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Perspective Piece

Chagas Disease in the United States: A Perspective on Diagnostic Testing Limitations and Next Steps

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Abstract. Chagas disease is a neglected tropical disease that affects an estimated 300,000 people in the United States. This perspective piece reviews diagnostic challenges and proposes next steps to address these shortfalls.

INTRODUCTION

More than 300,000 individuals in the United States are estimated to have chronic Chagas disease, a neglected tropical disease caused by infection with *Trypanosoma cruzi*.[1,2] Despite the prevalence and substantial long-term sequelae of Chagas disease, providers in the United States have demonstrated limited awareness and comfort with diagnosis and treatment.[3,4] The recent Food and Drug Administration (FDA) approval for benznidazole use in children aged 2–12 years[5] and nifurtimox use in children up to 18 years[6] has improved access to treatment for all age-groups. The question now is whom to test and with which assays. In the following text, we address key limitations and priorities in the field of Chagas disease diagnostics available in the United States, including assays used for acute Chagas disease, chronic Chagas disease, and blood/tissue screening.

DIAGNOSING ACUTE, CONGENITAL, AND REACTIVATED CHAGAS DISEASE

Acute, congenital, and reactivated Chagas disease (in the setting of immunosuppression, HIV infection, or transplantation) is encountered in the United States, although less frequently than chronic Chagas disease. In newborns, a diagnosis of congenital Chagas disease based on umbilical cord blood testing can be established through microscopic examination, PCR testing, and, in some countries, the IgM trypomastigote excreted–secreted antigens (TESA)-blot.[7,8] Each of these methods has been found to have low sensitivity (ranging from 16.7% to 68.7%),[8] whereas specificity in each has exceeded 99%[8]; sensitivity (as measured against a composite of various tests) rose when birth and 1-month peripheral blood tests were combined.[8] Antibody testing should be repeated at 9 months, and if positive, results can be interpreted as congenital Chagas disease. PCR and direct parasitologic examinations are also the modalities of choice for diagnosing acute and reactivated Chagas disease in immunocompromised patients. Of note, among PCR assays, there is considerable variability based on which gene is selected for amplification and other laboratory techniques[9]; a harmonization study of expert laboratories from 16 countries found that the four top-performing PCR assays had a sensitivity between 83 and 94% and specificity of 85–95%[10] compared with consensus results.

DIAGNOSING CHRONIC CHAGAS DISEASE

Chronic Chagas disease, which follows the brief acute phase and is characterized by low levels of parasitemia, poses additional challenges. In chronic disease, PCR has suffered from low sensitivity[11] likely because of low systemic trypomastigote levels. A variety of platforms exist for detecting IgG antibodies based on whole parasite or recombinant antigens, including both commercially available and laboratory-developed tests. These platforms include rapid immunochromatographic assays, immunofluorescent assay, indirect hemagglutination, Western blot, and ELISAs. Currently, four FDA-cleared assays for chronic Chagas disease are available in the United States (ORTHO *T. cruzi* ELISA Test System [Ortho Clinical Diagnostics, Raritan, NJ], Hemagen Chagas' kit [Hemagen Diagnostics, Inc., Columbia, MD], Wiener Chagatest Recombinante v.3.0 [Wiener Laboratories, Rosario, Argentina], and InBios Chagas Detect™ *Plus* Rapid Test [InBios International, Inc, Seattle, WA]).[12–15] Of note, the ORTHO *T. cruzi* ELISA Test System is currently only available for high-volume blood donor screening, although it is also FDA-cleared for patient diagnosis. The U.S. CDC Parasitic Diseases Branch supports commercial laboratories as well as state and local public health laboratories, offering the commercially available Wiener Chagatest Recombinante v.3.0 ELISA and laboratory-developed TESA immunoblot for confirmation of positive screening assays.[16] If these assays result in discordant results, an immunofluorescence assay is performed.

Test performance characteristics of the FDA-cleared assays available in the United States are summarized in Table 1. No single assay has been found to have ideal sensitivity and specificity.[17–22] The InBios Chagas Detect *Plus* Rapid Test assay has a fast turnaround time, and field testing in Bolivia was associated with a sensitivity greater than 99%,[20] but its

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TABLE 1

Characteristics of FDA-cleared assays for Chagas disease

	Testing platform	Antigen protein base	Sample type	Sensitivity performance characteristics from the FDA 510(k)	Specificity performance characteristics from the FDA 510(k)	Sensitivity within a U.S. population based on consensus reference	Specificity within a U.S. population based on consensus reference	Notes on performance characteristics
ORTHO *T. cruzi* ELISA (ORTHO Clinical Diagnostics)	ELISA	Native parasite	Serum and plasma	100% (CI: 96.6–100) compared with IFA (n = 106); 98.9% compared with IFA (n = 1,074)[12]	99.4% (CI: 98.7–99.8) compared with IFA (n = 1,074 patients)[12]	95.3% (CI: 93/0–97.0)[21]	99.7% (CI: 98.3–100.0)[21]	High rate of cross-reactivity with *Leishmania* (79/100 samples from India tested positive)[12]
Hemagen Chagas' kit ELISA (Hemagen Diagnostics, Inc)	ELISA	Native parasite	Serum	100% (CI: 97.7–100) compared with "commercial ELISA" (n = 160)[13]	98.7% (CI: 96.2–99.6) 226/229 compared with "commercial ELISA" comparator test (n = 394 kits)[13]	90.70% (CI: 87.8–93.1)[21]	99.68% (CI: 98.3–100.0)[21]	In the United States, testing has lower sensitivity but higher specificity
Wiener Chagatest Recombinante v.3.0 ELISA (Wiener Laboratories)	ELISA	Recombinant parasite proteins (shed acute-phase antigen)	Plasma or serum	97.9% (CI: 95.6–99.1) compared with IHA, IFA, or ELISA (n = 330)[14]	97.8% (CI: 97–98.5), compared with IHA, IFA, or ELISA (n = 1,507)[14]	96.3% (CI: 94.2–97.8)[21]	98.1% (CI: 95.9–99.3)[21]	There was a recall of this test in 2018 based on device not reaching expiration dates
InBios Chagas Detect *Plus* Rapid Test (InBios International, Inc)	Lateral flow immuno chromato graphic assay	Recombinant parasite proteins	Serum or whole blood (includes finger-prick)	96.6% (CI: 94.5–97.9) for serum, 97.0% (CI: 95.0–98.2) for finger-prick compared with IFA (n = 473)[15] 100.0% (CI: 95.2–100.0) for serum; 98.7% (CI: 93.0–99.8) for finger prick (n = 108 highly endemic Bolivian population)[15]	100% for both serum and whole blood (CI: 98.1–100) (n = 200 non-endemic U.S. population)[15] 87.1% (CI: 71.1–94.9) for serum and 96.8% (CI: 83.8–99.4) (n = 108 highly endemic Bolivian population)[15]	99.2% (CI: 97.9–99.8)[21]	90.5% (CI: 86.7–93.5)[21]	Finger-prick option has potential for screening in settings without trained phlebotomists. Based on U.S. testing, this has lower specificity and higher sensitivity[21]

FDA = Food and Drug Administration; IFA = immunofluorescence assay.

specificity of 90.5% was slightly lower than other assays on a comparative study of test characteristics among blood donors in the United States.[21] The Hemagen Chagas' kit was found to have lower sensitivity of approximately 90.7%,[21] whereas another U.S. study found this test to be associated with the highest sensitivity in a latent class analysis model (100%[22]). The Wiener Chagatest Recombinante v.3.0 ELISA, by contrast, was highly specific in both U.S.-based studies (98.1%[21] or 99.6%[22]) with a slightly lower sensitivity (96.3%[21] or 94.9%[22]). The practice of using a high-sensitivity initial test followed by a high-specificity confirmatory test could be used to optimize diagnostic accuracy for chronic Chagas disease in low-prevalence settings, such as the United States (where programs in Boston[23,24] and Los Angeles County[25] have found a prevalence of 0.9–1.2% among Latinx immigrants).

There are several challenges in comparing the performance characteristics of the different assays. There is no single gold standard reference test for establishing Chagas disease. Studies of sensitivity and specificity use varying comparator assays. Statistical methods such as latent class analysis may assist in interpreting different test performance outcomes but are not immune to bias. In addition, parasitic discrete typing units (DTUs) circulating in Central and North American countries have different antigenic profiles compared with their South American counterparts, which may influence the performance of diagnostic assays.[26,27] Thus, for U.S. immigrant populations from Mexico and Central America and local inhabitants in southern states with potential endemic transmission, commercially available tests that were primarily validated in South America may have important limitations.

Diagnosing Chagas cardiac disease and other end-organ sequelae poses parallel challenges as testing indeterminate Chagas disease. Diagnosis similarly relies on one of the four FDA-cleared assays followed by confirmatory testing usually performed through the CDC. Of note, these tests were not specifically validated among patients with Chagas heart disease or other end-organ sequelae. Pan American Health Organization guidelines define diagnostic standards as consisting of two serologic tests detecting different antibodies, with the use of a third test to resolve conflicting results.[28] However, there are no guidelines on diagnosis of Chagas disease from U.S.-based societies such as the Infectious Diseases Society of America (IDSA), which are more familiar to clinicians in the United States. The current diagnostic process in the United States may be perceived as complicated and resource-intensive.[29]

As a disease associated with rural poverty, Chagas disease poses other diagnostic barriers. Poverty, lack of job security, underinsurance, and discrimination due to immigration status may prevent individuals living with Chagas disease from accessing necessary medical care for diagnosis and consideration of treatment.[30] Testing protocols that require the patient to return for repeat phlebotomy may lead to drop-offs in the care continuum.

SCREENING BLOOD, TISSUE, AND ORGANS

A third major application of diagnostic assays for Chagas disease is donor blood and organ screening for recipient safety. Screening blood component, tissue, and organ donations requires exquisitely high sensitivity to minimize false-negatives and maintain high safety standards and is performed using assays assessed through distinct regulatory pathways in the United States. Two assays (ABBOTT PRISM[31] [Abbott Laboratories, Abbott Park, IL] and ORTHO *T. cruzi* ELISA Test System[32]) are licensed by the FDA for screening blood, blood components, and organ donors. A third FDA-licensed assay (ABBOTT ESA Chagas[33]) can be used for its higher specificity to confirm donor *T. cruzi* serologies. Across evaluations reported in their package inserts,[31–33] these assays have been shown to have high sensitivity and specificity.

DIAGNOSTICS TO ASSESS TREATMENT EFFICACY

Beyond the scope of disease confirmation, assessing response to treatment is problematic.[34] PCR results should turn negative after treatment of infected newborns (although this change may also be a reflection of transition to chronic Chagas disease), and PCR has utility for monitoring acutely infected and immunocompromised patients.[35] Direct microscopic examination of blood or buffy coat smears has shown some utility in identifying treatment failures in patients with congenital Chagas disease, though with lower sensitivity than PCR.[36] A test-of-cure assay for chronic infection, however, does not exist. PCR assays have been used in some treatment trials of chronic Chagas disease to assess response to therapy,[37–39] but systemically circulating trypomastigotes or parasitic DNA is of low yield in blood samples and can be transient, even in the absence of treatment,[40] making PCR invalid as a test of cure.[18,41] Decreased titers of anti–*T. cruzi* antibodies present one option for the assessment of response to therapy in patients with chronic Chagas disease, but decline in titers may take years.[42] Because available medications for treatment (benznidazole and nifurtimox) have poor safety profiles and often necessitate treatment interruption and/or discontinuation,[43–45] a test of cure is needed to help providers assess treatment efficacy and determine optimal treatment duration.

BIOMARKERS

Given the severity of cardiac complications in patients with chronic Chagas disease, there is a need for biomarkers that predict risk of disease progression and whether early-stage cardiomyopathy will worsen. A recently developed rapid diagnostic test (Chagas Sero K-SeT[46]) based on detection of antibodies to a trypomastigote small surface antigen in DTU II/V/VI epitopes may have prognostic value. However, this test is likely to have limited application in the United States where most of the immigrants originate from regions where DTU I dominates.[46] Microarray analysis using a panel of differentially expressed genes represents another potential test for risk stratification among individuals with chronic cardiac disease.[47] Improved understanding of the immunopathogenesis is necessary to enhance our ability to identify patients at risk. To have a meaningful impact on management decisions, potential biomarkers will also need to possess very high negative predictive values.

FUTURE DIRECTIONS

An ideal diagnostic test would have the following test characteristics as outlined by the proposed Target Product Profile of Pan American Health Organization/Medecins Sans

Frontieres/Drugs for Neglected Diseases Initiative/Special Programme for Research and Training in Tropical Diseases.[16] First, a rapid point-of-care test would eliminate diagnostic delay and decrease barriers to patient retention in care. Ideally, the sample would be processed individually, rather than batched. Second, improved sensitivity and specificity are needed compared with conventional assays. Third, low cost would be critical for uninsured individuals. Fourth, the ability to perform confirmatory testing on the same sample would expedite time to diagnosis. Finally, a saliva- or urine-based assay would be ideal to decrease discomfort to patients and reduce risk of needle sticks.

Short-term steps will have a direct impact on future Chagas disease clinical management in the United States. First, community-based studies are needed to define characteristics of existing diagnostics when compared with the best available assays (e.g., consensus determinations, as performed at the CDC[48]), particularly when applied to individuals infected with different DTUs. Second, we propose a renewed focus on and increased funding for new assays to meet the target product profile. Development of new rapid, sensitive, and specific assays could be facilitated by NIH support or potentially a prize competition such as used to develop assays for *chlamydia*.[49] Highly specific prognostic biomarkers that predict development of end-organ disease could reduce unnecessary side effects associated with treatment. Advances in the study of the immune response to other infectious diseases with long latency (e.g., tuberculosis) suggest that predictive biomarkers can be identified.[50,51] To achieve these aims, we propose establishing repositories with samples and associated clinical information (e.g., demographics, EKGs, and echocardiograms) for individuals from a range of endemic countries. In the meantime, improved access is needed for tests with superior test characteristics (e.g., ORTHO *T. cruzi* ELISA).

Clearly, there are barriers to addressing Chagas disease in the United States, but there is also reason for hope. Operating without easy access to effective diagnostics, Chagas disease testing in the United States has been successfully performed in cardiac patients, pregnant women, women of child-bearing age, and communities at large.[52,53] Now, we need to develop and implement more widespread testing for expectant mothers from endemic areas and consider universal testing for Chagas disease among all at-risk immigrants. We applaud the recent NIH meeting "Catalyzing the development of priority diagnostics for Chagas disease" which suggests an interest in addressing this neglected tropical disease and the efforts of the CDC in funding programs to increase awareness about Chagas disease.[54] It is time to harness this momentum.

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