# Evaluation of the performance of two Chagas disease antibody tests in patients from the Chaco region (Argentina)

## Romina Chiaraviglio[1], Carolina Aro[2] and Diego Mendicino[1, 3]

[1] Centro de Investigaciones sobre Endemias Nacionales, Faculty of Biochemistry and Biological Sciences, National University of the Littoral, Santa Fe Province, Argentina
[2] Cátedra de Parasitología, Faculty of Biochemistry and Biological Sciences, National University of the Littoral. Santa Fe Province, Argentina
[3] National Scientific and Technical Research Council, Santa Fe Province, Argentina

**Abstract**

Chagas disease (CD) is caused by the protozoan *Trypanosoma cruzi* and is a significant zoonotic disease affecting millions worldwide. The disease progresses from an often asymptomatic acute phase to a chronic phase, which can lead to severe cardiac or digestive issues in about 30% of untreated individuals after 20 to 30 years. Early diagnosis and treatment are crucial to managing the disease and preventing further transmission. Diagnosis typically involves serological tests to detect anti-*T. cruzi* antibodies, with the Indirect Hemagglutination Assay (IHA) and ELISA being the most common methods. Given the antigenic diversity of the parasite, the Pan American Health Organization (PAHO) recommends the use of two different tests for a reliable diagnosis. The objective of the present study was to evaluate the performance of two commercial diagnostic assays for chronic CD in the Chaco Region. Blood samples were collected from 388 patients from the Chaco Region between November 2019 and November 2023, and the results showed a high degree of agreement between the IHA and Lysate ELISA commercial tests. However, Lysate ELISA was found to produce more false negatives compared to the PAHO diagnostic algorithm, which could leave patients untreated and contribute to ongoing transmission. The study demonstrated very good performance of the commercial tests evaluated. However, the presence of some false results underscores the importance of continuing to use the PAHO algorithm based on two serological tests for reliable diagnosis in the region.

## Introduction

Chagas disease (CD), caused by the protozoan *Trypanosoma cruzi* (*T. cruzi*), is one of the leading zoonotic diseases worldwide (1). It can be transmitted by the triatomine bug (vector-borne), *Pastrongilus*, *Rhodnius*, or

*Triatoma*, as well as orally (food-borne), from mother to child (congenital), through blood/blood products, or organ transplantation. The World Health Organization estimates that more than 7 million people are currently infected with *T. cruzi* worldwide, including CD, with the neglected tropical diseases. In the Americas, the disease is endemic in 21 countries, with approximately 70 million people at risk of the infection. At least 30,000 new cases and approximately 12,000 deaths occur each year (2). The Chaco Region is particularly affected due to its social and ecological context, which hampers effective control efforts, especially in rural and remote areas (3, 4). CD progresses from an initial acute phase, often asymptomatic or oligosymptomatic, to chronic Chagas disease (CCD) if left untreated. After 20 to 30 years of asymptomatic progression, one-third of affected individuals will develop significant cardiac or digestive problems, which can lead to death. Therefore, key strategies to prevent CCD are timely diagnosis and treatment (5).

In the acute phase, parasitemia can be detected by direct parasitological examinations or by molecular tests. However, in the absence of clinical suspicion at this stage, most diagnoses are made during the chronic phase (5). In CCD, parasitemia drops to undetectable levels, with generally negative results in parasitological, cultural, antigenic, and even molecular tests. In this chronic phase, serological tests must be used for diagnosis (5). For the diagnosis of CCD, the most frequently used tests include indirect hemagglutination (IHA) and enzyme-linked immunosorbent assay (ELISA), the latter varying according to the type of antigens: total lysate antigens or recombinant antigens. There are seven *T. cruzi* variants, called Discrete Typing Units (DTUs), that generate different antigenic responses. The distribution of these DTUs varies by geographic region. Therefore, the accuracy and precision of the serological diagnostic tests vary depending on the antigens used in each assay and the geographic origin of the infection (6, 7). Since no test achieves 100% sensitivity and specificity, and no test is considered the gold standard, the PAHO recommends the use of at least two different tests for a reliable diagnosis (8). The present study provides an updated assessment of two commercial diagnostic tests for CCD conducted among patients in the Chaco Region of Argentina. The objective of the present study was to evaluate the performance of two commercial diagnostic assays for chronic CD in the Chaco Region, and to analyze their use within the diagnostic algorithm.

## Material and methods

### Study Area

Data were collected over ten three-day campaigns in Huanqueros (30°00'49.8"S 61°13'12.8" W) and Fortín Olmos (29°03'00.7"S 60°25'13.3"W), between November 2019 and November 2023. Huanqueros and Fortín Olmos are two semi-rural localities located in the province of Santa Fe (Argentina), in the southern Chaco Region (Figure 1). The environmental conditions in the region constitute the natural habitat of triatomine insects. The precarious housing conditions in the area, and the adaptation of *Triatoma infestans* to human habitation, increase the risk of *T. cruzi* infection.

### Participants

A non-probability convenience sampling method was used, including individuals aged over one year. Patients who had previously received treatment with anti-*T. cruzi* drugs were excluded from the study, as the serological test results in these individuals can be unreliable. Clinical evaluation of the patients was not part of the present study.

### Sample collection

Approximately 5 mL of blood samples were collected through venipuncture with sterile equipment and placed in sterile tubes containing a coagulation activator and separator gel. After allowing the samples to clot for 15 minutes at room temperature, they were centrifuged on-site at 3500 rpm for 10 minutes with a Rolco centrifuge

(Buenos Aires, Argentina). The samples were transported to the laboratory under refrigerated conditions at 4–8 °C and processed within a week.

*Laboratory analysis*

Each sample underwent analysis using the Indirect Hemagglutination Assay (IHA, Chagatest HAI, Wiener Lab, Rosario, Argentina) and the Lysate ELISA (Chagatest Lysate ELISA, Wiener Lab, Rosario, Argentina), adhering to the manufacturer's protocols. In cases where the test results were discordant, a recombinant ELISA (Chagatest recombinant ELISA v. 3. 0, Wiener Lab, Rosario, Argentina) was conducted. Micropar Washer (Rosario, Argentina) and Mindray MR-96A reader (Shenzhen, China) were employed for processing the ELISA tests. A positive result was determined when at least two of the tests returned positive outcomes.

*Statistical methods*

The total percentage agreement, positive percent agreement, and Kappa index were calculated to assess the agreement between the tests.
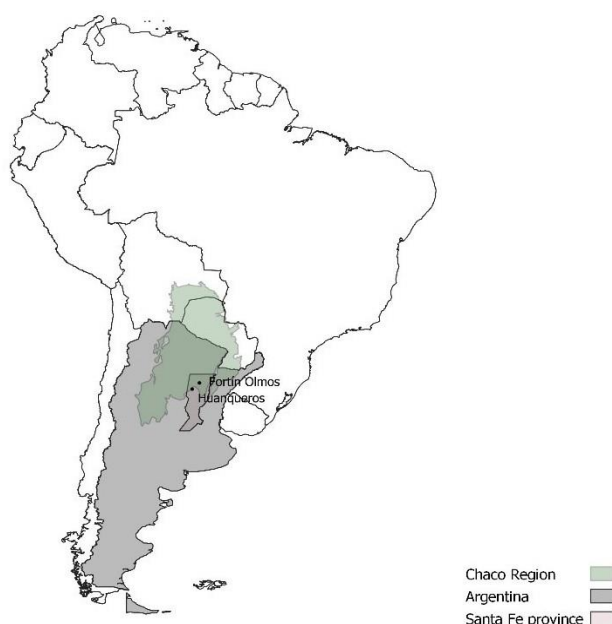


**Fig. 1.** Study area in Argentina. The green area shows the Chaco Region, Huanqueros, and Fortin Olmos are areas where samples were collected.

**Results**

A total of 283 blood samples were collected in Huanqueros and 105 in Fortín Olmos, encompassing 388 patients aged between 1 and 83 years (62% female and 38% male). Out of the total, 24 of the 283 samples (8.48%) were seropositive in Huanqueros, while 39 of the 105 samples (37.14%) tested positive in Fortín Olmos. The results of IHA and Lysate ELISA revealed a high degree of concordance between the two assays (Table 1).

The results of the comparison between IHA and Lysate ELISA with the PAHO algorithm, are presented in Table 2.

**Table 1.** Concordance between Indirect Hemagglutination and lysate ELISA.

|  |  | IHA | | |
|---|---|---|---|---|
|  |  | Positive | Negative | Total |
| Lysate ELISA | Positive | 58 | 9 | 67 |
|  | Negative | 3 | 318 | 321 |
|  | Total | 61 | 326 | 388 |

Global percentage of agreement= 96.90%
Positive percentage of agreement= 82.86%
Kappa index= 0.889

**Table 2.** IHA and ELISA lysate results compared with the official PAHO.

|  |  | IHA | | Lysate ELISA | | |
|---|---|---|---|---|---|---|
|  |  | Positive | Negative | Positive | Negative | Total |
| Algorithm result | Positive | 61 | 2 | 61 | 6 | 67 |
|  | Negative | 1 | 324 | 2 | 319 | 321 |

**Discussion**

A high seroprevalence of CCD was found in the studied populations, although this was not the objective of the study, which highlights the importance of having adequate diagnostic tests in the region. Controlling Chagas disease in the region requires ensuring access to available diagnostics; however, it is estimated that only 10% of those infected people receive a diagnosis (9). The high degree of concordance between IHA and lysate ELISA indicates that both tests could be used interchangeably. This result does not differ from other studies, despite the different origins of the patients (10, 11). However, the absence of a gold standard for CCD diagnosis limits the analysis to concordance statistics between the assays and prevents calculation of their sensitivity and specificity.

Compared with the PAHO diagnostic algorithm, the lysate ELISA tends to generate a higher number of false negatives, which could leave many patients without the necessary care. This situation could contribute to many patients with CCD remaining undiagnosed, allowing mother-to-child transmission and clinical progression that could affect cardiac and digestive health.

Discrepancies in serological test results can be caused by various reasons: differences in the host immune response, heterogeneity in T. cruzi strains, or factors dependent on the test used. In our case, since the tests were evaluated on samples from the same patients, differences in immune response would be ruled out. Furthermore, both HAI and lysate ELISA use the same antigens (total parasite culture lysate) from the same manufacturer, i.e., the same strain. Therefore, strain variability could also be ruled out. Therefore, the higher number of false negatives observed in lysate ELISA results would be due to factors specific to the test itself (12).

Although some studies suggest relying on a single test for serological diagnosis (13, 14), this alternative should be discarded for the moment since both tests leave patients undiagnosed. For resource-limited and difficult-to-access settings, previous publications recommend combining immunochromatographic testing (rapid test) with conventional serology (15), or direct combined use of two different rapid tests (16). This could be the best alternative for the diagnosis of CCD in the affected population.

**Conclusion**

The PAHO diagnostic algorithm, which uses both IHA and ELISA assays, remains the most reliable option for diagnosing Chagas disease in the Chaco Region.

**Ethical approval**

The study protocol was approved by the Advisory Committee on Ethics and Safety Research of the Biochemistry and Biological Sciences Faculty, National University of Littoral in Argentina (CAESI Acta 04.16, CE2018-71-C). Participants signed an informed consent form before sample collection. For those under 18 years of age, their parents signed the informed consent form. All test results were provided to each patient individually.

**Conflict of interest statements**

The authors declare that there is no conflict of interest.

**References**

1. Tarleton RL, Gürtler RE, Urbina JA, Ramsey J, Viotti R. Chagas disease and the London declaration on neglected tropical diseases. PLoS Negl Trop Dis. 2014;8(10):e3219. https://doi.org/10.1371/journal.pntd.0003219

2. World Health Organization. Chagas disease (also known as American trypanosomiasis) [Online]. Available from: https://www.who.int/news-room/fact-sheets/detail/chagas-disease-(american-trypanosomiasis. [Accessed 30th Jan 2025].

3. Colussi C, Stafuza M, Nepote M, Mendicino D. Seroprevalence of Chagas disease in urban and rural indigenous populations of the south of Gran Chaco. Rev Soc Bras Med Trop. 2022;55:e04792021. https://doi.org/10.1590/0037-8682-0479-2021

4. Bonney KM. Chagas disease in the 21st century: a public health success or an emerging threat?. Parasite. 2014;21:11. https://doi.org/10.1051/parasite/2014012

5. Pérez-Molina JA, Molina I. Chagas disease. Lancet. 2018;391(10115):82-94. https://doi.org/10.1016/S0140-6736(17)31612-4

6. Truyens C, Dumonteil E, Alger J, Cafferata M, Gibbons L, Ciganda A, et al. Geographic variations in test reactivity for the serological diagnosis of *Trypanosoma cruzi* infection. J Clin Microbiol. 2021;59(12):e0106221. https://doi.org/10.1128/JCM.01062-21

7. Padilla-Valdez J, Antonio-Campos A, Montes-Vergara Y, González-Quiroz J, Domínguez-López M, Martínez-Hernández F, et al. Serological determination of *Trypanosoma cruzi* in northern region of the State of Mexico. Parasitol Res. 2025;124(2):23. https://doi.org/10.1007/s00436-025-08464-6

8. Pan American Health Organization. Guidelines for the diagnosis and treatment of Chagas disease. 2019. [Online]. Available from: https://iris.paho.org/bitstream/handle/10665.2/49653/9789275120439_eng.pdf?sequence=6&isAllowed=y. [Accessed 18th April 2022].

9. Mendicino D, Bottasso O. Chagas disease in children from the Gran Chaco region: A bibliographic appraisal. Trop Doct. 2022;52(4):560-2. https://doi.org/10.1177/00494755221103002

10. Moser MS, Fleischmann CJ, Kelly EA, Prince HE, Bern C, Whitman JD. Concordance of results by three chagas disease antibody assays in US Clinical specimens. J Clin Microbiol. 2023;61(3):e0181422. https://doi.org/10.1128/jcm.01814-22

11. Caicedo Díaz RA, Forsyth C, Bernal OA, Marchiol A, Beltran-Duran M, Batista C, et al. Comparative evaluation of immunoassays to improve access to diagnosis for Chagas disease in Colombia. Int J Infect Dis. 2019;87:100-8. https://doi.org/10.1016/j.ijid.2019.07.022

12. Moure Z, Sulleiro E, Iniesta L, Guillen C, Molina I, Alcover M, et al. The challenge of discordant serology in Chagas disease: the role of two confirmatory techniques in inconclusive cases. Acta Tropica. 2018;185:144-8. https://doi.org/10.1016/j.actatropica.2018.05.010

13. Abras A, Gállego M, Llovet T, Tebar S, Herrero M, Berenguer P, et al. Serological diagnosis of chronic Chagas disease: is it time for a change?. J Clin Microbiol. 2016;54(6):1566-72. https://doi.org/10.1128/JCM.00142-16

14. Candia-Puma M, Machaca-Luque L, Roque-Pumahuanca B, Galdino A, Giunchetti R, Coelho E, et al. Accuracy of diagnostic tests for the detection of Chagas disease: A systematic review and meta-analysis. Diagnostics (Basel). 2022;12(11):2752. https://doi.org/10.3390/diagnostics12112752

15. Ardiles-Ruesjas S, Lesmo V, González-Romero V, Cubilla Z, Chena L, Huber C, et al. Prevalence and diagnostic accuracy of different diagnostic tests for Chagas disease in an indigenous community of the Paraguayan Chaco. PLoS Negl Trop Dis. 2025;19(2):e0012861. http://doi.org/10.1371/journal.pntd.0012861

16. Egüez KE, Alonso-Padilla J, Terán C, Chipana Z, García W, Torrico F, et al. Rapid diagnostic tests duo as alternative to conventional serological assays for conclusive Chagas disease diagnosis. PLoS Negl Trop Dis. 2017;11(4):e0005501. https://doi.org/10.1371/journal.pntd.0005501