ACIN
ASOCIACIÓN COLOMBIANA DE INFECTOLOGÍA

infectio

ARTÍCULO ORIGINAL

Diagnostic evaluation of two enzyme-linked immunosorbent assay tests for *Trypanosoma cruzi* infection in a Colombian population

Sandra Helena Suescún-Carrero[1,2,3]*, Ángela Liliana Monroy-Díaz[2,4], Philippe Ferdinand Tadger-Viloria[5], Carlos Ernesto Vargas-Niño[6], Mabel Idaliana Medina-Alfonso[1,7], Laura Ximena Ramírez-López[2,8]

Resumen

Objetivo: evaluar la precisión diagnóstica de dos pruebas de ensayos inmunoenzimáticos para determinar infección por *Trypanosoma cruzi* en una población colombiana.
Material y método: Estudio analítico con diseño de casos y controles, de evaluación diagnóstica. Se determinó la sensibilidad, especificidad y la eficacia de diagnóstico de las pruebas CHAGATEK ELISA total y CHAGATEK ELISA recombinante. Se calcularon el valor predictivo positivo, valor predictivo negativo y los índices de verosimilitud positivo y negativo. Se halló el coeficiente kappa para estimar la concordancia entre las pruebas índice y de referencia. La capacidad discriminatoria de las pruebas se expresó a través del área bajo la curva ROC.
Resultados: De las 184 muestras incluidas en el estudio, 99 fueron negativas para *T. cruzi* y 85 positivas según las pruebas de referencia utilizadas. La sensibilidad para la ELISA total y recombinante fue de 97.6% y 98.8% respectivamente, mientras que la especificidad fue 97.0% y 94.9%. El nivel de concordancia de la ELISA total y recombinante fue casi perfecto y la capacidad discriminatoria de las dos pruebas fue buena.
Conclusiones: Se demostró que las dos pruebas pueden confirmar y excluir el diagnóstico de la enfermedad de Chagas y que tienen un buen rendimiento diagnóstico.

Palabras clave: Enfermedad de Chagas, Trypanosoma cruzi, diagnóstico, sensibilidad y especificidad, ensayo inmunoenzimático ELISA.

Evaluación diagnóstica de dos pruebas de inmunoabsorción ligado a enzimas para la infección por Trypanosoma cruzi en una población Colombiana

Abstract

Objective: To evaluate the diagnostic accuracy of two enzyme-linked immunosorbent assay tests to determine *Trypanosoma cruzi* infection in a Colombian population.
Material and methods: Analytical study with case-control design of a diagnostic evaluation. Sensitivity, specificity and diagnostic efficacy of total CHAGATEK ELISA and recombinant CHAGATEK ELISA were determined. Positive predictive value, negative predictive value, and positive and negative likelihood ratios were calculated. The kappa coefficient was found to estimate the concordance between the index and reference tests. The discriminatory capacity of the tests was expressed by the area under the ROC curve.
Results: Of the 184 samples included in the study, 99 were negative for *T. cruzi* and 85 were positive according to the reference tests used. Sensitivity for total and recombinant ELISA was 97.6 % and 98.8 %, respectively; while specificity was 97.0% and 94.9%. The level of concordance of the total and recombinant ELISA was almost perfect and the discriminatory ability of the two tests were good.
Conclusions: It was demonstrated that the two tests can confirm and exclude the diagnosis of Chagas disease and have a good diagnostic performance.

Key words: Chagas disease; Trypanosoma cruzi; diagnosis; sensitivity and specificity; enzyme-linked immunosorbent assay ELISA.

Introduction

Chagas disease (CD) is the long-term clinical outcome of a chronic human infection by the protozoan parasite *Trypanosoma cruzi* that affects about 8-10 million people worldwide[1]. The diagnosis is a problem, due to the lack of knowledge of the disease by populations living in rural and urban environments; in addition, the infection does not present a marked symptomatology, which does not allow the infected population to suspect that they are suffering from the disease[2]. In addition to this, serious deficiencies have been identified in this health system for the disease, despite the success of the expansion of universal health insurance in Colombia, which directly affect the access to a treatment[3]. Similarly, CD can also go unnoticed, because the main areas of infection of this parasite are rural; this means that there are not enough measures for identification and thus generate a more appropriate diagnosis[4].

1 Boyacá Public Health Laboratory Research Group. Tunja, Boyacá, Colombia
2 Universidad de Boyacá. Tunja, Boyacá, Colombia
3 https://orcid.org/0000-0001-5821-4421
4 https://orcid.org/0000-0002-3420-9123
5 Independent researcher, Genk-Belgium. https://orcid.org/0000-0002-1453-4105
6 Sanidad Militar DISAN medical dispensary, Manizales, Caldas, Colombia. https://orcid.org/0000-0002-2088-5390
7 https://orcid.org/0000-0002-9264-6516
8 https://orcid.org/0000-0002-3585-3797

* Autor para correspondencia:
Correo electrónico: ssuescun27@uniboyaca.edu.co,
Phone number: +573134310618. Crra 2a Este # 64-169 Tunja, Boyacá, Colombia

Recibido: 20/12/2021; Aceptado: 17/03/2022

Cómo citar este artículo: S.H. Suescún-Carrero, *et al.* Diagnostic evaluation of two enzyme-linked immunosorbent assay tests for Trypanosoma cruzi infection in a Colombian population. Infectio 2022; 26(4): 389-393

Considering that the discriminatory capacity of serological tests varies depending on the type of antigen used, as well as the incidence and prevalence of the disease in the region, one of the most important applications of serological diagnostic tests is in their use in endemic areas, where the test must discriminate infected individuals from those who may have other conditions that generate false positives[5]. Although numerous studies have been carried out to evaluate different tests for the detection of specific anti-*T. cruzi* antibodies, there is no consensus on the choice of a reference technique. Some studies performed in South American countries, such as Argentina and Chile, report a high efficacy of commercial kits manufactured in this region; while others show that the use of antigens prepared from *T. cruzi* strains isolated in these areas increases the sensitivity of antibody detection assays. This is based on the predominance of the *T. cruzi* I genotype in these regions and the wide extension of the *T. cruzi* II genotype in the Southern Cone countries[6].

The use of ELISA tests for the diagnosis of CD with antigenic variability would increase the number of patients diagnosed; in addition, apart from being easy to acquire, of moderate cost and easily accessible to the clinical laboratory, it would achieve a rapid diagnosis that would lead to a treatment that would have more impact and prevent asymptomatic patients from presenting mostly cardiac complications[7]. On the other hand, the values reported, when proposing or evaluating a test from its sensitivity, specificity and predictive values, will be the result of the comparison of that test with another accepted or proposed as a gold standard for the diagnosis of the disease[8]. Therefore, it is very important to evaluate the serological methods routinely used in CD screening, in order to detect reliable techniques for accurate diagnosis in samples of patients from endemic areas, which, in turn, will be of general utility and will correct the diagnostic inconsistency recorded[9]. In addition, it is necessary to know the characteristics of diagnostic tests to support clinical decisions[10], especially in this disease, whose chronic phase requires validation of techniques that allow obtaining greater sensitivity and specificity. Considering the above, the objective of this article was to evaluate the diagnostic accuracy of two enzyme-linked immunosorbent assay tests to determine *T. cruzi* infection in a Colombian population.

Materials and methods

Study design, location and sampling of participants

An analytical, case-control, diagnostic evaluation study was designed to assess the accuracy of two enzyme-linked immunosorbent assay ELISA tests. The population consisted of a universe (population) of 50,038 individuals[11], from rural and urban areas of the municipalities of Soatá, Tipacoque, Moniquirá, Chitaraque, Otanche and Miraflores, in the department of Boyacá (Colombia). The sample calculation was estimated with a precision of 5 % and a confidence level of 99 %, a sensitivity and specificity of 93.4 %[12], plus 5 % loss, for a total of 184 sera selected from the macro project, entitled "Evaluation of the diagnostic validity of two rapid tests for the diagnosis of *Trypanosoma cruzi* infection in a Colombian population".

Reference tests

The diagnosis for CD in the chronic phase established by the World Health Organization indicates the use of a first ELISA test for total antigens and as a complementary test an ELISA test for recombinant antigens or synthetic peptides. Therefore, the Chagas ELISA III GrupoBios[13] and the Chagas ELISA IgG + IgM I Vircell[14] were used as reference tests in this study. In case of discordant results, a third indirect immunofluorescence test, an assay of a different principle from the first two, was processed to comply with the algorithm used for the diagnosis of chronic CD in Colombia. The tests were performed at the Departmental Public Health Laboratory of the Health Secretariat of Boyacá, CD Surveillance Program, following the recommendations of the commercial companies. The results were interpreted as positive or negative.

Index tests

The samples were processed with two enzyme-linked immunosorbent assays: one for total antigens (CHAGATEK ELISA®)[15] and the other for recombinant antigens (CHAGATEK ELISA®)[16], following the recommendations of the commercial company (Laboratorio Lemos, from Buenos Aires, Argentina). Samples with reactive results were defined as confirmed cases. The index tests were performed double-blind, i.e. the processing personnel were unaware of the results of the reference tests. The data were recorded in an information collection form, which included variables such as age, sex, origin, results of the evaluated tests and of the reference tests.

Data analysis

Sensitivity, specificity and diagnostic efficacy were determined to assess the validity of the index tests. Positive predictive value (PPV), negative predictive value (NPV), and positive and negative likelihood ratios were calculated. The kappa coefficient was found to estimate the concordance between the index and reference tests. The values were interpreted according to the recommendations of Landis and Koch: poor agreement equal to 0; slight between 0 and 0.20; low between 0.21 and 0.40; moderate between 0.41 and 0.60; considerable between 0.61 and 0.80; almost perfect between 0.81 and 1.00[17]. The discriminatory capacity of the tests was expressed through the area under the ROC curve.

Ethical aspects

The project was approved by the Bioethics Committee of the University of Boyacá, by memorandum CB 039-2019 of May 24, 2019. Informed consent was obtained from each participant and for those under 18 years of age the informed consent was signed by the legal representative.

Results

Of the 184 samples included in the study, 99 were negative for *T. cruzi*, and 85 were positive, according to the reference tests used. For the total antigen test (CHAGATEK ELISA®), two false negatives and three false positives were found (Table 1); while with the recombinant antigen test (CHAGATEK

ELISA®), one false negative and five false positives were found (Table 2). There were no disparities between the results, so there was no need for a third confirmatory indirect immunofluorescence test.

The sensitivity of the two index tests was 97.6 % for the total antigen test (CHAGATEK ELISA®) and 98.8 % for recombinant antigens (CHAGATEK ELISA®). On the other hand, the specificity was 97.0 % and 94.9 %, respectively. The LR+ and LR- analysis showed that the two index tests can confirm and exclude the diagnosis of CD (Table 3). A higher sensitivity was found for the recombinant ELISA but higher specificity for the total ELISA.

The level of agreement of the total CHAGATEK ELISA tests with the results of the reference tests was 97.3 %, and that of the recombinant CHAGATEK ELISA, 96.7 %, with a kappa index result thus κ = 0.945 (IC95 % = 0.891-0.989 and κ = 0.935 (IC95 % = 0.878-0.978), respectively. The results are considered near perfect, according to the Landis and Koch classification, demonstrating that the two tests have a high degree of reproducibility.

The area under the curve estimates the ability of the total and recombinant CHAGATEK ELISA tests to discriminate between those with and without CD. The efficacy of total CHAGATEK ELISA was expressed by the area under the ROC curve, which was 0.984 (95%CI = 0.964-1) and recombinant CHAGATEK ELISA 0.993 (95%CI = 0.983-1), as evidenced in Fig 1 and 2.

Discussion

In this research, the detection capacity of anti-*T. cruzi* antibodies of the CD diagnostic kits CHAGATEK ELISA total and CHAGATEK ELISA recombinant were compared with the ELISA Chagas III GrupoBios and Chagas ELISA IgG + IgM I Vircell, according to the reference standard proposed by the World Health Organization[2] and following the diagnostic algorithm of the National Institute of Health of Colombia[18]. Regarding sensitivity, it was found that recombinant CHAGATEK ELISA presented higher values than the total of CHAGATEK ELISA; while specificity was higher in total CHAGATEK ELISA than in recombinant CHAGATEK ELISA. The above differs from what has been reported in studies where it has been described that the diagnostic binomial for chronic CD should be constituted by serial tests that include total antigens as the first test and as a complementary test a recombinant antigen test, according to its configuration. Some tend to have better sensitivity and others better specificity[19]. In addition, recombinant ELISA tests have been implemented as a strategy to avoid cross-reactions that occur with conventional techniques with other parasites, mainly with *Leishmania* sp. and *Trypanosoma rangeli*, in order to reduce possible false positives and increase their specificity despite compromising their sensitivity[20].

Table 1. 2 × 2 table of the results of total CHAGATEK ELISA and conventional serology for the diagnosis of Chagas disease.

Total CHAGATEK ELISA	Reference test		
	Positive	Negative	Total
Positive	83	3	86
Negative	2	96	98
Total	85	99	184

Table 2. 2 × 2 table of the results of recombinant CHAGATEK ELISA and conventional serology for the diagnosis of Chagas disease.

Recombinant CHAGATEK ELISA	Reference test		
	Positive	Positive	Total
Positive	84	5	89
Negative	1	94	95
Total	85	99	184

Table 3. Performance of the index tests

	Total CHAGATEK ELISA	Recombinant CHAGATEK ELISA
Prevalence	46 %	46 %
Sensibility	97.6 (92.7-99.5)	98.8 (94.6-99.9)
Specificity	97.0 (92.1-99.1)	94.9 (89.3-98.0)
PPV % (IC 95 %)	96.5 (91-99)	94.4 (88.1-97.8)
NPV % (IC 95 %)	98.0 (93.6-99.6)	98.9 (95.2-99.9)
ROC area % (IC 95 %)	0.984 (0.964-1)	0.993 (0.983-1)
LR+	32.2 (10.6.-98.2)	19.6 (8,3-45.9)
LR−	0.24 (0.06-0.95)	0.12 (0.018-0.87)

LR+: Likelihood ratio positive
LR-: Likelihood ratio negative
NPV: Negative predictive value
PPV: Positive predictive value

The results obtained for PPV and NPV in the present study do not coincide with the data obtained in Colombia, where values for the detection of chronic CD with a Chagas BioELISA ELISA test are reported to be 100 % and 98 %, respectively; while for the Chagatest ELISA test the PPV and NPV values were 100 % and 91 %, respectively[21].

A rapid systematic review on immunoserology and molecular methods for CD indicated the accuracy found in the tests. Furthermore, an average sensitivity and specificity of 94.04 % and 97.5 % were identified for total ELISA tests, respectively, while for recombinant ELISA tests these data were, on average, 94.3 % and 98.5 %[22]. Such results differ from those found in this investigation. On the other hand, in an analysis of the external quality evaluation program carried out during 15 years in Chile, it was reported that the sensitivity of the total ELISA methods was in the range of 96.9 % to 100 % with a specificity that oscillated between 76.4 % and 100 %. As for recombinant ELISA methods, the sensitivity was 100 %, with a specificity ranging from 94 % to 98.8 %, ranges in which our results are found[23].

Regarding the results of the kappa index, a study which evaluated the reproducibility of serological tests for the diagnosis of *T. cruzi* infection in pregnant women in an endemic

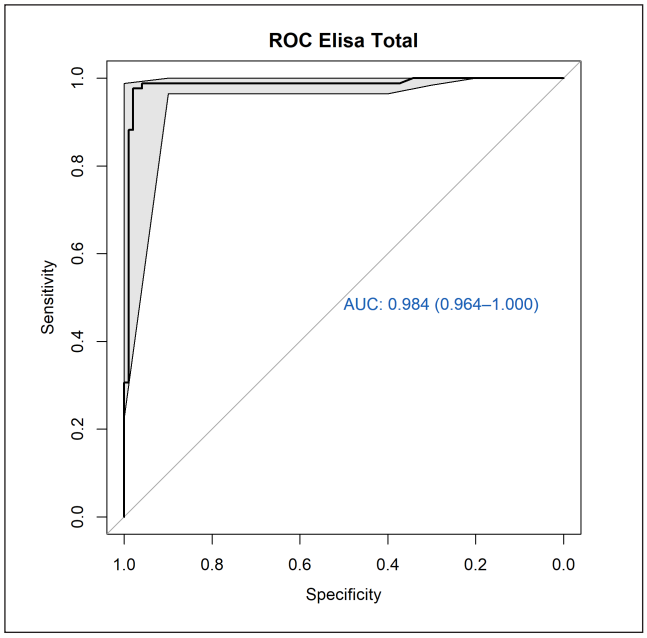

Fig 1. ROC curve for the total CHAGATEK ELISA test

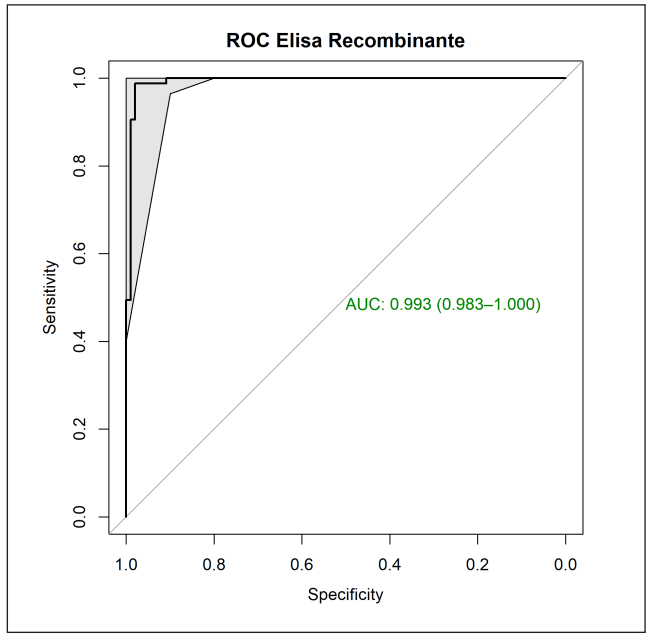

Fig 2. ROC curve for the recombinant CHAGATEK ELISA test

area of Santander (Colombia) reported a kappa index for the total ELISA tests performed in serum of 0.98 (IC95 % = 0.93-1.00) compared to the reference test[24], which was a lower value than that reported in the present study. The same occurs when comparing our results with a study performed in Paraguay, where excellent agreement was obtained between the ELISA Chagas test IICS V.1 and the commercial kits: Chagatest ELISA-Wiener (κ = 0.89; IC95 % = 0.76-1) and ELISA test for Chagas III-Grupo BiosChile (κ = 0.92; IC95 % = 0.82-1)[25].

The area under the curve estimates the ability of the total and recombinant CHAGATEK ELISA tests to discriminate between those with and without CD. The validity of the test was established by calculating the area under the ROC curve, whose result for total CHAGATEK ELISA was 0.984, a figure equal to that reported in a study conducted in patients from Aguazul and Maní (department of Casanare, Colombia), where the quality of a home ELISA test was 0.97[26]. On the other hand, in a study that evaluated the discriminatory capacity (capacity to differentiate between healthy and sick subjects) between a serological test (recombinant ELISA) and a molecular test to determine *T. cruzi* infection, the area under the curve was 0.62, a lower result in relation to that of this study, whose comparison was made with a commercial total ELISA test[27].

It was demonstrated that the CHAGATEK total ELISA and recombinant CHAGATEK ELISA tests can confirm and exclude the diagnosis of CD and that they have a good diagnostic performance.

Ethical disclosures

Acknowledgments. The authors would like to thank the health authorities of the municipalities who participated in the project.

Financial Support. The study was supported by Boyacá Public Health Laboratory and Universidad de Boyacá

Conflict of interest. The authors declare that they did not have a conflict of interest in the execution and writing of this research product.

Data availability. The data required to reproduce these findings is available upon reasonable request to the correspondence author

Author statement. Author contributions.

- Conceptualization: Sandra Helena Suescún-Carrero.
- Data curation: Sandra Helena Suescún-Carrero, Ángela Liliana Monroy-Día, , Carlos Ernesto Vargas-Niño Laura Ximena Ramírez López
- Formal analysis: Sandra Helena Suescún-Carrero, Ángela Liliana Monroy-Día, , Carlos Ernesto Vargas-Niño, Laura Ximena Ramírez López
- Funding acquisition: Sandra Helena Suescún-Carrero, Ángela Liliana Monroy-Día, Mabel Idaliana Medina-Alfonso, Laura Ximena Ramírez López
- Investigation: Sandra Helena Suescún-Carrero, Ángela Liliana Monroy-Díaz, Philippe Ferdinand Tadger-Viloria, Carlos Ernesto Vargas-Niño, Mabel Idaliana Medina-Alfonso, Laura Ximena Ramírez-López
- Methodology: Sandra Helena Suescún-Carrero, Ángela Liliana Monroy-Díaz, Laura Ximena Ramírez-López
- Project administration: Sandra Helena Suescún-Carrero, Laura Ximena Ramírez López.
- Resources: Sandra Helena Suescún-Carrero , Ángela Liliana Monroy-Díaz, , Mabel Idaliana Medina-Alfonso, Laura Ximena Ramírez-López
- Software: Philippe Ferdinand Tadger Viloria

- Supervision: Sandra Helena Suescún-Carrero
- Visualization: Sandra Helena Suescún-Carrero, Laura Ximena Ramírez López.
- Writing – original draft: Sandra Helena Suescún-Carrero, Ángela Liliana Monroy-Díaz, Philippe Ferdinand Tadger-Viloria, Carlos Ernesto Vargas-Niño, Mabel Idaliana Medina-Alfonso, Laura Ximena Ramírez-López
- Writing – review & editing: Sandra Helena Suescún-Carrero, Ángela Liliana Monroy-Díaz, Philippe Ferdinand Tadger-Viloria, Carlos Ernesto Vargas-Niño, Mabel Idaliana Medina-Alfonso, Laura Ximena Ramírez-López

Bibliography

1. Mills RM. Chagas disease: epidemiology and barriers to treatment. Am J Med. 2020 Nov 1;133(11):1262-5. https://doi.org/10.1016/j.amjmed.2020.05.022
2. Organización Mundial de la Salud. La enfermedad de Chagas (tripanosomiasis americana) [internet]. Organización Mundial de la Salud. 2021 abril 1. Disponible en: https://www.who.int/es/news-room/fact-sheets/detail/chagas-disease-(american-trypanosomiasis)
3. Cucunubá ZM, Manne-Goehler JM, Díaz D, Nouvellet P, Bernal O, Marchiol A, et al. How universal is coverage and access to diagnosis and treatment for Chagas disease in Colombia? A health systems analysis. Soc Sci Med. 2017 Feb 1;175:187-98. https://doi.org/10.1016/j.socscimed.2017.01.002
4. Moncayo Á. Cien años del descubrimiento de la enfermedad de Chagas. Infectio [internet]. 2009;13(4):243-5. Disponible en: http://www.scielo.org.co/scielo.php?script=sci_arttext&pid=S0123-93922009000400001
5. Llano M, Pavía P, Flórez AC, Cuéllar A, González JM, Puerta C. Evaluación preliminar de la prueba comercial Chagas (*Trypanosoma cruzi*) IgG-ELISA® en individuos colombianos. Biomédica. 2014;34(2):228-36. https://doi.org/10.7705/biomedica.v34i2.1580
6. Flores-Chávez M, Cruz I, Rodríguez M, Nieto J, Franco E, Gárate T, et al. Comparación de técnicas serológicas convencionales y no convencionales para el diagnóstico de la enfermedad de Chagas importada en España. Enferm Infecc Microbiol Clin. 2010 May 1;28(5):284-93. https://doi.org/10.1016/j.eimc.2009.07.005
7. Dias JCP. Evolution of Chagas disease screening programs and control programs historical perspective. Glob Heart. 2015 Sep 1;10(3):193-202. https://doi.org/10.1016/j.gheart.2015.06.003
8. Jaimes F. Pruebas diagnósticas: uso e interpretación. Acta Médica Colomb. 2007;32(1):29-33.
9. Añez N, Romero M, Crisante G, Bianchi G, Oarada H. Valoración comparativa de pruebas serodiagnósticas utilizadas para detectar enfermedad de Chagas en Venezuela. Bol Mal Salud Amb [internet]. 2010;50(1). Disponible en: http://ve.scielo.org/scielo.php?script=sci_arttext&pid=S1690-46482010000100003
10. Moratalla Rodríguez G. Lectura crítica de artículos de pruebas diagnósticas II: análisis de resultados. Radiología. 2015 Jan 1;57(S1):22-8. https://doi.org/10.1016/j.rx.2014.11.004
11. Departamento Administrativo Nacional de Estadística (DANE). Proyecciones de población [internet]. Bogotá: Gobierno de Colombia; 2021. Disponible en: https://www.dane.gov.co/index.php/estadisticas-por-tema/demografia-y-poblacion/proyecciones-de-poblacion
12. Enciso C, Montilla M, Santacruz MM, Nicholls RS, Rodríguez A, Mercado M, et al. Comparación de la prueba de inmunofluorescencia indirecta, un inmunoensayo enzimático y la prueba comercial Chagatek para la detección de anticuerpos anti-*Trypanosoma cruzi*. Biomedica. 2004 Mar 1;24(1):104-8. https://doi.org/10.7705/biomedica.v24i1.1254
13. Grupo BIOS. Test ELISA Chagas III [internet]. 2018. Disponible en: https://grupobios.cl/es/cotizacion-online/555-test-elisa-chagas-iii.html
14. Vircell. Chagas ELISA [internet]. 2020. Disponible en: https://www.vircell.com/producto/chagas-elisa/
15. Laboratorio Lemos. CHAGATEX ELISA [internet]. Buenos Aires; 2019. p. 38. Disponible en: https://9ed70faf-008a-484b-b316-c7044e4113af.filesusr.com/ugd/7c705f_ed4087e7c48c4c569dadd4c17d370053.pdf
16. Laboratorio Lemos L. CHAGATEK ELISA recombinante [internet]. Buenos Aires; 2019. p. 39. Disponible en: https://www.lab-lemos.com.arContenido
17. Landis JR, Koch GG. The measurement of observer agreement for categorical data. Biometrics. 1977 Mar;33(1):159.
18. Flórez Sánchez AC, Caicedo Díaz RA. Recomendación técnica sobre el uso de métodos ELISA para el diagnóstico de la enfermedad de Chagas en Colombia: nuevo algoritmo diagnóstico serológico [internet]. Bogotá: Instituto Nacional de Salud; 2017. Disponible en: https://www.ins.gov.co/buscador/Informacin de laboratorio/Recomendación técnica uso ELISA Chagas.pdf
19. Dias CPJ, Novaes Ramos Jr A, Dias Gontijo E, Luquetti A, Aparecida Shikanai-Yasuda M, Rodrigues Coura J, et al. II Consenso brasileiro em doença de Chagas, 2015. Epidemiol Serv Saúde. 2016;25 (esp):7-86. https://doi.org/10.5123/S1679-49742016000500002
20. Caicedo Díaz RA, Bernal OA, Flórez Sánchez AC. Evaluación del rendimiento diagnóstico de técnicas ELISA para la enfermedad de Chagas en Colombia [tesis de maestría en internet]. Universidad del Rosario y Universidad CES; 2017. Disponible en: https://repository.urosario.edu.co/bitstream/handle/10336/13423/Caicedo Andres Rendimiento ELISA Chagas.pdf;jsessionid=184F74A8CB3A3D5AE74C57C12B814BE2?sequence=1
21. Duarte LF, Flórez O, Rincón G, González CI. Comparison of seven diagnostic tests to detect *Trypanosoma cruzi* infection in patients in chronic phase of Chagas disease. Colomb Med. 2014;45(2):61-6.
22. González L, Scollo K, Bardach A, Sáez-Alquezar A, Ferlín C, Albajar-Viñas P, et al. Inmunoserología y métodos moleculares para el diagnóstico de Chagas: revisión sistemática rápida. Acta Bioquím Clín Latinoam [internet]. 2017;51(1):63-74. Disponible en: https://www.redalyc.org/pdf/535/53550497010.pdf
23. Instituto de Salud de Pública. Evaluación de reactivos comerciales, por el Laboratorio Nacional de Referencia de Parasitología, para el diagnóstico serológico de la infección por *Trypanosoma cruzi*, período 2000-2014 [internet]. Santiago; 2014. Disponible en: https://www.ispch.cl/sites/default/files/u20/info Evaluación de reactivos Comerciales_2014.pdf
24. Castellanos YZ, Cucunubá ZM, Flórez AC, Orozco-Vargas LC. Reproducibilidad de pruebas serológicas para el diagnóstico de infección por *Trypanosoma cruzi* en mujeres embarazadas de una zona endémica de Santander, Colombia. Biomédica [internet]. 2014 Jun 1;34(2):198-206. http://dx.doi.org/10.7705/biomedica.v34i2.1571
25. Aria L, Acosta ME, Guillen Y, Rojas A, Meza T, Infanzón B. Desempeño del Kit ELISA Chagas IICS V.1 para el diagnóstico de la enfermedad de Chagas. Mem Inst Invest Cienc Salud [internet]. 2016 Dec;14(3):7-13. Disponible en: http://scielo.iics.una.py/pdf/iics/v14n3/1812-9528-iics-14-03-00007.pdf
26. Farfán-García AE, Castellanos-Domínguez YZ, Luna-Marín KP, Angulo-Silva VM. Concordancia de dos pruebas serológicas para el diagnóstico de la enfermedad de Chagas. Rev Salud Pública [internet]. 2013;15(2):192-202. Disponible en: http://www.scielo.org.co/pdf/rsap/v15n2/v15n2a03.pdf
27. Gómez-Laiton ED, Polo-Ardila LA, Castellanos-Domínguez YZ, Herrera-Galindo VM, Villar-Centeno JC. Capacidad discriminatoria y concordancia entre el ELISA-F29 y la PCR en individuos con infección por *T. cruzi*. MedUNAB [internet]. 2015 Apr 12;18(1):27-33. Disponible en: https://revistas.unab.edu.co/index.php/medunab/article/view/2220