Published in final edited form as:
IEEE Trans Biomed Eng. 2011 March ; 58(3): 814–817. doi:10.1109/TBME.2010.2087334.

A Highly Sensitive Rapid Diagnostic Test for Chagas Disease That Utilizes a Recombinant *Trypanosoma cruzi* Antigen

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Abstract

Improved diagnostic tests for Chagas disease are urgently needed. A new lateral flow rapid test for Chagas disease is under development at PATH, in collaboration with Laboratorio Lemos of Argentina, which utilizes a recombinant antigen for detection of antibodies to *Trypanosoma cruzi*. To evaluate the performance of this test, 375 earlier characterized serum specimens from a region where Chagas is endemic were tested using a reference test (the Ortho *T. cruzi* ELISA, Johnson & Johnson), a commercially available rapid test (Chagas STAT-PAK, Chembio), and the PATH–Lemos rapid test. Compared to the composite reference tests, the PATH–Lemos rapid test demonstrated an optimal sensitivity of 99.5% and specificity of 96.8%, while the Chagas STAT-PAK demonstrated a sensitivity of 95.3% and specificity of 99.5%. These results indicate that the PATH–Lemos rapid test shows promise as an improved and reliable tool for screening and diagnosis of Chagas disease.

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Index Terms

Chagas disease; immunoassay; medical diagnosis; point of care (POC)

I. Introduction

Chagas disease (American trypanosomiasis), caused by infection with the parasite *Trypanosoma cruzi*, is one of the most significant neglected tropical diseases (NTDs) in the developing world [1]. It is found throughout Latin America, and is the NTD responsible for the largest health and economic burden in this region [2]. Chagas disease is primarily spread through parasite-carrying insect vectors, though it is also less frequently transmitted orally, congenitally, or through blood transfusion or organ transplantation [3], [4]. The highest incidence of Chagas disease are located in resource-constrained and rural settings, where a tremendous diversity of parasite reservoirs and vectors combine with insufficient housing conditions to greatly facilitate disease transmission. An estimated 10 million people are currently infected with *T. cruzi* and more than 25 million are at risk of being infected every year according to the World Health Organization (WHO) [5].

Frequently, Chagas disease does not produce immediate or easily observed symptoms in individuals, but over time, untreated Chagas disease can lead to serious cardiac and digestive complications, resulting in loss of productivity and ultimately death. The initial, acute phase of Chagas disease lasts for roughly two months post infection and many individuals have either no symptoms or only mild symptoms during this time. In the acute phase *T. cruzi* parasites can be found circulating in the bloodstream and microscopic identification of parasites is the recommended diagnostic practice. In the subsequent chronic phase, *T. cruzi* parasites primarily sequester in cardiac and digestive tissues, potentially causing gradual but severe damage to organs. Individuals may be asymptomatic, and diagnosis is based on serologic testing. Currently, the WHO recommends the use of two or more serological diagnostic tests for confirmation of infection [6].

Effective drug therapy is available for Chagas, and drugs for treatment are becoming more readily available and affordable in those Latin America countries that have mature Chagas control programs. Studies have shown that drug therapy is effective for use with chronic cases, especially in children and young adults [7], [8]. Unfortunately, case identification of those with *T. cruzi* infection has been severely hindered by a lack of timely, appropriate diagnosis. The gap in availability of diagnostic technologies is most prevalent and problematic in those settings that require them the most, such as rural and urban primary health care centers, where screening campaigns could be effectively implemented.

No combination of serological tests commonly used are appropriate for implementation at the point of care (POC). A number of good enzyme-linked immunosorbent assays (ELISA) are available, which are commonly used in settings where high throughput is needed and skilled laboratory staff and adequate equipment are available, such as screening in blood bank facilities. Many of these ELISA tests show good performance, demonstrating more than 99% sensitivity and specificity. However, ELISA tests are not appropriate to many settings, where diagnostic tools for Chagas are needed, since most Chagas patients live in peri-urban and rural areas that lack access to ELISA technology [9]. The need for an affordable and accessible Chagas diagnostic has been reiterated by every Chagas regional initiative [1], [3], [10]–[14].

One existing and widely used platform that has been successfully, economically, and sustainably used in the developing world is the immunochromatographic strip (ICS) test.

Being also known as lateral flow tests or rapid tests, ICS tests provide POC diagnosis in areas without access to well-equipped and -staffed clinical laboratories. Since they rely on inexpensive, off-the-shelf components and reagents, they can be affordable, in most cases costing less than $2 to the end user, with a cost of goods sometimes near $0.25. They can be formatted for detection of antigens or antibodies (and, more recently, nucleic acids) and are usable with many different specimen types, making them useful for a wide range of applications. ICS strips provide rapid results (typically less than 30 min), require relatively little, and sometimes no, sample processing, and can provide results without use of an external instrument. Additionally, ICS tests can be developed to use samples that are easily obtained in low-resource settings, such as capillary whole blood from finger sticks. Unfortunately, very little investment in the development of ICS tests for Chagas has occurred to date.

The ICS test that has been most significantly evaluated for performance and used in a public health context is the Chagas STAT-PAK by Chembio. The Chembio test shows good specificity (greater than 95%) in a variety of studies [15]–[17]. However, its sensitivity has been shown to be notably inadequate (less than 95%), with especially poor performance observed in whole blood samples [12], [18]. This is a crucial limitation, since a potentially important use for a POC test for Chagas is to accompany screening campaigns and the most essential aspect of these campaigns is to identify as many putative infected individuals as possible. Therefore, a screening test should be as sensitive as possible, ideally greater than 98%, without seriously compromising its specificity to below 95%.

Since very few ICS-based Chagas tests are available, there is still a great need for new and improved rapid tests with high sensitivity. Additionally, current literature suggests that recombinant *T. cruzi* antigens used in serological diagnostic tests can show differing performance depending on the variety of parasitic strains and geographic locations [9], [18]–[21], with some tests performing better than others. This highlights the need for a diversity of rapid test options, which utilize different antigens.

PATH and Laboratorio Lemos have been developing an ICS-based rapid diagnostic test for Chagas disease that could help address the need for improved POC diagnostic tests. We evaluated the performance of the PATH–Lemos rapid Chagas test in a single blind diagnostic comparator study, using clinical samples (n = 375) from a Chagas endemic region, as described in the following.

II. Materials and Methods

A. PATH–Lemos Rapid Test

The PATH–Lemos rapid test is an ICS test for the detection of human antibodies to *T. cruzi*. It is composed of an absorbent pad, a nitrocellulose membrane striped with a control line reagent (0.12 μg goat antimouse Ab, Jackson Immunologicals), a test line reagent (0.32 μg of recombinant *T. cruzi* antigen, Laboratorio Lemos), and a conjugate pad that contains dried colloidal gold detector reagent (see Fig. 1).

The test is performed by adding 125 μL of running buffer (50-mM Tris, 150-mM NaCl, 0.5% BSA, 0.1% Tween-20, 0.15% sodium caseinate, 0.05% NaN3, pH 8.3) and 10 μL of sample (serum or plasma) to a test tube into which a PATH–Lemos rapid test is then placed (see Fig. 2).

As the sample-running buffer mixture flows up the strip via capillary action, it rehydrates the detector reagent, then continues to migrate up the strip across the nitrocellulose membrane, and the detector reagent binds to human immunoglobulins in the sample. If the

sample contains antibodies to *T. cruzi*, the complex binds to the antigens in the test line, producing a red line and indicating a positive result. In the absence of antibodies to *T. cruzi*, no red line will form in the test line area, indicating a negative result.

B. Chagas STAT-PAK Test

The Chagas STAT-PAK (Chembio Diagnostic Systems, Medford, NY) is an ICS test for the detection of anti-*T. cruzi* antibodies in serum, plasma, or whole blood as described earlier [15].

C. Serum Specimens

A total of 375 previously collected and de-identified serum samples from Argentina were provided to PATH by Laboratorio Lemos. The samples were previously characterized by Laboratorio Lemos as reactive (positive) or nonreactive (negative) when tested serologically for antibodies against *T. cruzi* using two ELISA tests (BioMerieux ChagaTek ELISA and Laboratorio Lemos Biozima Chagas recombinant). These samples were characterized as positive, if both ELISA tests gave positive results, and negative, if both ELISA tests gave negative results. A sample with mixed ELISA results would be considered indeterminate. Out of the 375 serum samples, there were 185 samples characterized as negative and 190 samples characterized as positive, and none of them characterized as indeterminate. Upon receipt at PATH, all samples used in this evaluation were further blinded using a randomization key by staff uninvolved in the Chagas laboratory testing activities at PATH.

D. Evaluation Method

The samples were independently run on three separate assays: the Chagas STAT-PAK (Chembio), the PATH–Lemos rapid test, and the Ortho *T. cruzi* ELISA (Johnson & Johnson), an FDA-approved test for Chagas disease (see Fig. 3).

The Ortho ELISA was run according to the manufacturer's instructions. The Chagas STAT-PAK tests were run according to the manufacturer's instructions and independently interpreted by three readers at the standard 15-min time point and at a 20-min time point (which has been indicated to improve performance of the Chagas STAT-PAK test) [12]. The PATH–Lemos rapid test was run, and the results were independently interpreted by three readers at 15, 20, and 25 min from the start of the test.

III. Results

Quantitative absorbance values obtained from the Ortho *T. cruzi* ELISA were interpreted according to the manufacturer's instructions, and samples were classified as positive or negative. These results from the Ortho *T. cruzi* ELISA were then compared to the earlier sample classifications provided by Lab Lemos. Agreement between the Ortho *T. cruzi* ELISA results and results from Lab Lemos was 100% (185/185 negative samples, 190/190 positive samples).

The results from the Chagas STAT-PAK and PATH–Lemos rapid test were compared between the three independent readers for all read times, and agreement between readers was calculated (see Table I). If there was a discordant result between readers, the result with the majority interpretation (i.e., two out of three results) was considered the true result.

These results were then compared against results from the Ortho *T. cruzi* ELISA, and sensitivity and specificity were calculated (see Table II).

IV. Conclusion

The results of this evaluation indicate that the PATH–Lemos rapid test has great potential as a new and improved diagnostic test for Chagas disease. The sensitivity and specificity of the PATH test at the 20-min interpretation time (99.5% and 98.6%, respectively) is especially notable. Interpreting the test at the later time point of 25 min decreased the test performance, suggesting that read time is crucial.

The agreement between the readers was slightly better for the Chagas STAT-PAK test than for the PATH–Lemos test. This may be attributable to different manufacturing standards used for producing the two tests. The Chagas STAT-PAK test is a commercially available product manufactured under quality-controlled conditions, while the PATH–Lemos rapid test is currently a prototype test constructed on a small scale (batches of less than 500 tests) for research use. Further refinement of the PATH–Lemos rapid test may be needed to ensure improved reproducibility.

Greater availability of rapid tests for Chagas could dramatically improve the way public health systems battle the disease. Its utility fits with nascent strategies in all aspects of Chagas control—serological surveillance and targeted vector control, opportunistic case detection in primary health care (PHC) and secondary health systems, and in antenatal screening. The impact of new, highly sensitive POC tests would be notable in the increase in the number of Chagas cases detected in areas where the diagnostic is introduced.

Chagas disease afflicts the poorest, usually rural communities that are often served by the PHC system, where diagnostics that require more than the most basic of laboratory capabilities are not practical. While other, more recently developed platforms may have advantages over the immunochromatography format in terms of sensitivity and specificity, for many applications, including Chagas, cost, convenience, and simplicity, advantages generally outweigh them from a public health perspective. The ability of trained community health workers to utilize a rapid test has had remarkable success in case detection for HIV and for malaria clinical management throughout resource–poor communities around the globe. In much the same way, the provision of a rapid diagnostic test for Chagas will empower the PHC systems to identify and manage Chagas in those communities traditionally left without health services through outreach screening campaigns with a highly sensitive ICS test.

Additional development and validation of the PATH–Lemos rapid test is needed before it can be manufactured for use in clinical applications. Efforts are currently underway at PATH to continue development of the test and to add features that will make the test compatible with whole blood samples.

Acknowledgments

This work was supported by the Health Innovation Portfolio through funding from private foundations and individual donors.

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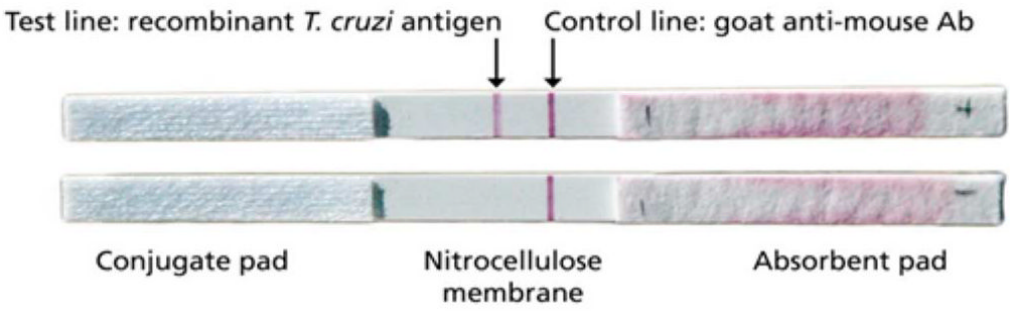

Fig. 1.
Examples of the PATH–Lemos rapid test, showing positive (two line, above) and negative (one line) results.

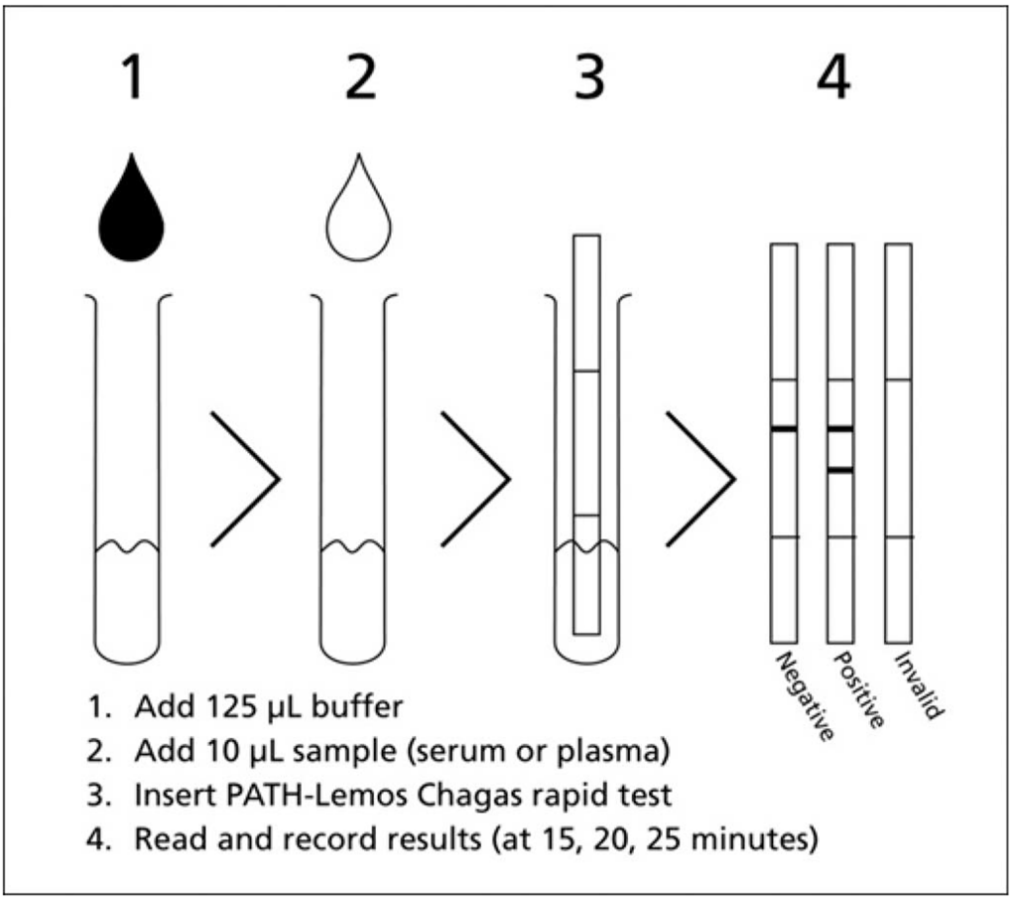

Fig. 2.
Illustration of the PATH–Lemos rapid test procedure.

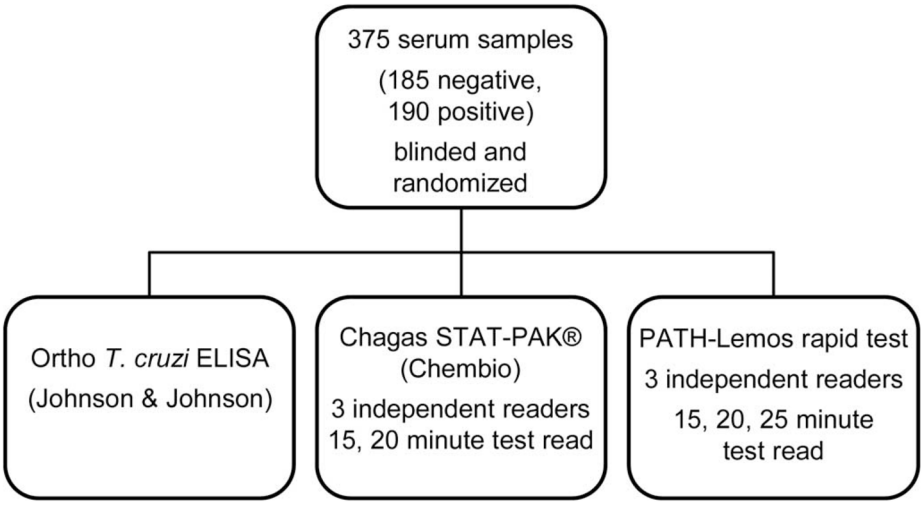

Fig. 3.
Evaluation method using three separate assays.

TABLE I

Agreement Between Three Independent Readers for the Chagas STAT-PAK and PATH–Lemos Rapid Test

Test Description	Time Read (min)	Kappa value (95%CI) N=375
Chagas STAT-PAK®	15	0.975 (0.961–0.979)
	20	0.975 (0.968–0.989)
PATH-Lemos rapid test	15	0.900 (0.872–0.922)
	20	0.900 (0.865–0.931)
	25	0.889 (0.861–0.893)

TABLE II

Performance of the Chagas STAT-PAK and PATH–Lemos Rapid Test Compared to Ortho *T. cruzi* Elisa

Test Description	Time Read (min)	Sensitivity (95%CI)	Specificity (95%%)
Chagas STAT-PAK®	15	181/190 95.3% (92.3–98.3)	184/185 99.5% (98.5–100)
	20	182/190 95.8% (92.9–98.7)	184/185 99.5% (98.5–100)
PATH-Lemos rapid test	15	186/190 97.9% (95.3–99.7)	178/185 96.2% (93.4–99.0)
	20	189/190 99.5% (98.7–100)	179/185 96.8% (94.3–99.3)
	25	188/190 98.9% (97.4–100)	174/185 94.0% (90.1–97.4)