

ORIGINAL ARTICLE

Evaluation of commercial serological tests for the diagnosis of Chagas disease

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Keywords:

Chagas disease; ELISA tests; indirect hemagglutination; diagnostic tests; *Trypanosoma cruzi*; sensitivity and specificity (Source: MeSH - NLM).

ABSTRACT

Objective. To evaluate commercial serological tests for the diagnosis of Chagas disease by determining the sensitivity and specificity of indirect hemagglutination (IHA), the Chagas POLYCHACO test (Argentina), and the ELISA Chagas III® test (Laboratorios BiosChile), using the Chagatest ELISA recombinant v.3.0 (Laboratorios Wiener) as the reference standard. **Methods.** A total of 105 positive and 40 negative serum samples for Chagas disease were analyzed. Additionally, two serum samples from patients with leishmaniasis and two from patients with toxoplasmosis were included. All samples were tested using indirect hemagglutination, the ELISA Chagas III test, and the Chagatest ELISA recombinant v.3.0. **Results.** The indirect hemagglutination test showed 100% sensitivity and 55.56% specificity, while the ELISA Chagas III test demonstrated 86.30% sensitivity and 98.61% specificity. Agreement between tests ranged from moderate to very good (Kappa coefficient: 0.56–0.85). Cross-reactivity was observed in the ELISA tests with sera from patients with leishmaniasis and toxoplasmosis. **Conclusions.** The evaluated commercial serological tests showed variable sensitivity and specificity, with moderate to very good concordance between them.

Evaluación de pruebas serológicas comerciales para el diagnóstico de la enfermedad de Chagas

Palabras clave:



enfermedad de Chagas; pruebas de ELISA; hemaglutinación indirecta; pruebas de diagnóstico; *Trypanosoma cruzi*; sensibilidad y especificidad (Fuente: DeCS - BIREME).

RESUMEN

Objetivo. Evaluar las pruebas serológicas comerciales para el diagnóstico de la enfermedad de Chagas, determinando la sensibilidad, especificidad de hemaglutinación indirecta (HAI), del Chagas POLYCHACO (Argentina) y del test ELISA para Chagas III® (Laboratorios BiosChile), utilizando como prueba de referencia el Chagatest ELISA recombinante v.3.0 (Laboratorios Wiener). **Métodos.** Se analizaron 105 sueros positivos y 40 negativos para la enfermedad de Chagas. Adicionalmente, se incluyeron 2 sueros de pacientes con leishmaniasis y 2 sueros con pacientes con toxoplasmosis. Los sueros fueron analizados mediante hemaglutinación indirecta mediante, el test ELISA Chagas III y el Chagatest ELISA recombinante v.3.0. **Resultados.** La prueba de hemaglutinación indirecta presentó una sensibilidad del 100 % y una especificidad del 55,56 %, mientras que el test ELISA para Chagas III mostró una sensibilidad del 86,30 % y una especificidad del 98,61 %. La concordancia entre las pruebas fue moderada y muy buena (coeficiente Kappa: 0,56-0,85). Se observó reacciones cruzadas con sueros de pacientes con leishmaniasis y toxoplasmosis en los test ELISA. **Conclusiones.** Las pruebas serológicas comerciales evaluados mostraron una sensibilidad y especificidad variable y se observó una concordancia moderada y muy buena entre las pruebas.

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INTRODUCTION

Chagas disease is a parasitic pathology caused by the hematozoan *Trypanosoma cruzi* (*T. cruzi*), a flagellated parasite whose life cycle alternates between a triatomine insect and a mammalian host, including humans ⁽¹⁻³⁾.

It is estimated that by 2025, more than seven million people worldwide will be infected with *T. cruzi*, causing approximately 10,000 deaths annually ⁽⁴⁾. This disease is predominantly found in endemic areas of 21 Latin American countries, where the main mode of transmission occurs through the bite of infected triatomines (vector transmission) ⁽⁵⁾. However, other non-vectorial transmission routes also exist, such as transplacental transmission, blood transfusions, organ transplantation, ingestion of contaminated food, and accidents in laboratory settings ^(6,7).

In Bolivia, despite strategies implemented to control Chagas disease, it remains one of the main causes of chronic conditions affecting the heart and digestive system. Early detection is therefore essential to prevent severe complications and improve patients' quality of life ^(8,9). For diagnosis, commercial serological tests that detect specific antibodies against the parasite in the blood are commonly used, being particularly useful in the chronic phase of the disease ^(10,11).

Several studies have evaluated and compared commercial serological tests for the diagnosis of Chagas disease. In one of them, the ELISA IgG+IgM® assay, conducted by Viettri et al. ⁽¹²⁾, reported a sensitivity of 93.4%. Meanwhile, the Speed Oligo Chagas® test showed a sensitivity of 92.6% and a specificity of 100%. In another study by Kelly et al. ⁽¹³⁾, the Wiener Lisado, Wiener v.4.0, and Abbott PRISM tests demonstrated sensitivities of 97.1% and a specificity of 100%. Similarly, in a study by Whitman

et al. ⁽¹⁴⁾, variations in sensitivity and specificity among different tests were observed.

The most common serological tests are indirect immunofluorescence (IIF), indirect hemagglutination (IHA), and enzyme-linked immunosorbent assay (ELISA). However, these tests present significant differences in sensitivity and specificity, which may depend on factors such as geographic location, the antigen used, and the characteristics of the population under study ⁽¹⁵⁻¹⁷⁾. In this context, the objective of this study was to evaluate commercial serological tests for the diagnosis of Chagas disease, determining the sensitivity and specificity of the indirect hemagglutination (IHA) Chagas POLYCHACO test (Argentina) and the ELISA test for Chagas III (BiosChile Laboratories), using the Chagatest recombinant ELISA v.3.0 (Wiener Laboratories) as the reference standard.

METHODS

Study type and area

A quantitative, comparative, observational, and cross-sectional study was conducted at the OMEGALAB laboratory, located in the city of Cochabamba, Bolivia, during the period from January to May 2024.

Population and sample

The study sample consisted of 145 serum samples obtained from patients suspected of having Chagas disease. Of these, 105 sera were positive and 40 were negative. In addition, 2 positive controls and 2 negative controls were included. To evaluate potential cross-reactions, 2 sera from patients with toxoplasmosis and 2 sera from patients with leishmaniasis were incorporated. The samples were selected through non-probabilistic convenience sampling, chosen based on the availability and quality of sera in the context of the research.

Table 1. Comparison between the IHA Chagas POLYCHACO kit and the Chagatest recombinant ELISA v.3.0

		Chagatest recombinant ELISA v.3.0			Total
		Positive	Negative		
IHA Chagas POLYCHACO kit	Positive	fi	73	32	105
		%	50.3	22.1	72.4
	Negative	fi	0	40	40
		%	0.0	27.6	27.6

Table 2. Comparison between the ELISA Chagas III test and the Chagatest recombinant ELISA v.3.0

		Chagatest recombinant ELISA v.3.0			Total
		Positive	Negative		
ELISA Chagas III test	Positive	fi	63	1	64
		%	43.4	0.7	44.1
	Negative	fi	10	71	81
		%	6.9	49.0	55.9

Table 3. Cross-reactivity of commercial serological tests

Disease	IHA Chagas POLYCHACO kit		ELISA Chagas III test		Chagatest recombinant ELISA v.3.0	
	Positive	Negative	Positive	Negative	Positive	Negative
Leishmaniasis	0	2	2	0	0	2
Toxoplasmosis	0	2	1	1	1	1

Techniques and data collection instruments

Sera were analyzed using the following commercial serological tests: the IHA Chagas POLYCHACO kit (Argentina) ⁽¹⁸⁾, the ELISA for Chagas III[®] developed by BiosChile Laboratories (Chile) ⁽¹⁹⁾, and the Chagatest recombinant ELISA v.3.0 developed by Wiener Laboratories (Argentina), which was used as the reference test ⁽²⁰⁾.

All samples and control sera were processed according to the manufacturers’ instructions for each kit, and results were recorded in a results logbook, including all study variables such as sensitivity, specificity, and agreement between tests.

Data analysis

Data obtained were organized in an Excel database and analyzed using SPSS Statistics v.23. Contingency tables (2x2) were constructed to calculate the sensitivity and specificity of the commercial serological tests. Correlation between methods was determined using Pearson’s chi-square test, with p-values <0.05 considered statistically significant. Agreement between methods was evaluated using Cohen’s Kappa index, interpreted according to Landis and Koch’s criteria: poor (<0.40), moderate (0.41-0.60), good (0.61-0.80), and very good (0.81-1.0).

Ethical considerations

The study was conducted in accordance with the ethical guidelines established in the Declaration of Helsinki. Participants voluntarily provided informed

consent, ensuring the confidentiality of their data, which were used exclusively for scientific purposes.

 **RESULTS**

Out of a total of 145 samples analyzed, the IHA Chagas POLYCHACO kit detected 105 positives and 40 negatives, whereas the Chagatest recombinant ELISA v.3.0 detected 73 positives and 72 negatives (see Table 1).

Out of a total of 145 samples analyzed, the ELISA Chagas III test detected 64 positives and 81 negatives, whereas the Chagatest recombinant ELISA v.3.0 detected 73 positives and 72 negatives (see Table 2).

Regarding cross-reactions, out of 2 sera from toxoplasmosis patients and 2 sera from leishmaniasis patients, cross-reactivity was observed in 2 leishmaniasis sera and 1 toxoplasmosis serum with the ELISA Chagas III test, whereas with the Chagatest recombinant ELISA v.3.0 only 1 toxoplasmosis serum showed cross-reactivity (see Table 3).

The IHA kit showed 100% sensitivity and 55.56% specificity. In turn, the ELISA Chagas III test showed 86.30% sensitivity and 98.61% specificity (see Table 4).

Agreement between the IHA Chagas POLYCHACO kit and the Chagatest recombinant ELISA v.3.0 was 0.56, with a 95% confidence interval (CI), indicating

Table 4. Sensitivity and specificity of the IHA Chagas POLYCHACO kit and the ELISA Chagas III test, using the Chagatest recombinant ELISA v.3.0 as the reference test

Commercial serological test	Sensitivity (%)	Specificity (%)	Positive predictive value (PPV) (%)	Negative predictive value (NPV) (%)
IHA Chagas POLYCHACO kit	100.00	55.56	69.52	100.00
ELISA Chagas III test	86.30	98.61	98.44	87.65

Table 5. Agreement between the IHA Chagas POLYCHACO kit, the ELISA Chagas III test, and the Chagatest recombinant ELISA v.3.0

Commercial serological test	Cohen's Kappa index	p-value
IHA Chagas POLYCHACO kit	0.56	0.000
ELISA Chagas III test	0.85	0.000

moderate agreement between both tests. In contrast, agreement between the ELISA Chagas III test and the Chagatest recombinant ELISA v.3.0 was 0.85, with a 95% CI, representing very good agreement between the two tests. Pearson's chi-square analysis yielded a value of $p < 0.000$ ($p < 0.05$ at 95% CI), indicating a statistically significant relationship between the IHA Chagas POLYCHACO kit, the ELISA Chagas III test, and the Chagatest recombinant ELISA v.3.0 (see Table 5).



DISCUSSION

In this study, discrepancies were observed among the results of the different serological tests. The IHA Chagas POLYCHACO test detected 105 positive and 40 negative cases, while the ELISA Chagas III test detected 64 positives and 81 negatives. In turn, the Chagatest recombinant ELISA v.3.0 identified 73 positives and 72 negatives. These differences reflect that, although useful, serological tests have varying detection capacities.

Although serological tests generally exhibit high sensitivity, their specificity may be affected by cross-reactions with parasites such as *Leishmania* spp., *T. rangeli*, *Plasmodium* spp., and *T. gondii*. In this study, cross-reactions were observed with sera from patients with leishmaniasis and toxoplasmosis. The presence of these coinfections may compromise the accuracy of serological tests, potentially leading to erroneous or uncertain diagnoses. Therefore, it is important to consider diagnostic alternatives, such as combining serological assays with molecular techniques or incorporating other methods that allow for more accurate differentiation of these diseases, thereby improving diagnostic precision in cases of coinfection.

In terms of performance, the IHA Chagas POLYCHACO test showed 100% sensitivity and 55.56% specificity, making it highly sensitive but with a significant risk of false positives. This means it may be useful for identifying nearly all infected patients but

is limited in its ability to distinguish between *T. cruzi* and other parasites. Conversely, the ELISA Chagas III test showed 86.30% sensitivity and 98.61% specificity, indicating greater accuracy in identifying uninfected cases. These findings are comparable to those of other studies conducted by Hurtado et al. ⁽²²⁾, Añez et al. ⁽²³⁾, and Mamani ⁽⁵⁾, although variations in results may be attributed to the use of non-native antigens in commercial kits, which affects sensitivity and specificity ⁽²⁴⁾.

Finally, the concordance analysis between the IHA Chagas POLYCHACO test and the Chagatest recombinant ELISA v.3.0 showed moderate agreement (kappa index = 0.56), indicating that although both tests can detect the disease, they present certain discrepancies in their results. In contrast, the agreement between the ELISA Chagas III test and the Chagatest recombinant ELISA v.3.0 was very good (kappa index = 0.85), suggesting that both tests yield similar results and could be used complementarily to improve diagnostic accuracy. Moreover, the p-value of 0.000 indicated a statistically significant relationship among the evaluated tests, reinforcing the relevance of using these serological assays in combination for a more reliable diagnosis. The findings are consistent with previous research conducted by Hurtado et al. ⁽²²⁾ and Aria et al. ⁽²⁴⁾.

One limitation of this study was the inability to include samples from different regions of Bolivia, which restricted the generalization of the results at the national level. This lack of geographical representativeness limits the external validity of the study, as the results cannot be guaranteed to apply to the entire Bolivian population, particularly in areas with different prevalence rates or specific socio-environmental conditions.

Further comparative studies are recommended, including diverse populations and local conditions, to optimize diagnostic protocols and minimize the observed limitations.

Conclusions

In conclusion, the results of this study highlight the importance of combining different serological tests to improve the diagnosis of Chagas disease. The IHA Chagas POLYCHACO test (Argentina), with its high sensitivity, is useful for detecting positive cases, but its low specificity may lead to false positives. The ELISA Chagas III test (Laboratorios BiosChile), on the other hand, offers higher specificity but lower sensitivity. Cross-reactions with other parasites remain a challenge, although ELISA-based tests perform better in this regard. The observed agreement between the tests suggests that combining assays could enhance diagnostic accuracy, especially in regions where multiple parasitic infections coexist.

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