681 blood donor samples (0.17%) derived from non-endemic and endemic regions, a significant lower percentage than observed with new-generation competitors (28). Since there is no established gold standard for the detection of anti- *T. cruzi* antibodies we used a neutralization test to further investigate such discrepant reactive results obtained in the Elecsys® test. Application of a neutralization test has successfully been described for verification of discrepant reactive results in a highly sensitive assay detecting anti-*T. gondii* antibodies (41) and could successfully be applied here for *T. cruzi*. The relative specificity (99.74%) observed in the present study for the subgroup of blood donors from Latin America may therefore be even higher due to the reconfirmed presence of specific antibodies in Elecsys®-discrepant reactive samples. The in-house neutralization method to resolve discrepant reactive findings with state-of-the-art assays was used here for the first time within a multicenter study, and was deemed to be an additional specific and valuable method. The use of the heterologous native *T. cruzi* antigen extract for supplemental neutralization testing avoids an inbreeding confirmatory situation to the recombinant antigens used by the Elecsys® assay. We were however, unable to perform neutralization testing in all samples with qualitative discrepant results due to a lack of sufficient sample volume in some cases. Due to the use of residual blood
donor samples, there was also no possibility of a serological donor follow-up to clarify questionable results.

The high analytical sensitivity of the Elecsys® assay is reflected by comparison of cut-off sensitivity based on the use of material accessible to the public, such as WHO reference material from NIBSC. Such reference material may help to better benchmark the dilutional sensitivities and the individual cut-off settings of the respective assays. This approach is widely used to assess the performance of screening assays and is also an inherent part of the Common Technical Specifications (CTS) of European Commission Directive 98/79, for screening assays.

The herein described recombinant assay format is highly sensitive, which is in contradiction to the notion that solely whole-parasite using techniques are sufficiently sensitive (42). Sensitivity assessment regarding different Distinct Typing units of *T. cruzi* was covered during the development of the assay by analysis of *n* = 1370 suspected Chagas samples (all investigated samples ≥ 3 assays reactive) derived from 13 countries (Argentina, Bolivia, Chile, Spain, Ecuador, El Salvador, Honduras, Mexico, Nicaragua, Paraguay, Uruguay, USA and Venezuela) revealing 100% reactive results with the Elecsys® Chagas assay (Roche internal data, not shown; lowest observed S/CO value of all samples: > 2). Thus the assay is suitable in blood donor management as well as in diagnostic use.

The development of highly sensitive and specific new generation assays for the detection of anti-*T. cruzi* antibodies thus helps to reduce expenses for additional second line testing for diagnosis of the disease and safeguards the sensitivity needed for blood screening purposes.
CONCLUSIONS

The automated Elecsys® Chagas assay demonstrated a robust and favorable performance under routine conditions at multiple sites in Europe and Latin America. In contrast to other available Chagas assays, the Elecsys® assay uses a reduced number of recombinant *T. cruzi* antigens, resulting in a significantly smaller number of cross-reactions with improved analytical specificity, while still being highly sensitive and with high discriminatory power.
MATERIALS AND METHODS

Study design. Analytical performance of the Elecsys® Chagas assay was evaluated at five independent laboratories in Europe and Latin America, and at the Roche Diagnostics Assay Development facility. The study was performed between August 2015 and July 2016. All samples were anonymized or pseudonymized, residual fresh or frozen serum/plasma samples, either from daily routine or from blood donor testing (Table 1).

Prior to study start, ethical approval (or waiver) was obtained from the relevant local authorities. The study was conducted in accordance with the principles of the Declaration of Helsinki and International Conference on Harmonisation guidelines for Good Clinical Practice. Where necessary, donors/participants provided written informed consent.

Elecsys® Chagas and comparator assays. The Elecsys® Chagas assay is an automated electrochemiluminescence immunoassay for the qualitative determination of antibodies to T. cruzi for use on cobas e analyzers (43) in equipped laboratory settings. The assay is based on a double-antigen sandwich principle, utilizing soluble forms of recombinant T. cruzi antigens derived from flagellar calcium binding protein, flagellar repetitive antigen, and cruzipain (the major cysteine proteinase of T. cruzi).

During the first incubation, 18 µL of sample is added to a reaction mixture containing biotin-labeled and ruthenium-labeled T. cruzi antigens to form antibody-antigen immune complexes (one antigen binding site of the patient’s specific IgG binding the Biotinylated antigen and the other paratope binding the Ruthenium-labeled antigen). In a second incubation, the immunoglobulin G (IgG)-double-antigen sandwich complex is bound via the biotin to streptavidin-coated beads and subsequently
transferred to the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Chemiluminescent emission from the Ruthenium-label is induced by application of a voltage to the electrode and measured by a photomultiplier. The results are automatically determined by software based on comparison of the electrochemiluminescence signal obtained from the reaction product with the cut-off value previously obtained during system calibration. The total assay time is 18 minutes.

The following comparison assays were performed according to the manufacturers’ recommendations (including calibration and respective control runs): whole cell lysate ORTHO® T. cruzi ELISA (Ortho Clinical Diagnostics, Johnson & Johnson, High Wycombe, UK); Abbott ARCHITECT Chagas and Abbott PRISM Chagas (Abbott Diagnostics, Illinois, USA); DiaSorin Liaison® XL MUREX Chagas (DiaSorin S.p.A, Saluggia, Italy); Wiener lab Chagastest ELISA recombinante v.4.0 (Wiener lab Group, Rosario, Argentina), NovaTech NovaLisa® Chagas (NovaTech Immundiagnostica GmbH, Dietzenbach, Germany) and the Biokit Bioelisa Chagas IgG (BIOKIT, S.A., Barcelona, Spain).

Relative sensitivity. The relative sensitivity of the Elecsys® Chagas assay was evaluated at sites in Europe (n = 1) and Latin America (n = 1) using pre-characterized Chagas-positive samples obtained from Chagas-infected patients in both regions. Comparison assays were the whole cell lysate ORTHO® T. cruzi ELISA and the Abbott ARCHITECT Chagas. Samples tested with the ORTHO® T. cruzi ELISA were previously characterized using in-house assays (ELISA-Centro Nacional de Microbiología [CNM] and IFI-CN) (44), Wiener lab Chagastest ELISA recombinante v.4.0 and PCR (45), while samples tested with the Abbott ARCHITECT Chagas were previously characterized with at least two of the following
serology assays: Wiener lab Chagatest ELISA recombinante v. 4.0, Wiener lab Chagatest HAI and in-house IFI (antigens and controls provided by the National Institute of Parasitology “Dr. Mario Fatala Chaben”, Buenos Aires, Argentina).

Samples with non-reactive results in the comparison assay were repeated in triplicate.

Relative specificity. The relative specificity of the Elecsys® Chagas assay was evaluated at four sites in Europe ($n = 2$) and Latin America ($n = 2$) using samples from blood donors, pregnant women and hospitalized patients, obtained in both regions.

For the testing of samples from blood donors, comparison assays were the ORTHO® $T. cruzi$ ELISA, DiaSorin Liaison® XL MUREX Chagas, Abbott PRISM Chagas and the Abbott ARCHITECT Chagas. For the testing of samples from pregnant women and hospitalized patients, comparison assays were the DiaSorin Liaison® XL MUREX Chagas and the Abbott ARCHITECT Chagas.

Initial determinations were carried out in single measurements. Samples with discrepant and concordant reactive results were repeated in duplicate on the respective assays and were considered to be RR if either of the retest results had a signal/cut-off ratio of $\geq 1.00$.

IR samples with incomplete retesting, or without retesting, were considered RR. IR and RR grayzone samples for the Abbott ARCHITECT Chagas assay were considered as reactive. Furthermore, an aliquot of discrepant and concordant reactive samples was stored for further resolution testing at two reference centers according to their local diagnostic algorithms (representing the surrogate ‘gold standard’; see below and Fig. 1).
Analytical specificity. Analytical specificity of the Elecsys® Chagas assay was evaluated at two sites using potentially cross-reacting samples from other infectious diseases (Table 4) e.g., (leishmaniasis, malaria, Epstein–Barr virus, dengue, syphilis, toxoplasmosis and human African trypanosomiasis).

Analytical sensitivity at the cut-off. Serial dilutions of two anti- T. cruzi antibody preparations from the NIBSC were measured in a single run (single determination per sample) using the Elecsys® Chagas assay and comparison assays. The WHO 1st International Standard for Chagas (TcI) antibody in Human Plasma (NIBSC code: 09/188) freeze-dried preparation contains anti- T. cruzi antibodies and consists of seropositive samples from autochthonous individuals living in Mexico, the region where T. cruzi I is endemic. The WHO 1st International Standard for Chagas (TcII) antibody in human plasma (NIBSC code: 09/186) freeze-dried preparation contains anti- T. cruzi antibodies and is representative for seropositive samples from autochthonous individuals living in Brazil, the region where T. cruzi II is endemic.

Each standard was dissolved in deionized water to a final concentration of 0.5 IU/ml. Serial 1:2 pool-dilutions were performed using Chagas-negative serum and distributed to the laboratories for analysis (dilutions ranged from 1:2 to 1:8192, corresponding to theoretical concentrations of 250 to 0.0610 mIU/ml of the respective antibody standards).

Comparison assays were the Abbott PRISM Chagas, Abbott ARCHITECT Chagas, DiaSorin Liaison® XL MUREX Chagas, Wiener lab Chagatest, ORTHO® T. cruzi ELISA whole cell lysate, NovaTech NovaLisa® Chagas and the Biokit Bioelisa Chagas IgG.
Confirmatory testing – Resolution testing. Discrepant and concordant reactive results from relative specificity testing underwent resolution testing at two independent reference centers using CE-labeled or in-house methods representing state-of-the-art Chagas antibody assays (Fig. 1). Final interpretation for each sample was used as the basis for assessment of relative specificity.

Confirmatory testing – Neutralization testing. If sufficient sample volumes remained after resolution testing (Fig. 1), Elecsys® Chagas discrepant reactive samples from the blood donor cohort were re-tested using an in-house neutralization method similar to that previously reported (41). Briefly, antigen extract (aqueous ultrasonic lysate supernatant) from native *T. cruzi* (CL Brener or DM28c) was added to the samples to a final concentration of 50 µg/ml *T. cruzi* antigen extract to generate a competitive situation between the recombinant antigens used in the Elecsys® Chagas assay and the native *T. cruzi* antigen extract. After this pre-treatment procedure to form stable antigen-antibody complexes, samples were subsequently re-run with the Elecsys® Chagas assay. Results of the COI values were compared with those derived using the untreated sample and recovery of the neutralized sample was calculated. A recovery of ≤25% of the initial concentration was interpreted as reconfirmation of the presence of anti-*T. cruzi* antibodies.

Data analyses. Measurements were captured using WinCAEv software; statistical analyses were performed using R-package VCA (version 1.2.1) and SAS (version 9.3; SAS Institute). Interpretation of assay results was performed according to the package inserts of the respective assays.

Relative sensitivity and specificity, and analytical sensitivity, are expressed as point estimates and two-sided 95% CI. For the Elecsys® Chagas assay, samples with a
signal/COI ratio of ≥1.0 were considered reactive (i.e. positive for antibodies to *T. cruzi*); <1.0 were considered non-reactive (i.e. negative for antibodies to *T. cruzi*).

Results of the comparator assays were interpreted as follows: whole cell lysate

ORTHO® *T. cruzi* ELISA (reactive: ≥cut-off [CO]; non-reactive: <CO); Abbott

ARCHITECT Chagas (reactive: ≥1.0; grayzone ≥0.8 to <1.0; non-reactive: <0.8);

Abbott PRISM Chagas (reactive: ≥1.0; non-reactive: <1.0); DiaSorin Liaison® XL

MUREX Chagas (reactive: ≥1.0; non-reactive: <1.0); Wiener lab Chagatest (reactive: ≥CO; non-reactive: <CO), NovaTech NovaLisa® Chagas (reactive: ≥CO absorbance +10%, grayzone: ± 10% CO signal; negative: <CO signal –10%) and the Biokit

Bioelisa Chagas IgG (positive: ≥1.0; equivocal: ≥0.9 —<1.0; negative: <0.9).

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| Site          | Cohort       | Number of samples | Source       | Condition | Matrix | Comparator assays tested               |
|--------------|--------------|-------------------|--------------|-----------|--------|----------------------------------------|
| Hagen, Germany | Blood donors | 4391              | Blood screening | Fresh | Serum  | Abbott PRISM Chagas                   |
| Pievesestina, Italy | Blood donors | 5244              | Blood screening | Fresh | Serum  | ORTHO® T. cruz/ ELISA;                 |
|              | Hospitalized patients | 500               | Daily routine | Frozen | Serum  | DiaSorin Liaison® XL                   |
|              | Pregnant women       | 239               | Daily routine | Frozen | Serum  | DiaSorin Liaison® XL                   |
| Location          | Type                  | Count | Sample Type | Storage | Method                        |
|-------------------|-----------------------|-------|-------------|---------|-------------------------------|
| Bucaramanga, Colombia | Blood donors        | 2707  | Fresh Plasma |        | Abbott ARCHITECT Chagas     |
| Buenos Aires, Argentina | Blood donors       | 1056  | Fresh Serum  |        | Abbott ARCHITECT Chagas     |
| Buenos Aires, Argentina | Blood donors     | 1283  | Frozen Serum |        | Abbott ARCHITECT Chagas     |
|                    | Hospitalized patients | 17    | Fresh Serum  |        | Abbott ARCHITECT Chagas     |
|                    | Pregnant women      | 74    | Fresh Serum  |        | Abbott ARCHITECT Chagas     |

**RELATIVE SENSITIVITY**

| Location          | Type                  | Count | Sample Type | Storage | Method                        |
|-------------------|-----------------------|-------|-------------|---------|-------------------------------|
| Madrid, Spain     | Chagas positive      | 500   | Collection of stored samples | Frozen Serum plasma | ORTHO® T. cruzi ELISA |
| Buenos Aires, Chagas positive |               | 174   | Serotheque | Frozen Serum | Abbott ARCHITECT Chagas     |
Argentina

Samples collected at the Universidad Nacional del Litoral, Santa Fe, Argentina
### TABLE 2 Relative sensitivity cohort characterization

|                     | Chagas positive samples cohort n=674 | Endemic region\(^a\) n=174 | Non – Endemic region\(^b\) n=500 | PCR + Serological characterization n=158 |
|---------------------|--------------------------------------|-----------------------------|----------------------------------|----------------------------------------|
| Serological characterization | Serological characterization plus clinical stage characterization n=135\(^c\) | ELISA                      | In house CNM IFAT                | Anti T. cruzi – kDNA – PCR             |
|                     |                                      | HAI                         | In house CNM ELISA               | In house CNM IFAT                      |
|                     |                                      | Antibodies induced by T. cruzi | Wiener lab Chagatest ELISA recombinante v.4.0 | In house CNM ELISA |
|                     |                                      | Anti T. cruzi homogenate    |                                  | Wiener lab Chagatest ELISA recombinante v.4.0 |
|                     |                                      | Anti FRA                    |                                  |                                        |
|                     |                                      | Anti p28                    |                                  |                                        |
|                     |                                      | Anti B13                    |                                  |                                        |
|                     |                                      | Chest and abdominal X ray   |                                  |                                        |
|                     |                                      | Electrocardiogram           |                                  |                                        |
|                     |                                      | Echocardiogram              |                                  |                                        |

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\(^a\) Samples provided by Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) – Argentina

\(^b\) Samples provided by Instituto de Salud Carlos III – Spain

\(^c\) Chronic Chagas stage I (n=66), stage II (n=44) and stage III (n=25)
Table 3: Relative specificity of the Elecsys® Chagas assay in blood donor samples

|                      | Elecsys® Chagas | ORTHO® T. cruzi ELISA | DiaSorin Liaison® XL MUREX Chagas | Abbott PRISM Chagas | Abbott ARCHITECT Chagas |
|----------------------|-----------------|-----------------------|------------------------------------|---------------------|------------------------|
| Overall cohort       |                 |                       |                                    |                     |                        |
| Sample number, N     | 14681           | 5241                  | 5244                               | 4391                | 5046                   |
| Confirmed positive   | 8               | 0                     | 0                                  | 0                   | 8                      |
| Negative             | 14673           | 5241                  | 5244                               | 4391                | 5038                   |
| IR                   | 25              | 0                     | 2                                  | 6                   | 15                     |
| RR*                  | 23              | 0                     | 2                                  | 3                   | 14                     |
| RR confirmed positive| 8/23            | 0/0                   | 0/2                                | 0/3                 | 8/14                   |
| Specificity % IR (95% CI) | 99.88         | 100                   | 99.96                              | 99.86               | 99.78                  |
| Specificity, % RR (95% CI) | 99.90 | 100 | 99.96 | 99.93 | 99.78 |
|---------------------------|-------|-----|-------|-------|-------|
|                         | (99.83–99.94) | (99.93–100) | (99.86–100) | (99.80–99.99) | (99.61–99.89) |

**European subgroup**

| Sample number, n | 9635 | 5241 | 5244 | 4391 | NA |
|------------------|------|------|------|------|----|
| IR               | 2    | 0    | 2    | 6    | NA |
| RR               | 2    | 0    | 2    | 3    | NA |
| RR confirmed positive | 0/2 | 0/0 | 0/2 | 0/3 | NA |

| Specificity, % IR (95% CI) | 99.98 | 100 | 99.96 | 99.86 | NA |
|---------------------------|-------|-----|-------|-------|----|
|                         | (99.93–100) | (99.93–100) | (99.86–100) | (99.70–99.95) |

| Specificity, % RR (95% CI) | 99.98 | 100 | 99.96 | 99.93 | NA |
|---------------------------|-------|-----|-------|-------|----|
|                         | (99.93–100) | (99.93–100) | (99.86–100) | (99.80–99.99) |

**Latin America subgroup**
| Sample number, n | 5046 | NA | NA | NA | 5046 |
|------------------|------|----|----|----|------|
| IR               | 23   | NA | NA | NA | 15   |
| RR               | 21   | NA | NA | NA | 14   |
| RR confirmed positive | 8/21 | NA | NA | NA | 8/14 |
| Specificity, % IR (95% CI) | 99.70 | NA | NA | NA | 99.78 |
|                  | (99.51–99.83) | (99.61–99.89) |
| Specificity, % RR (95% CI) | 99.74 | NA | NA | NA | 99.78 |
|                  | (99.56–99.86) | (99.61–99.89) |

*3 reactive samples (in at least one assay) with incomplete retesting were considered RR for specificity calculation.

CI, confidence interval; IR, initially reactive; NA, not applicable; RR, repeatedly reactive.
### TABLE 4 Analytical specificity of the Elecsys® Chagas assay with potentially cross-reactive samples

| Potentially cross-reacting condition or disease state | N  | Non-reactive n (%) | Reactive n (%) |
|-----------------------------------------------------|----|--------------------|---------------|
| Epstein–Barr virus                                  | 26 | 26 (100)           | 0             |
| Dengue                                              | 87 | 87 (100)           | 0             |
| Leishmaniasis<sup>a</sup>                           | 241| 241 (100)          | 0             |
| Malaria<sup>b</sup>                                 | 204| 203 (99.5)         | 1 (0.5)       |
| Syphilis                                            | 19 | 19 (100)           | 0             |
| Toxoplasmosis                                      | 15 | 15 (100)           | 0             |
| Human African trypanosomias                         | 2  | 2 (100)            | 0             |
| **TOTAL**                                           | 594| 593                | 1             |
Samples were tested at Roche Diagnostics Centralized and Point of Care Solutions (Penzberg, Germany), unless stated otherwise.

a164 Leishmania-positive and 100 malaria-positive serum/plasma samples were tested in Madrid, Spain; samples (serotheque) were previously stored frozen.

bSamples used for the analytical specificity study were derived from commercial vendors (Acess Biologicals, USA; Slieagen LLC, USA, Cerba Specimens Services, France; TRINA Bioreactives AG, Switzerland; BioClinical Partner Inc., USA, DiaServe GmbH, Germany), routine laboratories and Institutions (Instituto de Salud, Carlos III, Madrid, Spain). Characterization of the samples was either by serological analysis, parasitological certificate, or clinical definition.
TABLE 5  Detection limit of the Elecsys® Chagas and comparison assays using World Health Organization-standard National Institute for Biological Standards and Control reference panels 09/188 (T. cruzi I) (A) and 09/186 (T. cruzi II) (B).

| Dilution | Conc. [mIU/ml] | Elecsys® Chagas | Abbott PRISM Chagas | Abbott ARCHITECT Chagas | DiaSorin Liaison® XL MUREX Chagas | Wiener lab Chagatest | ORTHO® T. cruzi ELISA | NovaTech NovaLisa® Chagas | Biokit bioelisa Chagas |
|----------|----------------|----------------|---------------------|------------------------|---------------------------------|---------------------|---------------------|---------------------|---------------------|
| 1:8192   | 0.06           | 0.14           | 0.06                | 0.26                   | 0.03                            | 0.20                | 0.31                | 0.52                | –                   |
| 1:4096   | 0.12           | 0.21           | 0.08                | 0.13                   | 0.03                            | 0.14                | 0.23                | 0.61                | –                   |
| 1:2048   | 0.24           | 0.32           | 0.06                | 0.21                   | 0.03                            | 0.19                | 0.25                | 0.56                | 0.04                |
| 1:1024   | 0.49           | 0.57           | 0.07                | 0.19                   | 0.03                            | 0.20                | 0.38                | 0.61                | 0.04                |
| 1:512    | 0.98           | 1.02           | 0.07                | 0.20                   | 0.03                            | 0.20                | 0.38                | 0.53                | 0.07                |
| 1:256    | 1.95           | 2.00           | 0.11                | 0.31                   | 0.04                            | 0.26                | 0.36                | 0.53                | 0.02                |
| 1:128    | 3.91           | 3.87           | 0.14                | 0.47                   | 0.05                            | 0.34                | 0.38                | 0.54                | 0.07                |

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| Dilution | Conc. [mIU/ml] | Elecsys® Chagas | Abbott PRISM Chagas | Abbott ARCHITECT Chagas | DiaSorin Liaison® XL Chagas | Wiener lab Chagatest | ORTHO® T. cruzi ELISA | NovaLisa® Chagas | Bioelisa Chagas |
|----------|----------------|------------------|--------------------|------------------------|---------------------------|---------------------|---------------------|----------------|----------------|
| 1:8192   | 0.06           | 0.15             | 0.06               | 0.14                   | 0.03                      | 0.22                | 0.27                | 0.55           | –              |
| 1:4096   | 0.12           | 0.21             | 0.05               | 0.14                   | 0.03                      | 0.21                | 0.32                | 0.64           | –              |
| Dilution | Value 1 | Value 2 | Value 3 | Value 4 | Value 5 | Value 6 | Value 7 | Value 8 |
|----------|---------|---------|---------|---------|---------|---------|---------|---------|
| 1:2048   | 0.24    | 0.34    | 0.06    | 0.13    | 0.03    | 0.21    | 0.59    | 0.58    | 0.06    |
| 1:1024   | 0.49    | 0.61    | 0.07    | 0.14    | 0.03    | 0.23    | 0.43    | 0.54    | 0.06    |
| 1:512    | 0.98    | 1.10    | 0.06    | 0.19    | 0.03    | 0.21    | 0.33    | 0.54    | 0.05    |
| 1:256    | 1.95    | 2.08    | 0.06    | 0.20    | 0.03    | 0.27    | 0.31    | 0.54    | 0.06    |
| 1:128    | 3.91    | 4.00    | 0.09    | 0.27    | 0.04    | 0.29    | 0.30    | 0.52    | 0.08    |
| 1:64     | 7.81    | 7.89    | 0.09    | 0.37    | 0.04    | 0.38    | 0.41    | 0.56    | 0.12    |
| 1:32     | 15.60   | 15.60   | 0.10    | 0.74    | 0.07    | 0.73    | 0.50    | 0.60    | 0.16    |
| 1:16     | 31.30   | 30.70   | 0.27    | 1.49    | 0.11    | 0.95    | 0.87    | 0.70    | 0.27    |
| 1:8      | 62.50   | 60.20   | 0.95    | 2.54    | 0.27    | 1.43    | 1.41    | 0.92    | 0.51    |
| 1:4      | 125     | 112     | 2.84    | 4.56    | 0.54    | 2.54    | 1.92    | 1.09    | 1.01    |
| 1:2      | 250     | 192     | 2.21    | 6.52    | 1.10    | 3.41    | 2.96    | 1.76    | 1.86    |
| Undiluted| 500     | 229     | 5.04    | 8.49    | 1.90    | 4.43    | 4.12    | 2.95    | 1.50    |
Data are expressed as COI (bold = reactive; no bold = non-reactive). *Data in italics represents results within the ‘grayzone’ for the NovaTech NovaLisa® or the Abbott ARCHITECT assays respectively.
TABLE 6 Neutralization results of Elecsys® Chagas assay-discrepant repeatedly reactive samples from blood donors

| Study site | Comparator [COI] | Elecsys® [COI] | Elecsys® [COI] after neutralization | Recovery® [%] |
|------------|-----------------|----------------|-------------------------------------|---------------|
| Italy      | 0.016           | 2.94           | 1.87                                | 64            |
| Colombia   | 0.023           | 1.16           | 0.298                               | 26            |
| Colombia   | 0.037           | 1.09           | 0.300                               | 28            |
| Colombia   | 0.554           | 1.47           | 0.515                               | 35            |
| Argentina  | 0.230           | 12.6           | 0.348                               | 3             |
| Argentina  | 0.99/0.97/1.14c | 40.8           | 2.04                                | 5             |
| Argentina  | 0.040           | 2.92           | 0.227                               | 8             |
| Argentina  | 0.080           | 1.60           | 0.132                               | 8             |
| Argentina  | 0.510           | 1.47           | 0.152                               | 10            |
| Country | COI | Titer | Titer | % Neutralization |
|---------|-----|-------|-------|-----------------|
| Argentina | 0.030 | 1.03 | 1.01 | 98 |

*COI as determined by Roche Diagnostics Centralized and Point of Care Solutions (Penzberg, Germany) prior to neutralization procedure.

A percentage recovery ≤25 was assessed as successful neutralization (i.e. positive for anti- *T. cruzi* antibodies); values in **bold** represent neutralizable/borderline and neutralizable samples.

COI was repeatedly in the grayzone.

COI, cut-off index.
TABLE 7 Evaluation of seroconversion sensitivity with a commercially available seroconversion panel: SeraCare Chagas (*T. cruzi*) AccuVert™ Seroconversion Panel 0615-0038.

| Panel member | Bleed date | Days since 1<sup>st</sup> bleed | Roche Diagnostics Elecsys<sup>®</sup> Chagas<sup>1</sup> S/CO | Interpretation |
|--------------|------------|---------------------------------|-------------------------------------------------|----------------|
| 1            | 31.07.2012 | 0                               | 0.071                                           | Non-reactive   |
| 2            | 10.09.2012 | 41                              | 117                                             | Reactive       |
| 3            | 17.09.2012 | 48                              | 118                                             | Reactive       |
| 4            | 24.09.2012 | 55                              | 127                                             | Reactive       |
| 5            | 01.10.2012 | 62                              | 143                                             | Reactive       |
| 6            | 08.10.2012 | 69                              | 151                                             | Reactive       |
| 7            | 15.10.2012 | 76                              | 146                                             | Reactive       |
| 8            | 29.10.2012 | 90                              | 178                                             | Reactive       |
| 9            | 12.11.2012 | 104                             | 169                                             | Reactive       |
| 10           | 26.11.2012 | 118                             | 210                                             | Reactive       |
FIGURE LEGENDS

**FIG 1a** Sample workflow for evaluation of relative specificity.

**FIG 1b** Resolution algorithm for evaluation of samples found repeatedly reactive during specificity testing. Resolution testing was performed at two independent reference centers. ELISA, enzyme-linked immunosorbent assay; IF, immunofluorescence.

**FIG 2** Distribution of COI values in reactive and non-reactive samples from blood donors, pregnant women, hospitalized patients, and Chagas-positive patients, measured with the Elecsys® Chagas assay ($n = 16,185$; suitable COI-increments were chosen).
Screening of blood donations at measurement site

Interpretation

Non-reactive

Negative

Reactive

Repeat in duplicate

1 or 2 x reactive

Repeatedly reactive (RR)

2 x non-reactive

Initially reactive (IR)

Resolution testing at Independent Reference Centers

Evaluation sites

Figure 1A
Figure 1B

Resolution testing at Independent Reference Centers

ELISA Ortho®
T. cruzi

ELISA bioelisa
Chagas

ELISA alphaWell
Chagas IgG

ELISA Inhouse
T. cruzi

IF Inhouse
T. cruzi

ELISA WienerLab
Chagatest

ELISA Inhouse
T. cruzi

IF Inhouse
T. cruzi

Reference centers

Final interpretation

Negative

Positive

Indeterminate

Test performed at National Reference Centre for Tropical Infectious Agents, Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany

Test performed at Unit of Leishmaniasis and Chagas Disease, Department of Parasitology, National Microbiology Centre (CNM), Instituto de Salud Carlos III, Madrid, Spain
Figure 2

Elecsys® Chagas CoI

Number of samples

Europe
Latin America