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# Comparative evaluation of immunoassays to improve access to diagnosis for Chagas disease in Colombia

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ABSTRACT

*Objective:* Chagas disease affects over six million people, but less than 1% are diagnosed and treated. Complicated diagnostic processes are a major barrier. Colombia's previous diagnostic algorithm, using in-house tests, was difficult to scale up, creating significant access barriers for patients. A new algorithm using commercially manufactured immunoassays would potentially improve access, but these tests' performance in Colombian patients with Chagas disease is not well known.
*Methods:* We assessed seven commercially available assays. Samples (n = 501), 93.8% originating from Colombia, were characterized as positive or negative based on standard procedure at the National Reference Laboratory. Performance characteristics were calculated for individual assays and hypothetical test pairings, then compared to the existing algorithm.
*Results:* Five of seven assays exhibited sensitivity >98% while six showed specificity >97%. A total antigen ELISA paired with a recombinant assay provided similar performance to the current diagnostic process. Six of six assays tested proved capable of detecting different *Trypanosoma cruzi* genetic lineages.
*Conclusions:* The study indicated that several commercial assays accurately detect *T. cruzi* infection in Colombian patients. A simplified testing process with two commercial assays could perform comparably to the previous process, reducing cost and accessibility barriers and facilitating national scale-up.


## Introduction

Chagas disease (CD), caused by the protozoan *Trypanosoma cruzi*, is a vector-borne infection of significant public health concern in Latin America, where nearly six million people are infected and 70 million are at risk of infection (World Health Organization, 2015). Large numbers of people also live with CD in the United States (>300,000) and Europe (68,000–122,000) (Basile et al., 2011; Manne-Goehler et al., 2016). The World Health Organization (WHO) considers CD a neglected tropical disease; it primarily affects marginalized populations with limited access to healthcare and is largely overlooked by both governments and market-driven pharmaceutical research and development (2018). Up to 99% of CD cases in the Americas and >90% in Europe are undiagnosed (Basile et al., 2011; Cucunuba et al., 2017; Manne-Goehler et al., 2015; Manne et al., 2013). A key barrier is the complexity of the diagnostic process, which is hampered by the lack of a gold standard, availability of multiple types of assays with varying sensitivity and specificity, and the sheer difficulty of detecting the parasite in the chronic phase of the disease.

In Colombia, 438,000 people are infected by *T. cruzi* and 131,000 suffer from CD-induced cardiomyopathy (World Health Organization, 2015). Estimated CD healthcare costs reach $175,000,000 annually (Castillo-Riquelme et al., 2008). Despite progress in vector control, active transmission by domestic and sylvatic

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triatomine species is an ongoing threat (Hernández et al., 2016a; Parra-Henao et al., 2016), and several oral outbreaks have occurred (Hernández et al., 2016b; Rueda et al., 2014). Moreover, Colombia confronts various barriers to diagnosis and treatment; only 1.2% of the at-risk population has been screened, while only 0.3–0.4% of expected cases have received etiological treatment (Cucunuba et al., 2017).

*T. cruzi* is primarily transmitted by blood-sucking triatomine bugs, but also congenitally, through blood transfusion and organ transplant, and via consumption of food contaminated with triatomine faeces. After infection there is an acute phase with variable symptoms which is usually not recognized as CD, followed by a lifelong chronic phase. The majority remain in an asymptomatic, indeterminate form, but after years or decades, 30–40% progress to an advanced chronic form with potentially life-threatening cardiac, gastrointestinal, or other complications (Rassi et al., 2010).

Treatment of CD with antitrypanosomal drugs (benznidazole or nifurtimox) is more effective the more recent the infection, with high cure rates in infants, acute cases, and chronically infected children (Alonso-Vega et al., 2013; Cerisola, 1977; Sosa Estani et al., 1998). In adults with indeterminate CD, treatment can improve clinical outcomes (Cardoso et al., 2018; Fabbro et al., 2007; Viotti et al., 2006) and prevent congenital transmission (Fabbro et al., 2014; Moscatelli et al., 2015; Murcia et al., 2017; Sosa-Estani et al., 2009), but is less effective after the onset of clinical complications (Morillo et al., 2015). Early screening and diagnosis help ensure patients receive treatment before developing chronic complications, potentially preventing a substantial burden of morbimortality.

CD diagnosis in the chronic phase relies on detection of antibodies and poses various technical and operational challenges. Because no single test is sufficiently accurate, the WHO recommends confirming positive diagnosis on two different types of assays (2002). If the results are discordant, a third assay is needed. While diverse tests are available, their performance varies considerably, which may arise from the different antigens employed (Umezawa et al., 1999), the genetic diversity of the parasite (Zingales, 2018), varying immune responses in patients (Balouz et al., 2017; Martin et al., 2014), and variation in disease prevalence (Leeflang et al., 2013). *T. cruzi* comprises seven discrete typing units (DTUs), labelled TcI-TcVI and Tcbat, whose prevalence varies geographically (Zingales, 2018; Zingales et al., 2009). In Colombia, TcI predominates in sylvatic and domestic transmission cycles, yet coinfection with other DTUs or infection solely with TcII has also been documented (Ramírez et al., 2010). DTUs involved in creating antigens for commercially available tests may not correspond with those found in Colombia, potentially impacting performance. Moreover, immune responses vary geographically, which could impact a test's ability to detect *T. cruzi* antibodies (Messenger et al., 2015). For these reasons, tests which perform well in Argentina or Bolivia may not reproduce comparable results in Colombia.

The National Reference Laboratory (*Laboratorio Nacional de Referencia*, or LNR), part of the National Health Institute (*Instituto Nacional de Salud*, or INS) used an in-house enzyme-linked immunosorbent assay (ELISA) as an initial test and an in-house indirect immunofluorescence assay (IFA) as a complementary test until 2017. Both are derived from Colombian strains of *T. cruzi*. While these tests have high sensitivity and specificity (Enciso et al., 2004), their use on a wide scale proved challenging. It was difficult for the LNR to produce enough reagents for the whole country, so departmental laboratories used several different commercial ELISAs for screening. The extensive resources, training, and equipment required for the IFA made it difficult to implement outside the LNR. Testing created substantial barriers for patients, who had to give blood samples on two or even three separate visits. Samples from rural areas where most CD patients live were sent to the LNR in Bogotá or reference laboratories in other cities for confirmation (Martinez-Parra et al., 2018). Patients incurred high out-of-pocket expenses for the IFA (often not covered by insurers), plus travel to distant cities where the IFA was available. Delays between positive screening results and diagnostic confirmation often exceeded a year, while a third of patients never received confirmation (Cucunuba et al., 2017).

A new algorithm utilizing two different commercial immunoassays would potentially accelerate diagnosis, reduce costs, and remove an important barrier to diagnosis and treatment of CD. Twenty-four immunoassays for CD are commercially available in Colombia, require less technical infrastructure and inputs than the in-house tests, and can therefore be used in a wider range of laboratories. However, their performance capabilities in Colombian patients are largely unknown. The purpose of this study was to (1) validate several immunoassays currently used throughout Colombia's health system, including public health laboratories, private laboratories, and blood banks; (2) determine their sensitivity and specificity and identify the best tests to use for screening and confirmation; and (3) establish a simplified diagnostic algorithm to make CD testing more accessible for Colombian patients. The new diagnostic algorithm would initially be piloted as part of a new, patient-centered roadmap for CD validated by the Ministry of Health and Social Protection (MSPS) in April, 2017 (Marchiol et al., 2017).

## Materials and methods

### *Specimens*

A panel of 501 serum samples obtained from whole blood was utilized (Figure 1). Samples reflected a broad spectrum of CD patients in Colombia, originating from the following sources: the INS, departmental public health laboratories, blood donors, and CD research. Patients giving blood at public health laboratories or blood banks in Colombia undergo an informed consent process encompassing future research. Patients ranged in age from 1 to 81, but only seven were under 18 years old. Mean patient age was 39.3 and was younger in blood donors (22.3) than patients tested in public health laboratories (49.4). All samples originated in Colombia, excluding a reference panel acquired from a commercial laboratory (SeraCare, Milford, Massachusetts, USA), which comprised ten samples taken from a single patient at different stages following infection, plus a mixed titer panel (n = 21).

Samples were characterized as positive (n = 256) or negative (n = 245) using standard procedures at the LNR, based on four serological assays: 1) in-house ELISA, optic density $\geq$ 0·300, 2) in-house IFA, dilution $\geq$ 1:32, 3) indirect haemagglutination (Wiener Chagatest HAI $\geq$ 1:16), and 4) trypomastigote excreted-secreted antigens (TESA, bioMérieux Immunoblot). All samples were obtained from 2014 to 2016 and stored at the LNR at a temperature of $-70\,^{\circ}C$ ($\pm 5$). As a control measure, a reference panel with antibodies against TcI and TcII from The National Institute for Biological Standards and Control (NIBSC, Potters Bar, UK) was utilized, in accordance with WHO recommendations for evaluation of new assays (Otani et al., 2011). The panel consisted of lyophilized human plasma samples containing antibodies responding to infection by TcI or TcII. The samples came from patients with documented autochthonous transmission in endemic areas.

### *Commercial tests*

Manufacturers of commonly used immunoassays for detecting *T. cruzi* IgG antibodies in Colombia's public health laboratories and

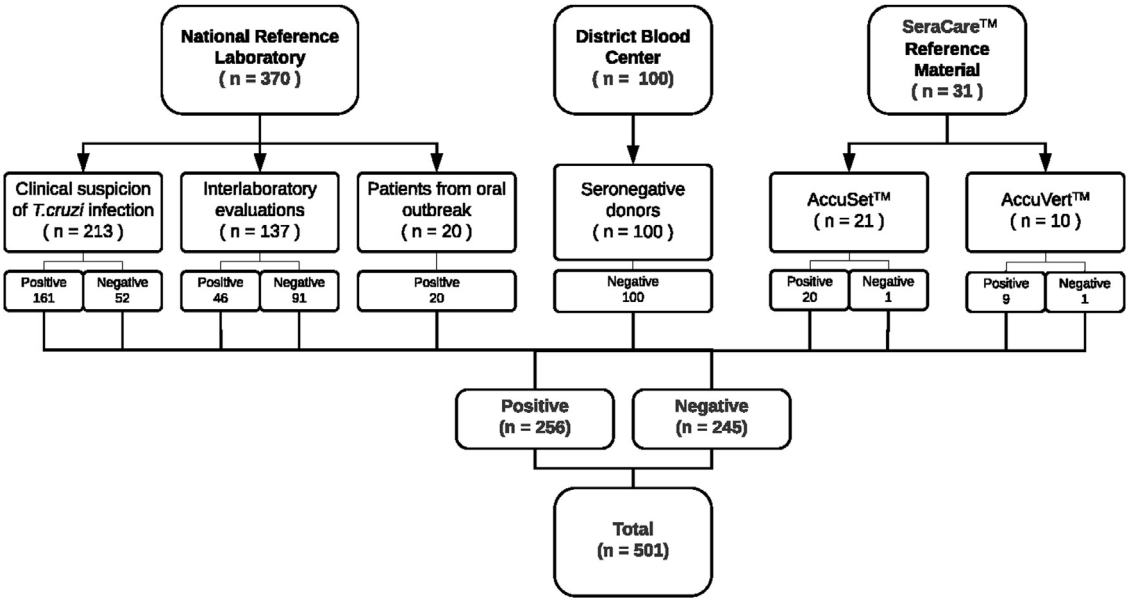


**Figure 1.** Sources of serum samples used in the study.

blood banks were identified through an annual quality control exercise. All manufacturers were invited to participate in a validation study and seven accepted; we refer to them using the abbreviations in Table 1. All seven immunoassays were registered with Colombia's National Institute of Medication Surveillance. All samples were tested with each immunoassay at the LNR in Bogotá. Technicians were blinded to any prior classification of samples. Testing was conducted according to the instructions for each immunoassay and its corresponding reader.

*Statistical analysis*

Sample size was calculated in an Excel spreadsheet to compare sensitivity and specificity in binomial tests ($\alpha = 0.05$, $\beta = 0.8$, maximum CI = 7.5%) (Hajian-Tilaki, 2014). Data from testing with each immunoassay were validated according to the criteria determined by the manufacturer, entered into an Excel spreadsheet, and analyzed in Epidat v. 3.0 (Dirección Xeral de Saúde Pública, Galicia). Percentages of positive and negative results were tabulated for each type of test and compared in contingency tables to calculate sensitivity and specificity.

To compare different scenarios for a new diagnostic algorithm, we estimated how different combinations of immunoassays with different antigenic principles would perform in either a two-stage or simultaneous testing process. We projected outcomes in a hypothetical population of 10,000 with 10% prevalence of *T. cruzi* infection, based on the reported prevalence for Casanare (Moncayo and Silveira, 2009), one of the departments where the new algorithm would be piloted. For two-stage screening, either a total antigen (BIOS) or synthetic peptide assay (SUMA) would be used initially. Any positive results would then be run against a second test based on recombinant antigens. For comparative purposes, we included estimates using the IFA as a second assay. In an alternate scenario, samples would be simultaneously tested with two assays (based on different antigenic principles), potentially providing an immediate diagnosis. Using Excel, net sensitivity and specificity for both scenarios was calculated. However, because these measures fail to account for the totality of the CD testing process,

**Table 1**
Commercial *T. cruzi* immunoassays evaluated in the study.

| Immunoassay | Manufacturer | Abbreviation[a] | Antigens | Reader used | Sensitivity (%)[b] | Specificity (%)[b] |
|---|---|---|---|---|---|---|
| Conventional method (*total antigen*) | | | | | | |
| Test ELISA Chagas III | Grupo BIOS (Santiago, Chile) | BIOS | Total antigen + membrane antigens | spectrophotometer | 100 | 100 |
| Nonconventional methods | | | | | | |
| Synthetic peptides | | | | | | |
| Umelisa Chagas | Tecnosuma International (Havana, Cuba) | SUMA | Peptide 17 &18 | Fluorometer (SUMA Technology) | 100 | 100 |
| Recombinant antigens | | | | | | |
| Architect System Chagas | Abbott (Germany) | ARCHI | FP3, FP6, FP10 & TcF | Abbot i1000SR immunoassay analyzer | 99-100 | 99–100 |
| BioELISA Chagas | Biokit (Barcelona, Spain) | BIOKIT | TcD, TcE, PEP2, TCL1, TCL2 | spectrophotometer | 100 | 97·4–99·5 |
| Chagatest ELISA recombinante v. 4 | Wiener Lab. (Rosario, Argentina) | WIENER | SAPA, Ag1, Ag2, Ag13, Ag30 & Ag 36 | spectrophotometer | 99·13-100 | 98·30–99·66 |
| *T. cruzi* AB | Diagnostics Bioprobes (Milan, Italy) | DIAPRO | No data | Gemini XPS microplate reader | 100 | >99·5 |
| Chagas ELISA IgM + IgG | Vircell Microbiologists (Granada, Spain) | VIRCELL | FRA, B13, MACH (PEP2, TcD, TcE, SAPA) | spectrophotometer | 100 | 98 |

[a] These abbreviations are used throughout the article.
[b] Values from manufacturer inserts.

which often results in discordant results on the first two tests, we calculated three additional measures of public health importance: false negatives, false positives, and discordant results requiring a third test.

## Results

### *Operational characteristics of the seven immunoassays*

Of the 501 samples, 85.22% were correctly classified by all seven immunoassays; 222/256 positive samples (86.71%) and 202/245 (83.67%) negative samples. There were 74 discordant results on at least one assay: 40 false positives and 34 false negatives (Supplementary Table S1). In total, UMELISA had the most discordant results (n = 25), followed by BIOKIT (n = 18) and DIAPRO (n = 18). SUMA had the most false negatives (n = 19), while BIOKIT had the most false positives (n = 13) (Table 2). All immunoassays demonstrated sensitivity and specificity superior to 90%. Five of seven sensitivity point estimates exceeded 98% and six of seven specificity point estimates exceeded 97%. VIRCELL registered the highest sensitivity (99.61; 95% CI = 98.65–100) followed by BIOS (99.22; 95% CI = 97.94–100). The highest specificity (97.96; 95% CI = 95.98–99.3) was observed in three tests: ARCHI, BIOS, and WIENER. Following WHO guidelines, the LNR's two in-house techniques (ELISA and IFA) and six of the seven immunoassays were cross-checked using the reference panel from the NISBC. The seventh (Abbot Architect) could not be evaluated due to a lack of reagents. All six immunoassays as well as the in-house ELISA and IFA correctly detected antibodies on the reference panel (Supplementary Table S2).

### *Operational characteristics of paired tests*

Results of a hypothetical two-stage testing scenario in a population of 10,000 with 10% prevalence of *T. cruzi* infection are estimated in Table 3. Net specificity approached 100% for all combinations. Figure 2 projects the performance of the entire testing process for BIOS followed by WIENER, with the IFA as a third test for discordant results. As complementary tests, most of the recombinant assays were projected to provide similar results to the IFA. Simultaneous testing performance was also estimated under the same hypothetical parameters, pairing SUMA or BIOS with the other assays (Table 4). Simultaneous testing reduced false negatives but entailed higher numbers of discordant results requiring a third test. The bulk of samples with discordant results in both two-stage and simultaneous testing were projected to be true negatives.

## Discussion

This study demonstrates that several commercial assays have high sensitivity and specificity for detecting *T. cruzi* antibodies in Colombian blood samples. Further, a diagnostic process using two commercial assays of different types performs comparably to Colombia's previous algorithm (using an in-house ELISA and IFA). This finding has significant public health importance because the IFA posed a barrier to diagnosis due to costs for patients and the public health system. A simplified diagnostic algorithm would facilitate scaling up diagnosis by providing results more quickly and easily to patients. Based on these results, a new diagnostic algorithm was proposed using a total antigen ELISA (BIOS) and a recombinant ELISA (WIENER). Of the remaining six assays, five exhibited specificity >97% and were of a different type (recombinant, chemiluminescent, or synthetic peptides) than BIOS, suggesting all would be good candidates for a complementary test. The IFA was retained as a tiebreaker for discordant results.

We calculated net sensitivity and net specificity of different test combinations, finding several could perform comparably to the old algorithm. Even though simultaneous testing minimizes false negatives and can potentially provide an immediate diagnosis, the trade-off is higher costs for reagents, more tests to process, and more discordant results requiring confirmation in a reference laboratory, which could create delays in confirmation. Two-stage testing is projected to produce fewer discordant results, entailing less cost for the public health system and significantly reducing a potential source of delays. In a two-stage scenario, the false negative rate is dependent on the initial test, necessitating an assay with very high sensitivity such as BIOS.

The main goal of this study was to select two serological tests of different antigen types, per WHO recommendations, to reduce barriers, simplify diagnosis, and facilitate confirmation at the primary health care level. The new algorithm provides several advantages over the previous system (Table 5). Screening and diagnosis can be conducted in a much wider range of clinics and laboratories, allowing greater coverage of infected individuals and ensuring more patients receive a timely, accurate diagnosis, facilitating treatment initiation before the onset of chronic complications. The new algorithm was successfully incorporated into a new patient-centered roadmap for CD with official validation by the MSPS, and insurance coverage of testing is therefore now mandated. Because patients no longer must travel to urban laboratories to give a second or third blood sample, patient costs are reduced, eliminating an important barrier. Diagnosis only requires one reader, which provides an automated result, reducing bias from subjective interpretation.

**Table 2**
Sensitivity and specificity of *T. cruzi* immunoassays evaluated.

| Immunoassay[a] | Sensitivity % (CI) | Specificity % (CI) | False negatives | False positives |
|---|---|---|---|---|
| BIOS | 99·22 (97·94–100) | 97·96 (95·98–99·93) | 2 | 5 |
| VIRCELL | 99·61 (98·65–100) | 97·55 (95·41–99·69) | 1 | 6 |
| WIENER | 98·83 (97·31–100) | 97·96 (95·98–99·93) | 3 | 5 |
| ARCHI | 98·44 (96·72–100) | 97·96 (95·98–99·93) | 4 | 5 |
| BIOKIT | 98·05 (96·16–99·94) | 94·69 (91·68–97·70) | 5 | 13 |
| DIAPRO | 95·70 (93·02–98·38) | 97·14 (94·85–99·43) | 11 | 7 |
| SUMA | 92·58 (89·17–95·98) | 97·55 (95·41–99·69) | 19 | 6 |

[a] See Table 1 for test abbreviations.

**Table 3**
Estimated outcomes for the new *T. cruzi* diagnostic algorithm in a hypothetical population of 10,000 with 10% prevalence of Chagas disease; two-stage testing process.

| First test (type) | Second test (type) | Net sensitivity (95% CI) | Net specificity (95% CI) | False positives | False negatives | Discordant results (TP, TN)[a] |
|---|---|---|---|---|---|---|
| BIOS | WIENER | 98·06 (96·37–99·75) | 99·96 (99·71–100) | 4 | 8 | 192 (12, 180) |
| | ARCHI | 97·67 (95·82–99·52) | 99·96 (99·71–100) | 4 | 8 | 195 (15, 180) |
| | BIOKIT | 97·29 (95·30–99·28) | 99·89 (99·48–100) | 10 | 8 | 193 (19, 174) |
| | DIAPRO | 94·95 (92·27–97·63) | 99·94 (99·63–100) | 5 | 8 | 221 (43, 178) |
| | VIRCELL | 98·83 (97·51–100) | 99·95 (99·67–100) | 4 | 8 | 183 (4, 179) |
| | SUMA | 91·86 (88·51–95·21) | 99·95 (99·67–100) | 4 | 8 | 253 (74, 179) |
| | IFA | 97·24 (95·23–99·25) | 99·96 (99·71–100) | 4 | 8 | 198 (18, 180) |
| SUMA | ARCHI | 91·14 (87·66–94·62) | 99·95 (99·67–100) | 4 | 74 | 230 (14, 216) |
| | BIOKIT | 90·77 (87·22–94·32) | 99·87 (99·42–100) | 12 | 74 | 227 (18, 209) |
| | DIAPRO | 88·60 (84·71–92·49) | 99·87 (99·42–100) | 6 | 74 | 254 (40, 214) |
| | WIENER | 91·50 (88·08–94·92) | 99·95 (99·67–100) | 4 | 74 | 227 (11, 216) |
| | BIOS | 91·86 (88·51–95·21) | 99·95 (99·67–100) | 4 | 74 | 223 (7, 216) |
| | VIRCELL | 92·22 (88·94–95·50) | 99·94 (99·63–100) | 5 | 74 | 219 (4, 215) |
| | IFA | 90·73 (87·18–94·28) | 99·96 (99·71–100) | 4 | 74 | 233 (17, 216) |

[a] TP = true positive, TN = true negative, as determined by the standard INS process.

Our study corroborates prior research indicating that total antigen techniques produce high sensitivity for detecting *T. cruzi* infection (Duarte et al., 2014; Flores-Chávez et al., 2010). In this study, BIOS demonstrated high sensitivity (99.22%) and specificity (97.96%). This is similar to summary estimates of sensitivity (97.7%) and specificity (98.7%) reported in a recent meta-analysis (do Brasil et al., 2016) and a study of Argentinian blood donors (se = 95.6, sp = 99.8) (Remesar et al., 2009). An earlier version of this test exhibited excellent performance (se = 99.4%, sp = 99.62%) in a WHO comparative evaluation with samples from ten Latin American countries, including Colombia (Otani et al., 2009).

Recombinant assays can detect antigens prevalent during *T. cruzi* infection, including mucine associate surface proteins, cytoplasmic repetitive antigens, and flagellar repetitive antigens (Longhi et al., 2012; Marcipar and Lagier, 2012; Umezawa et al., 1999). However, since not all infected patients produce antibodies against all these antigens, use of various recombinant antigens, ideally representing different stages of the parasite, can reinforce sensitivity (Balouz et al., 2017). In this study, three recombinant assays exhibited specificity and sensitivity superior to 97%. WIENER exhibited high specificity (99%) but lower sensitivity (93.7%) in a metanalysis (do Brasil et al., 2016). However, sensitivity was 98.81% and specificity 99.62% in the WHO's comparative evaluation (Otani et al., 2009). Other investigations corroborated high sensitivity and specificity for ARCHI (do Brasil et al., 2016; Iborra-Bendicho et al., 2012; Otani et al., 2009). A Spanish study found sensitivity and specificity approaching 100% (Abras et al., 2016). In a Colombian study with 205 patients, BIOKIT and WIENER demonstrated specificity of 100%, but sensitivity was higher in BIOKIT (98 vs. 90%) (Duarte et al., 2014). Nonetheless, we found sensitivity >98% in both tests, yet higher specificity in WIENER. We are not aware of prior published research on characteristics of VIRCELL or DIAPRO.

Synthetic peptides are less complex than proteins but can be utilized as antigens in ELISAs. Combining recombinant multiepitopic proteins results in high sensitivity and specificity (Duthie et al., 2016; Marcipar and Lagier, 2012). Nonetheless, in this study a synthetic peptide technique proved more specific than sensitive. This could be due to small quantities of synthetic peptides, a limited range of epitopes due to the synthesis process, or the purity of the synthesized peptides, whereas recombinant antigens can have various repetitions in their epitopes, increasing their sensitivity. SUMA, which had often been used in Colombia as a screening test, proved better suited as a complementary test.

Another goal of the study was to ensure that commercially available immunoassays developed in diverse settings from different parasite strains would perform adequately in the Colombian population, given previously documented geographic variation in assay performance (Martin et al., 2014; Messenger et al., 2015; Umezawa et al., 2004). Five of seven immunoassays exhibited sensitivity above 98% and showed good concordance with in-house techniques developed from Colombian parasite strains by the LNR. We were unable to characterize *T. cruzi* DTUs within the sample using molecular techniques due to limitations in cost and available equipment, and because polymerase chain reaction (PCR) has not been validated for diagnosis of *T. cruzi* infection in Colombia. As a control measure, we used the WHO reference panel, which included both TcI and TcII lineages, to determine if the *T. cruzi* lineage with which the assays were developed could have affected the results. The six tests we validated and the two in-house techniques were 100% concordant, suggesting that different lineages did not significantly impact assay performance in our investigation, similar to findings in a study of Mexican sera (Luquetti et al., 2009).

Our study has certain limitations. To minimize overestimating sensitivity and specificity, we included samples representing the range of the Colombian CD patient population in addition to a seroconversion panel. Nevertheless, due to our inclusion criteria (true positive/negative samples were confirmed on four tests), sensitivity and specificity could have been overestimated. We

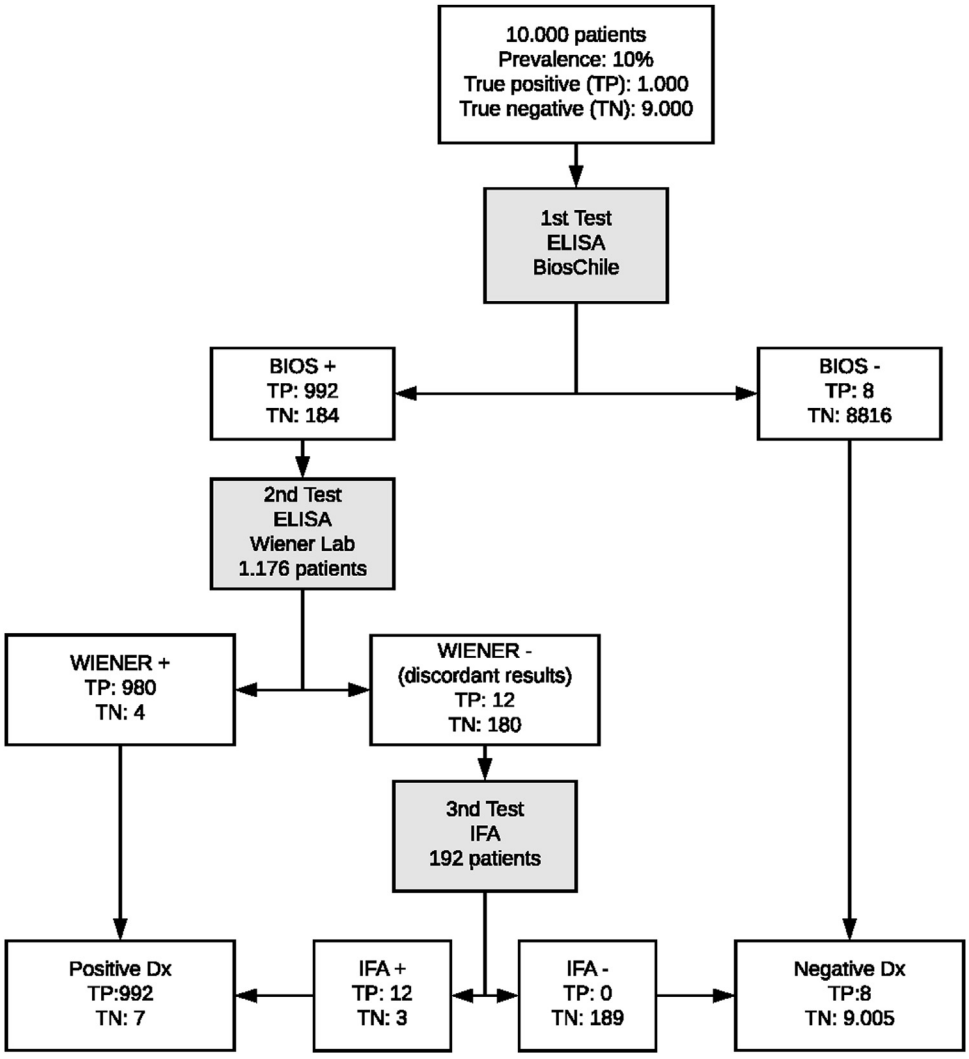


**Figure 2.** Predicted outcomes for the new 2-stage algorithm.
Estimated pathways for a hypothetical sample of 10,000 patients with 10% *T. cruzi* infection prevalence with BIOS (Bioschile Test ELISA Chagas III) and WIENER (Chagatest ELISA recombinante v. 4) in a 2-stage process. The immunofluorescence assay (IFA) is used as a tiebreaker in case of discordant results. TP = true positive, TN = true negative, dx = diagnosis, "+" = positive, "−" = negative.

included seven samples (five positive and two negative) which were initially in the "grey zone", which we defined as within 20% of the cut-off value (Ferreira et al., 2001), using the in-house ELISA. The new algorithm is being prospectively evaluated, which will provide insight as to how it performs with more challenging samples with weak levels of antibodies. Additionally, because of potential geographic variation in assay performance, our results are not generalizable to other countries/contexts. Another important limitation is that we were unable to determine the possible impact of cross-reaction due to coinfection with *Leishamania spp.* or *Trypanosoma rangeli.* This was due in part to technical limitations (insufficient volume of blood and lack of *T. rangeli* reference samples), and partly because manufacturer inserts indicated these assays had a low probability of cross-reaction. However, in a posterior internal validation of BIOS and WIENER prior to implementing the new algorithm, neither presented cross-reaction with *Leishmania spp.* Our calculations of net sensitivity and specificity for test pairings are purely analytical exercises intended as a reference tool, which do not reflect clinical or other factors which could impact outcomes in practice.

Our Colombian study indicated a simplified algorithm utilizing commercially available tests, requiring only one sample and basic laboratory inputs, would have comparable accuracy to the previously used in-house techniques. Similar simplified diagnostic algorithms have been described in Mexico (INDRE 2019) and Ministry of Health and Sports, Bolivia (2007), while other research indicates that rapid diagnostic tests provide adequate sensitivity and specificity (Sanchez-Camargo et al., 2014). The new algorithm is currently being implemented in a pilot project to eliminate barriers to diagnosis and treatment for people with CD in the departments of Boyacá, Casanare, Santander, and Arauca (Marchiol et al., 2017). It was also employed in 2016–2017 in 23 municipalities to assess levels of seropositivity in children as part of the MSPS's official process of certification of interruption of transmission by *Rhodnius prolixus*, the main domestic vector.

Gaps in screening and diagnosis are one of the main reasons CD remains a hidden, neglected disease (Cucunuba et al., 2017; Manne-Goehler et al., 2015). This study demonstrates the utility of systematically evaluating *T. cruzi* assays, using WHO standards, to validate national guidelines for CD diagnosis, strengthen quality control of diagnostic tools, and promote international standardization. In Colombia, further study is needed of other commercially available assays not included in this investigation. Simplified diagnostic algorithms will facilitate the provision of diagnosis and

**Table 4**
Estimated outcomes for new *T. cruzi* diagnostic algorithm in a hypothetical population of 10,000 with 10% prevalence of Chagas disease; simultaneous testing process.

| Tests pairings, simultaneous testing | | Net sensitivity[a] (95% CI) | Net specificity (95% CI) | False positives | False negatives[b] | Discordant results (TP, TN)[c] |
|---|---|---|---|---|---|---|
| BIOS | WIENER | 99·99 (99·87–100) | 95·96 (93·49–98·43) | 4 | 0 | 379 (19, 360) |
| | ARCHI | 99·99 (99·87–100) | 95·96 (93·49–98·43) | 4 | 0 | 383 (23, 360) |
| | BIOKIT | 99·99 (99·87–100) | 92·76 (89·51–96·01) | 10 | 0 | 669 (27, 642) |
| | DIAPRO | 99·97 (99·76–100) | 95·16 (92·47–97·85) | 5 | 0 | 481 (50, 431) |
| | VIRCELL | 99·99 (99·87–100) | 95·56 (92·98–98·14) | 4 | 0 | 407 (12, 395) |
| | SUMA | 99·94 (99·64–100) | 95·56 (92·98–98·14) | 4 | 1 | 476 (81, 395) |
| | IFA | 99·99 (99·87–100) | 96·00 (93·55–98·45) | 4 | 0 | 382 (26, 356) |
| SUMA | ARCHI | 99·88 (99·46–100) | 95·56 (92·98–98·14) | 4 | 1 | 483 (88, 395) |
| | BIOKIT | 99·86 (99·4–100) | 94·05 (91·09–97·01) | 12 | 1 | 766 (91, 675) |
| | DIAPRO | 99·68 (98·98–100) | 94·76 (91·97–97·55) | 6 | 3 | 576 (111, 465) |
| | WIENER | 99·91 (99·54–100) | 95·56 (92·98–98·14) | 4 | 1 | 479 (84, 395) |
| | VIRCELL | 99·97 (99·76–100) | 95·56 (92·98–98·14) | 5 | 0 | 508 (78, 430) |
| | IFA | 99·87 (99·42–100) | 95·56 (92·98–98·14) | 4 | 1 | 482 (90, 392) |

[a] Net sensitivity in this case measures tests which are either confirmed positive or sent for confirmatory testing, where based on simulations virtually all true positives are projected to be confirmed.
[b] Zero is a function of rounding and simply implies extremely low probability. Integers are used in order to represent individual patients.
[c] TP = true positive, TN = true negative, as determined by the standard INS process.

**Table 5**
Comparison of old and new *T. cruzi* diagnostic algorithms, Colombia.

| Previous Algorithm | New Algorithm |
|---|---|
| Not covered by insurance. | Covered by insurance. |
| 2–3 blood draws in different facilities; patients travel to departmental capitals for second and third tests. | Only one blood draw in a facility closer to patients, eliminating need for costly travel. |
| In-house production of reagents. | Commercially available reagents. |
| Extensive training of personnel; complex, expensive equipment. | Use of automated readers available in most private and public laboratories. |
| Different equipment for each test. | Use of same equipment for both tests. |
| Subjective interpretation of results. | Automated results. |
| Unclear guidelines for screening tests. | Evidence-based guideline for screening, complementary tests. |

treatment for CD in primary healthcare, improving resource allocation, increasing cost-effectiveness, and ultimately ensuring patients with *T. cruzi* infection receive proper care, reducing CD's heavy burden.

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## Conflict of interest statement

The authors report no conflicts of interest.

## Ethical statement

The study met the ethical requirements of Colombia's Ministry of Health and Social Protection. Samples used in the study were obtained from patients who gave informed consent for use in future research, and no patient identifying information has been included in the study.

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## Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.ijid.2019.07.022.

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