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Modalities Allowing For Early Detection and Diagnosis of Chagas Disease: A Systematic Review

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ABSTRACT

Background: Early detection and diagnosis of Chagas disease remains challenging despite its significant health burden. Identifying effective diagnostic modalities is important for timely treatment and prevention of chronic complications.

Objective: This review examines diagnostic modalities that enable early detection and diagnosis of Chagas disease.

Materials and Methods: This systematic review was conducted according to modified PRISMA guidelines and included studies investigating diagnostic modalities for early detection and diagnosis of Chagas disease.

Results: Among 400 identified publications, 21 studies with 3926 participants were selected for inclusion. The review identifies multiple diagnostic modalities that support early detection of Chagas disease. Molecular techniques, particularly PCR, were the most frequently reported methods (7 of 21 studies), while one study examined loop-mediated isothermal amplification (LAMP) as a rapid molecular alternative. Additional approaches included immunological assays (4 of 21 studies) and cardiac diagnostic tools used to identify infection and early disease-related complications (13 of 21 studies).

Conclusion: In summary, this review demonstrates that multiple diagnostic modalities contribute to the early detection and diagnosis of Chagas disease, with molecular techniques playing a particularly important role.

INTRODUCTION

Chagas disease, also known as American trypanosomiasis, is a parasitic infection caused by the protozoan *Trypanosoma cruzi.*[1] Transmission occurs most commonly through the bite of infected triatomine insects, often called "kissing bugs." Additional routes include blood transfusions, organ transplants, congenital transmission, and ingestion of contaminated foods or drinks.[2] If left untreated, Chagas disease can progress to include serious cardiac and gastrointestinal complications, significantly increasing morbidity and mortality. Chagas is endemic in Latin America, particularly in rural areas of Bolivia, Argentina, Brazil, and Mexico, but migration has introduced cases to North America, Europe, and Asia.[3] Despite its global relevance, Chagas disease remains classified as a neglected tropical disease, disproportionately affecting low-income populations and receiving limited attention from public health initiatives. First described in 1909 by Brazilian physician Carlos Chagas, the disease has since become a persistent public health challenge in affected communities. If left untreated, Chagas disease can progress from an often mild acute phase to a chronic stage associated with serious cardiac and gastrointestinal complications, significantly increasing morbidity and mortality.[4]

Chagas disease progresses through two distinct phases: acute and chronic.[5] The acute phase occurs in the first few weeks after infection and is often mild or asymptomatic. When symptoms do appear, they may include fever, fatigue, and localized swelling at the parasite entry site, sometimes presenting as Romana's sign near the eye.[4] During this period, the parasite circulates in the blood, allowing for early detection through microscopic examination or molecular techniques such as polymerase chain reaction (PCR), which detects circulating parasite DNA, as well as emerging alternatives such as loop-mediated isothermal amplification (LAMP), a rapid and low-resource method with potential for use in decentralized settings. Early treatment with antiparasitic therapy, such as benznidazole or nifurtimox, is highly effective in preventing progression to the chronic phase.[2] The chronic phase is usually asymptomatic, further preventing timely diagnosis. Over time, chronic infection can cause severe cardiac and gastrointestinal complications, including cardiomyopathy, arrhythmias, heart failure, megaesophagus, and megacolon.[5] Serological assays are commonly used for diagnosis during this stage, but treatment effectiveness declines compared with the acute phase, highlighting the critical importance of early intervention.

Cardiac involvement is the most serious manifestation of chronic Chagas disease, an estimated 300,000 to over 1 million people in the United States are infected with chronic Chagas disease, with approximately 30,000 to 45,000 of them developing severe chronic chagasic cardiomyopathy.[1] Persistent parasite activity and immune-mediated inflammation progressively damage the myocardium and disrupt the heart's conduction system.[6] Common electrical abnormalities detectable on electrocardiograms (ECGs) include right bundle branch block (RBBB), left anterior fascicular block (LAFB), and bifascicular block, these abnormalities show that the heart's electrical system has been damaged, which can disrupt normal signaling, cause irregular heartbeats or heart block, and increase the risk of heart failure and sudden cardiac death. Patients may also experience atrioventricular (AV) blocks, sinus node dysfunction, and fragmented QRS complexes, reflecting underlying myocardial fibrosis. Arrhythmias, such as ventricular tachycardia, premature ventricular contractions, and atrial fibrillation, increase the risk of sudden cardiac death. Non-specific ECG changes, including ST-T wave abnormalities, QRS prolongation, and

low-voltage complexes, indicate myocardial damage and disease progression. Early detection of these electrical disturbances is crucial, as they often precede overt heart failure and guide timely intervention and monitoring. Echocardiography complements ECG findings by revealing structural changes, chamber enlargement, and functional impairment. Understanding these cardiac manifestations provides the rationale for focusing on early detection modalities that can prevent progression to severe complications.[7]

This review aims to investigate strategies for the early detection and timely treatment of Chagas disease to prevent severe chronic complications. Specifically, it will examine diagnostic tools that allow identification of infection during the acute phase, including microscopic, molecular, and serological techniques, and assess the effectiveness of early antiparasitic interventions in improving patient outcomes. By highlighting effective early detection methods and treatment protocols, this project seeks to provide actionable insights that could reduce morbidity and mortality, enhance quality of life, and inform public health strategies in at-risk populations. Ultimately, this study emphasizes the critical importance of proactive healthcare measures in managing Chagas disease and preventing its long-term consequences.[5]

METHODS

The systematic review was conducted according to modified PRISMA[8] guidelines. MEDLINE (through pubmed) was searched for the MeSH filters " chagas disease detection and diagnosis." The search strategy included studies published in English from any country between January 1, 2000 and February 1, 2026. Studies that assessed the detection abilities of each modality were included. Case reports, magazines or news articles, COVID-19–SPECIFIC literature and review papers were excluded. Studies with patients younger than 18 years and congenital chagas were also excluded.

Data, including number of participants, gender, mean age, study setting, and modality were extracted. Modalities within each study were then classified under Disease manifestation, pathological testing and immunologic testing. Exclusion and inclusion criteria can be found in **Table 1.**

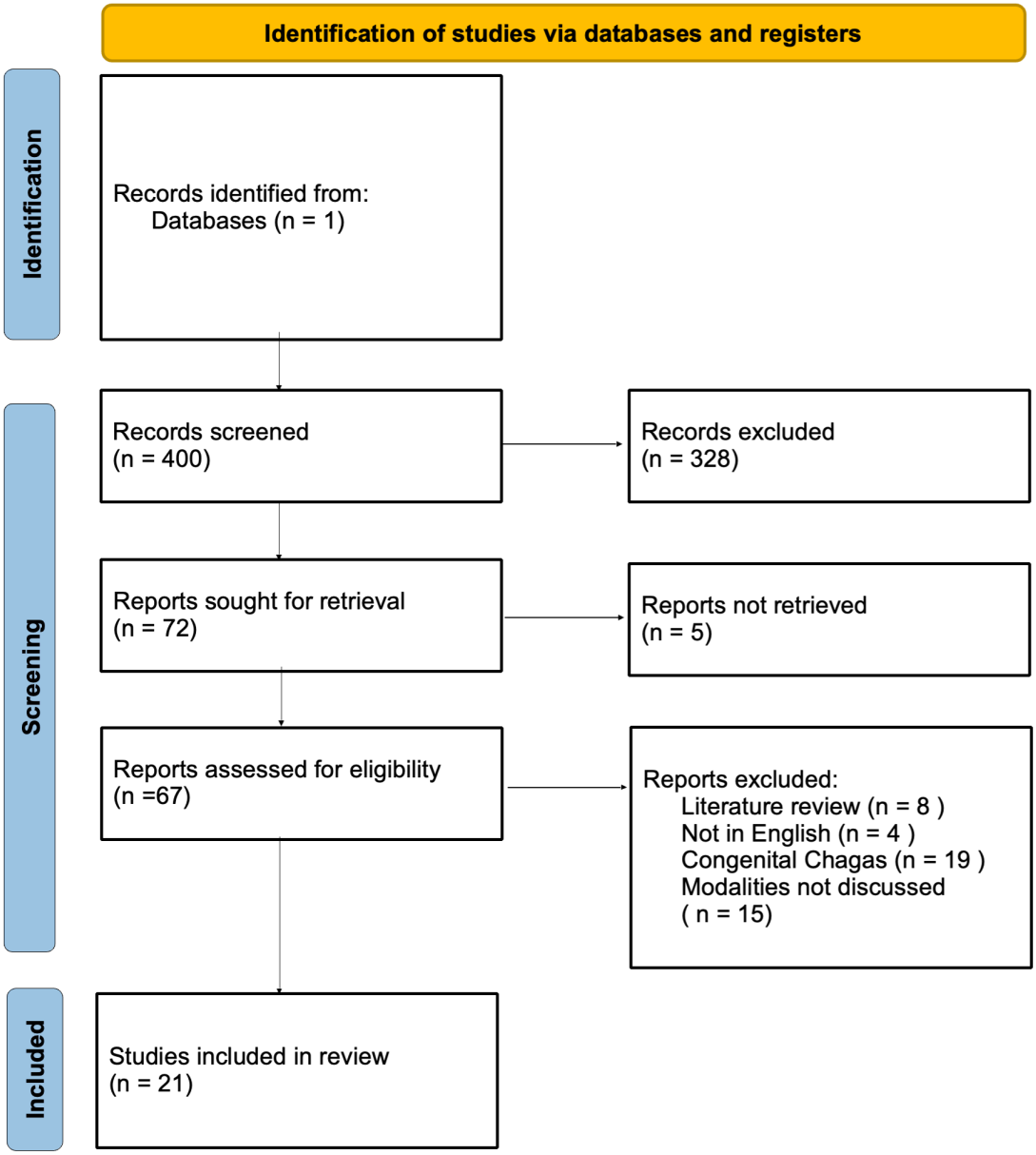
Table 1

Inclusion criteria	Exclusion criteria
People with chagas disease	Case reports, magazine articles, news articles, COVID-19 specific literature
Screening modalities	Youth (under 18 years)
Public healthy interventions	Review papers
Early detection	
Endemic to chagas	

RESULTS

Literature Search and Study Characteristics

The initial search yielded a total of 400 articles. After filtering the papers by the inclusion and exclusion criteria in table 1, 67 articles were assessed for full text review. Twenty one studies were selected for inclusion, corresponding to 3926 study participants from around the world. Most studies were observational studies.


Identification of studies via databases and registers
Identification
Records identified from:
Databases (n = 1)
Screening
Records screened
(n = 400)
Records excluded
(n = 328)
Reports sought for retrieval
(n = 72)
Reports not retrieved
(n = 5)
Reports assessed for eligibility
(n =67)
Reports excluded:
Literature review (n = 8)
Not in English (n = 4)
Congenital Chagas (n = 19)
Modalities not discussed
(n = 15)
Included
Studies included in review
(n = 21)

Pathological testing

Seven studies explore pathological testing as a modality for early detection and diagnosis of Chagas disease.[11, 12, 20, 21, 22, 23, 24] With polymerase chain reaction (PCR) being the most frequently reported technique, appearing in six of these studies.[11,12, 20, 21, 22, 23] Additionally, one study investigated loop-mediated isothermal amplification (LAMP) as a PCR alternative for molecular detection of *Trypanosoma cruzi*.[24] PCR and loop-mediated isothermal amplification (LAMP) were identified as molecular diagnostic techniques used for detection of *Trypanosoma cruzi*. PCR detects *Trypanosoma cruzi* DNA directly in blood or tissue sample by amplifying parasite-specific genetic material during periods of detectable parasitemia, particularly in the acute phase and during reactivation; offering high sensitivity and specificity, particularly during the acute phase when parasitemia is detectable.[20] Several studies emphasize that PCR can identify infection earlier than traditional serological assays, which rely on antibody formation and may only become positive weeks to months after exposure.[20] PCR demonstrated high sensitivity and specificity for detecting active infection, although sensitivity decreased during the chronic indeterminate stage when parasite levels in blood are lower. Serial blood sampling was reported to improve detection yield in these cases.Early detection via PCR is therefore critical for timely initiation of antiparasitic therapy.

Histopathological evaluation provides insight into both detection and disease progression. Cardiac tissue biopsies can reveal parasite nests, inflammatory infiltrates, and fibrosis, demonstrating myocardial damage associated with progression to chronic Chagas cardiomyopathy. correlating with conduction abnormalities such as right bundle branch block, left anterior fascicular block, atrioventricular blocks, and other arrhythmias. These structural and cellular findings not only confirm infection but also elucidate the pathophysiological mechanisms leading to chronic Chagas cardiomyopathy. The combination of histopathology with PCR or serology strengthens diagnostic accuracy, particularly in patients with suspected cardiac involvement.

Immunologic testing

Four studies investigated immunological testing as a modality for early detection and monitoring of Chagas disease,[11, 25, 26, 27] with three of these focusing on enzyme-linked immunosorbent assay (ELISA) techniques.[11, 25, 27] ELISA offers several advantages, including high sensitivity and specificity, the capacity to process large numbers of samples rapidly, and the provision of objective quantitative results.[11,25] ELISA detects host antibodies directed against T. cruzi antigens and is primarily used during the chronic phase of disease, when parasitemia is low and molecular detection becomes less reliable. The specific antigens used in ELISA can significantly influence diagnostic performance. For instance, the combination of FRA

and CRA antigens in a single ELISA assay achieved 100% sensitivity and specificity in the studies reviewed.[25] This represented the highest reported diagnostic performance among the immunological assays included in the review. Additionally, recombinant and multiepitope recombinant proteins, such as TcF, CP1, CP2, TcBDE, and the IBMP series, have been engineered to enhance assay performance, demonstrating exceptional diagnostic capacity by improving antibody recognition and reducing cross-reactivity with non–T. cruzi pathogens in both ELISA and lateral flow format.[25]

Serological testing remains the standard for chronic Chagas disease. Conventional assays, including indirect immunofluorescence assay (IFA), ELISA, and indirect hemagglutination assay (IHA), are widely used.[27] IFA provides high sensitivity by detecting fluorescent antibody binding to parasite antigens under microscopy and is commonly used during chronic infection. It also provides qualitative and quantitative information through immunofluorescence patterns and titers, but it requires an expensive fluorescence microscope, is time-consuming, and depends on operator expertise.[27] ELISA, in addition to its high sensitivity and specificity ,demonstrated greater overall diagnostic reliability compared with IHA due to improved sensitivity and reduced false-positive results. It also allows large-scale testing but necessitates a cold chain, skilled technicians, and specialized equipment. IHA offers relatively rapid results through detection of antibody-mediated red blood cell agglutination, but showed lower sensitivity than ELISA and IFA across reviewed studies without sophisticated equipment, yet it has lower sensitivity compared to ELISA and IFA, is prone to false positives, and also requires careful storage and handling.[27]

Immunological testing is also valuable for monitoring treatment outcomes. Serological evaluation of 94 patients treated with benznidazole at 5 mg/kg for 60 days at least ten years prior showed that 75.5% remained ELISA-positive, 12.8% had results in the gray zone, and 11.7% tested negative.[11] Among positive patients, 23.4% exhibited high reactivity indices (RI > 2), and 52.1% had low reactivity indices ($1.2 \leq RI \leq 2$).[11] These findings indicate persistent antibody responses years after treatment, highlighting the need for sensitive biomarkers to assess disease clearance.

Recent advances have focused on glycan-based biomarkers, such as the neoglycoprotein NGP11b, which consists of bovine serum albumin linked to multiple copies of the Galα1,2[Galα1,6]Galβ glycotope derived from T. cruzi tGPI-MUC.[11] Chemiluminescent ELISA (CL-ELISA) using NGP11b demonstrated strong differential antibody reactivity between Chagas patients and healthy controls, confirming its diagnostic potential.[11] Furthermore, NGP11b shows promise for monitoring serological responses after chemotherapy, as changes in anti-α-Gal antibody levels can indicate treatment efficacy.[11] Ongoing research aims to develop additional glycan-based biomarkers that improve antibody differentiation and extend post-treatment monitoring, enabling more accurate assessment of long-term therapeutic outcomes.[11]

Cardiac Manifestations

Of the 21 included studies, 13 evaluated disease manifestations as a modality for early detection or diagnosis of Chagas disease.[6, 7, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19] Six of the 13 studies evaluated ECG findings in individuals with serologically confirmed Chagas disease.[7,10, 12, 15,18 19] Across these cohorts, cardiac

conduction disturbances were reported among seropositive cases, reflecting early involvement of the cardiac conduction system. The most frequent abnormalities were right bundle branch block (RBBB), followed by left anterior fascicular block (LAFB) and left bundle branch block (LBBB), which are associated with increased risk of arrhythmias, heart block, and progression to Chagas cardiomyopathy. While only a portion of infected individuals develop cardiac disease, ECG abnormalities are highly prevalent among those with cardiac involvement.[7,18,19]

Several studies reported that the combination of right bundle branch block (RBBB) and left anterior fascicular block (LAFB) was the most common conduction pattern observed. In some cases, LAFB was identified through typical ECG features in leads III and aVF, including small R waves and deep S waves. In one large cohort (n = 1910), abnormal ECG findings were present in 93.4% of individuals with Chagas disease.[12] Overall, the presence of ECG abnormalities was associated with a higher likelihood of Chagas disease, although these findings were not specific to the condition.

Where stage distribution was described, conduction abnormalities were more common in early cardiomyopathy compared with indeterminate disease.[7] However, ECG findings were not specific to Chagas disease and were also observed in non-Chagas cardiac populations. Overall, ECG abnormalities increased clinical suspicion but demonstrated variable sensitivity depending on disease stage. Taken together, these findings suggest that ECG has limited value for detecting early infection in the indeterminate stage but is useful for identifying early cardiac involvement.

Four studies assessed conventional Doppler echocardiographic parameters to detect early myocardial involvement.[9,13,16,19] Even in patients with preserved left ventricular ejection fraction, markers of impaired relaxation were identified.[16] Compared with control groups, patients with Chagas disease demonstrated higher peak A velocity (0.55 m/s vs 0.44 m/s), reduced E/A ratio (1.22 vs 1.45), and prolonged deceleration time of early diastolic filling (167.9 ms vs 138.7 ms).[9] These findings indicated impaired ventricular relaxation despite preserved global systolic function. Importantly, diastolic dysfunction was observed before the development of left ventricular dilation, regional wall motion abnormalities, or significant elevation in filling pressures as assessed by E/e' an echocardiographic index of left ventricular filling pressure that can detect early diastolic dysfunction and identify subclinical myocardial involvement before structural heart disease develops.[16] Across studies, these Doppler indices suggested that subclinical myocardial involvement could be detected prior to overt structural remodeling.

Two studies evaluated myocardial deformation using speckle-tracking echocardiography, an imaging technique that measures myocardial strain by tracking tissue motion, in patients without overt systolic dysfunction.[10,14] Even in the absence of reduced left ventricular ejection fraction or detectable fibrosis, regional strain abnormalities were identified.[10] Reduced longitudinal strain was observed in the basal inferior, basal inferoseptal, mid inferoseptal, and mid inferolateral segments.[14] These abnormalities were present despite preserved global systolic performance, indicating early regional myocardial impairment.

Further evaluation of right ventricular performance revealed prolonged isovolumetric relaxation time and increased right ventricular index of myocardial performance, suggesting combined systolic and diastolic dysfunction even in early or indeterminate stages of disease.[10,14] Although STE and advanced right

ventricular indices demonstrated increased sensitivity for detecting subclinical dysfunction, their implementation required specialized equipment and technical expertise, limiting widespread applicability in resource-constrained endemic settings.[10,14]

DISCUSSION

Early detection and diagnosis of Chagas disease relies on multiple diagnostic modalities with effectiveness varying by disease stage.[11, 12, 20, 21, 22, 23, 24] Molecular diagnostics, particularly polymerase chain reaction (PCR), quantitative PCR (qPCR), and loop-mediated isothermal amplification (LAMP), show high diagnostic utility during the acute phase and during reactivation, where parasite DNA is detectable in blood.[22] In contrast, serological assays such as enzyme-linked immunosorbent assays (ELISA) demonstrate high sensitivity and specificity for identifying chronic infection. Cardiac evaluation tools including electrocardiography (ECG), conventional echocardiography, and speckle-tracking echocardiography (STE) identify electrical and functional myocardial abnormalities associated with early Chagas cardiomyopathy, the leading cause of mortality amongst chagas patients.[6 , 7, 9 ,10 ,11 ,12, 13, 14 , 15 , 16, 17 , 18, 19] Among the studies reviewed, ECG abnormalities were observed in approximately,[10] while STE detected early myocardial strain abnormalities of patients without overt structural disease. Collectively, these findings suggest a stage-specific diagnostic approach in which molecular testing is most useful for detecting active infection, while serological and cardiac assessments contribute to identifying chronic disease and early cardiac involvement.

ECG remains one of the most widely available screening tools in endemic settings because it is inexpensive and simple to perform. However, many electrical abnormalities associated with Chagas disease such as bundle branch blocks, conduction delays, and arrhythmias are not specific and may occur in other cardiac conditions. More advanced imaging methods, particularly speckle-tracking echocardiography, provide greater sensitivity for detecting early myocardial dysfunction. Several studies included in this review reported that STE could detect subtle myocardial strain abnormalities before structural changes were visible on conventional echocardiography, suggesting that functional impairment may precede overt cardiomyopathy. Molecular diagnostics provide complementary information by identifying circulating *Trypanosoma cruzi* DNA. PCR demonstrates high sensitivity during acute infection, when parasitemia becomes detectable within the first weeks after exposure, but lower detection rates during the chronic indeterminate stage, when parasite levels in blood are typically low. Serial sampling strategies have been shown to improve detection yield in these cases.

Loop-mediated isothermal amplification (LAMP) represents a promising alternative to conventional PCR for molecular detection of *Trypanosoma cruzi*. Unlike PCR, LAMP operates under constant temperature conditions, enabling rapid amplification and detection of parasite DNA in approximately 30 minutes while requiring minimal laboratory infrastructure. Recent developments targeting the highly conserved heat shock protein 70 (HSP70) gene, present in multiple copies within the parasite genome, have demonstrated strong diagnostic performance, with reported sensitivity of approximately 97% and specificity of 100%. In addition, LAMP assays have shown the ability to detect very low parasite loads, down to a few genomic copies per reaction. These features, combined with substantially lower cost and

reduced equipment requirements, make LAMP particularly suitable for decentralized or near–point-of-care testing in resource-limited endemic settings. However, earlier LAMP assays have reported limitations, including occasional false positives and cross-reactivity with related trypanosomatids, indicating that further validation and standardization are required before widespread clinical implementation. Loop-mediated isothermal amplification (LAMP) has emerged as a potential alternative molecular approach because it can be performed more rapidly and with less complex equipment, making it potentially suitable for decentralized or near–point-of-care testing in endemic regions. Serological assays such as ELISA remain widely used for diagnosing chronic infection because they detect host antibody responses and can process large numbers of samples efficiently. Together, these modalities suggest that early detection of Chagas disease may benefit from a combined diagnostic strategy in which ECG functions as an accessible screening tool, cardiac imaging detects early myocardial dysfunction, and molecular or serological tests confirm infection depending on disease stage.

Implementation considerations are particularly relevant in regions where Chagas disease remains endemic. Many molecular diagnostics require specialized laboratory equipment, trained technicians, and reliable supply chains, which may limit accessibility in rural or resource-limited settings. Echocardiography and speckle-tracking imaging also require trained personnel and advanced equipment that may not be universally available. In settings where STE is not feasible, focused Doppler indices or portable echocardiography systems may provide more pragmatic alternatives for early cardiac evaluation. Screening strategies may also vary depending on the population being tested, including community-based screening in endemic areas, blood donor screening programs, and testing of pregnant individuals to prevent congenital transmission. Integrating these modalities into practical diagnostic pathways may therefore require consideration of local infrastructure, training capacity, and healthcare system resources.

Several strengths support the methodological rigor of this review. A modified PRISMA framework guided the systematic identification and screening of studies,[8] and clear inclusion and exclusion criteria were applied to define the study population and diagnostic modalities of interest. Data extraction was conducted using a structured framework, allowing consistent comparison across multiple diagnostic categories. Physician mentorship also contributed to the interpretation of clinical findings and methodological oversight. Nevertheless, several limitations should be acknowledged. The review relied on a single reviewer for study screening and data extraction, which may introduce selection bias. Language restrictions may have excluded relevant studies published in other languages. Additionally, substantial heterogeneity existed across included studies with respect to diagnostic platforms, assay protocols, and reported thresholds for positive results, limiting direct comparison across modalities. The absence of a formal risk-of-bias assessment tool and the relatively small sample sizes in several studies may further limit generalizability.

Future research should focus on improving both the accuracy and accessibility of early diagnostic strategies for Chagas disease. Large prospective studies are needed to validate emerging tools such as artificial intelligence assisted ECG and advanced echocardiographic techniques in patients with early or indeterminate disease. Standardization of molecular diagnostic protocols, particularly qPCR assays, would help establish consistent detection thresholds and improve comparability across laboratories. Additional research is also needed to evaluate decentralized molecular testing approaches, including

LAMP or portable PCR platforms, in resource-limited endemic settings. Integrated diagnostic algorithms that combine symptom screening, ECG, cardiac imaging, and molecular or serological testing may help optimize stage-specific diagnosis and streamline referral pathways for positive cases. Future work should also evaluate the cost-effectiveness and feasibility of implementing such algorithms in endemic clinics, particularly in populations at increased risk of transmission or disease progression, such as pregnant individuals, transplant recipients, and immunocompromised patients. Strengthening these diagnostic frameworks may improve early detection and reduce the progression to chronic Chagas cardiomyopathy.

CONCLUSION

This review demonstrates that a combination of diagnostic modalities, including molecular, serological, and electrocardiographic methods, contributes to the early detection and diagnosis of Chagas disease. Molecular techniques such as PCR and LAMP are most effective for identifying acute or reactivated infection, while serological assays provide reliable diagnosis in chronic infection. Cardiac evaluation tools, including ECG and echocardiography, support the identification of early myocardial involvement and disease progression. These findings highlight the importance of a stage-specific, integrated diagnostic approach to improve early detection and guide clinical management of Chagas disease.

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