Technology Landscape of Diagnostic Products for Chagas Disease

Challenges with diagnosing patients at risk of chronic infection in Latin America

CUIDA CHAGAS

Technology Landscape

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Technology Landscape of Diagnostic Products for Chagas Disease

This report was developed by FIND, the global alliance for diagnostics, to fulfill one of FIND's objectives within the CUIDA Chagas consortium. We would like to thank all those who contributed to the development and review of this technology landscape:

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Disclaimer

Although all efforts have been made to ensure that the present landscape provides an accurate and comprehensive overview of diagnostic tools for Chagas disease, some devices may not have been identified.

The mention of specific companies or of certain manufacturers' products does not imply that they are endorsed or recommended by FIND, the CUIDA Chagas consortium, or any global humanitarian aid organization or individual involved in defining the scope and content of this report.

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About CUIDA Chagas Project

The CUIDA Chagas project ("Communities United for Innovation, Development and Attention for Chagas disease") is sponsored by Unitaid and the Ministry of Health in Brazil. The CUIDA Chagas consortium is led by the Oswaldo Cruz Foundation (Fiocruz) from Brazil; it includes other government organizations such as Instituto Nacional de Laboratorios de Salud "Néstor Morales Villazón" (INLASA) from Bolivia, Instituto Nacional de Salud (INS) from Colombia, and Servicio Nacional de Erradicación del Paludismo (SENEPA) from Paraguay, and the international non-governmental organization FIND, the global alliance for diagnostics. The CUIDA Chagas consortium aims to contribute to the elimination of congenital transmission of Chagas disease by scaling-up and enhancing access to diagnosis, treatment and comprehensive care, through innovative and sustainable approaches in Bolivia, Brazil, Colombia and Paraguay. More information about the consortium project can be found on https://cuidachagas.org/.

One of our objectives within the CUIDA Chagas consortium is to provide market shaping and supply chain interventions to ensure equitable access to innovative diagnostic products. This technology landscape report is the first milestone and aims to help health sector stakeholders, at global and national levels, understand the market landscape of priority diagnostic tools for chronic *Trypanosoma cruzi* infection that meet availability, affordability, appropriateness for use in low- and middle-income countries (LMICs), and adoption criteria in the four countries of interest (Bolivia, Brazil, Colombia and Paraguay).

About FIND

FIND, the global alliance for diagnostics, seeks to ensure equitable access to reliable diagnosis around the world. We connect countries and communities, funders, decision-makers, healthcare providers and developers to spur diagnostic innovation and make testing an integral part of sustainable, resilient health systems. We are co-convener of the Access to COVID-19 Tools (ACT) Accelerator diagnostics pillar, and a WHO Collaborating Centre for Laboratory Strengthening and Diagnostic Technology Evaluation. Founded in Geneva, Switzerland, in 2003, we have regional hubs in Kenya, India, South Africa and Viet Nam. With partners across the public and private sectors, we are working to make sure that everyone who needs a test can get one.

Table of contents

1. Abbreviations

CCD	congenital Chagas disease
CD	Chagas disease
CI	confidence interval
CLIA	chemiluminescent immunoassay
DTU	discrete typing unit
EIA	enzyme immunoassay
ELISA	enzyme-linked immunosorbent assay
Fiocruz	Fundação Oswaldo Cruz
Fiotec	Fundação para o Desenvolvimento Científico e Tecnológico em Saúde
IFU	instructions for use
IgA	immunoglobulin A
IgG	immunoglobulin G
IgM	immunoglobulin M
IFA	immunofluorescence assay
IIF	indirect immunofluorescence (*IFI in Spanish*)
INLASA	Instituto Nacional de Laboratorios de Salud "Néstor Morales Villazón"
INS	Instituto Nacional de Salud de Colombia
IHA	indirect haemagglutination assay (*HAI in Spanish*)
IVD	in vitro diagnostic
LFA	lateral flow assay
LAMP	loop-mediated isothermal amplification
LMIC	low- and middle-income country
NHI	National Health Institute
NTD	neglected tropical disease
PAHO	Pan American Health Organization
PCR	polymerase chain reaction, qPCR: real-time or quantitative PCR
POC	point-of-care
R&D	research and development
RDT	rapid diagnostic test
RPA	recombinase polymerase amplification
RUO	research use only
SENEPA	Servicio Nacional de Erradicación del Paludismo
USD	US dollars
WB	western blot
WHO	World Health Organization

Regulatory Agencies (country or region):

AGEMED	Agencia Estatal De Medicamentos Y Tecnologías En Salud (Bolivia)
ANMAT	Administración Nacional de Medicamentos, Alimentos y Tecnología Médica (Argentina)
ANVISA	Agência Nacional de Vigilância Sanitária (Brazil)
ARCSA	Agência Nacional de Regulación, Control y Vigilancia Sanitaria (Ecuador)

CE	European Conformity (Conformité Européenne) (EU)
COFEPRIS	Comisión Federal para la Protección contra Riesgos Sanitarios (Mexico)
DGVMN	Dirección General de Vigilancia del Marco Normativo (Honduras)
DGVRCS	Dirección General de Regulación, Vigilancia y Control de la Salud (Guatemala)
DIGEMID	Dirección General de Medicamentos (Peru)
DINAVISA	Dirección Nacional de Vigilancia Sanitaria (Paraguay)
FDA	Food and Drug Administration (USA)
HSA	Health Sciences Authority (Singapore)
INVIMA	Instituto Nacional de Vigilancia de Medicamentos (Colombia)
MFDS	Ministry of Food and Drug Safety (South Korea)
MHLW	Ministry of Health, Labour and Welfare (Japan)
NMPA	National Medical Products Administration (China)
RMH	Russian Ministry of Health (Russia)
TGA	Therapeutic Goods Administration (Australia)

2. List of figures

3. Annex contents

4. Executive summary

Chagas disease (CD), also known as American trypanosomiasis, is endemic in 21 continental Latin American countries, with around 6 to 7 million people infected; 70 million people are at risk of infection worldwide. The annual economic burden of the disease is estimated to be USD 630 million in healthcare costs and USD 7.19 billion in economic losses. CD is caused by the parasite *Trypanosoma cruzi*. Diagnosis and treatment of chronic *T. cruzi* infection is essential for the control of CD, by preventing the development of symptoms and subsequent spread of the parasite, particularly via congenital transmission. However, fewer than 10% of individuals with chronic *T. cruzi* infection are diagnosed, and only 1% of those diagnosed receive etiological treatment.

Diagnosing chronic *T. cruzi* infection is complex, as it is based on the agreement of at least two conventional immunoassays, such as an enzyme-linked immunosorbent assay (ELISA), indirect haemagglutination assay (IHA) or immunofluorescence assay (IFA). While these immunoassays show high clinical performance, they are impractical in many CD-endemic regions due to their complexity. Easy-to-use rapid diagnostic tests (RDTs) for CD are commercially available in Latin America, but they have not been widely implemented in public health systems. Despite the lower performance of individual RDTs compared with conventional non-RDTs, studies have highlighted the potential value of new algorithms that combine several RDTs. If validated, the use of RDTs in resource-limited settings has the potential to revolutionize the diagnosis of chronic CD.

Here, we provide a diagnostic landscape analysis, in which 103 immunoassays were identified (39 RDTs and 64 conventional non-RDTs). Most have received regulatory approval and are currently on the market (25 RDTs and 35 non-RDTs), with 8 RDTs that appear the most promising for implementation as part of a diagnostic algorithm as they meet the following selection criteria: (1) stringent regulatory approval, (2) market availability in the four countries of interest (Bolivia, Brazil, Colombia and Paraguay), and (3) high or acceptable clinical performance. These RDTs are (i) Accu-Tell Chagas Cassette, by AccuBiotech; (ii) SD Bioline Chagas Ab Rapid test, by Abbott; (iii) Chagas Stat-Pak, by Chembio Diagnostic Systems Inc.; (iv) TR Chagas Bio-Manguinhos by Bio-Manguinhos/Fiocruz; (v) Onesite Chagas Ab Rapid test, by CTK Biotech; (vi) Chagas Rapid Test Cassette - S/P and (vii) Chagas Rapid Test Cassette - WB/S/P, both by Acro Biotech Inc. and others; and (viii) WL Check Chagas test, by Wiener Lab. Ideally, criteria such as cross-reactivity and clinical evaluation in relevant subpopulations e.g. pregnant women and infants should have been considered when comparing the various RDTs, but these data were lacking for most of them.

Diagnosis of acute CD is performed using either parasitological or molecular methods. While algorithms for diagnosis in children vary depending on the country, in general, children born to infected mothers are tested using parasitological methods (mainly microscopy) shortly after birth and serological methods 8 to 12 months after birth. However, this multistep approach delays prompt access to treatment due to the low sensitivity of microscopy and the

loss to follow-up of children at 8 to 12 months. As a result, a considerable number of congenital Chagas disease (CCD) cases are missed in endemic countries. Although molecular tests (polymerase chain reaction, PCR and quantitative real-time PCR, qPCR) are more effective for diagnosing CCD than the current diagnostic algorithm, their implementation is limited, primarily due to their complexity and high cost. Among endemic countries, only Chile routinely uses PCR as part of its national diagnostic strategy for CD. New technologies in development could overcome some of these hurdles, particularly molecular point-of-care (POC) diagnostic tools, including isothermal DNA amplification methods such as loop-mediated isothermal amplification (LAMP) or recombinase polymerase amplification (RPA). In this landscape analysis, we identified seven commercialized qPCR tests and one LAMP prototype currently under evaluation in endemic countries.

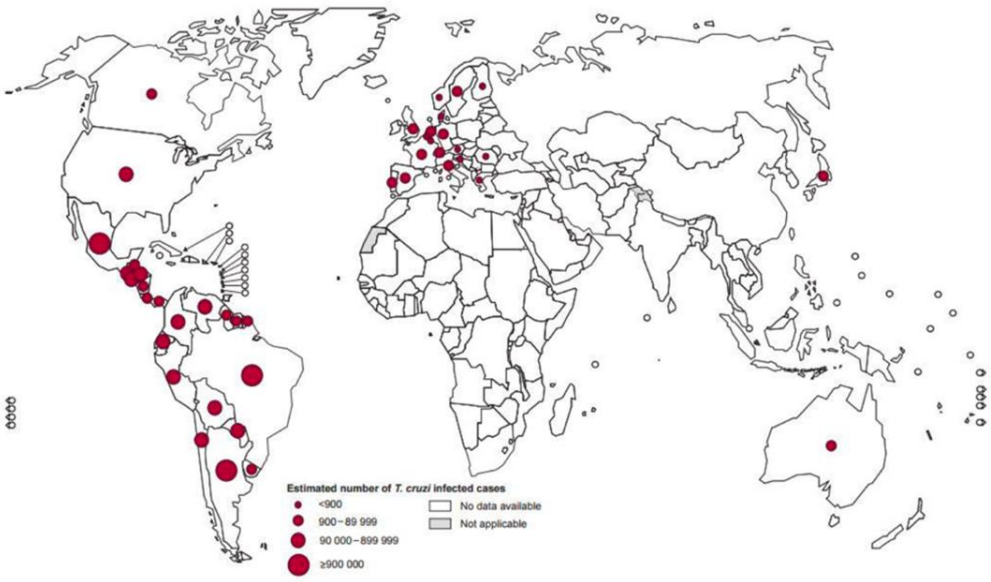
Further evaluation of diagnostic technologies such as RDT immunoassays and POC molecular tests should be conducted in endemic countries to explore their adaptability and utility. It will also be necessary to create collections of samples to test these new technologies and/or new diagnostic algorithms. However, even if easy-to-use POC diagnostics could be validated and made commercially available, some challenges in accessing these diagnostics may remain for people at risk of infection.

5. Introduction

5.1 Chagas disease burden

Chagas disease (CD) is a neglected tropical disease caused by the parasite *Trypanosoma cruzi* (*T. cruzi*). It is endemic in 21 continental Latin American countries, with around 6 to 7 million people afflicted by the disease. Due to migration of infected individuals, 70 million people are at risk of infection worldwide. The World Health Organization (WHO) estimates that 39 000 new cases occur annually, with more than 12 000 related deaths. Argentina, Brazil, Mexico and Bolivia are the countries with the highest estimated numbers of CD-infected individuals (1 505 235, 1 156 821, 876 458 and 607 186, respectively). The Andean region accounts for 958 453 infected individuals, 45.7% of whom (437 960) are from Colombia. The global prevalence of CD is shown in **Figure 1**. Congenital transmission is now considered to be the main source of incident cases. The estimated annual numbers of cases of *T. cruzi* infection due to congenital transmission were highest in Mexico (1788), Argentina (1457) and Colombia (1046), followed by Venezuela (665), Bolivia (616), Brazil (571), Ecuador (696) and Paraguay (525) (WHO, 2015a).

Figure 1. Global distribution of cases of Chagas disease

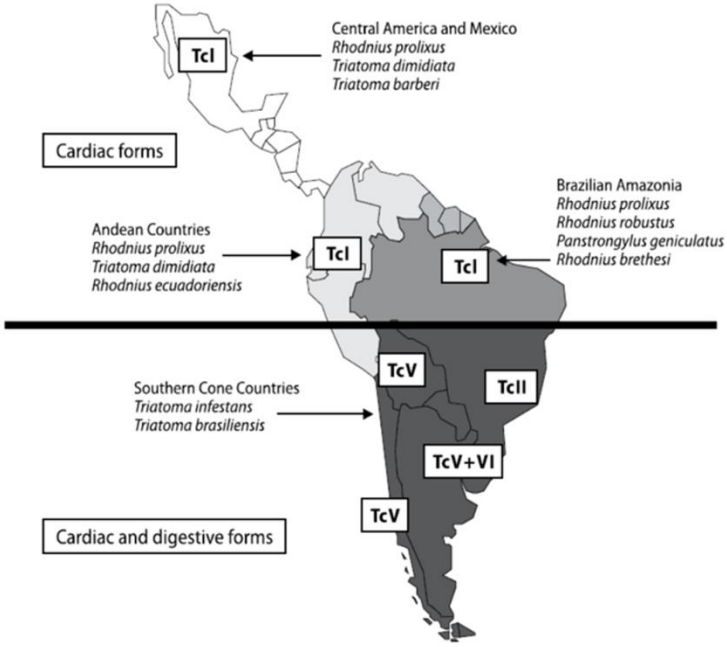


Source of figure: PATH, 2016
Source of data: WHO, Control of Neglected Tropical Diseases, 2006–2010

The parasite *T. cruzi* exhibits wide genetic diversity, with more than 6000 strains currently classified into 7 discrete typing units (DTUs), referred to as TcI to TcVI, with another named

Tcbat; their prevalence in human samples varies among endemic countries in Latin America (**Figure 2** (PATH, 2016)). *Trypansoma cruzi* parasites can be contracted via contact with the faeces or urine of infected blood-sucking triatomine bugs, the main vector of CD; they can also be transmitted via contaminated food, blood transfusion, organ transplantation, an infected mother to her baby during pregnancy or delivery, and laboratory accidents. CD presents in two phases: acute and chronic. The acute phase lasts for around 2 months following infection. During the chronic phase, the parasites hide, mainly in the heart and digestive muscles. An estimated 30% to 40% of infected and untreated individuals will develop severe and sometimes life-threatening medical problems over the course of their lives, including cardiomyopathy, gastrointestinal disorders, and neurological or mixed symptoms, all of which require specific treatment; thus, if untreated, infection is lifelong and can lead to severe complications that in some cases are fatal (Coura & Borges-Pereira, 2010).

Figure 2. Geographical distribution of *Trypanosoma cruzi* DTUs in humans



Source of figure: PATH, 2016

Although there has been a considerable reduction in vectorial transmission in recent years, the chronic form of CD remains a long-lasting challenge for the prevention and control of non-vectorial transmission (WHO, 2015a). Symptomatic CD imposes a substantial financial burden on societies and healthcare systems. With an estimated USD 630 million in healthcare costs annually and USD 8 billion in annual economic losses, the economic burden due to CD equals

or exceeds that caused by other prominent infectious diseases, such as Zika virus disease (USD 3.7 billion).

Despite the high morbidity and mortality of CD, and the considerable associated economic burden, substantial numbers of CD cases are missed, with fewer than 10% of individuals chronically infected with *T. cruzi* being diagnosed and only about 1% of those receiving etiological treatment (Basile et al., 2011; Cucunubá et al., 2017). In most cases, symptoms are absent and, due to its diverse and nonspecific manifestation, a confirmatory diagnosis is based largely, if not exclusively, on laboratory tests. Timely identification and treatment of CD has important benefits, including prevention of future congenital transmission in treated mothers, serological cure in infants and children, and a reduction in progression to advanced forms of the disease in adults (Moscatelli et al., 2015). Once the disease has progressed to an advanced phase, with severe cardiac or digestive disease, etiological treatment does not appear to have any clinical benefits. This supports the need for improved diagnostics and early access to safe and effective treatment.

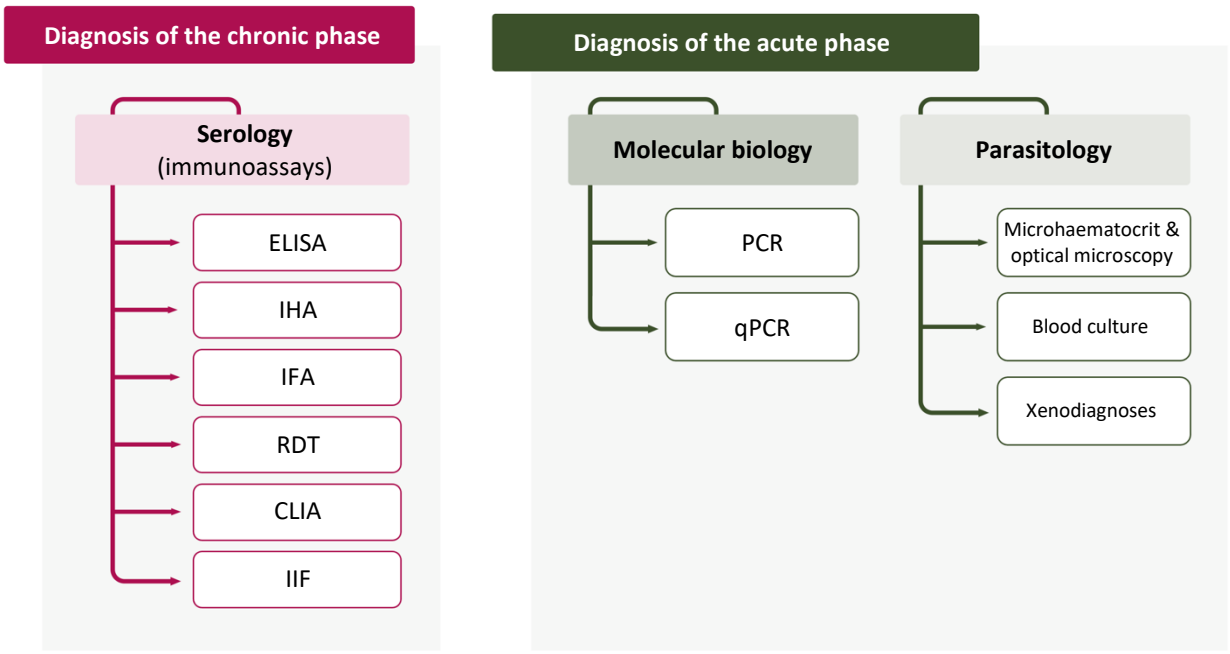
5.2 Diagnostics for Chagas disease

According to the Pan American Health Organization (PAHO), the current diagnostic algorithm suggested for patients with suspected chronic *T. cruzi* infection is the agreement of two serological tests for detecting antibodies against *T. cruzi*, such as an enzyme-linked immunosorbent assay (ELISA), indirect haemagglutination assay (IHA) or indirect immunofluorescence assay (IFA). If the results of the two tests are conflicting, a third test based on a different antigenic principle should be used (ELISA, IHA or IFA) (PAHO, 2018). However, each country has its own guidelines, as outlined below.

Highly sensitive kits, such as ELISAs or chemiluminescent immunoassays (CLIAs), are recommended to screen for the disease in haemotherapy services. ELISAs and CLIAs can detect different antibodies against *T. cruzi*, with good analytical performance, but require a laboratory and specialized personnel, and the results can take hours to obtain. Rapid diagnostic tests (RDTs) for CD are commercially available; however, these tools have not been widely implemented in public health systems in Latin America.

In patients with suspected acute *T. cruzi* infection, it is recommended to perform either parasitological or molecular tests. Parasitological methods include microhaematocrit, optical microscopy, blood culture, and xenodiagnosis. Molecular techniques such as conventional polymerase chain reaction (PCR) and quantitative real-time PCR (qPCR) are considered to be supportive diagnostic tests due to their ability to determine *T. cruzi* parasite loads; however, their implementation in endemic regions remains limited due to several factors, including a lack of clinical evidence and standardization, complexity, high costs and the need for cold-chain transport (Moreira et al., 2013; Picado et al., 2018). Diagnostic tests for CD are summarized in **Figure 3.**

Figure 3. Types of diagnostic tests for Chagas disease



5.3 Diagnostic algorithms for chronic Chagas disease in the four countries of interest

The majority of countries in the Latin American region have achieved universal blood screening for CD, where haemovigilance at blood banks is performed using double screening for infectious markers in 100% of samples, by ELISA or chemiluminescence, following the indications for the diagnosis of a chronic case (WHO, 2015b). Serological tests for the detection of immunoglobulin G (IgG) antibodies are also used during and after detection, as the clinical criterion for cure is seroconversion. Seroconversion is considered to have occurred if an individual receives negative results for at least two consecutive serological tests, by two methods using different antigenic principles, in two samples taken with a minimum interval of three weeks (the two methods may be an ELISA, an indirect immunofluorescence (IIF) assay, or an IHA, plus a third test in cases of conflicting results), and performed periodically (1 year in cases of congenital transmission, 3 to 5 years in acute cases, and 5 to 10 years in chronic cases).

The healthcare levels cited below are defined in **Table 2**, **Annex I**.

In **Bolivia**, the diagnostic methods validated for use in patients (Doctors without borders, 2016; Ministerio de Salud y Deportes de Bolivia, 2007) are as follows:

- In suspected acute *T. cruzi* infection and in neonates (<9 months of age) born to infected women, parasitological diagnosis is used (microscopy, microhaematocrit, Strout method or thick film, or smear); in cases of positive parasitological results, two serological tests for IgG detection (ELISA or IHA) are performed after 9-months of age, plus a third test in cases of discordant results.
- In suspected chronic *T. cruzi* infection:

  a) Two serological tests with different antigenic principles, first conventional ELISA for screening, then a second serological test (either recombinant ELISA or IHA) for confirmation, performed serially, plus a third, recombinant ELISA if there are conflicting results. These guidelines are followed in those facilities that have an ELISA reader (healthcare level 2).
  b) A rapid test (lateral flow assay, LFA) for screening (healthcare level 1), followed by confirmation at the nearest laboratory (healthcare level 2) using a serological test with different antigenic principles (ELISA or IHA), plus a third recombinant ELISA (healthcare level 2) or IIF (healthcare level 3) if there are conflicting results. This methodology is followed in endemic areas with limited access to laboratory-based tools, using samples from pregnant women at their first prenatal care visit. In addition, for every ten negative results from rapid tests (LFAs), quality control is performed on 10% of the negative results, using a venous sample.

- PCR technology is used for research purposes.

The algorithm used in **Brazil** (CONITEC Brazil, 2018) is as follows:

- In patients with suspected chronic CD, it is recommended that confirmation be based on at least two serological tests for IgG detection with different antigenic principles (ELISA, IIF, IHA, CLIA or WB) with the same blood sample. If the results are discordant, a third IgG test is performed on a different sample. Rapid tests are recommended only for patients who have limited access to health services and for pregnant women with suspected CD during their prenatal care or when they are in labour.

- For any probable case of acute CD (clinical symptoms), samples are analysed using one of the following:
  - Direct parasitological methods (microscopy, microhaematocrit, Strout method or thick film, or smear), with a repeated test in the case of negative results and confirmation of seroconversion using an IgG test.
  - Serology for IgM detection; in the case of a negative result, confirmation with IgG serology.
  - Detection of seroconversion via IgG detection in two serum samples taken at a minimum interval of 15 days.
- In neonates (<9 months of age) born to women with CD, it is recommended to perform parasitological diagnosis and two serological tests for IgG detection based on different

antigenic principles, plus a third test in cases of discordant results after 9-months of age.

In **Colombia**, based on the guidelines for the management of CD patients (Instituto Nacional De Salud – INS. Colombia, 2017), the algorithm used is as follows:

- Patients with suspected chronic CD need to give just one blood sample, which is evaluated at a local laboratory (level 2). The first technique recommended is an ELISA for total extract antigens with a sensitivity greater than or equal to 98%. If the result is positive, a confirmatory test should be performed with a second recombinant antigen or synthetic peptide ELISA/CLIA, with a specificity greater than or equal to 98%. If the results are discordant, a third serological test (IIF or an immunoblot performed at level 3) must be performed.

- For any probable cases of acute CD showing clinical symptoms, a sample should be analysed using either a parasitological method (microscopy, microhaematocrit, Strout method or thick film, smear) or determination of seroconversion via detection of IgG antibodies in two serum samples taken with a minimum interval of three weeks (21 days). PCR or blood culture are recommended in the guidelines but are usually not performed as they require healthcare level 3.
- For pregnant women living in endemic areas and during their prenatal care visit, and for neonates (>10-months of age) born to infected women, it is recommended that two serological tests for IgG detection with different antigenic principles (ELISA) be performed, plus a third test (IIF) in the case of discordant results. In neonates <10 months of age, parasitological diagnosis (microscopy, microhaematocrit, Strout method or thick film, smear) should be performed.
- The use of rapid tests for diagnosis or confirmation is not allowed.

In **Paraguay**, the algorithm (DGVS Paraguay, 2015; Paraguay. Ministerio de Salud Pública y Bienestar Social., n.d., 2021) used is as follows:

- Diagnosis in the acute phase of CD is confirmed by parasitological methods or the detection of genetic material in the blood using molecular tools. In the case of a negative result, the parasitological test is repeated and a serological test for IgM antibodies against *T. cruzi* is performed.

For diagnosis of chronic cases, at least two reactive serological methods for detecting anti-*T. cruzi* IgG antibodies should be used (usually ELISA or IHA). In the event that the results are discordant, a third technique must be performed or the sample should be referred to a more sophisticated laboratory (e.g. to perform IIF at level 2). Rapid tests are recommended only in pregnant women with suspected CD during their prenatal care or who are in labour, confirmed by a second reactive serological test.

6. Scope and methodology

The aim of this assessment is to help health sector stakeholders, at both global and national levels, understand the technological landscape and build a sustainable market for RDTs for chronic *T. cruzi* infection in CD-endemic countries. It is essential to ensure the availability, affordability and suitability of the tests for use in low- and middle-income countries (LMICs), to make their adoption more likely in such settings. A preliminary analysis by FIND (unpublished, 2021) found that the diagnostic market for CD is highly fragmented, with more than 90 in vitro diagnostic (IVD) tests produced by at least 50 manufacturers worldwide. Furthermore, CD diagnostic testing needs and practices are not well known. Market size and readiness to use RDTs to diagnose chronic *T. cruzi* infection have also not been elaborated. Therefore, by preparing this report we aimed to build a comprehensive technology landscape of diagnostic products for chronic CD, including commercialized tests (especially in Bolivia, Brazil, Colombia and Paraguay) and tests in development under the framework of the CUIDA Chagas project ("Communities United for Innovation, Development and Attention for Chagas disease – Towards elimination of congenital transmission of Chagas disease in Latin America") (Sousa et al., 2022).

This report includes the following information for all diagnostic tests:

- Competitive analysis of CD diagnostic tests identified, including information on commercially available CD diagnostic products (serological and molecular tests).
- Challenges for diagnosing patients at risk of CD in Latin America, along with solutions provided by new technologies and point-of-care (POC) diagnostic tests such as RDTs.
- General information about the manufacturers of CD tests (research and development (R&D) pipeline; products commercialized in the Latin American market).

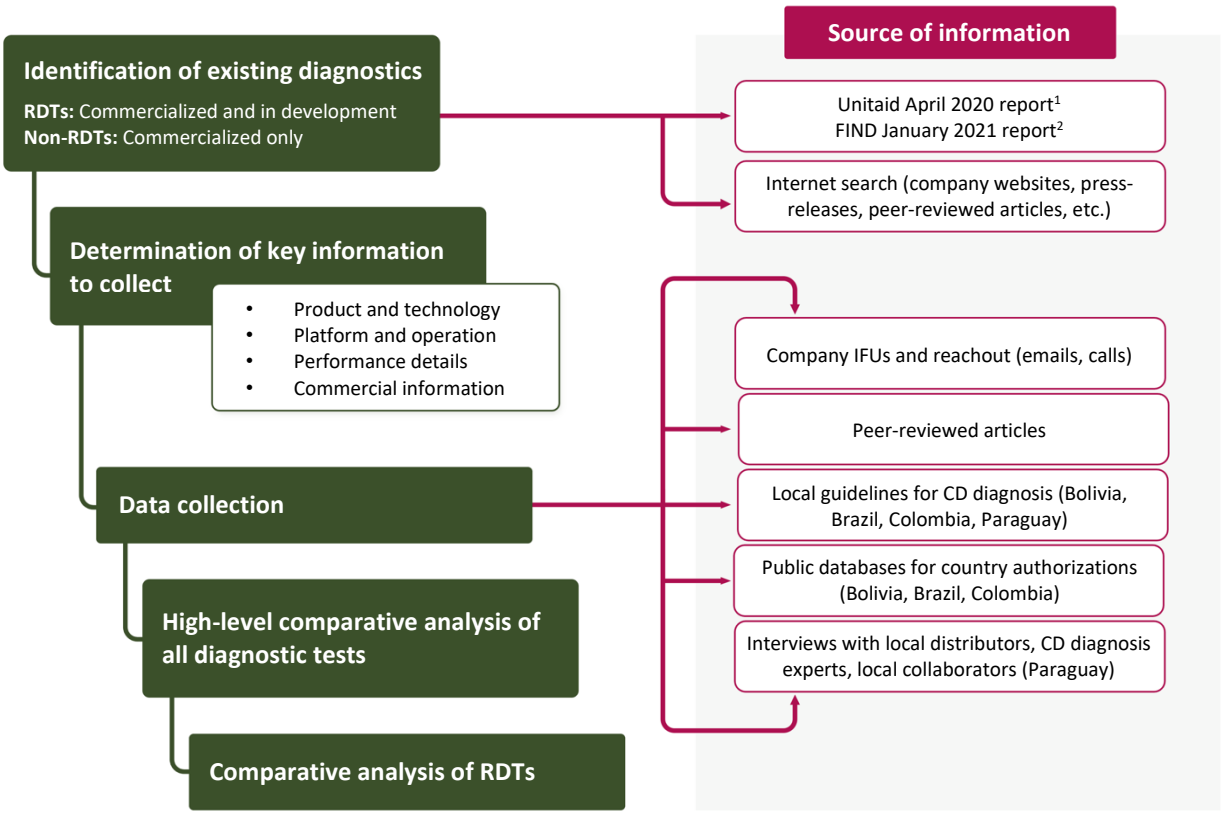
A flow diagram of the methodology we used is shown in **Figure 4**. Briefly, the technology landscape was built up by following five steps:

1. Identification of existing RDTs and other, non-RDT technologies for the diagnosis of CD. The project was initially conducted using previous landscape reports on diagnostics for chronic CD (FIND 2021 - unpublished; UNITAID, 2020), which led to the identification of 14 different RDTs and some non-RDTs. An internet search was conducted, and the panel was completed with 24 additional RDTs and 6 non-RDTs.
2. Determination of key data to be collected: product (stage of development, regulatory approval and primary use case), technology (type, assay target and sample type), platform and operation (instrument requirement), performance details (cross-reactivity and clinical data) and commercial information (commercialization).
3. Collection of data from a variety of sources, including product instructions for use (IFUs), peer-reviewed articles, interviews with manufacturers or local distributors who had agreed to share their information, interviews with experts in CD diagnosis and local researchers who collaborate with FIND, and a study of local guidelines for the

diagnosis of CD in the four countries of interest. Public-domain databases that store information about country authorizations were screened for commercialized RDTs in Bolivia, Brazil and Colombia. Certificates of registration were requested for authorized products that did not appear in these country databases. Paraguay does not have any publicly available information regarding sanitary registrations; import license certificates were requested as proof of marketing approval in the country. Authorizations from other regulatory authorities cited in this report are based solely on information provided by manufacturers or distributors.

4. An overview was created of all CD diagnostic tests identified, and a high-level comparison was conducted.
5. A comparative analysis of CD RDTs was made based on regulatory approval(s), market availability (globally and in the countries of interest), and clinical performance.

Figure 4. A flow diagram of the methodology used



[1] Screening and treatment for Chagas Disease: Technology and market landscape (UNITAID April 2020)
[2] The Landscape for Chagas Disease Rapid Diagnostic Tests in Latin America (FIND January 2021 – not published)

7. Results of the analysis

It is important to note the following:

- All RDTs listed in this landscape assessment were assigned a specific "test number" that will be used throughout this report. Please refer to **Table 1** in the **Annex** to find the corresponding test names and detailed information about the tests.
- This report focuses on commercialized diagnostic products for CD. All technologies, including immunoassays and molecular tests, were investigated. However, more thorough research was carried out into RDTs, as they were the main focus of this report.

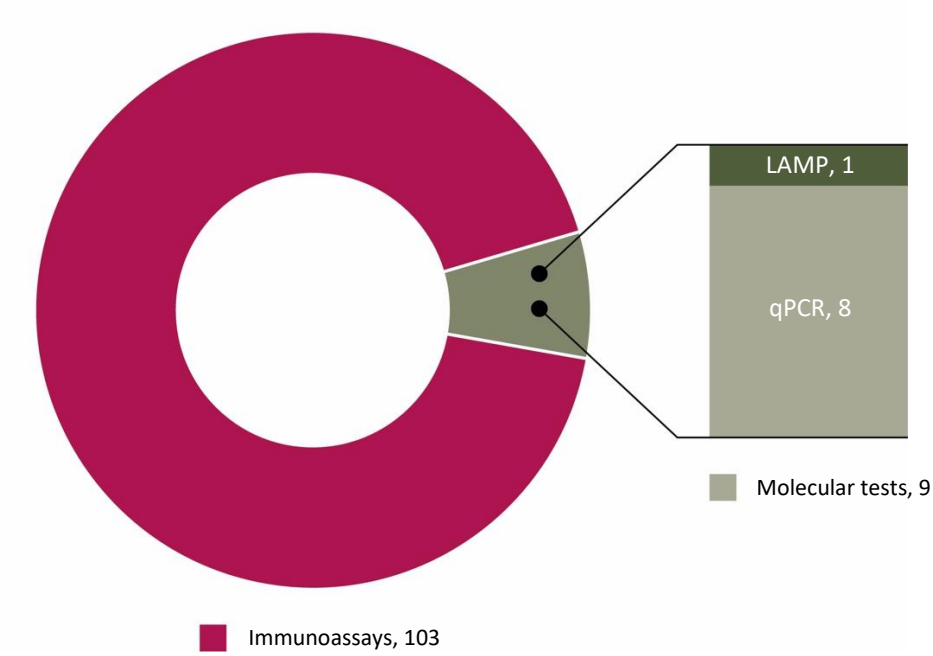
Regulatory approvals recognized to be stringent are based on the Global Harmonization Task Force and the International Medical Device Regulators Forum. Stringent regulatory authorities include Australia TGA, Brazil ANVISA, Health Canada, China NMPA, Europe CE-IVD, Japan MHLW, Russia RMH, Singapore HSA, South Korea MFDS, and United States FDA clearance.

7.1 Overview of data collected

We identified a total of 112 tests for the diagnosis of CD, most of which were immunoassays (92%). Only nine molecular assays were detected, including eight qPCR assays and one loop-mediated isothermal amplification (LAMP) assay prototype (**Figure 5**). All qPCR tests are laboratory-based. The NAT Chagas kit by IBMP (Brazil ANVISA approval in 2022) includes a reagent to preserve whole blood samples extracted in remote regions. The LAMP prototype (Eiken Chemicals Co. Ltd) is under development and currently being tested as a molecular POC test in endemic regions.

Molecular assays are recommended in the guidelines of endemic countries, for diagnosis in patients with suspected acute infection, as they allow detection of *T. cruzi* in the blood. Molecular methods might have clear advantages for the early detection of congenital CD, but they are currently primarily used in research laboratories. This report will focus on immunoassays, as they are mainly used for the diagnosis of patients suspected of having chronic CD infection.

Figure 5. Overview of all diagnostic tests for Chagas disease



7.2 Immunoassays

Definition of RDTs and non-RDTs

Immunoassay technologies can be grouped into two categories, RDTs and non-RDTs. We defined RDTs as tests that can be performed in less than 30 minutes and do not require sample preparation. All RDTs except one were LFAs. The full list and details of the 39 RDTs and 64 non-RDTs for CD included in the landscape assessment are shown in **Table 1, Annex**.

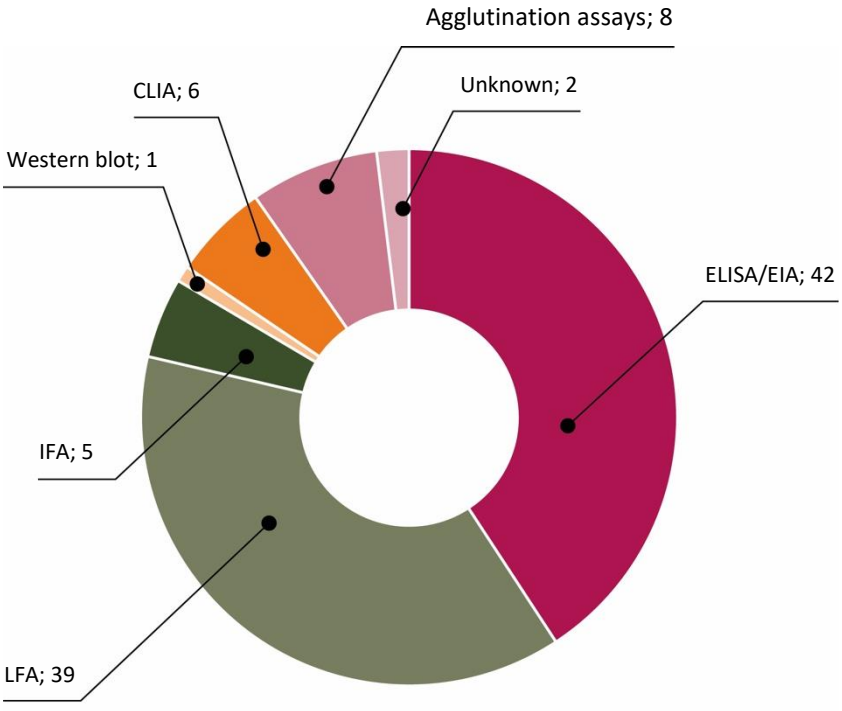
The sample type required for most of the RDTs is a drop of whole blood, in contrast to the non-RDTs, which often require a processed sample such as serum or plasma. As expected, most (66%) of the RDTs are easy to use and could be performed in a community facility by a lay person; 24% required trained staff in a primary healthcare facility. The non-RDT immunoassays are more complex, and most must be performed by a laboratory technician in a district hospital laboratory or a reference laboratory, although a few of them (less than 10%)

could be used by a trained staff member in a healthcare facility. Full details of this information are shown in **Table 2, Annex**.

Overview of immunoassay technologies

We identified 103 immunoassays, including 39 LFAs, 42 ELISAs/EIAs, 8 agglutination assays (6 of which were IHAs), 6 CLIAs, 5 IFAs, and 1 western blot (see **Figure 6**). Other immunoassays are described in the literature (e.g. radioimmunoprecipitation assay, RIPA) but no commercial kits for them were identified; they were therefore not included in this analysis.

Figure 6. Overview of all immunoassay technologies



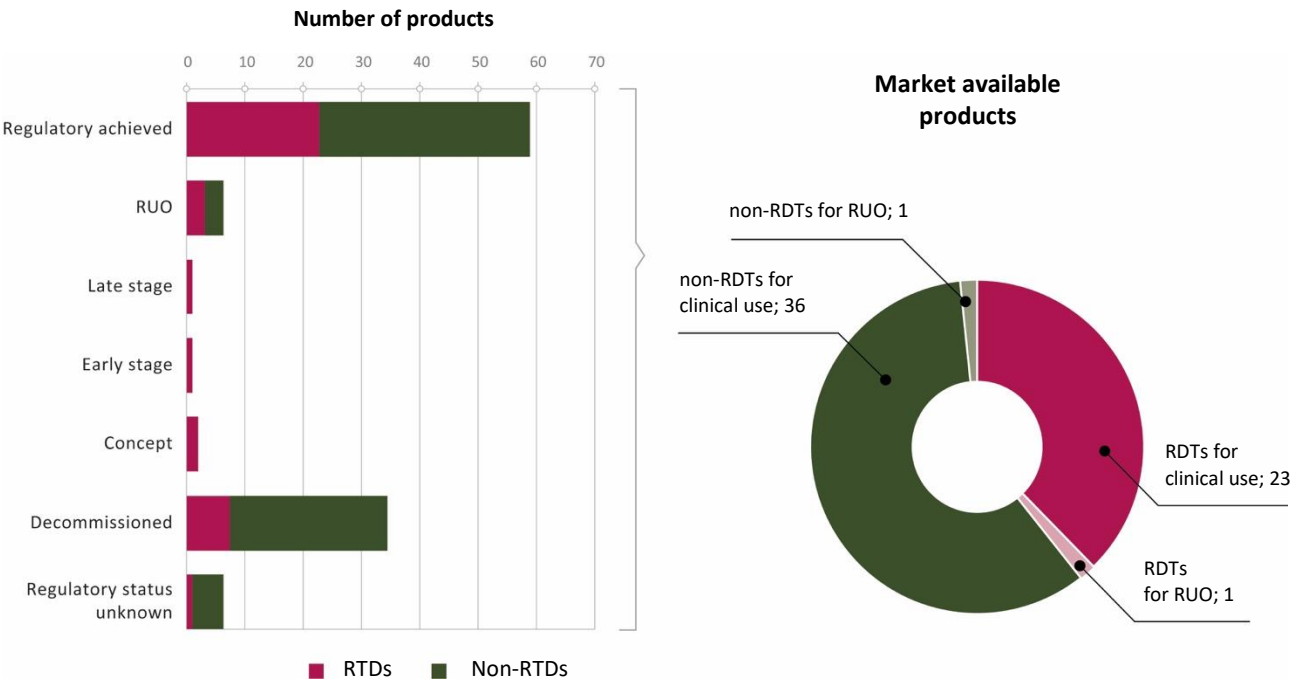
Stage of development

Among the 39 RDTs identified, 23 tests are currently on the market and have received regulatory approval for clinical use. One RDT is on the market for research use only (RUO). Eight RDTs had been decommissioned because they no longer had regulatory approval and were not on the market anymore (e.g. the two tests from InBios International Inc. were

discontinued in May 2022). Only a few RDTs in development were identified, including two in the concept phase, one in early-stage development (partial prototype) and one in late-stage development (functional prototype). Three RDTs are for RUO, and the regulatory status was not available for one test.

Regarding the non-RDT immunoassays, 37 out of the 64 identified are on the market; 36 have received regulatory approval for clinical use, while one is for RUO. Twenty non-RDTs had been decommissioned. Three of these were for RUO, while the regulatory status was not available for five tests (Figure 7).

Figure 7. Stage of development and market availability of all immunoassays

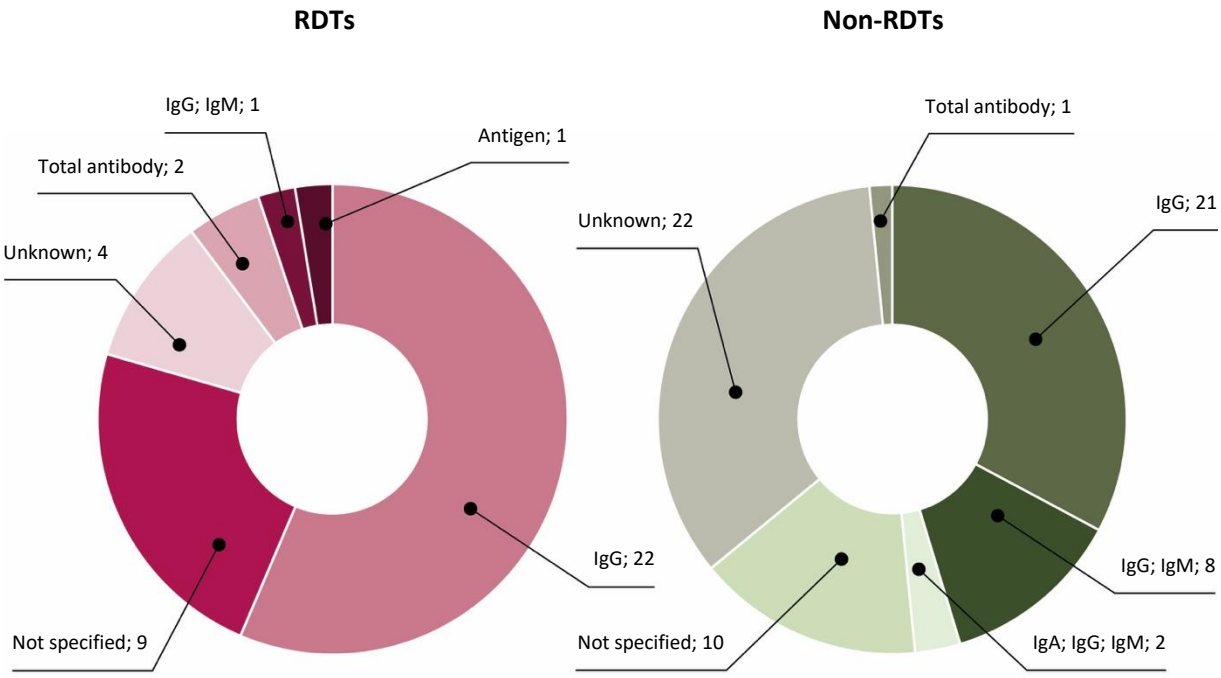


Assay target

The assay target was IgG antibodies for most of the immunoassays that specified this information (22 and 21 for RDTs and non-RDTs, respectively). Although eight non-RDT tests target IgG and IgM, only one RDT with the same target was developed (Chagas Antibody Test

Cassette by Artron Laboratories, test #14). Tests targeting IgG, IgM and IgA exist only among the non-RDT immunoassays (two tests), while three assays have total antibodies as assay targets (two RDTs and one non-RDT). Only one RDT that targets antigens was identified (CD-Rapid Test by Kephera Diagnostics, test 33; **Table 1, Annex**), and this was in development. The remaining tests did not specify this information, or the IFUs were not available (labelled as unknown) (**Figure 8**).

Figure 8. Overview of targets for all immunoassays



General discussion of clinical performance based on the systematic review

The clinical data reported by manufacturers on the IFUs were analyzed to compare the performance of the various immunoassays. This approach had some limitations, as the study design on which the IFUs were based may have differed in terms of sample type, study site (endemic versus non-endemic) and reference assays used. However, it was possible to obtain an overview of the performance of the tests. In general, non-RDTs perform better than RDTs,

based on the available IFU data. More than 50% of non-RDTs exhibit high performance (sensitivity and specificity both >98%) versus around 20% of the RDTs.

Several publications were identified that provide evidence to support the good clinical performance of the non-RDTs, including independent comparative evaluation studies that tested various assays in parallel and were performed using samples from CD-endemic countries. One independent report documented the operational characteristics and a retrospective comparative evaluation of 19 assays that were commercially available in 2004. This was performed by WHO, using 430 serum samples from 10 blood transfusion centres in Latin America, including in the four countries of interest, and characterized the assays using IIF, WB and RIPA as reference methods (WHO, 2004). The results revealed that most of the tests displayed a clinical sensitivity of >97% (95% confidence interval (CI) 91–100), except for three tests, two of which are no longer on the market, and one ELISA that had a sensitivity of 94.09% (95%CI 89.3–97.1). In terms of specificity, most of the assays showed values >95.8% (95%CI 92–100), except for three tests, two of which are no longer on the market and one IHA test that had a specificity of 78.62% (95%CI 77.2–83.4).

Similarly, in 2019, a retrospective comparative evaluation of seven commercially available immunoassays in Colombia, using 501 serum samples (>90% of which originated in the country) previously characterized using a reference method (an in-house ELISA, IIF, IHA or WB), was performed by the Colombian National Reference Laboratory (Caicedo Díaz et al., 2019). The authors showed that five assays exhibited a sensitivity of >98% (95%CI 96.16–100), while six assays showed a specificity of >97% (95%CI 91.68–100); they concluded that a total antigen ELISA paired with a recombinant assay provided similar performance to the previous diagnostic process (reference method). The assays were also capable of detecting different genetic lineages of *T. cruzi* (Caicedo Díaz et al., 2019).

Finally, five commercially available ELISAs, one in-house ELISA, and two IHAs were evaluated in two retrospective studies with samples from donors in Brazil (187 samples) and Panama (120 samples) and using WB with *T. cruzi* trypomastigote excreted–secreted antigens as the reference test. In the first study, the ELISA kits showed 100% sensitivity (95%CI 94.5–100), but the specificities ranged from 82.84% to 100% (95%CI 75–100) when leishmaniasis cases were included, and from 95.57% to 100% (95%CI 90–100) when leishmaniasis cases were excluded. In the second study, the assays showed a sensitivity of 75% to 100% (95%CI 50–100) and a specificity of 97.12% to 100% (95%CI 92–100) (Caballero et al., 2007).

A few LFAs have been validated in third-party clinical studies and the results published in peer-reviewed articles; this information is discussed in further detail below, in the section titled *Selection of RDTs*. Although LFAs exhibited lower performance compared with other immunoassays, especially in terms of sensitivity, these tests are of interest for implementation in regions with limited resources and in POC settings for the detection of chronic CD cases; we therefore focus on their analysis for the remainder of this report.

7.3 RDTs for detection of chronic CD

Antibody target analytes

The analyte target of the antibody was not specified for most of the RDTs or was claimed to be "recombinant *T. cruzi* antigens" or "specific recombinant antigens from epimastigote and trypomastigote stages of *T. cruzi*". However, we found the specific antibody target for five of the RDTs, as shown in **Table 3**, **Annex**. These included the biomarkers *T. cruzi* trypomastigote excretion–secretion antigens (TESAs), shed acute-phase antigens (SAPAs), recombinant antigens representing cytoplasmic and flagellar protein (TcF), lineage-specific epitopes of the trypomastigote small surface antigen (TSSA) (TSSApep specific for lineages TcII, TcV and TcVI), and others, such as peptides 30, 36, Kmp-11, peptide 1, B13; 1F8; H49/JL7, peptide 2, TcD and TcE.

Market availability and regulatory status of RDTs

An overview of the RDTs that are or were commercially available in Bolivia, Brazil, Colombia and Paraguay is shown in **Figure 9**. All 16 RDTs listed had received at least one regulatory approval and most had received a stringent regulatory approval (SRA) European Union (EU) CE marking (i.e. CE-IVD) or Brazil ANVISA. In total, 11 tests are currently commercialized in Bolivia (including 8 with SRA), 4 in Brazil (all with SRA), 6 in Colombia (5 with SRA) and 2 in Paraguay with SRA.

Figure 9. Overview of commercially available RDTs for Chagas disease in Bolivia, Brazil, Colombia and Paraguay

	Product name	Company name (country of headquarters)	Product with SRA[1]	RDT not sold anymore	Bolivia	Brazil	Colombia	Paraguay[3]
2	Onsite Chagas Ab Rapid test	CTK Biotech (United States)	CE-IVD, ANVISA		x	x	x	
5	Accu-Tell Chagas Cassette	AccuBiotech (China)	CE-IVD		x			
7	Chagas Ab cassette	Linear Chemicals S.L. (Spain)	CE-IVD	x			(x)	
8	Chagas AC Cassette	Xerion (Colombia)	-				x	
10	T. cruzi IgG Chagas Test cassette	Atlas Link Technology (China)	-		x			
13[2]	Chagas Rapid Test Cassette (S/P)	Hangzhou AllTest Biotech Co., Ltd (China)	CE-IVD		x			
13[2]	Chagas Rapid Test Cassette - S/P	Acro Biotech, Inc. (United States)	CE-IVD		x			
14	Chagas Antibody Test Cassette	Artron Laboratories (Canada)	CE-IVD				x	x[3]
15	Chagas Stat-Pak	Chembio Diagnostic Systems, Inc. (USA)	CE-IVD		x		x	
17	SD Bioline Chagas AB rapid test	Abbott (standard Diagnostic) (USA)	CE-IVD, ANVISA		x	x	x	x[4]
19	WL Check Chagas test	Wiener Lab (Argentina)	CE-IVD, ANVISA		x	x	x	
23	TR Chagas - Bio-Manguinhos	Bio-Manguinhos/Fiocruz (Brazil)	ANVISA			x		
24	Chagas Rapido First Response	Lemos (Argentina)	-		x			
26[2]	Chagas Rapid Test Cassette (WB/S/P)	Hangzhou AllTest Biotech Co., Ltd (China)	CE-IVD		x			
26[2]	Chagas Rapid Test Cassette - WB/S/P	Acro Biotech, Inc. (United States)	CE-IVD		x			
27	Chagas Ab Combo Rapid Test	Zhuhai Encode Medical Engineering Co., Ltd (China)	-	x	(x)		(x)	
34	Chagas Quick Test	Cypress Diagnostic (Belgium)	CE-IVD	x	(x)			
35	Hexagon Chagas	Human Diagnostics (Germany)	-	x			(x)	
			Number of RDTs currently available in each country		**11**	**4**	**6**	**2**

The test number corresponds to the one in **Table 1**.

[1] SRA = stringent regulatory approval
[2] Indicate duplicate tests, i.e. the same product reference number commercialized under different brand companies.
[3] For Paraguay, the import license certificate was requested as a proof of marketing approval in the country
[4] Chagas Stat-Pak from Chembio Diagnostic is potentially marketed in Paraguay, but the import license certificate could not be obtained as proof
(x) indicates that RDTs were sold in these countries previously

Analysis of RDT performance

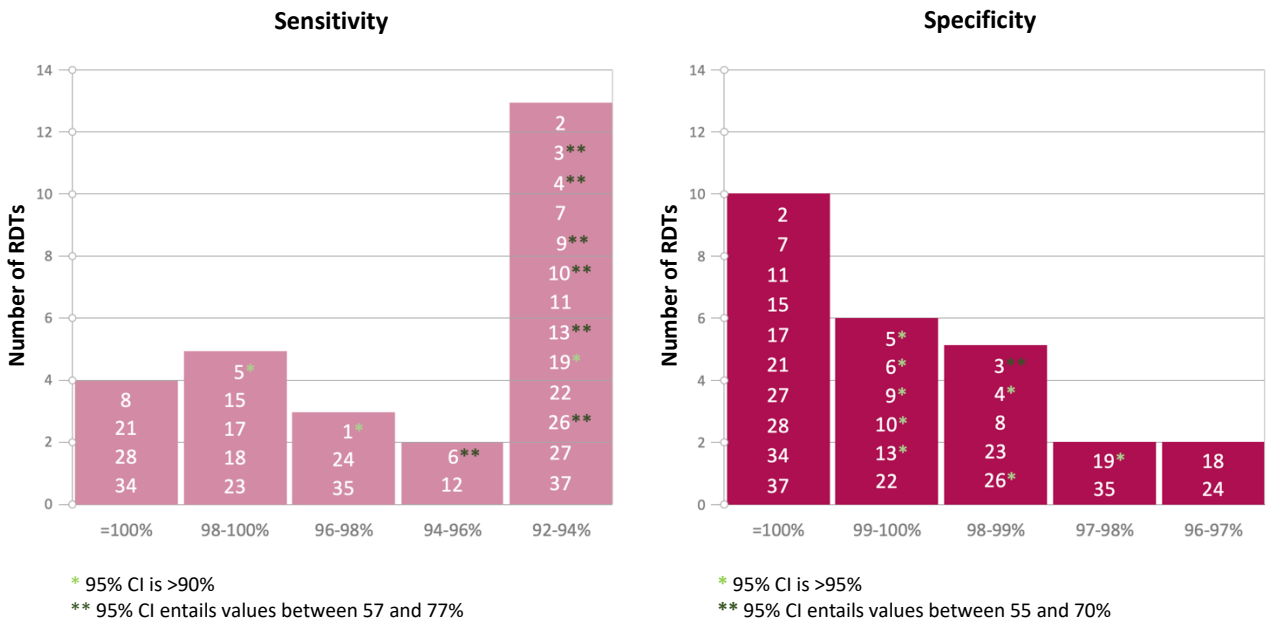
Comparing the performance of RDTs is difficult, as the information for each test varies. Ideally, high-performing tests exhibit excellent performance (>98% sensitivity and >98% specificity) and have no cross-reactivity against co-endemic parasites, such as *Leishmania* spp. and *Plasmodium* spp., and the non-pathogenic *Trypanosoma rangeli*. Additionally, RDT performance data for pregnant women and infants (especially children aged more than 10 months) should be provided, as these subpopulations are of special interest for testing for chronic CD. Available data for these three criteria are presented below.

Clinical data declared by manufacturers on their Instructions for Use

Figure 10 shows the clinical performance of the RDTs in terms of their sensitivity and specificity as claimed by the manufacturers in the IFUs provided. Out of the 39 RDTs, 27 provided sensitivity (just 10 reported confidence intervals (CI)) and 26 included specificity data (just 10 reported CI). In general, most RDTs exhibited an acceptable performance, with clinical sensitivity >92% and specificity >98%. The RDTs can be categorized into three groups according to their performance (for additional test details, including manufacturer names, please refer to the relevant test number in **Table 1, Annex**):

1. **High performance** (sensitivity >98% and specificity >98%): this group comprised eight RDTs, including Accu-Tell Chagas Cassette by AccuBiotech (test #5), Chagas AC Cassette by Xerion (test #8), Chagas Stat-Pak by Chembio Diagnostic Systems Inc (test #15), SD Bioline Chagas Ab Rapid test by Abbott (test #17), Trypanosoma Detect™ Rapid Test by InBios International Inc. (test #21), TR Chagas Bio-Manguinhos by Bio-Manguinhos/Fiocruz (test #23), Chagas Antibody Test Card by LumiQuick Diagnostics (test #28), and Chagas Quick Test by Cypress Diagnostic (test #34). Of note, 95%CI data was only provided for the Accu-Tell Chagas Cassette by AccuBiotech (test #5).
2. **Acceptable performance with high sensitivity** (sensitivity >98% and specificity 96–98%): Simple Chagas/Stick Chagas by Operon (test #18).
3. **Acceptable performance with high specificity** (sensitivity 92–98% and specificity >98%): this group comprised 13 RDTs: Onsite Chagas Ab Rapid test by CTK Biotech (test #2), Chagas Rapid Test Cassette by Certum® Diagnostics (test #3), TruQuick™ Chagas 40T by Meridian Bioscience (test #4), Rapid Response Chagas Antibody Test Cassette by BTNX Inc. (test #6), Chagas Ab cassette by Linear Chemicals S.L. (test #7), Amunet prueba rapida Chagas by Amunet (test #9), T. cruzi IgG Chagas Test cassette by Atlas Link Technology (test #10), One-Step Chagas Ab Rapid Test by Span Biotech (test #11), Chagas Rapid Test Cassette (S/P) by Hangzhou AllTest Biotech CO., LTD. (test #13), Simple Chagas WB test by Operon (test #22), Chagas Rapid Test Cassette - WB/S/P by Acro Biotech, Inc. and others (test #26, see **Table 1**), Chagas Ab Combo Rapid Test by Zhuhai Encode Medical Engineering Co., Ltd. (test #27), and NADAL® Chagas IgG, test cassette by nal von minden GmbH (test #37).

Figure 10. Clinical performance of RDTs for Chagas disease



The numbers inside the columns correspond to the numbers in **Table 1**; absence of asterisks means that the 95% CI was not reported.

IFU performance	Sensitivity	Specificity	
High	>98%	>98%	8 RDTs (5, 8, 15, 17, 21, 28, 23, 34)
Acceptable	>98%	96%–98%	1 RDT (18)
Acceptable	92%–98%	>98%	13 RDTs (2, 3, 4, 6,7, 9, 10, 11, 13, 22, 26, 27, 37)

Independent studies

The tests (see **Table 1, Annex**) with more publicly available data were Chagas Detect™ Plus Rapid Test by InBios International Inc (test #1) and Chagas Stat-Pak by Chembio Diagnostic Systems (test #15), with 12 and 11 publications, respectively. Both tests were included in a considerable number of studies performed in the countries of interest. Other tests, such as SD Bioline Chagas Ab Rapid test by Abbott (test #17), Simple Chagas by Operon (test #18), WL Check Chagas test by Weiner Lab (test #19), and Trypanosoma Detect™ Rapid Test by InBios International Inc (test #21) each had five studies published, at least one of which had been performed in one of the countries of interest. The remaining tests had between zero and five publications (**Table 4, Annex**). For the tests of most interest, the clinical data extracted from

the publications are discussed below (see "*Selection of RDTs according to regulatory status, clinical performance and availability in the countries of interest*").

Cross-reactivity

Due to the diverse and nonspecific manifestations of CD, it is frequently misdiagnosed. Indeed, the co-endemicity of *Leishmania* spp. and *T. cruzi* parasites is one of the factors that could potentially affect the performance of CD antibody-detection tests, as they are caused by related kinetoplastid protozoan pathogens. Therefore, providing evidence in relation to cross-reactivity is of great importance when assessing the ability of RDTs for CD to distinguish between leishmaniasis and CD and to ensure appropriate case management. We found that just five RDTs had been tested for cross-reactivity with samples from *Leishmania*-infected individuals; these were Chagas Stat-Pak by Chembio Diagnostic Systems Inc (test #15), Simple Chagas/Stick Chagas by Operon (test #18), WL Check Chagas test by Wiener Lab (test #19), WL Check Chagas test by Lemos (test #24) and Chagas Ab Rapid test by Creative Diagnostics (test #30). Possible cross-reactivity with *Leishmania* in *Leishmania*-infected individuals was only reported for the WL Check Chagas test by Lemos (test #24). Three tests were investigated for possible malaria cross-reactivity with samples from malaria-infected individuals; these were Chagas Detect™ Plus Rapid Test by InBios International Inc (test #1), Simple Chagas/Stick Chagas by Operon (test #18) and Chagas Ab Rapid test by Creative Diagnostics (test #30). Cross-reactivity with malaria was reported for the second and third of these tests.

Performance in subpopulations of interest (pregnant women and infants aged >10 months)

The subpopulations pregnant women and infants (especially children aged more than 10 months) are of special interest when testing for chronic CD. RDTs could enable the implementation of a test-and-treat strategy targeted at these populations during pre- and post-natal care. However, most of the studies that validated the performance of RDTs for CD only used samples from adult populations. We found that just three tests had been evaluated in pregnant women: Chagas Detect™ Plus Rapid Test and Trypanosoma Detect™ Rapid Test from InBios International Inc (tests #1 and #21) and Chagas Sero K-SeT rapid diagnostic test by Coris Bioconcept (test #29). For Chagas Detect™ Plus Rapid Test by InBios International Inc, four studies included samples from pregnant women (one reported in the IFUs and three in third-party studies). As for infants, only Trypanosoma Detect™ Rapid Test by InBios International Inc. (test #21; however, the manufacturer has just announced that the product has been discontinued from May 2022) and Chagas Sero K-SeT rapid diagnostic test by Coris Bioconcept (test #29) had been evaluated in neonates and infants (in two third-party studies). However, these tests are not available on the Latin American market, which highlights the need to have the RDTs that are available in the target countries validated among these subpopulations.

Performance of tests in different endemic countries (discrete typing units, DTUs)

T. cruzi comprises seven discrete typing units (DTUs). Some DTUs are more common than others in human infections in different endemic countries, and there is a theoretical distribution of the DTUs, as shown in Figure 2. The DTUs corresponding to the antigens used in commercially available tests may not correspond with those found in specific endemic countries, potentially impacting test performance. Moreover, immune responses vary geographically, which could impact the ability of a test to detect *T. cruzi* antibodies. FIND is currently developing a new RDT with DCN Dx (USA), which theoretically will be more accurate than commercially available RDTs across all endemic regions (i.e. with improved detection of various DTUs). Studies will need to be conducted in multiple countries to evaluate the performance of LFAs and their utility for case management in different epidemiological settings, to take into account the diversity of DTUs.

Selection of RDTs

Selection of RDTs according to regulatory status, clinical performance and availability in the countries of interest

A tentative selection of the most promising RDTs was made based on the following three criteria: (i) regulatory approval and market availability status, (ii) clinical performance (comparing data from IFUs and some independent studies), and (iii) commercialization in the four countries of interest (**Figure 11** and **Table 4, Annex**). In total, 17 RDTs were identified as being available on the market and having received stringent regulatory approval (FDA clearance, CE-mark, China NMPA or Brazil ANVISA). Among them, four RDTs showed "high performance", with reported sensitivity and specificity >98% in the IFUs or at least two independent studies. Only Chagas Stat-Pak by Chembio Diagnostic Systems Inc (test #15) exhibited a high performance in independent studies (Angheben et al., 2019; Egüez et al., 2017; Lozano et al., 2019; Suescún-Carrero et al., 2021). All four of these high-performance tests are currently on the market in at least one of the countries of interest (Brazil, Bolivia, Colombia or Paraguay). The potentially most interesting RDTs (RDTs in group 1 in **Figure 11**) were therefore:

- Accu-Tell Chagas Cassette by AccuBiotech (test #5)
- Chagas Stat-Pak by Chembio Diagnostic Systems Inc (test #15)
- SD Bioline Chagas Ab Rapid test by Abbott (test #17)
- TR Chagas Bio-Manguinhos by Bio-Manguinhos-Fiocruz (test #23)

Of these RDTs, only Chagas Stat-Pak by Chembio Diagnostic Systems Inc (test #15) had data showing it was not cross-reactive in *Leishmania*-infected individuals. The IFU of TR Chagas Bio-Manguinhos by Bio-Manguinhos-Fiocruz (test #23) declared that there was 4% cross-reactivity but did not mention against which pathogens this was tested. The other two RDTs

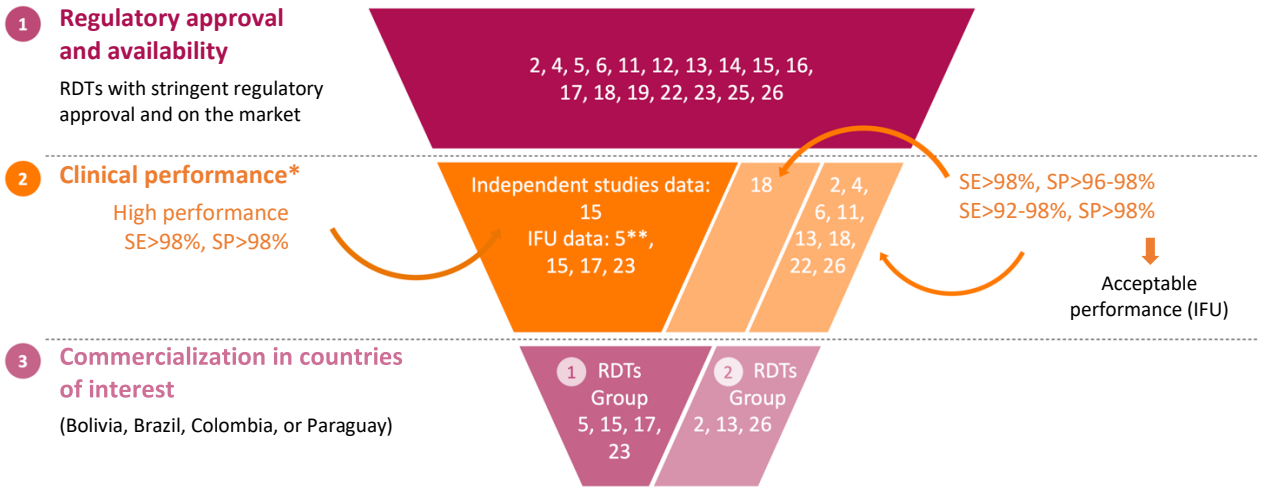
had no cross-reactivity data, and none of the four RDTs had any data relating to cross-reactivity in malaria-infected individuals.

Although a total of nine other RDTs displayed acceptable performance (sensitivity >98% and specificity 96–98%, or sensitivity 92–98% and specificity >98%), only three were currently available on the market in at least one of the four countries of interest and fell into the "RDTs group 2" category (**Figure 11**); these were:

- Onesite Chagas Ab Rapid test by CTK Biotech (test #2)
- Chagas Rapid Test Cassette - S/P by Acro Biotech Inc and Hangzhou AllTest Biotech CO., LTD (test #13)
- Chagas Rapid Test Cassette - WB/S/P, by Acro Biotech Inc and others (test#26, see **Table 1**)

No cross-reactivity data for *Leishmania* spp. or malaria were available for any of these RDTs.

Figure 11. Identification of the most promising RDTs for CD according to regulatory approval, clinical performance, and commercialization status



RDTs fitting all criteria (including high performance)

5 - Accu-Tell Chagas Cassette by AccuBiotech (China)
15 - Chagas Stat-Pak by Chembio Diagnostic Systems, Inc. (USA)
17 - SD Bioline Chagas AB rapid test by Abbott (US)
23 - TR Chagas - Bio-Manguinhos by Bio-Manguinhos/Fiocruz (Brazil)

The test numbers correspond to the ones in **Table 1.**

Of note, 8-Chagas AC Cassette by Xerion (Colombia) fit all criteria except approval, it has Colombia INVIMA
*SE=sensitivity, SP=specificity. RDTs 14, 16, 25 are potentially interesting; however, clinical data were not accessible
**RDT showing data on 95% CI >90%

Clinical performance data from the literature were reviewed in detail for the potentially most interesting products (RDT groups 1 and 2, **Figure 11**). Of particular interest were studies in which several RDTs were evaluated in parallel. The main findings of this review were as follows:

1. The high performance of Chagas Stat-Pak by Chembio Diagnostic Systems Inc (test #15) based on its IFU data was well supported by two independent clinical studies and one meta-analysis. Both clinical studies compared the performance of this RDT with local algorithms for CD diagnosis and were prospective studies conducted in Bolivia (342 patients) and Colombia (305 patients) (Egüez et al., 2017; Suescún-Carrero et al., 2021). Both studies showed excellent results for the test, with sensitivities of 100% (95%CI 99.8–100% and 95.9–100%) and specificities of 99.3% (95%CI 99.3–99.8%) and 100% (95%CI 98.3–100%) in Bolivia and Colombia, respectively. Another publication confirmed that this RDT could be used as screening tool even if used as a stand-alone test, due to the high number of individuals tested in endemic areas (Angheben et al., 2019). This meta-analysis compared more than 4574 records of performance data individually evaluated from endemic and non-endemic countries and found that the test displayed an overall sensitivity of 97% (95%CI 87.6–99.3%) and specificity of 99.4% (95%CI 98.6–99.8%).
2. The high performance of the SD Bioline Chagas Ab Rapid test by Abbott (test #17) was also supported by evidence from the literature. First, one study compared the performance of this RDT with local algorithms for CD diagnosis in a prospective study in Argentina, based on more than 600 whole-blood samples. The RDT displayed a sensitivity of 97.2% (95%CI 93.5–100%) and specificity of 99.7% (95%CI 96.2–99.2%) (Lopez-Albizu et al., 2020). Second, a retrospective study compared 10 other RDTs in parallel, using very challenging samples (medium or low antibody loads) from endemic countries. The performance of the RDT was lower than that provided by the manufacturer but still acceptable, given the difficult samples tested (sensitivity and specificity >90%, 95%CI not reported) (Sánchez-Camargo et al., 2014).
3. The Accu-Tell Chagas Cassette by AccuBiotech (test #5) was only identified in one performance evaluation study, which was executed at the National Reference Laboratory for CD diagnosis in Bolivia. Its performance was low in terms of specificity, at 84.6%, but it had a sensitivity of 100%. This study compared the performance of this RDT with local algorithms for CD diagnosis, using 44 stored samples (*Technical Report from NHI in Bolivia – INLASA – Informe Tecnico MS/INLASA/IUD/IT/47/2018*).
4. The Onesite Chagas Ab Rapid test by CTK Biotech (test #2) showed acceptable performance in an independent evaluation, involving a retrospective study using samples from endemic countries (sensitivity and specificity >90%, 95%CI not reported), but lower than that declared by the manufacturer (Sánchez-Camargo et al., 2014).
5. No independent studies were identified for one of the tests with high performance, declared on the IFU, TR Chagas – Bio-Manguinhos by Bio-Manguinhos/Fiocruz (test

#23), or for the other two potentially interesting RDTs with moderate performance: Chagas Rapid Test Cassette - S/P by Acro Biotech Inc and Hangzhou AllTest Biotech CO., LTD (test #13) and Chagas Rapid Test Cassette - WB/S/Pby Acro Biotech Inc and others (test #26, **Table 1**).

RDTs manufactured in Latin America but not previously selected (based on **Figure 11** criteria):

- Chagas AX Cassette by Xerion (test #8), manufactured in Colombia
- Chagas Rapido First Response by Lemos (test #24), manufactured in Argentina
- WL Check Chagas test by Wiener Lab (test #19), manufactured in Argentina, with local distributors in Bolivia and Argentina

Of these locally manufactured RDTs, only WL Check Chagas test by Wiener Lab (test #19) had received stringent regulatory approval (CE-mark and Brazil ANVISA). This RDT was not previously selected due to the strict criteria we applied for the clinical data declared in the IFU (**Figure 11**), but its performance was close to the acceptable criteria, with a sensitivity of 93.8% (95%CI 91.1–96.6%), specificity of 97.89% (95%CI 97.1–98.7%), and negative cross-reactivity with *Leishmania*. Its performance has also been validated in two independent studies. The first was a prospective study performed in Argentina using more than 600 whole-blood samples, comparing the performance of the test against the local algorithm for CD diagnosis, where the test displayed a sensitivity of 93.4% (95%CI 88.2–98.6%) and specificity of 99.1% (95%CI 98.1–100%) (Lopez-Albizu et al., 2020). The second was a retrospective validation study comparing 10 different RDTs in parallel, using very challenging samples (medium or low antibody loads) from endemic countries. The test sensitivity and specificity were 88.7% and 97%, respectively (95%CI not available) (Sánchez-Camargo et al., 2014).

The other RDTs produced locally, Chagas AC Cassette by Xerion (test #8) and Chagas Rapido First Response by Lemos (test #24), had not received stringent regulatory approval, as the former was registered only in Colombia and the latter was registered only in Bolivia and Argentina. According to the IFU data, Chagas Rapido First Response by Lemos (test #24) may potentially cross-react with *Leishmania* but exhibited good performance in a prospective study of almost 600 samples from patients in Argentina, showing a sensitivity and specificity 96.4% and 96%, respectively (95%CI not reported). This high performance was also supported by a third-party retrospective study, where the test was found to have a sensitivity of 99.5% (95%CI 95.3–99.7%) and specificity of 96.2% (95%CI 94.3–99.3%), compared with composite local reference tests (Barfield et al., 2011).

We also included as a test of interest the RDT WL Check Chagas test by Wiener Lab (test #19) (performance close to acceptable).

Additional considerations for RDT selection: Healthcare level of use

A further aspect that could be key to the final selection of RDTs is the ease of use of a test, its related healthcare level of use, and targeted user profile(s) (see **Annex**, **Table 1** for the list of RDTs and **Table 2** for healthcare level definitions). Of note, among the tentatively selected RDTs shown in **Figure 11** (Groups 1 and 2), only the SD Bioline Chagas Ab Rapid test by Abbott (test #17) and Chagas Rapid Test Cassette - S/P by Acro Biotech Inc (test #13) could not be used with a finger-prick blood sample. The Chagas Rapid Test Cassette - S/P by Acro Biotech Inc (test #13) requires a primary healthcare facility (L1, healthcare level 1), as serum or plasma must be extracted from whole blood prior to the use of the RDT. The SD Bioline Chagas Ab Rapid test by Abbott (test #17) can only be used in community facilities that can process a 100-µL whole-blood sample and was therefore classified as healthcare level L0/L1. All of the other tentatively selected RDTs, including the TR Chagas Bio-Manguinhos by Bio-Manguinhos/Fiocruz (test #23) and WL Check Chagas test by Wiener Lab (test #19), can be used at the community level (L0, healthcare level 0) by a lay person.

7.4 Innovative technologies

A few interesting new technologies that could help in the diagnosis of CD were identified during our research. Three of them are currently in development and were briefly mentioned above; some more details are given below.

Molecular technologies

One of the promising assays in development is a LAMP prototype from Eiken Chemical Co. Ltd. LAMP is a method for nucleic acid amplification that does not require an expensive thermocycler instrument (Besuschio et al., 2017). This molecular POC assay is currently being tested in Argentina, Bolivia and Paraguay to validate its implementation for the control of CCD. This study will also evaluate the platform used to obtain the purified DNA required for the assay (funded by the Global Health Innovative Technology Fund (GHIT), 2021–2023).

Recombinase polymerase amplification (RPA) tests are described in the literature as useful for the detection of parasites such as *Leishmania* spp. (Cossio et al., 2021). As with LAMP assays, RPA tests enable sensitive, specific and rapid amplification of DNA under isothermal conditions and are potentially applicable as POC tests (Castellanos-Gonzalez et al., 2018; Rivero et al., 2017). One prototype of an RPA test, coupled with a lateral flow test (LF-RPA), was able to identify *T. cruzi* infection in dogs (Jimenez-Coello et al., 2018) and COVID-19 infection in humans (technology acquired by Abbott).

Imaging technologies

Researchers at the University of California, Los Angeles have developed an artificial intelligence-based device that can detect moving *T. cruzi* parasites in whole blood samples.

This label-free technology is based on a deep-learning algorithm that analyses the motility of parasites; it does not require any sample processing or refrigeration. The device could potentially be very useful in low-resource settings due to its high sensitivity (detection limit of 10 parasites per mL of blood), portability and ease of use. The current state of this technology does not allow it to automatically differentiate various parasites (Zhang et al., 2018); however, deep learning-based methods are beginning to be used in research to identify parasites in blood smears. The aim is to improve their detection sensitivity based on microscopy images (Jung, 2021; Pereira et al., 2020).

Antigen-based technologies

A lateral-flow RDT based on antibodies that bind to *T. cruzi* antigens in the blood of infected individuals is currently under development. The presence of these targeted antigens is indicative of an active infection, while successful treatment is anticipated to result in their diminution or disappearance. Kephera Diagnostics is developing this assay, with the aim of providing a method to monitor the treatment of CD (CD-Rapid Test, test #33).

7.5 General information on manufacturers of tests for CD

We identified a total of 95 different manufacturers of diagnostic tools for CD. Just two manufacturers produced more than one RDT for CD: InBios International Inc (USA), with Chagas Detect™ Plus Rapid Test and Trypanosoma Detect™ Rapid Test (tests 1 and 21, Table 1); and Operon (Spain), with Simple Chagas/Stick Chagas and Simple Chagas WB test (tests 18 and 22, Table 1). In both cases, the RDTs differed in the type of platform used (strip versus cassette), with each platform requiring a different sample type. However, InBios International Inc has announced that the two products (test #1, test #21) were discontinued in May 2022. Operon does not market their products in Latin America.

Regarding local manufacturers in the Latin American region, we found a total of 19 manufacturers with headquarters in Argentina (3), Brazil (7), Chile (1), Colombia (1), Cuba (1), Mexico (3), Paraguay (1) and Uruguay (1). Lemos and Wiener Lab, in Argentina, produce and procure both RDT and non-RDT immunoassays. Wiener Lab not only provides serological tests but is also developing molecular tests. Lemos provides tests directly from their headquarters in two countries of interest (Bolivia and Colombia), whereas Wiener Lab uses local distributors in three countries of interest (Bolivia, Brazil and Colombia). Bio-Manguinhos/Fiocruz, from Brazil, produce and procure an RDT in Brazil. These three companies have previously engaged with FIND for test performance evaluation studies.

8. Discussion

8.1 Competitive analysis of CD diagnostic tests identified

Diagnosing chronic CD in Latin America is complex, as it currently requires at least two laboratory-based tests such as ELISA, IHA or IFA. Despite their potential for improving test-and-treat strategies, RDTs for CD have not been widely implemented in public health systems in endemic regions of Latin America.

This report aims to help health sector stakeholders select appropriate RDTs that are commercially available in endemic regions, especially in the four countries of interest (Bolivia, Brazil, Colombia and Paraguay). In total, there are 39 different RDTs for CD. The majority are LFAs that target antibodies against *T. cruzi*, with 68% of the RDTs suitable for use at the community level (requiring just one drop of whole blood). Most of these RDTs provide clinical evidence in their IFUs, and some of them are supported by evidence obtained in independent third-party studies, showing that they could be sufficiently accurate to be recommended for CD diagnosis in endemic areas. The use of RDTs in resource-limited settings has the potential to revolutionize the diagnosis of chronic CD, although new algorithms that combine several RDTs remain to be validated (Angheben et al., 2019; Barfield et al., 2011; Egüez et al., 2017; Lopez-Albizu et al., 2020; Lozano et al., 2019; Sánchez-Camargo et al., 2014; Suescún-Carrero et al., 2021).

We selected those RDTs with the greatest potential, based on stringent regulatory approval status, market availability, clinical performance and commercialization in the four countries of interest. Ideally, other criteria should also be considered when comparing the various RDTs, such as cross-reactivity and clinical evaluations conducted in relevant subpopulations, i.e. pregnant women and infants. However, such data were lacking for most of the RDTs. In summary, none of the RDTs fulfills all of the criteria in addition to possessing a short procurement timeline, being low in cost, and being easy to use at the community level. However, we identified eight promising LFAs for the diagnosis of chronic CD in the four countries of interest and classified these RDTs into three categories:

1. Four RDTs with high performance (sensitivity and specificity >98%), stringent regulatory approval and commercialized in the countries of interest: TR Chagas Bio-Manguinhos by Bio-Manguinhos/Fiocruz, Accu-Tell Chagas Cassette by AccuBiotech, SD Bioline Chagas Ab Rapid test by Abbott, and Chagas Stat-Pak by Chembio Diagnostic Systems Inc. However, neither TR Chagas Bio-Manguinhos by Bio-Manguinhos/Fiocruz or Accu-Tell Chagas Cassette by AccuBiotech have been validated in any independent studies. SD Bioline Chagas Ab Rapid test by Abbott can only be used in community facilities that can process samples of 100 µL of whole blood. Chagas Stat-Pak by Chembio Diagnostic Systems Inc is the only test that exhibits no cross-reactivity against *Leishmania.*

2. Three RDTs with acceptable performance (sensitivity >98% and specificity 96% to 98% or sensitivity 92% to 98% and specificity >98%), stringent regulatory approval and commercialized in at least one of the four countries of interest: Onesite Chagas Ab Rapid test by CTK Biotech; Chagas Rapid Test Cassette - S/P by Acro Biotech Inc and Hangzhou AllTest Biotech Co., Ltd; and Chagas Rapid Test Cassette - WB/S/P by Acro Biotech and others. Only Onesite Chagas Ab Rapid test by CTK Biotech has been investigated in an independent study and showed moderate/acceptable performance (sensitivity and specificity >90%) with samples from endemic countries. Except for Chagas Rapid Test Cassette - S/P by Acro Biotech Inc, which requires a serum or a plasma sample, the other RDTs only require finger-prick blood samples and are appropriate for use at the community level.
3. Another RDT, WL Check Chagas test by Wiener Lab, did not meet the selection criteria due to a slightly low performance; however, it has a close to acceptable performance (93.9% sensitivity and 97.9% specificity). In addition, it shows no cross-reactivity with *Leishmania* and can be used at the community level.

8.2 Challenges for diagnosing patients at risk of CD in Latin America

There are several challenges facing the diagnosis of chronic CD in endemic countries, including (i) access to healthcare in remote areas within each country; (ii) limited awareness and knowledge of physicians in some countries, because CD has been mainly associated with poverty and sylvatic regions due to the presence of vectors; thus, CD is not considered a risk in larger municipalities or cities, where migration of people from rural areas has been increasing in recent years and where congenital transmission is the main mode of transmission; and (iii) limited access to laboratories able to perform confirmatory tests. Although the inclusion of RDTs in diagnostic algorithms for chronic CD in LMICs could help reduce the diagnostic gap in areas with limited resources and limited access, it will not solve all these issues (Olivera 2018).

One of the largest barriers to controlling CD is the lack of prompt diagnosis and treatment of neonates born to CD-infected women. Serological tests cannot be used to diagnose *T. cruzi* infection in neonates, as maternal antibodies can produce false-positive results (Abras et al., 2017; Rodriguez et al., 2005). While algorithms for the diagnosis of CD in children vary in the four countries of interest, in general, children born to infected mothers are tested using parasitological methods (mainly microscopy) shortly after birth and again at 8 to 12 months using serological methods. Children who test positive by parasitological and/or serological methods are considered to be positive for CCD and are treated. However, this approach also delays prompt access to treatment due to the low sensitivity of microscopic methods and the loss to follow-up of children aged 8 to 12 months (Bern et al., 2009; Bua et al., 2013); thus, considerable numbers of CCD cases are missed in endemic countries (Picado et al., 2018).

Despite their availability and the recommendations in some of the guidelines produced in the countries of interest (e.g. Colombia and Paraguay) (DGVS Paraguay, 2015; Instituto

Nacional De Salud – INS. Colombia, 2017), molecular tests are rarely used to diagnose CCD. Although molecular tools outperform the current diagnostic algorithm used for CCD (Diez et al., 2008; Messenger et al., 2017; Montes-Rincón et al., 2016; Mora et al., 2005; Virreira et al., 2003), only Chile among endemic countries routinely uses PCR as part of its national CD diagnostic strategy. However, this has been shown to be a highly cost-effective approach (Gobierno de Chile, 2014). In addition, the first prospective evaluation of a standardized qPCR method in Argentina was recently published (Benatar et al., 2021). This study showed that the qPCR *T. cruzi* DNA test by Wiener Lab achieves high sensitivity compared with the current parasitological tests for detecting CCD within the first few months of age. Based on these results, the algorithm used for diagnosis of CCD in Argentina is currently under revision. In contrast, in some non-endemic, high-income countries, such as Spain and Switzerland, PCR forms part of the diagnostic algorithm for CCD (Conselleria de sanitat – Comunitat Valenciana., 2009; Jackson et al., 2009). We identified PCR kits for the detection of *T. cruzi* that are commercially available, such as RealCycler CHAG (Progenie, Spain) and TCRUZIDNA.CE (Diagnostics Bioprobes Srl, Italy). However, their implementation in endemic regions remains limited due to factors such as a lack of clinical evidence, a lack of standardization, complexity, high costs and the need for cold-chain transport.

Promising technologies that could overcome some of these hurdles, such as the Loopamp *Trypanosoma cruzi* Detection Kit being developed by Eiken Chemical Co. Ltd, or an RPA method, are currently under development (Besuschio et al., 2017; Castellanos-Gonzalez et al., 2018; Rivero et al., 2017). Some interesting new parasitological methods that could improve the sensitivity of parasite detection using a microscope, and which have recently been shown to be cost-effective, rapid screening tools suitable for implementation in LMICs, were also found in the literature (Zhang et al., 2018).

8.3 Challenges for manufacturers

We identified several challenges faced by manufacturers. At the market level, the availability and affordability of priority diagnostics in Latin America should be improved. The manufacturers were in general willing to discuss these issues and shared the relevant information requested. From the manufacturers' perspective, several issues hinder availability: (i) the extremely low price of CD tests in some countries in Latin America, (ii) recent changes in stringent regulatory approval processes that are complex and therefore costly (such as obtaining a CE-mark), (iii) a preference for the manufacture of serological tests for COVID-19 rather than RDTs for CD, as the demand is currently higher and the market more favourable, and (iv) the COVID-19 pandemic has also had an impact on global logistics and supplies, with subsequent overbooking of the freight market and delayed delivery of products as transportation fees have skyrocketed.

Interestingly, we found a manufacturer from Brazil, Bio-Manguinhos/Fiocruz, that produces and procures a CD RDT, and two Argentinian manufacturers, Lemos and Wiener Lab, that produce and procure both CD RDTs and other technologies. Bio-Manguinhos/Fiocruz and

Wiener Lab hold stringent regulatory approval, ANVISA and CE-mark, respectively, for their products.

9. Conclusion

The use of RDTs could revolutionize the diagnosis of chronic CD in resource-limited settings, especially if they are included as part of an algorithm that recommends using a combination of several tests. In this landscape analysis, a total of 39 different RDTs for CD were identified, the majority of which are serological LFAs that target antibodies against *T. cruzi*. Of these tests, 11 are currently marketed in Bolivia, 4 in Brazil, 6 in Colombia and 2 in Paraguay. In this report, we have made a tentative selection of the eight most promising RDTs based on several criteria, including stringent regulatory approval, clinical performance, and market availability in the four countries of interest.

Innovative tests are also in development for the diagnosis of congenital CD. Those at the most advanced stage are POC tests, e.g. LAMP and RPA, which greatly reduce the complexity and cost of molecular diagnostics. Deep learning-based methods are also being developed to improve the sensitivity of parasitological tests based on blood-smear images.

This analysis has highlighted the lack of evidence relating to the performance of new diagnostic products across several CD-endemic regions, a circumstance that inhibits the implementation of such products. Additionally, many factors can potentially affect a test's performance, including the genetic variability of *T. cruzi*, co-endemicity of other parasites such as *Leishmania*, and different prevalence levels between rural and urban areas. Therefore, we call attention to the need to create a sample repository representing the different epidemiological and clinical scenarios in the countries of interest, which can then be used to test new tools that are currently in development.

10. References

Abras, A., Gállego, M., Muñoz, C., Juiz, N. A., Ramírez, J. C., Cura, C. I., Tebar, S., Fernández-Arévalo, A., Pinazo, M.-J., de la Torre, L., Posada, E., Navarro, F., Espinal, P., Ballart, C., Portús, M., Gascón, J., & Schijman, A. G. (2017). Identification of Trypanosoma cruzi Discrete Typing Units (DTUs) in Latin-American migrants in Barcelona (Spain). *Parasitology International*, *66*(2), 83–88. https://doi.org/10.1016/j.parint.2016.12.003

Angheben, A., Buonfrate, D., Cruciani, M., Jackson, Y., Alonso-Padilla, J., Gascon, J., Gobbi, F., Giorli, G., Anselmi, M., & Bisoffi, Z. (2019). Rapid immunochromatographic tests for the diagnosis of chronic chagas disease in at-risk populations: A systematic review and meta-analysis. *PLoS Neglected Tropical Diseases*, *13*(5). https://doi.org/10.1371/journal.pntd.0007271

Barfield, C. A., Barney, R. S., Crudder, C. H., Wilmoth, J. L., Stevens, D. S., Mora-Garcia, S., Yanovsky, M. J., Weigl, B. H., & Yanovsky, J. (2011). A highly sensitive rapid diagnostic test for Chagas disease that utilizes a recombinant Trypanosoma cruzi antigen. *IEEE Transactions on Bio-Medical Engineering*, *58*(3), 814–817. https://doi.org/10.1109/TBME.2010.2087334

Barnabé, C., De Meeûs, T., Noireau, F., Bosseno, M.-F., Monje, E. M., Renaud, F., & Brenière, S. F. (2011). Trypanosoma cruzi discrete typing units (DTUs): microsatellite loci and population genetics of DTUs TcV and TcI in Bolivia and Peru. *Infection, Genetics and Evolution : Journal of Molecular Epidemiology and Evolutionary Genetics in Infectious Diseases*, *11*(7), 1752–1760. https://doi.org/10.1016/j.meegid.2011.07.011

Basile, L., Jansa, J. M., Carlier, Y., Salamanca, D. D., Angheben, A., Bartoloni, A., Seixas, J., Van Gool, T., Canavate, C., Flores-Chavez, M., Jackson, Y., Chiodini, P. L., & Albajar-Vinas, P. (2011). Chagas disease in European countries: the challenge of a surveillance system. *Euro Surveillance : Bulletin Europeen Sur Les Maladies Transmissibles = European Communicable Disease Bulletin*, *16*(37).

Benatar, A. F., Danesi, E., Besuschio, S. A., Bortolotti, S., Cafferata, M. L., Ramirez, J. C., Albizu, C. L., Scollo, K., Baleani, M., Lara, L., Agolti, G., Seu, S., Adamo, E., Lucero, R. H., Irazu, L., Rodriguez, M., Poeylaut-Palena, A., Longhi, S. A., Esteva, M., … Schijman, A. G. (2021). Prospective multicenter evaluation of real time PCR Kit prototype for early diagnosis of congenital Chagas disease. *EBioMedicine*, *69*, 103450. https://doi.org/10.1016/j.ebiom.2021.103450

Bern, C., Verastegui, M., Gilman, R. H., Lafuente, C., Galdos-Cardenas, G., Calderon, M., Pacori, J., Del Carmen Abastoflor, M., Aparicio, H., Brady, M. F., Ferrufino, L., Angulo, N., Marcus, S., Sterling, C., & Maguire, J. H. (2009). Congenital Trypanosoma cruzi transmission in Santa Cruz, Bolivia. *Clinical Infectious Diseases : An Official Publication of the Infectious Diseases Society of America*, *49*(11), 1667–1674. https://doi.org/10.1086/648070

Besuschio, S. A., Llano Murcia, M., Benatar, A. F., Monnerat, S., Cruz, I., Picado, A., Curto, M. de L. Á., Kubota, Y., Wehrendt, D. P., Pavia, P., Mori, Y., Puerta, C., Ndung'u, J. M., & Schijman, A. G. (2017). Analytical sensitivity and specificity of a loop-mediated isothermal amplification (LAMP) kit prototype for detection of Trypanosoma cruzi DNA in human blood samples. *PLoS Neglected Tropical Diseases*, *11*(7), e0005779. https://doi.org/10.1371/journal.pntd.0005779

Bua, J., Volta, B. J., Perrone, A. E., Scollo, K., Velázquez, E. B., Ruiz, A. M., De Rissio, A. M., & Cardoni, R. L. (2013). How to improve the early diagnosis of Trypanosoma cruzi infection: relationship between validated conventional diagnosis and quantitative DNA amplification in congenitally infected children. *PLoS Neglected Tropical Diseases*, *7*(10), e2476. https://doi.org/10.1371/journal.pntd.0002476

Caballero, Z. C., Sousa, O. E., Marques, W. P., Saez-Alquezar, A., & Umezawa, E. S. (2007). Evaluation of serological tests to identify Trypanosoma cruzi infection in humans and determine cross-reactivity with Trypanosoma rangeli and Leishmania spp. *Clinical and Vaccine Immunology : CVI*, *14*(8), 1045–1049. https://doi.org/10.1128/CVI.00127-07

Caicedo Díaz, R. A., Forsyth, C., Bernal, O. A., Marchiol, A., Beltrán Duran, M., Batista, C., Herazo, R., Vera, M. J., Pachón Abril, E., Valencia-Hernández, C. A., & Flórez Sánchez, A. C. (2019). Comparative evaluation of immunoassays to improve access to diagnosis for Chagas disease in Colombia. *International Journal of Infectious Diseases : IJID : Official Publication of the International Society for Infectious Diseases*, *87*, 100–108. https://doi.org/10.1016/j.ijid.2019.07.022

Castellanos-Gonzalez, A., White, A. C. J., Melby, P., & Travi, B. (2018). Molecular diagnosis of protozoan parasites by Recombinase Polymerase Amplification. *Acta Tropica*, *182*, 4–11. https://doi.org/10.1016/j.actatropica.2018.02.002

CONITEC Brazil. (2018). *Protocolo Clínico e Diretrizes Terapêuticas Doença de Chagas Outubro/2018 No 397*.

Conselleria de sanitat - Comunitat Valenciana. (2009). *Enfermedad De Chagas Importada Protocolo De Actuación En La Comunitat Valenciana*.

Cossio, A., Jojoa, J., Castro, M. D. M., Castillo, R. M., Osorio, L., Shelite, T. R., Gore Saravia, N., Melby, P. C., & Travi, B. L. (2021). Diagnostic performance of a Recombinant Polymerase Amplification Test-Lateral Flow (RPA-LF) for cutaneous leishmaniasis in an endemic setting of Colombia. *PLoS Neglected Tropical Diseases*, *15*(4), e0009291. https://doi.org/10.1371/journal.pntd.0009291

Coura, J. R., & Borges-Pereira, J. (2010). Chagas disease: 100 years after its discovery. A systemic review. *Acta Tropica*, *115*(1–2), 5–13. https://doi.org/10.1016/j.actatropica.2010.03.008

Cucunubá, Z. M., Manne-Goehler, J. M., Díaz, D., Nouvellet, P., Bernal, O., Marchiol, A., Basáñez, M.-G., & Conteh, L. (2017). How universal is coverage and access to diagnosis and treatment for Chagas disease in Colombia? A health systems analysis. *Social Science & Medicine (1982)*, *175*, 187–198. https://doi.org/10.1016/j.socscimed.2017.01.002

DGVS Paraguay. (2015). *Guía Nacional de Vigilancia y Control de Enfermedades Sistema Nacional de Vigilancia Epidemiológica. Paraguay, 2015.* https://dgvs.mspbs.gov.py/files/guiaNacional/Guia-Vigilancia-2015.pdf

Diez, C. N., Manattini, S., Zanuttini, J. C., Bottasso, O., & Marcipar, I. (2008). The value of molecular studies for the diagnosis of congenital Chagas disease in northeastern Argentina. *The American Journal of Tropical Medicine and Hygiene*, *78*(4), 624–627.

Doctors without borders. (2016). *Manual De Atención Integral De Chagas En Zona Rural. Bolivia. 2016. Médicos Sin Fronteras España*. https://www.doctorswithoutborders.ca/sites/default/files/manual_de_atencion_integral_de_chagas_en_zona_rural_de_msf_en_bolivia.pdf

Egüez, K. E., Alonso-Padilla, J., Terán, C., Chipana, Z., García, W., Torrico, F., Gascon, J., Lozano-Beltran, D. F., & Pinazo, M. J. (2017). Rapid diagnostic tests duo as alternative to conventional serological assays for conclusive Chagas disease diagnosis. *PLoS Neglected Tropical Diseases*, *11*(4). https://doi.org/10.1371/journal.pntd.0005501

FIND (not published). (2021). *The Landscape for Chagas Disease Rapid Diagnostic Tests in Latin America*.

Gobierno de Chile. (2014). *Norma general técnica control y prevención nacional de la enfermedad de Chagas. Departamento de Enfermedad de Enfermedades Transmisibles División de Prevención y*

Control de Enfermedades Subsecretaría de Salud Pública.

Instituto Nacional De Salud – INS. Colombia. (2017). *Protocolo De vigilancia En Salud Pública. Chagas*. https://www.ins.gov.co/buscador-eventos/Informesdeevento/CHAGAS 2017.pdf

Jackson, Y., Myers, C., Diana, A., Marti, H. P., Wolff, H., Chappuis, F., Loutan, L., & Gervaix, A. (2009). Congenital transmission of Chagas disease in Latin American immigrants in Switzerland. *Emerging Infectious Diseases*, *15*(4), 601–603. https://doi.org/10.3201/eid1504.080438

Jimenez-Coello, M., Shelite, T., Castellanos-Gonzalez, A., Saldarriaga, O., Rivero, R., Ortega-Pacheco, A., Acevedo-Arcique, C., Amaya-Guardia, K., Garg, N., Melby, P., & Travi, B. L. (2018). Efficacy of Recombinase Polymerase Amplification to Diagnose Trypanosoma cruzi Infection in Dogs with Cardiac Alterations from an Endemic Area of Mexico. *Vector Borne and Zoonotic Diseases (Larchmont, N.Y.)*, *18*(8), 417–423. https://doi.org/10.1089/vbz.2017.2258

Jung, T. (2021). Automatic Detection of Trypanosomosis in Thick Blood Smears Using Image Pre-processing and Deep Learning. In *Intelligent Human Computer Interaction*. https://doi.org/10.1007/978-3-030-68452-5_27

Lopez-Albizu, C., Danesi, E., Piorno, P., Fernandez, M., Campos, F. G., Scollo, K., & Crudo, F. (2020). Rapid diagnostic tests for trypanosoma cruzi infection: Field evaluation of two registered kits in a region of endemicity and a region of nonendemicity in argentina. *Journal of Clinical Microbiology*, *58*(12). https://doi.org/10.1128/JCM.01140-20

Lozano, D., Rojas, L., Méndez, S., Casellas, A., Sanz, S., Ortiz, L., Pinazo, M. J., Abril, M., Gascón, J., Torrico, F., & Alonso-Padilla, J. (2019). Use of rapid diagnostic tests (RDTs) for conclusive diagnosis of chronic Chagas disease - Field implementation in the Bolivian Chaco region. *PLoS Neglected Tropical Diseases*, *13*(12). https://doi.org/10.1371/JOURNAL.PNTD.0007877

Messenger, L. A., Gilman, R. H., Verastegui, M., Galdos-Cardenas, G., Sanchez, G., Valencia, E., Sanchez, L., Malaga, E., Rendell, V. R., Jois, M., Shah, V., Santos, N., Abastoflor, M. D. C., LaFuente, C., Colanzi, R., Bozo, R., & Bern, C. (2017). Toward Improving Early Diagnosis of Congenital Chagas Disease in an Endemic Setting. *Clinical Infectious Diseases : An Official Publication of the Infectious Diseases Society of America*, *65*(2), 268–275. https://doi.org/10.1093/cid/cix277

Ministerio de Salud y Deportes de Bolivia. (2007). *Manual de procesos para la deteccion, diagnostico, tratamiento y seguimiento de la enfermedad de Chagas infantil*. https://www.minsalud.gob.bo/38-libros-y-normas/fichas-bibliograficas/1575-area-chagas

Montes-Rincón, L. M., Galaviz-Silva, L., González-Bravo, F. E., & Molina-Garza, Z. J. (2016). Trypanosoma cruzi seroprevalence in pregnant women and screening by PCR and microhaematocrit in newborns from Guanajuato, Mexico. *Acta Tropica*, *164*, 100–106. https://doi.org/10.1016/j.actatropica.2016.08.029

Mora, M. C., Sanchez Negrette, O., Marco, D., Barrio, A., Ciaccio, M., Segura, M. A., & Basombrío, M. A. (2005). Early diagnosis of congenital Trypanosoma cruzi infection using PCR, hemoculture, and capillary concentration, as compared with delayed serology. *The Journal of Parasitology*, *91*(6), 1468–1473. https://doi.org/10.1645/GE-549R.1

Moreira, O. C., Ramírez, J. D., Velázquez, E., Melo, M. F. A. D., Lima-Ferreira, C., Guhl, F., Sosa-Estani, S., Marin-Neto, J. A., Morillo, C. A., & Britto, C. (2013). Towards the establishment of a consensus real-time qPCR to monitor Trypanosoma cruzi parasitemia in patients with chronic Chagas disease cardiomyopathy: a substudy from the BENEFIT trial. *Acta Tropica*, *125*(1), 23–31. https://doi.org/10.1016/j.actatropica.2012.08.020

Moscatelli, G., Moroni, S., García-Bournissen, F., Ballering, G., Bisio, M., Freilij, H., & Altcheh, J. (2015). Prevention of congenital chagas through treatment of girls and women of childbearing age. *Memorias Do Instituto Oswaldo Cruz*, *110*(4), 507–509. https://doi.org/10.1590/0074-02760140347

PAHO. (2018). *Guidelines for the diagnosis and treatment of Chagas disease*. https://iris.paho.org/bitstream/handle/10665.2/49653/9789275120439_eng.pdf

Paraguay. Ministerio de Salud Pública y Bienestar Social. (n.d.). *Guía práctica para manejo de transmisión congénita de Chagas y Chagas crónico reciente infantil*.

Paraguay. Ministerio de Salud Pública y Bienestar Social. (2021). *Guía de manejo de pacientes adultos con enfermedad de Chagas. Asunción*.

PATH. (2016). *Diagnostic Gaps and Recommendations for Chagas: Assessment of User Needs, Use Cases, and the Diagnostic Landscape.* https://path.azureedge.net/media/documents/2016-04-08_Report_Chagas-disease_Dx-Gap-analysis_final.pdf

Pereira, A., Pyrrho, A., Vanzan, D., Mazza, L., & Gomes, J. G. (2020). *Deep Convolutional Neural Network applied to Chagas Disease Parasitemia Assessment*. *May*, 1–8. https://doi.org/10.21528/cbic2019-119

Picado, A., Cruz, I., Redard-Jacot, M., Schijman, A. G., Torrico, F., Sosa-Estani, S., Katz, Z., & Ndung'u, J. M. (2018). The burden of congenital Chagas disease and implementation of molecular diagnostic tools in Latin America. *BMJ Global Health*, *3*(5), e001069. https://doi.org/10.1136/bmjgh-2018-001069

Ramírez, J. D., Guhl, F., Rendón, L. M., Rosas, F., Marin-Neto, J. A., & Morillo, C. A. (2010). Chagas cardiomyopathy manifestations and Trypanosoma cruzi genotypes circulating in chronic Chagasic patients. *PLoS Neglected Tropical Diseases*, *4*(11), e899. https://doi.org/10.1371/journal.pntd.0000899

Rivero, R., Bisio, M., Velázquez, E. B., Esteva, M. I., Scollo, K., González, N. L., Altcheh, J., & Ruiz, A. M. (2017). Rapid detection of Trypanosoma cruzi by colorimetric loop-mediated isothermal amplification (LAMP): A potential novel tool for the detection of congenital Chagas infection. *Diagnostic Microbiology and Infectious Disease*, *89*(1), 26–28. https://doi.org/10.1016/j.diagmicrobio.2017.06.012

Rodrigues-Dos-Santos, Í., Melo, M. F., de Castro, L., Hasslocher-Moreno, A. M., do Brasil, P. E. A. A., Silvestre de Sousa, A., Britto, C., & Moreira, O. C. (2018). Exploring the parasite load and molecular diversity of Trypanosoma cruzi in patients with chronic Chagas disease from different regions of Brazil. *PLoS Neglected Tropical Diseases*, *12*(11), e0006939. https://doi.org/10.1371/journal.pntd.0006939

Rodriguez, P., Truyens, C., Alonso-Vega, C., Flores, A., Cordova, M., Suarez, E., Torrico, F., & Carlier, Y. (2005). [Serum levels for IgM and IgA antibodies to anti-trypanosoma cruzi in samples of blood from newborns from mothers with positive serology for Chagas disease]. *Revista da Sociedade Brasileira de Medicina Tropical*, *38 Suppl 2*, 62–64.

Sánchez-Camargo, C. L., Albajar-Viñas, P., Wilkins, P. P., Nieto, J., Leiby, D. A., Paris, L., Scollo, K., Flórez, C., Guzmán-Bracho, C., Luquetti, A. O., Calvo, N., Tadokoro, K., Saez-Alquezar, A., Palma, P. P., Martin, M., & Flevaud, L. (2014). Comparative evaluation of 11 commercialized rapid diagnostic tests for detecting Trypanosoma cruzi antibodies in serum banks in areas of endemicity and nonendemicity. *Journal of Clinical Microbiology*, *52*(7), 2506–2512. https://doi.org/10.1128/JCM.00144-14

Sousa, A. S. de, Vermeij, D., Parra-Henao, G., Lesmo, V., Fernández, E. F., Aruni, J. J. C., Mendes, F. de S. N. S., Bohorquez, L. C., & Luquetti, A. O. (2022). The CUIDA Chagas Project: towards the elimination of congenital transmission of Chagas disease in Bolivia, Brazil, Colombia, and Paraguay. *Revista Da Sociedade Brasileira de Medicina Tropical*, *55*, e01712022. https://doi.org/10.1590/0037-8682-0171-2021

Suescún-Carrero, S. H., Salamanca-Cardozo, L. P., Pinazo, M.-J., & Armadans-Gil, L. (2021). Sensitivity and Specificity of two rapid tests for the diagnosis of infection by Trypanosoma cruzi in a Colombian population. *PLOS Neglected Tropical Diseases*, *15*(6), e0009483. https://doi.org/10.1371/journal.pntd.0009483

UNITAID. (2020). *Screening and treatment for Chagas Disease: Technology and market landscape*. https://unitaid.org/assets/210726_BLS21015_Chagas-Landscape_EN.v0272.pdf

Virreira, M., Torrico, F., Truyens, C., Alonso-Vega, C., Solano, M., Carlier, Y., & Svoboda, M. (2003). Comparison of polymerase chain reaction methods for reliable and easy detection of congenital Trypanosoma cruzi infection. *The American Journal of Tropical Medicine and Hygiene*, *68*(5), 574–582. https://doi.org/10.4269/ajtmh.2003.68.574

WHO. (2004). *Anti-Trypanosoma cruzi Assays: Operational Characteristics Report 1*. https://www.who.int/diagnostics_laboratory/publications/anti_t_cruzi_assays.pdf

WHO. (2015a). Chagas disease in Latin America: an epidemiological update based on 2010 estimates. *Weekly Epidemiological Record*, *90*(6), 33–44. https://doi.org/10.2147/IBPC.S70402

WHO. (2015b). *Supply of blood for transfusion in Latin America and Caribbean countries 2012 and 2013*.https://www.paho.org/hq/dmdocuments/2015/Blood-supplies-transfusions-ENG-2015.pdf

Zhang, Y., Ceylan Koydemir, H., Shimogawa, M. M., Yalcin, S., Guziak, A., Liu, T., Oguz, I., Huang, Y., Bai, B., Luo, Y., Luo, Y., Wei, Z., Wang, H., Bianco, V., Zhang, B., Nadkarni, R., Hill, K., & Ozcan, A. (2018). Motility-based label-free detection of parasites in bodily fluids using holographic speckle analysis and deep learning. *Light, Science & Applications*, *7*, 108. https://doi.org/10.1038/s41377-018-0110-1

11. Annex

Table 1. Overview of the diagnostic test products for Chagas disease.

RDTs were defined as tests that could be performed in less than 30 minutes and without sample preparation (see the section Results of the analysis - Immunoassays). Most RDTs are LFAs that work with a drop of blood and can be performed at the community level. However, a few RDTs require serum or plasma and therefore need a higher level of user training, i.e. trained staff in healthcare facilities. These RDTs are indicated by an asterisk next to the test number.

RDTs								
	Product Name	**Company name (location of headquarters)**	**Product reference**	**Stage of development**	**Stringent regulatory approval[1]**	**Planned market entry**	**Validated sample type(s)**	**End user profile (training level)**
1	Chagas Detect™ Plus Rapid Test	InBios International, Inc. (USA)	CP050	Decommissioned	Yes	No longer on the market	Finger-prick blood; venous blood; serum	Community level (lay person)
2	Onsite Chagas Ab Rapid test	CTK Biotech (USA)	R0171C	Regulatory approval achieved	Yes	Already on the market	Finger-prick blood; venous blood; serum	Community level (lay person)
3	Chagas Rapid Test Cassette	Certum® Diagnostics (Mexico)	ICHA-402	Regulatory approval achieved		Already on the market	Finger-prick blood; venous blood; serum	Community level (lay person)
4	TruQuick™ Chagas 40T	Meridian Bioscience (USA)	TQ2540	Regulatory approval achieved	Yes	Already on the market	Finger-prick blood; venous blood; serum; plasma	Community level (lay person)
5	Accu-Tell Chagas Cassette	AccuBiotech (China)	ABT-IDT-B219	Regulatory approval achieved	Yes	Already on the market	Finger-prick blood; venous blood; serum; plasma	Community level (lay person)
6	The Rapid Response Chagas Antibody Test Cassette	BTNX Inc. (Canada)	CHA-13C25	Regulatory approval achieved	Yes	Already on the market	Finger-prick blood; venous blood; serum; plasma	Community level (lay person)

7	Chagas Ab cassette	Linear Chemicals S.L. (Spain)	4272240	Decommissioned	Yes	No longer on the market	Finger-prick blood; venous blood; serum; plasma	Community level (lay person)
8	Chagas AC Cassette	Xerion (Colombia)	CHAPT0002	Regulatory approval achieved		Already on the market	Finger-prick blood; venous blood; serum; plasma	Community level (lay person)
9*	Amunet prueba rapida Chagas	Amunet (Mexico)	746321	Regulatory approval achieved		Already on the market	Serum; plasma	Trained staff in healthcare facilities
10*	T. cruzi IgG Chagas Test cassette	Atlas Link Technology (China)	CHAG 492	Regulatory approval achieved		Already on the market	Serum; plasma	Trained staff in healthcare facilities
11	One-Step Chagas Ab Rapid Test	Span Biotech (China)	na	Regulatory approval achieved	Yes	Already on the market	Finger-prick blood; venous blood; serum; plasma	Community level (lay person)
12	Chagas Antibody Rapid Test	Healgen Scientific LLC/Zhejiang Orient Gene Biotech Co (China)	GCCHA-302a/GCCHA-402a	Regulatory approval achieved	Yes	Already on the market	Finger-prick blood; venous blood; serum; plasma	Community level (lay person)
13*	Chagas Rapid Test Cassette (S/P)	Hangzhou AllTest Biotech CO., LTD (China)	ICHA-302	Regulatory approval achieved	Yes	Already on the market	Serum; plasma	Trained staff in healthcare facilities
	Chagas Rapid Test Cassette - S/P	Acro Biotech, Inc. (USA)	ICHA-302					
14	Chagas Antibody Test Cassette	Artron Laboratories (Canada)	A03-33-222	Regulatory approval achieved	Yes	Already on the market	Whole blood; serum; plasma	Unknown
15	Chagas Stat-Pak	Chembio Diagnostic Systems, Inc. (USA)	CG101 (60-9550-0)	Regulatory approval achieved	Yes	Already on the market	Finger-prick blood; venous blood; serum; plasma	Community level (lay person)

16	OneStep Chagas (Trypanosoma cruzi) Serum/WB/ Plasma RapiDip™ InstaTest	Cortez Diagnostics (USA)	146119-25	Regulatory approval achieved	Yes	Already on the market	Finger-prick blood; venous blood; serum; plasma	Community level (lay person)
17	SD Bioline Chagas AB rapid test	Abbott (standard Diagnostic) (USA)	49FK10	Regulatory approval achieved	Yes	Already on the market	Whole blood; serum; plasma	Trained staff in healthcare facilities
18*	Simple Chagas/Stick Chagas	Operon (Spain)	9.035.050.15 .000	Regulatory approval achieved	Yes	Already on the market	Serum	Trained staff in healthcare facilities
19	WL Check Chagas test	Wiener Lab (Argentina)	1690011	Regulatory approval achieved	Yes	Already on the market	Finger-prick blood; venous blood; serum; plasma	Community level (lay person)
20*	Chagas Instantest	Silanes (Mexico)	na	Regulatory approval achieved		Already on the market	Serum; plasma	Trained staff in healthcare facilities
21	Trypanosoma Detect™ Rapid Test	InBios International, Inc. (USA)	ITC015	Decommissioned	Yes	No longer in the market	Finger-prick blood; serum; whole blood	Community level (lay person)
22	Simple Chagas WB test	Operon (Spain)	9.131.020.00 .000	Regulatory approval achieved	Yes	Already on the market	Finger-prick blood; venous blood; serum; plasma	Community level (lay person)
23	TR Chagas Bio-Manguinhos	Bio-Manguinhos/Fiocruz (Brazil)	na	Regulatory approval achieved	Yes	Already on the market	Finger-prick blood; venous blood; serum; plasma	Community level (lay person)
24	Chagas Rapido First Response	Lemos (Argentina)	na	Regulatory approval achieved		Already on the market	Finger-prick blood; venous blood; serum; plasma	Community level (lay person)
25	Chagas (Trypanosoma cruzi)	Veda Lab (France)	47053 - 47083	Regulatory approval achieved	Yes	Already on the market	Whole blood; serum; plasma	Community level (lay person)

26	Chagas Rapid Test Cassette (WB/S/P)	Hangzhou AllTest Biotech CO., LTD (China)	ICHA-402	Regulatory approval achieved	Yes	Already on the market	Finger-prick blood; venous blood; serum; plasma	Community level (lay person)
	Chagas Rapid Test Cassette - WB/S/P	Inzek / Biozek medical (Netherlands)	BCHA-402					
	Chagas Rapid Test Cassette - WB/S/P	Acro Biotech, Inc. (USA)	ICHA-402					
	Chagas Ab Rapid Test	Rapid Labs (United Kingdom)	D-CHABD20					
27	Chagas Ab Combo Rapid Test	Zhuhai Encode Medical Engineering Co., Ltd (China)	ICS-402	Decommissioned		No longer on the market	Finger-prick blood; venous blood; serum; plasma	Community level (lay person)
28	Chagas Antibody Test Card	LumiQuick Diagnostics (USA)	71078	Early-stage development		Not yet on the market	Finger-prick blood; venous blood; serum; plasma	Community level (lay person)
29*	Chagas Sero K-SeT rapid diagnostic test (RUO)	Coris Bioconcept (Belgium)	na	Research Use Only		Not yet on the market	Serum; plasma	Trained staff in healthcare facilities
30*	Chagas Ab Rapid Test	Creative diagnostics (USA)	DTS204	Research Use Only		Unknown	Serum	Trained staff in healthcare facilities
31	Chagas Ab Rapid Test	Zhejiang Quark Biotechnology Co., Ltd. (KWORK) (China)	CTNI-C41	Regulatory status unknown		Already on the market	Finger-prick blood; venous blood; serum; plasma	Community level (lay person)
32	ViroTrack Chagas test	BluSense Diagnostics Aps (Denmark)	na	Concept		Not yet on the market	Finger-prick blood; venous blood; serum; plasma	Unknown
33	Chagas Disease - Rapid Test	Kephera Diagnostics (USA)	na	Concept		Unknown	Whole blood	Unknown

34	Chagas Quick Test	Cypress Diagnostic (Belgium)	na	Decommissioned	Yes	No longer on the market	Whole blood; serum	Community level (lay person)
35	Hexagon Chagas	Human Diagnostics (Germany)	58002	Decommissioned		No longer on the market	Finger-prick blood; venous blood; serum; plasma	Community level (lay person)
36	Immu-Sure Chagas	Millennium Biotech/Teakeda (USA)	na	Decommissioned		No longer on the market	Finger-prick blood; venous blood; serum; plasma	Community level (lay person)
37	NADAL® Chagas IgG, test cassette	Nal von minden GmbH (Germany)	652001N-30	Decommissioned	Yes	No longer on the market	Finger-prick blood; venous blood; serum; plasma	Community level (lay person)
38	Chagas Disease - Rapid Test based on TESA antigen	Kephera Diagnostics (USA)	na	Early-stage development		Not yet on the market	Unknown	Unknown
39	FIND-DCN Chagas RDT	FIND (Switzerland)	na	Late-stage development		Not yet on the market (planned 2025)	Finger-prick blood; venous blood; serum; plasma	Trained staff in healthcare facilities

non-RDT immunoassays								
	Product name	**Company name (location of headquarters)**	**Product reference**	**Stage of development**	**Stringent regulatory approval**	**Planned market entry**	**Validated sample type**	**End user profile (training level)**
1	Chagatek ELISA	Laboratório Lemos SRL (Argentina)	BCH96 BCH192	Regulatory approval achieved		Already on the market	Serum	Laboratory technician
2	Chagatek ELISA Recombinant	Laboratório Lemos SRL (Argentina)	R96 R192	Regulatory approval achieved		Already on the market	Serum; plasma	Laboratory technician

3	Chagatest ELISA lisado	Wiener Laboratórios (Argentina)	1293096 1293192	Regulatory approval achieved	Yes	Already on the market	Serum; plasma	Laboratory technician
4	Chagatest ELISA recombinante v.4.0	Wiener Laboratórios (Argentina)	12930	Regulatory approval achieved	Yes	Already on the market	Serum; plasma	Laboratory technician
5	Cellabs T. cruzi IgG CELISA II	Cellabs Pty Ltd (Australia)	KT4	Regulatory approval achieved	Yes	Already on the market	Serum; plasma	Laboratory technician
6	Chagas ELISA (IHA)	Ebram (Brazil)		Decommissioned		Not on the market anymore	Serum	Laboratory technician
7	ELISA Chagas III	GrupoBios (Chile)		Regulatory approval achieved	Yes	Already on the market	Serum; plasma	Laboratory technician
8	Chagas (Trypanosoma cruzi) IgG ELISA	IBL International GmbH (Germany)	RE58691	Regulatory approval achieved	Yes	Already on the market	Serum; plasma	Laboratory technician
9	NovaLisa Chagas (Trypanosoma cruzi)	NovaTec Immundiagnostica (Germany)	TRYP0570	Regulatory approval achieved	Yes	Already on the market	Serum; plasma	Laboratory technician
10	Celquest Chagas ELISA	ATGen Diagnostica (Uruguay)		Regulatory approval achieved		Already on the market	Serum; plasma	Laboratory Technician
11	Chagas Test ELISA	Research Institute for Health Sciences (Paraguay)		Regulatory approval achieved		Already on the market	Whole blood; serum	Laboratory technician
12	Chagas V2-IICS	Research Institute for Health Sciences (Paraguay)		Regulatory approval achieved		Already on the market	Serum	Laboratory technician
13	BioELISA Chagas	Werfen (Spain)		Regulatory approval achieved		Already on the market	Serum; plasma	Laboratory technician

14	Chagas ELISA IgG + IgM	Vircell (Spain)	T1020	Regulatory approval achieved	Yes	Already on the market	Serum; plasma	Laboratory technician
15	Chagas TESA ELISA IgG + IgM	Vircell (Spain)	T1023	Regulatory approval achieved	Yes	Already on the market	Serum; plasma	Laboratory technician
16	Anti-Chagas IgG ELISA Kit	Abcam (UK)	ab178637	Research Use Only		Already on the market	Serum; plasma	Laboratory technician
17	Chagas IgG ELISA CE	CTK Biotech (USA)	E170	Regulatory approval achieved		Already on the market	Serum; plasma	Laboratory technician
18	AccuDiag™ Chagas ELISA Kit	Diagnostic Automation/Cortez Diagnostics, Inc. (USA)	8100-35	Regulatory approval achieved	Yes	Already on the market	Serum; plasma	Laboratory technician
19	DRG Trypanosoma cruzi IgG	DRG International Inc. (USA)	EIA-5813	Regulatory approval achieved	Yes	Already on the market	Serum; plasma	Laboratory technician
20	Hemagen Chagas	Hemagen Diagnostics Inc (USA)	66101	Regulatory status unknown		Already on the market	Serum	Laboratory technician
21	Premier Chagas IgG ELISA	Meridian Bioscience (USA)		Decommissioned		No longer on the market	Serum	Laboratory technician
22	ORTHO T. cruzi ELISA Test System	Ortho Diagnostics & Johson and Jhonson (USA)	6902594 6901968 6901969	Regulatory approval achieved	Yes	Already on the market	Serum; plasma	Laboratory technician
23	T. cruzi Ab - ELISA	Diagnostic Bioprobes (Italy)	TCAB.CE	Regulatory approval achieved	Yes	Already on the market	Serum; plasma	Laboratory technician
24	Tryanosoma cruzi IgG ELISA Kit	MyBiosource (Canada)	MBS495311	Research use only		Unknown	Serum; plasma	Laboratory technician

25	Chagas (Trypanosoma cruzi) IgG ELISA	DEMEDITEC Diagnostics GmbH (Germany)	DENO0114	Regulatory approval achieved	Yes	Already on the market	Serum; plasma	Laboratory technician
26	Chagas (Trypanosoma cruzi) IgG ELISA	GenWay Biotech, Inc. (USA)	GWB-A4E22B	Research Use Only		Unknown	Serum; plasma	Laboratory technician
27	Umelisa Chagas	Tecnosuma International (Havana, Cuba) (Cuba)	UM 2014	Regulatory approval achieved	Yes	Already on the market	Serum; plasma; whole blood in filter paper	Laboratory technician
28	ELISA Anti-Chagas	Symbiosys (Brazil)		Regulatory status unknown		Unknown	Unknown	Laboratory technician
29	Chagas Rec ELISA	Human Diagnostics Worldwide (Germany)		Regulatory approval achieved		Already on the market	Unknown	Laboratory technician
30	cruziTEST ELISA	GenCell Biosystems (Ireland)		Decommissioned		No longer on the market	Unknown	Laboratory technician
31	EIAgen T cruzi IgG + IgM	Adaltis (Italy)		Regulatory status unknown		Unknown	Unknown	Laboratory technician
32	Pathozyme Chagas	Omega Diagnostics (Scotland)	OD147	Regulatory approval achieved	Yes	Already on the market	Serum	Laboratory technician
33	ELISA BLK	BLK Diagnostics (Spain)		Regulatory status unknown		Unknown	Unknown	Laboratory technician
34	Gull ELISA (Chagas IgG ELISA)	Meridian Bioscience Inc (USA)		Decommissioned		No longer on the market	Unknown	Laboratory technician
35	BIOELISACRUZI	Biolab Mérieux (Brazil)		Decommissioned		No longer on the market	Serum; plasma	Laboratory technician

36	Abbott Chagas Antibody EIA	Abbott Laboratorios do Brasil Ltda (Brazil)	7A007-26	Decommissioned		No longer on the market	Serum; plasma	Laboratory technician
37	CHAGAS HEMAGEN	Hemagen Diagnosticos Ltda (Brazil)	66101-01	Decommissioned		No longer on the market	Serum	Laboratory technician
38	HBK 740 IMUNOBLOT LINHAS anti-T.cruzi	EMBRABIO - Empresa Brasileira de Biotecnologica S.A. (Brazil)		Decommissioned		No longer on the market	Serum; plasma	Laboratory technician
39	IVD ELISA	IVD Research Inc. (USA)		Decommissioned		No longer on the market	Unknown	Laboratory technician
40	ELISA cruzi	bioMérieux SA (France)		Decommissioned		No longer on the market	Unknown	Laboratory technician
41	ImmunoComb II Chagas Ab	Orgenics/Abbot-Alere (Israel)		Decommissioned		No longer on the market	Unknown	Laboratory technician
42	Chagastest HAI	Wiener Laboratórios (Argentina)	12932	Regulatory approval achieved	Yes	Already on the market	Serum	Trained staff in healthcare facilities
43	Imuno-HAI Chagas	Wama Diagnóstica (Brazil)	34096, 34192, 34380	Regulatory approval achieved	Yes	Already on the market	Serum	Trained staff in healthcare facilities
44	HAI CHAGAS POLYCHACO	Laboratorio Lemos SRL (Argentina)		Regulatory approval achieved		Already on the market	Serum	Trained staff in healthcare facilities
45	Serodia Chagas	Serodia Chagas (Japan)	227442	Decommissioned		No longer on the market	Serum; plasma	
46	Hemacruzi	bioMérieux Brasil (Brazil)	029.015	Decommissioned		No longer on the market	Serum	
47	Chagas-HAI	EBRAM (Brazil)	200	Decommissioned		No longer on the market	Serum	

48	Inmunofluor Chagas	Biocientífica (Argentina)	NF09-90	Regulatory approval achieved	Yes	Already on the market	Serum	Laboratory technician
49	Imuno-CON Chagas	Wama Diagnóstica (Brazil)	1430, 1460, 14100, 14200	Regulatory approval achieved	Yes	Already on the market	Serum	Laboratory technician
50	Chagas IFA IgG + IgM	Vircell (Spain)	PCHAG	Regulatory approval achieved	Yes	Already on the market	Serum; plasma	Laboratory technician
51	ImunoCruzi	Biolab Mérieux (Brazil)	022.002	Decommissioned		No longer on the market	Serum	
52	Trypanosoma cruzi IFA Test System	Trinity biotech (Ireland)	20-03648	Regulatory approval achieved	Yes	Already on the market	Serum; plasma	Laboratory technician
53	Chagas Virclia IgG + IgM MONOTEST	Vircell (Spain)	VCM008	Regulatory approval achieved	Yes	Already on the market	Serum; plasma	Laboratory technician
54	Chagas TESA Virclia IgG + IgM MONOTEST	Vircell (Spain)	VCM099	Regulatory approval achieved	Yes	Already on the market	Serum; plasma	Trained staff in healthcare facilities
55	PRISM Chagas	Abbott Laboratories (USA)	7K35-68	Regulatory approval achieved	Yes	Already on the market	Serum; plasma	Trained staff in healthcare facilities
56	Elecsys Chagas	Roche Diagnostic (USA)	7092563	Regulatory approval achieved		Already on the market	Serum; plasma	Trained staff in healthcare facilities
57	VITROS Immunodiagnostic Anti-T.cruzi (Chagas) Controls	Ortho Diagnostics & Johson and Jhonson (USA)		Regulatory approval achieved	Yes	Already on the market	Not specified	Trained staff in healthcare facilities

58	Architect Chagas	Abbott Laboratories (USA)		Regulatory approval achieved		Already on the market	Unknown	
59	ESA Chagas	Abbott Laboratories (USA)	8L34-68	Decommissioned	Yes	No longer on the market	Serum; plasma	Laboratory technician
60	ID-Chagas antibody test	DiaMed-ID (Aqquired by Biorad) (USA)	20022	Decommissioned		No longer on the market	Serum; plasma	Laboratory technician
61	TESA-blot	Biolab Mérieux (Brazil)		Decommissioned		No longer on the market	Serum; plasma	Laboratory technician
62	HBK 401 HEMOBIO Chagas	EMBRABIO - Empresa Brasileira de Biotecnologica S.A. (Brazil)		Decommissioned		No longer on the market	Serum; plasma	Laboratory technician
63	Inno-Lia Chagas	Fuijrebio (previously Innogenetics) (USA)		Decommissioned		No longer on the market	Serum	Laboratory technician
64	Immulite Chagas IgG	Siemens Healthcare (USA)		Regulatory status unknown		Unknown	Unknown	Laboratory technician

non-RDT molecular assays								
	Product name	**Company name (location of headquarters)**	**Product reference**	**Stage of development**	**Stringent regulatory approval**	**Planned market entry**	**Validated sample type**	**End user profile (training level)**
1	RealStar® Chagas PCR Kit	Altona Diagnostics (Germany)	611013	Regulatory approval achieved	Yes	Already on the market	Depends on nucleic acid extraction kit	Laboratory technician

2	VIASURE Trypanosoma cruzi Real Time PCR Detection Kit	Certest Biotec (Spain)		Regulatory approval achieved	Yes	Already on the market	Whole blood; plasma; serum	Laboratory technician
3	TCRUZIDNA.CE	Diagnostic Bioprobes (Italy)	TCRUZIDNA. CE.25 / E.100 / E.150	Regulatory approval achieved	Yes	Already on the market	Whole blood	Laboratory technician
4	T. cruzi DNA qPCR kit	Wiener laboratories (Argentina)		Regulatory approval achieved	Yes	Already on the market	Whole blood	Laboratory technician
5	ViPrimePLUS Trypasoma cruzi qPCR kit	Vivantis (Malaysia)	QM4018	Research Use Only		Already on the market	Whole blood	Laboratory technician
6	RealCycler CHAG	Progenie Molecular Emelca (Possible manufacturer) (Spain)		Regulatory approval achieved		Already on the market	Unknown	Laboratory technician
7	LAMP assay	Eiken Chemical Company (Japan)		Early-stage development		Not yet on the market	Unknown	
8	T. cruzi primers (Kit format)	Genesig Primerdesign (United Kingdom)		Regulatory status unknown		Unknown	Unknown	Laboratory technician
9	Kit NAT Chagas	INSTITUTO DE BIOLOGIA MOLECULAR DO PARANÁ – IBMP (Brazil)		Regulatory approval achieved	Yes	Not yet on the market (planned 2022)	Whole blood	Laboratory technician

Table 2. Definitions used for healthcare level classification.

	Level 0 (L0) – Community	Level 1 (L1) – Primary Care	Level 2 (L2) – District Hospital Laboratory	Level 3 (L3) – Reference Laboratory
Use setting	• Community outreach • Home testing	• Primary care facility	• Near-patient laboratory • Referral hospital laboratory	• Reference laboratory
Laboratory infrastructure	• No mains power • No water • No laboratory equipment • No temperature control	• No mains power (unreliable) • Minimal laboratory equipment (may not support cold-chain) • BSL-1 containment	• Mains power (may be intermittent) • Basic laboratory equipment (biosafety cabinet, centrifuge, calibrated pipettes, fridge) • BSL-2/1 containment	• Mains power (reliable) • High level of laboratory infrastructure • BSL-2/3 containment
Test complexity	• RDT	• True-POC PCR • Basic microscopy • RDT	• Near-POC PCR • ELISA with simple reader • Microscopy • RDT	• Laboratory PCR • ELISA/EIA/CLIA • Fluorescence microscopy • Culture (some) • Sequencing (some)
Operator skill	• Nurse/pharmacist • Community health worker • Self-testing (some cases) • Simple reagent/sample transfer	• Nurse • Trained laboratory worker • Minimal sample processing (≤3 steps)	• Laboratory technician (1–2-year certification) • Sample processing with calibrated volumes (≤3 steps)	• Science research specialists • Laboratory technician (1–2-year certification)

Specimen capacity	• Can process minimally invasive samples: fingerstick blood, nasal swabs, saliva, urine	• Can process upper respiratory specimens; clinic may not have capacity for lower respiratory, venipuncture, plasma	• Can process most BSL-2 specimens; depends on clinic sample capacity	• Can process most BSL2/3 specimens
Test demand (throughput)	• One-at-a-time testing (STAT test)	• STAT test or end-of-day batch • Up to 10 patients/day	• STAT test or end-of-day batch • May require random access • Up to 50–100 patients/day	• Up to hundreds of samples per run • Random access
Desired time to result (Turnaround time	• Test results in <30 min • While-you-wait results	• Test results in 30–90 min • While-you-wait or same day results	• Test results in 30–90 min • Same day or next day results	• Batch run; next day test results • Batch and return of results ≤2 weeks

Table 3. Antibody targets of RDTs for Chagas disease.

	Product name	Company name	Antibody target*
1	Chagas Detect™ Plus Rapid Test	InBios International, Inc.	Recombinant protein ITC8.2 (multiepitope: TcF; SAPA; Pep30; Pep36; Kmp-11; Pep1)
15	Chagas Stat-Pak	Chembio Diagnostic Systems, Inc.	B13; 1F8; H49/JL7
17	SD Bioline Chagas AB rapid test	Standard Diagnostic (Abbott)	H49; 1F8
18	Simple Chagas/Stick Chagas	Operon	Recombinant protein (multiepitope: Pep2; TcD; TcE; SAPA)
21	Trypanosoma Detect™ Rapid Test	InBios International, Inc.	Recombinant protein ITC8.2 (multiepitope: TcF; SAPA; Pep30; Pep36; Kmp-11; Pep1)
22	Simple Chagas WB test	Operon	Recombinant protein (multiepitope: Pep2; TcD; TcE; SAPA)
29	Chagas Sero K-SeT rapid diagnostic test	Coris Bioconcept	TSSApep (specific for lineages TcII, TcV, and TcVI)

* Antibody target information was not available for the other RDTs

Table 4. Marketing of RDTs in Bolivia, Brazil, Colombia and Paraguay and number of clinical studies.

	Product name	Company name (Country of headquarters)	Product commercialized in:				Product with stringent regulatory approval[1]	Not on the market	Number of clinical studies				
									In IFU	In publications			Others
			Bolivia (BO)	Brazil (BR)	Colombia (CO)	Paraguay (PA)				Company-sponsored	Independent studies performed in BO, BR, CO, PA	Independent studies performed in other countries	Poster, independent report by regulatory agency
1	Chagas Detect™ Plus Rapid Test	InBios International, Inc. (United States)						x	6	1	3	2	
2	Onsite Chagas Ab Rapid test	CTK Biotech (United States)	x	x	x		CE-IVD, Brazil ANVISA		1		1	1	
3	Chagas Rapid Test Cassette	Certum® Diagnostics (Mexico)							1				
4	TruQuick™ Chagas 4T	Meridian Bioscience (United States)					CE-IVD		1				
5	Accu-Tell Chagas Cassette	AccuBiotech (China)	x				CE-IVD		1				1
6	The Rapid Response Chagas Antibody Test Cassette	BTNX Inc. (Canada)					CE-IVD		1				
7	Chagas Ab cassette	Linear Chemicals S.L. (Spain)						x	1				
8	Chagas AC Cassette	Xerion (Colombia)			x				1				

9	Amunet prueba rapida Chagas	Amunet (Mexico)							1				
10	T.cruzi IgG Chagas Test cassette	Atlas Link Technology (China)	x						1				
11	One-Step Chagas Ab Rapid Test	Span Biotech (China)					China NMPA		1				
12	Chagas Antibody Rapid Test	Healgen Scientific LLC/Zhejiang Orient Gene Biotech Co (China)					CE-IVD						
13[5]	Chagas Rapid Test Cassette (S/P)	Hangzhou AllTest Biotech CO.,LTD (China)	x				CE-IVD		1				
13[5]	Chagas Rapid Test Cassette - S/P	Acro Biotech, Inc. (United States)	x				CE-IVD		1				
14	Chagas Antibody Test Cassette	Artron Laboratories (Canada)			x	x	CE-IVD		0	1			
15	Chagas Stat-Pak	Chembio Diagnostic Systems, Inc. (United States)	x		x	X[4]	CE-IVD		3	1	5	2	
16	OneStep Chagas (Trypanosoma cruzi) S/WB/P RapiDip InstaTest	Cortez Diagnostics (United States)					CE-IVD, FDA						
17	SD Bioline Chagas AB rapid test	Abbott (standard Diagnostic) (United States)	x	x	x	x	CE-IVD, Brazil ANVISA		1		1	2	1
18	Simple Chagas/Stick Chagas	Operon (Spain)					CE-IVD		4			1	

19	WL Check Chagas test	Wiener Lab (Argentina)	x	x	x		CE-IVD, Brazil ANVISA		1		1	3	
20	Chagas Instantest	Silanes (Mexico)									1		
21	Trypanosoma Detect™ Rapid Test	InBios International, Inc. (United States)						x	1		2	2	
22	Simple Chagas WB test	Operon (Spain)					CE-IVD		2		1	1	
23	TR Chagas	Bio-Manguinhos/Fiocruz (Brazil)		x			Brazil ANVISA		1				
24	Chagas Rapido First Response	Lemos (Argentina)	x						1	1			
25	Chagas (Trypanosoma cruzi)	Veda Lab (France)					CE-IVD						
26[5]	Chagas Rapid Test Cassette (WB/S/P)	Hangzhou AllTest Biotech CO.,LTD (China)	x				CE-IVD		1				
26[5]	Chagas Rapid Test Cassette - WB/S/P	Inzek / Biozek medical (Netherlands)					CE-IVD						
26[5]	Chagas Rapid Test Cassette - WB/S/P	Acro Biotech, Inc. (United States)	x				CE-IVD		1				
26[5]	Chagas Ab Rapid Test	Rapid Labs (United Kingdom)					CE-IVD						
27	Chagas Ab Combo Rapid Test	Zhuhai Encode Medical Engineering Co.,Ltd (China)						x	1				
28	Chagas Antibody Test Card	LumiQuick Diagnostics (United States)						unknown	1				

29	Chagas Sero K-SeT rapid diagnostic test (RUO)	Coris Bioconcept (Belgium)						x			2	2	
30	Chagas Ab Rapid Test	Creative diagnostics (United States)						unknown					
31	Chagas Ab Rapid Test	Zhejiang Quark Biotechnology Co., Ltd. (KWORK) (China)											
32	ViroTrack Chagas test	BluSense Diagnostics Aps (Denmark)						x					
33	Chagas Disease - Rapid Test	Kephera Diagnostics (United States)						unknown					
34	Chagas Quick Test	Cypress Diagnostic (Belgium)					CE-IVD	x	1		1	1	
35	Hexagon Chagas	Human Diagnostics (Germany)						x	2				
36	Immu-Sure Chagas	Millennium Biotech/Teakeda (United States)						x			1		
37	NADAL® Chagas IgG, test cassette	Nal von minden GmbH (Germany)					CE-IVD	x	1				
38	Chagas Disease- Rapid Test based on TESA antigen	Kephera Diagnostics						x					

[1]Regulatory approvals recognized as stringent are based on the Global Harmonization Task Force and the International Medical Device Regulators Forum. The stringent regulatory authority list comprises Australia TGA, Brazil ANVISA, Health Canada, China NMPA, Europe CE-IVD, Japan MHLW, Russia RMH, Singapore HSA, South Korea MFDS, and USA FDA clearance.

[4]Chagas Stat-Pak from Chembio Diagnostics is potentially marketed in Paraguay, but the import license certificate could not be obtained as proof

[5]Products that are commercialized by different distributors