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ORIGINAL ARTICLE

Reproducibility of rapid diagnostic tests for *Trypanosoma cruzi* infection in endemic areas of Colombia

Reproducibilidad de pruebas de diagnóstico rápido de la infección por *Trypanosoma cruzi* en áreas endémicas de Colombia

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ABSTRACT

Introduction: Chagas disease is a neglected tropical disease of interest to public health because of its social and economic burden. Identifying infected and sick people with Chagas disease constitutes the first step towards achieving World Health Ooganization's goals for 2020.

Objective: To evaluate the reproducibility with gold standard of a rapid diagnostic test for detection of antibodies to *T. cruzi*; and to propose a diagnostic algorithm for Chagas disease under the point-of-care concept in an area with limited access to health care coverage.

Material and Methods: A cross-sectional study was performed to detect antibodies to *T. cruzi* in 151 indigenous volunteers belonging to three ethnic groups of the Sierra Nevada de Santa Marta, Colombia. Rapid tests-PDR *SD BIOLINE Chagas Ab* were implemented in the field versus confirmation in the laboratory using two standardized serological methods (ELISAs).

Results: The results show that 19,2 % seroreactivity for *T. cruzi* was found among the entire population screening. The highest rate of human infection with *T. cruzi* was detected in the Wiwa community. No significant differences between rapid diagnostic test and the standard techniques (ELISAs) were found. Sensitivity, specificity and concordance for RDT were 100 % (*Kappa: 1,0*).

Conclusions: The Sierra Nevada de Santa Marta continues to be a hyperendemic area for Chagas disease. The area is difficult to access and has low or no primary health care coverage, making the assessed rapid diagnostic test a useful tool for screening programs and defining treatment and control plans, which represents the first approach at establishing a point-of-care testing strategy for endemic countries for Chagas disease.

Keywords:
Epidemiology, Chagas Disease, *T. cruzi*, Indigenous population.

RESUMEN

Introducción: La enfermedad de Chagas es una enfermedad desatendida de interés en salud pública por su carga social y económica. Identificar personas infectadas y enfermas con el mal de Chagas constituye el primer paso para alcanzar los objetivos de la Organización Mundial de la Salud para el 2020.

Objetivo: Evaluar la reproducibilidad de una prueba de diagnóstico rápida para la detección de anticuerpos contra *T. cruzi*; y proponer un algoritmo de diagnóstico para enfermedad de Chagas bajo el concepto de uso de tecnologías en el lugar de atención en áreas de acceso limitados a los servicios de salud.

Materiales y métodos: Se realizó un estudio de corte transversal para la detección de anticuerpos para *T. cruzi* a 151 indígenas voluntarios pertenecientes a tres grupos étnicos de la Sierra Nevada de Santa Marta, Colombia. Se implementó pruebas rápidas-PDR *SD BIOLINE Chagas Ab* en campo versus la confirmación en el laboratorio mediante dos métodos serológicos estandarizados (ELISAs).

Resultados: Se encontró el 19,2 % de seroreactividad para *T. cruzi* entre toda la población estudiada. La tasa más alta de infección humana por *T. cruzi* se detectó en la comunidad Wiwa. No hubo diferencias significativas entre la prueba de diagnóstico rápida y las técnicas estandarizadas (ELISAS). La sensibilidad, especificidad y concordancia para la PDR fue del 100 % (*Kappa: 1,0*).

Conclusiones: La Sierra Nevada de Santa Marta continúa siendo un área hiperendémica para la enfermedad de Chagas. Dado que es un área de difícil acceso, con baja o nula cobertura en atención primaria en salud, la prueba de diagnóstico rápida evaluada se convierte en una herramienta útil como prueba de elección para programas de tamización y definir planes de acción de tratamiento y control, y representa el primer acercamiento de uso de tecnologías en el sitio de atención para el diagnóstico rápido en países endémicos para la enfermedad.

Palabras claves:
Epidemiología, Enfermedad de Chagas, *T. cruzi,* Población indígena.

INTRODUCTION

More than 100 years after its discovery, American trypanosomiasis or Chagas disease (CD)[1] continues to be a neglected tropical disease, being one of the most prevalent infections in rural populations of poor and developing countries in the Americas.[2] There are approximately 6 to 7 million people worldwide infected with the protozoan *Trypanosoma cruzi*, the etiologic agent of CD.[3] This disease is endemic in 21 countries in Latin America where susceptible people coexist with insect vectors (*Reduviidae: Triatominae*) and mammal reservoirs of *T. cruzi*, and have cultural food-consumption practices that may promote infection with the pathogen.[4] Similarly, the transmission of infection can occur by secondary pathways such as blood transfusions, vertical transmission, and organ transplantation.[5,6,7]

Through its London declaration about neglected tropical diseases, the WHO laid out the elimination or control of ten diseases, amongst them Chagas, as a priority for 2020 (https://sites.google.com/site/chagasddc/home/chagas-disease-milestones). Several objectives were proposed to reach this goal, including: (i) 100% of countries with no intra-domiciliary transmission; (ii) 100 % of countries with access to antiparasitic treatments; (iii) 100 % of countries with control of congenital transmission; (iv) 100 % of countries with a surveillance and prevention system for oral infection; and (v) 100 % care for infected/sick patients.[8]

Identifying infected and sick people with Chagas constitutes the first step towards achieving WHO's goals for 2020. The real incidence of CD in endemic countries is still unknown, although estimated infection rates for some Latin American countries range from 0,95 % to 6,1 %.[9] There continues to be reports of human cases with sustained increases year after year, with 13 397 new cases in 2011 increasing to 58 894 cases in 2016 (https://unitingtocombatntds.org/ntds/chagas-disease/). Many of these cases were detected late, in the chronic phase, while less than 0,5 % were in the acute phase.

Chagas disease is endemic to Colombia, where it is estimated that more than 437 000 people are infected with *T. cruzi*, and more than 4,8 million remain at risk.[10]

In studies done in different departments of Colombia, the following prevalences have been reported: Sierra Nevada de Santa Marta 36,9 %;[11] Casanare 16,9 %,[11] Boyacá 7,8 %,[12] Santander 3,2 %,[13] Guaviare 2,07 %, Vaupés 0,79 %, and Amazonas 0,09 %.

From 2008 to 2014, only 1,2 % of these people had access to screening tests and only 0,8 % had access to treatment.[12] Limited understanding of epidemiology and low coverage of trypanocidal treatment are associated with current barriers that remain in Colombia's health system, along with: (i) limited diagnosis and lack of laboratories that perform confirmatory tests; (ii) absence of epidemiological surveillance methods in areas with difficult access and low coverage of promotion and preventive actions; (iii) lack of validation of fast and reliable diagnostic methods; (iv) lack of institutional financing for patient care activities; and (v) gaps in national clinical guidelines for care in indigenous communities.[13,14,15,16]

Diagnosis and treatment of CD vary depending on the clinical phase of the patient. If the patient is diagnosed during the acute phase, direct diagnostic methods such as peripheral blood smear or polymerase chain reaction (PCR) can be performed. If the patient is diagnosed in the chronic phase, indirect immunofluorescence (IIF), indirect hemagglutination test (IHA), and indirect test using enzyme-linked immunosorbent assay (ELISAs) would be performed.[17]

According to the WHO,[18] and adopted in Colombia,[14,17] diagnosis in chronic phase should be performed using two serological diagnostic tests with different antigens for confirmation of infection, such as ELISAs and/or IIF, which are considered the gold standard for diagnosis.[18] Several studies have shown that the sensitivity of tests is higher if local strains of *T. cruzi* are used.[15,19]

Among the serological tests, the rapid diagnostic tests (RDTs), which are easy to apply and handle, require small amounts of sample without the need of refrigeration and have a turnaround time of less than 20 minutes, thus avoiding setbacks and potentiating laboratory diagnosis.[15]

The **objective** of the present study is to evaluate the reproducibility of RDTs with two gold-standard diagnostic tests (ELISAs) for the diagnosis of Chagas disease with the aim of providing new tools for timely diagnosis of CD in endemic areas.

MATERIALS AND METHODS

A descriptive cross-sectional study was performed from 2017-2018 by medical brigades in three different indigenous subpopulations: The Wiwa with 115 inhabitants in the Barcino shelter; the Kogui with 85 inhabitants in the Surivaca shelter; and the Arhuaco with 300 inhabitants in the Bunkwimake shelter. During the medical brigade, we used a non-probability sampling for convenience where an indigenous translator from the communities explained and asked them if they wanted to participate as volunteers in the screening project; therefore, we included 151 participants older than two years of age. The first two communities do not have access to primary health services, while Bunkwimake has a school and a primary health care center with surveillance from a health promoter.

The study took place in Sierra Nevada de Santa Marta (SNSM), a pyramid-shaped territory located in northern Colombia that encompasses 17 000 km^2 with altitudes of 200-5 775 meters above sea level, including the highest mountain in the country. The territory is the cradle of the Tayronas, the most monumental and unique indigenous civilization of Colombia, comprising about 60 000 indigenous people of the Arhuaco (28 092 members), the Kogui (9 191) the Kankuamo (12 533) and the Wiwa (8 559) (http://www.colparques.net/SIERRA). Studies performed in SNSM from 2000 to 2010, reported a CD prevalence of 47,0 %.[13,20] A program carried out by the National Institute of Health of Colombia (INS) in 2014 reported a CD prevalence rate of 16,5 % among children of SNSM.[20]

Serological study

Paired blood samples, obtained by venipuncture and finger puncture, were taken from patients. Blood was collected in 4ml Vacutainer tubes without anticoagulant, preserved at 4 to 8 °C and transported to Universidad Cooperativa de Colombia-Santa Marta where they were maintained at -20 °C for subsequent diagnostic testing for antibodies of *T. cruzi.* For the finger puncture, approximately 100 µl of blood was collected for in situ *anti-T. cruzi* diagnosis with RDT SD BIOLINE Chagas Ab. This RDT is composed of recombinant antigen with different protozoan epitopes (H49, JL7, A13, B13, JL8 and 1F8), and has shown good performance with 99,2 % sensitivity and 100 % specificity. The RDT was carried out following the manufacturer´s instructions (https://www.alere.com/en/home/product-details/sd-bioline-chagas-ab.html). The tests implemented here are registered by the National Institute for Drug and Food Surveillance (INVIMA) of Colombia (http://web.sivicos.gov.co/registros/pdf/15665087_2018046786.pdf).

Gold-standard laboratory confirmation tests

Confirmatory tests were performed following the national guide for *T. cruzi* detection in the laboratory.[17,21] Two ELISA tests, which are considered the gold-standard for CD diagnosis, were employed: ELISA-lysate and ELISA-recombinant. First, an ELISA-lysate kit from Wiener-Lab was used following the manufacturer's recommendations.[22] The 96 wells plate are coated with a lysate of the *T. cruzi* parasite, which corresponds to highly conserved zones of proteins among different strains of the parasite. The enzymatic reaction is stopped by the addition of sulfuric acid, which produces a light blue to yellow color change in reactive samples. The optical density is measured bichromatically at 430/450 nm by a Mindray MR 96A ELISA reader. All samples with a cut-off value greater than 0.210 were considered positive.

ELISA recombinant v.4.0 Wiener-Lab was used, following the manufacturer´s recommendations[23] as a second confirmatory test. The sample is diluted in a well in which six recombinant antigens are immobilized (SAPA, 1, 2, 13, 30 & 36). These antigens are from specific proteins of epimastigote and trypomastigote stages of *T. cruzi* and correspond to highly conserved zones among different strains. All samples with a cut-off value greater than 0,220 were considered positive.

Statistical analysis

Data were processed and analyses of frequency and central tendencies for epidemiological variables (age, gender, occupation and seroreactivity analyzed separately for each indigenous population) were performed using Microsoft Excel. Reproducibility, sensibility, specificity and concordance were evaluated by comparative statistical analysis of binomial results (positive and negative) of seroreactivity between RTD and the gold-standard laboratory ELISA tests in a 2x2 table using Openepi online diagnostic test evaluation platform (https://www.openepi.com/Menu/OE_Menu.html). Linear regression analysis and Pearson´s coefficient of optical measurements of cut-off values between the two ELISAs were developed in OpenEpi.

Ethical considerations

The present study was in accordance with the guidelines of Resolution N° 008430 of October 4th, 1993 issued by the Ministry of Health of Colombia, and is considered a minimum risk investigation according to Article II of Chapter I.[24] Approvals were granted by the technical research committee and ethics research board at Fundación Salud para El Trópico (Tropical Health Foundation), protocol Ch-SNSM01, on April 14th, 2017. All indigenous people voluntarily participated in the study.

Adults were required to sign an informed consent form while underage participants were required to have permission from their legal representative.[24,25] Gonawindua Ette Ennaka, the institution that provides health services for the indigenous communities of SNSM, was responsible for etiological treatment of all persons with a confirmed positive diagnostic.

RESULTS

A total of 151 indigenous people were screened: 73,5 % Arhuacos (111/151), 15,0 % Koguis (23/151) and 10,5 % Wiwas (17/151). Their ages ranged from 2 to 60 years of age, with an average of 16 years. A total of 19,2 % (29/151) of the participants had reactive results with the RDT and ELISAs.

Of the participants, 55,6 % were women and 44,4 % were men; the youngest participant was 3 years old and the oldest participant was 66 years old. The distribution by age range was: between 3-14 years old, 16,5 %; 15-30 years old, 31,7 %; 31-45 years old, 13,1 %; 46-60 years old, 8,5 %; and more than 61 years old 3,8 %. The percentages of participants from each Ethnic group were: Arhuaco 73,4 %, Kogui 10,5 %, and Wiwa 15,8 %. The highest rate of human infection with *T. cruzi* was detected in the Wiwa community (62,5 %; 15/24), where seroprevalence was found to increase with age. (**Table 1**).

Table 1- Demographic distribution of seroreativity to *T. cruzi*

Variables	Negative		Positive		Total	Seroreactivity (%)	95% CI
	No.	%	No.	%			
Ethnic group							
Arahuaco	103	68,2	8	5,2	111	7,2	3,2-13,7
Wiwa	9	5,9	15	9,9	24	62,5	40,5-81,2
Kogui	10	6,6	6	3,9	16	37,5	15,2-64,6
Gender							
Male	53	35,1	14	9,2	67	20,8	11,9-32,6
Female	69	45,7	15	9,9	84	17,8	10,4-27,7
Age (years)							
3-14	60	13,9	4	2,6	64	6,3	3,0-20,9
15-30	37	24,5	11	7,2	48	22,9	12,7-36,3
31-45	14	9,2	6	3,9	20	30	18,9-24,8
46-60	8	5,2	5	3,3	13	38,5	15,7-65,9
>60	3	1,9	3	1,9	6	50	14,7-85,3

The frequency of CD in the Wiwa population was concentrated among people younger than 30 years of age and is the only area where the seroreactivity of antibodies to *T. cruzi* was demonstrated in underage participants. The participants who were positive to CD were remitted to a CD observation program that is administered by their institution indigenous providing health services for medical evaluation and management.

Concordance between seroreactivity results with RDT and with standard laboratory tests for antibodies to *T. cruzi* was 100 % (Cohen's *Kappa* = 1). (**Table 2**).

Table 2- Concordance test of RDT among gold standard tests

Type of test/reactivity		Gold-standard ELISA diagnostic tests		Total
		Positive	Negative	
RDT BIOLINE SD Chagas Ab	**Negative**	122	0	122
	Positive	0	29	29
Total		122	29	151
Sensitivity, Specificity, Positive predictive value, Negative predictive value and Diagnostic precision: 100 %				

No significant differences were found between the optical density values of the ELISA-lysate and ELISA-recombinant serologies ($F_{1,300}$ = 0,05; Pv = 0,825), with a positive regression and Pearson correlation coefficient of 0,96. (**Figure 1**).

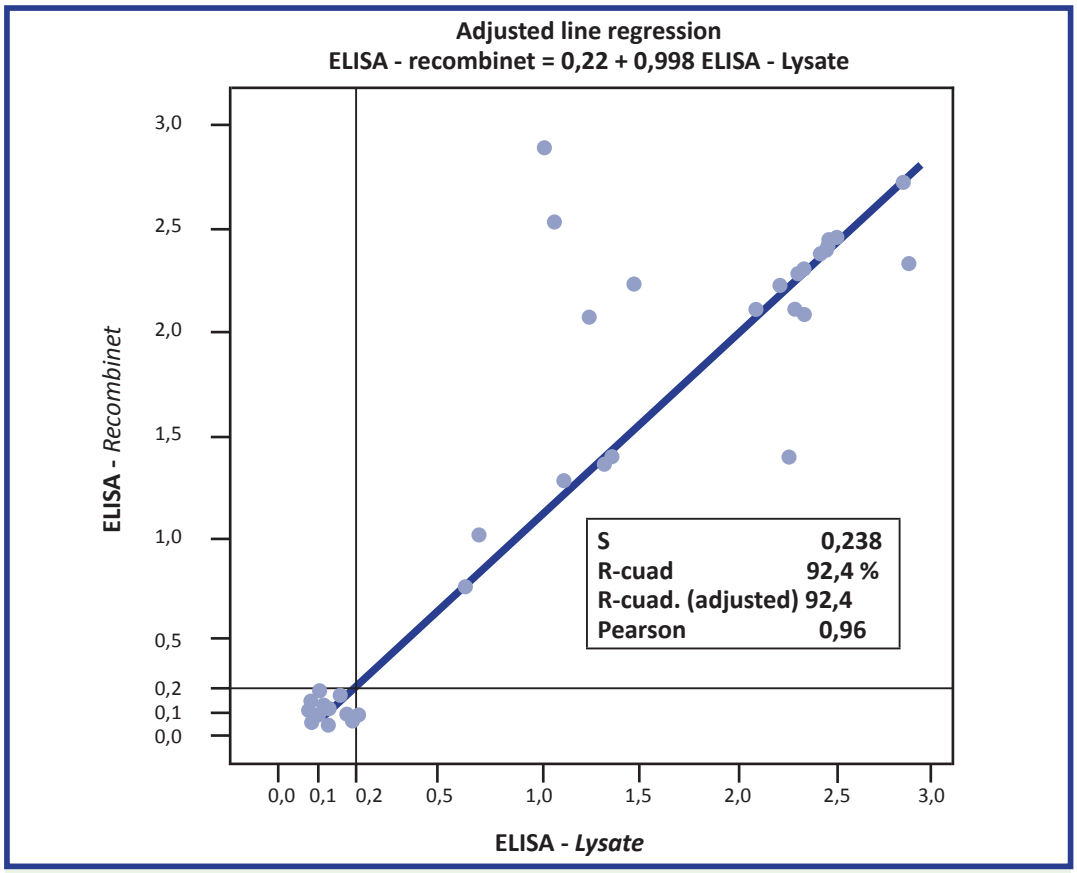

Figure 1- Concordance of optical density values of the gold standard tests for Chagas disease

DISCUSSION

The diagnosis of chronic phase of CD is performed through the detection of specific IgG antibodies against *T. cruzi,* using conventional ELISA or IIF. Consequently, the lack of means in difficult to access zones becomes one of the main obstacles for initiating a timely treatment. Due to this, the development and use of diagnostic techniques such as RDT applicable in remote areas is necessary.[26]

The results of this study demonstrate that SNSM remains a hyperendemic area for Chagas disease. The seroreactivity ranges reported here (7,2 % to 62,5 %) are higher than those reported by studies conducted in SNSM between 2000 and 2014.[13,27,28] Nevertheless, history demonstrates that the Wiwa indigenous community has the highest epidemiological burden (62,5 % in the present study). Furthermore, the Wiwa community is the only community to have had someone under 14 years of age infected with *T. cruzi.* Some authors[13] have suggested that the Wiwa community is genetically more susceptible to infection by this parasite.

The applicability of the RDT in others studies have shown excellent results in validation terms. In Bolivia, a high reproducibility was demonstrated, which had a sensitivity of 96,2 % and a specificity of 98,8 %. In endemic and non-endemic areas from Boyacá department, Suescún et al. obtained a sensitivity and specificity with InBios Chagas Detect Plus (CDP) of 100 % and 99,1 %, respectively. In Switzerland, the use of the RDT was implemented in a study to determine Latin-American migrant population with CD, obtaining similar results,[26,29] which coincides with the values found in the present study, which demonstrates a sensitivity and specificity of 100 %.

The predisposing factors for the persistence of foci of infection and human reinfection with *T. cruzi* that are evident in SNSM are related to overlap of the wild and domestic cycles of parasite transmission[30,31,32] and the cultural settings of the indigenous settlements: insufficient housing conditions and traditional customs and ancestral knowledge that facilitates disease transmission.[13,17,30] The homes of indigenous people in SNSM have a precarious structure usually consisting of a thatched roof, wooden walls, and cane or mud and dirt floor, which facilitate the establishment of the main vectors *(Rhodnius prolixus, Triatoma dimidiate and Panstrongilus geniculatus).* Coexistence with domestic animals that serve as a reservoir of *T. cruzi* is another risk factor.[13,32]

The sensitivity and specificity of RDT SD BIOLINE Chagas Ab was notable (100 %) when compared with the two gold-standard ELISA tests.[17] These reproducibility results are higher than those found by blood bank screenings of endemic and non-endemic areas for CD using the same RDT[33] and slightly above the pilot study of the manufacturer.[34]

Among the **limitations** of this study, it was not taken into account whether the participants had infections by other protozoa such as *Leishmania spp.* However, Suescún and Lorca agreed that RDTs did not present

crossing reaction, highlighting the importance of performing studies in regions where this parasite coexists with *T. cruzi*.[26,35]

This is the first study to validate the efficiency of RDT SD BIOLINE Chagas Ab applied in rural areas of Colombia with difficult access to health care centers. The results suggest that this test could be included in the initial diagnosis or screening for CD, particularly in zones with deficiencies of laboratories and well-trained professionals.[26]

Based on the newest diagnostic algorithm proposals for CD in Colombia[15,17,36] and following the cost-effectiveness study for CD screening in blood banks in Colombia[37] we propose the following diagnostic algorithm for endemic areas with difficult access and/or low or no installed health care capacity, as is the case of SNSM. (**Figure 2**).

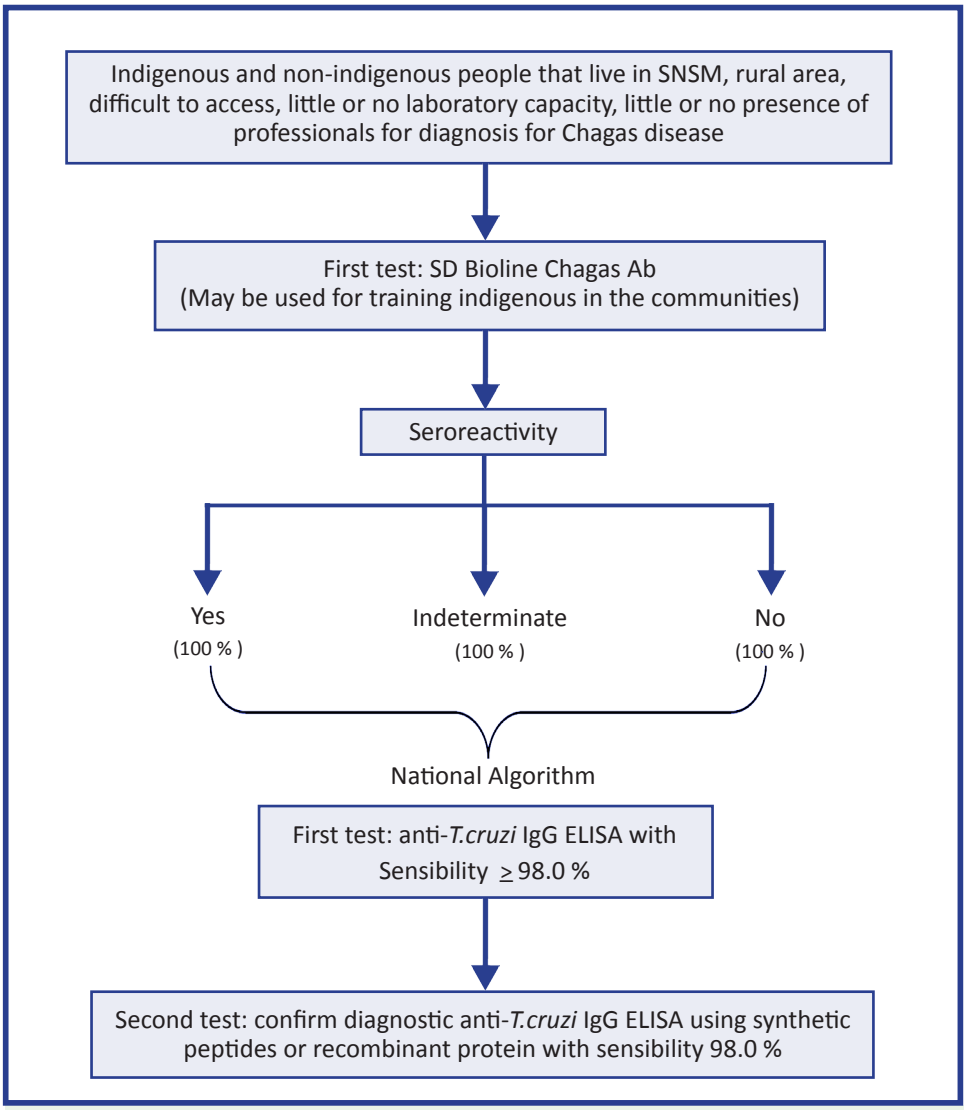

Figure 2- Diagnostic algorithm for chronic Chagas disease in indigenous population from Sierra Nevada de Santa Marta, Colombia

The advantages of RDT guarantee individual diagnosis in situ,[38] which avoids having to wait to batch samples to perform conventional tests, thus minimizing turnaround time for results and optimizing timely treatment. It also avoids long displacements of these populations and increases the benefits of laboratory diagnosis by applying the confirmation of gold-standard tests (ELISA IgG) only for seroreactive people as indicated by initial RDT.

CONCLUSIONS

The implementation of these tests reduces the current social, geographic, economic and technical-scientific gaps in Chagas disease diagnosis and treatment and is an approach to the point-of-care testing concept, to which the fight against infectious diseases should be focused.

RECOMMENDATIONS

We propose an algorithm as a first approach to a point-of-care diagnosis of Chagas disease that can be adapted and/or adopted by public health policies in Colombia and other endemic countries for CD.

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Conflict of interests

The authors declare that they have prepared the manuscript in accordance with the standards of the journal, have the exclusive responsibility for the accuracy and correctness of the contents of the article submitted, and declare that they have no conflicts of interest.

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