Evaluation of two lateral flow rapid tests in the diagnosis of Chagas disease in the Washington Metropolitan Area

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ABSTRACT

We compared the accuracy of the Stat-Pak and Chagas Detect Plus to a Latent Class Analysis. A sensitivity of 89.7% and 91.9% and specificity of 97.1% and 80.3%, respectively was seen in the serodiagnosis of Chagas disease in Hispanic immigrants, revealing the limitations of these tests in diverse populations.

Keywords: Lateral flow assay; serodiagnosis; Chronic Chagas disease; Trypanosoma cruzi; Latin American immigrants
The diversity of Hispanics in the U.S. from varying Chagas endemic countries provides challenges in the diagnosis of *Trypanosoma cruzi* infection [1,2]. The anti-*T. cruzi* antibody levels and sensitivity detected by Food and Drug Administration (FDA)-cleared tests are lower in Mexicans and Central Americans as compared to South Americans [1,2]. These variations align with the geographic distribution of *T. cruzi* Discrete Typing Units (DTUs) [2] with TcI being predominant in Mexico, Central America, and northern South America and TcII/V/VI in southern South America [3].

The inadequate specificity of some FDA-cleared tests leads to a high ratio of false-positives to true-positives in this low prevalence population [1]. These limitations create the need for further follow-up in this population that is mainly uninsured and are usually taken care of by non-profit organizations or community clinics.

The use of rapid tests that can be performed in a community setting facilitates the detection and follow-up of hard-to-reach populations. Most rapid tests are performed in 15-20 minutes, so potentially infected individuals can be informed of their results during their first contact, which increases adherence to follow-up testing of rapid test positive individuals [4]. Since the World Health Organization (WHO) recommends the use of at least two different tests, researchers in low-resource settings have suggested the parallel use of two rapid tests based on different antigens to facilitate detection [5].

The Chagas Detect Plus (CDP, InBios International, Inc, WA), is based on a multiepitope fusion antigen ITC 8.2 (TcF, SAPA, Pep30, Pep36, KMP11, KMP11, Pep1), and was cleared by the FDA in 2016. Studies in the U.S. suggest that CDP has suboptimal specificity but adequate sensitivity (≥ 97.0%), even for infections acquired in Mexico and Central America [1].

The Stat-Pak (Chembio Diagnostic Systems, NY) is not FDA-cleared but has been extensively evaluated in southern South America [6]. The assay uses recombinant proteins (B13, 1F8, and H49/JL7). In a meta-analysis, Stat-Pak sensitivity in Bolivians was 97.0% (95% CI: 87.6–99.3%) and specificity was 99.4% (98.6–99.8%) when compared to at least two different diagnostic tests [6]. Studies in TcI-predominant areas found sensitivity range from 95.0% to 100.0% in repository samples.
from Central Americans [7,8] and 62.5% in umbilical cord blood samples in Mexico [9], but evaluations were predominantly in comparison to only one reference test [7,9].

Due to the observed geographic variability in test performance likely due to the differences in the geographic distribution of *T. cruzi* DTUs, we conducted this evaluation to generate more evidence of test accuracy in at-risk immigrants. To determine if the use of two different rapid tests can facilitate early diagnosis as recommended by the WHO, we evaluated the CDP and the Stat-Pak in serum samples of Latin Americans living in the Washington Metropolitan Area (WMA).

**METHODS**

A random sample of seronegatives (n=350) was selected from a cross-sectional study that enrolled healthy Hispanic adults from any of the 21 Chagas endemic countries via recruitment in churches, community centers, consulate events, and health fairs in the WMA [2]. Individuals were classified as: being from TcI-predominant (Mexicans and Central Americans, n=16) and TcII/V/VI-predominant areas (Bolivians, n=21) [3]. Asymptomatic Latin American immigrants from any of the 21-Chagas endemic countries were enrolled (Supplementary methods) [2].

The two rapid tests were compared against the conditional probability of class membership to determine Chagas status using a modified version of a Latent Class Analysis (LCA) previously described [2] (Supplementary methods and Table S1). The modified LCA used area of origin and the results of two FDA-cleared immunoassays: Hemagen Chagas kit (Hemagen Laboratories, MD), Chagatest recombinant v.3.0 (Wiener Laboratories SAIC, Argentina), and the IgG-TESA-blot (a western blot that uses the Trypomastigote Excretory-Secretory Antigen) (Supplementary methods and Table S2) [2,10,11]. The three assays are based on different antigens and were run in parallel on all samples (Table S3). The statistical software Mplus Version 8 (Muthén & Muthén, Los Angeles, CA) was used for LCA.

The assays were read by two observers blinded to each other’s reading and the LCA specimen classification. The weighted kappa index comparing positive and negative results between the two
readers was 0.87 (p<0.001) for the CDP and 0.89 (p<0.001) for the Stat-Pak. Samples with any reactions in the test line were considered positive.

The band intensities were recorded at the end of the incubation time by the two observers using an intensity card elaborated by our research group (Supplementary Figure 1), the mean value of band intensities was used for future analysis. To determine time-to-positive, results were also read every five minutes after the addition of the sample and reagents and until the incubation time (15 and 20 minutes for the Stat-Pak and the CDP, respectively).

The sensitivity, specificity, and Youden Index (YI) of the test were calculated using 2x2 tables against the results of the LCA. The chi-square test and the Kruskal Wallis test were used to determine statistical associations. We used receiver operating characteristic curve (ROC) to determine cutoffs of band intensities that provide the highest YI. A two-sided p-value of < 0.05 was considered significant. These statistical analyses were done using Stata version 15 (Stata Corp, TX).

Patient Consent Statement

The Institutional Review Board of the Johns Hopkins School of Public Health approved the protocol. All participants provided written informed consent.

RESULTS

CDP sensitivity was lower in TcI-predominant (87.5%) compared to TcII/V/VI-predominant (95.2%, p=0.39) areas, but the difference was not statistically significant, which could be due to the small number of seropositives. CDP specificity was low in both endemic regions (80.3%, 241/300). Significantly lower Stat-Pak sensitivity was observed in TcI-predominant compared to TcII/V/VI-predominant areas (75.0% vs. 100.0%, p=0.03) (Table 1).

False-positives in the two rapid tests had higher time-to-positive and lower band intensities compared to true-positives. No differences were observed in time-to-positivity and band intensities between seropositives in TcI-predominant and TcII/V/VI-predominant areas in either of the two tests, possibly because our seropositives from TcI-predominant areas were mainly Central Americans, where reactivity seems to be lower than South Americans but higher than Mexicans [1].
A very low YI was observed for the Stat-Pak in TcI-predominant areas (YI at cutoff ≥ 2 in TcI-predominant: 0.77 vs. TcII/V/VI-predominant areas: 0.99) (Table 2).

DISCUSSION

CDP specificity in serum was lower in our study than previously reported in U.S. blood donors (UBD) (87.5-92.3%) [1]. A high CDP sensitivity (≥97.0%) without significant differences among Mexicans, Central or South Americans was reported in UBD [1]. The CDP sensitivity in serum was also lower than our estimate (94.7%, 95% CI: 92.4-95.0) in whole blood in community settings [2]. A study in Bolivia also showed slightly lower CDP specificity in serum than whole blood (96.9%, 95% CI:94.2-98.6% vs. 98.8%, 95% CI:95.9-99.9%) [12]. Serum samples are evaluated under controlled laboratory conditions where health workers may be more likely to observe faint bands. Health workers in areas such as Bolivia where high antibody levels prevail [2] might be accustomed to high band intensities and more likely to miss faint bands, resulting in higher specificities in TcII/V/VI-predominant countries. However, a recent study in Bolivia demonstrated suboptimal specificity (91.9%, 95% CI: 88.6–94.5), similar to the current data as well as the UBD evaluation [13]. The differences in the results of the CDP specificity between oldest and recent publications in Bolivia could be due to the differences in the methodology between studies or differences in the performance of the test over time.

The low Stat-Pak sensitivity is similar to the one reported in Mexicans when the test was compared only to the Chagatree recombinant v3.0 [9]. The Stat-Pak specificity is comparable with the one calculated in a meta-analysis in Bolivians (99.4%, 95% CI: 98.6–99.8). However, cross-reactions of the Stat-Pak with leishmaniasis (22.2%, 2/9) and hepatitis B (18.2%, 2/11) have been reported [8]. Assay validation only in TcII/V/VI-predominant areas, where most seropositives have high antibody levels may lead to the use of low antigen concentrations that are enough to achieve good sensitivity and specificity in TcII/V/VI-predominant but when the test is used in TcI-predominant areas a lower sensitivity could be observed without affecting the specificity.

One limitation of this study is the small sample size of seropositive samples, especially from Mexicans, where most patients have low antibody levels [1], this could result in even lower assay
sensitivity. Our results cannot conclude that test performance is due to the differences in geographic
distribution of DTUs because we have not conducted genotyping analysis.

While the Stat-Pak had high specificity, its variable sensitivity limits its utility for screening in
community settings with highly diverse populations. However, its accuracy is high in TcII/V/VI-
predominant areas where its use could be justified. Conversely, the low specificity of the CDP in
serum samples leads to a high ratio of false positives to true positives in both areas. For both rapid
tests, standardized intensity scoring and validation of a borderline category for low intensity scores
could provide a better balance of sensitivity and specificity; however, the need for a second test to
confirm or refute borderline results will remain (Table 2). Effective screening of low prevalence
populations will require availability of assays with better performance characteristics.
Conflict of Interest

YECS reports non-financial support from InBios International Inc. during the conduct of the study and outside the submitted work. CB reports grants from Mundo Sano Foundation, personal fees from UpToDate, both outside the submitted work. RHG reports grants, non-financial support and other from InBios International during the conduct of the study and outside the submitted work. All other authors report no potential conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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Table 1. Performance of the two lateral flow assays, Chagas Detect Plus (InBios International) and Stat-Pak (Chembio Diagnostic Systems), for the diagnosis of chronic Chagas disease in two endemic areas.

|                      | Both areas | TcI-predominant | TcII/V/VI-predominant | P-value (TcI- vs. TcII/V/VI-predominant) |
|----------------------|------------|-----------------|------------------------|-----------------------------------------|
| **CDP**              |            |                 |                        |                                         |
| Sensitivity %, (n/N) | 91.9 (34/37) | 87.5 (14/16)\(^a\) | 95.2 (20/21)\(^b\) | 0.39                                    |
| Specificity %, (n/N) | 80.3 (241/300) | 79.8 (142/178) | 81.2 (99/122) | 0.77                                    |
| **Time-to-positive, minutes** |            |                 |                        |                                         |
| Seropositives, median (IQR) | 5.0 (5.0-5.0) | 5.0 (5.0-5.0) | 5.0 (5.0-5.0) | 0.35                                    |
| False positives, median (IQR) | 10.0 (5.0-10.0) | 10.0 (5.0-10.0) | 10.0 (5.0-10.0) | 0.93                                    |
| P-value (Difference between seropositives and false positives) | \(<0.001\) | 0.010 | \(<0.001\) | -                                       |
| **Band intensity, score** |            |                 |                        |                                         |
| Seropositives, median (IQR) | 5.0 (4.0-6.0) | 4.0 (3.0-5.5) | 5.5 (4.8-6.0) | 0.02                                    |
| False positives, median (IQR) | 2.0 (1.0-2.5) | 2.0 (1.0-2.3) | 2.0 (1.5-3.0) | 0.23                                    |
| P-value (Difference between seropositives and false positives) | \(<0.001\) | \(<0.001\) | \(<0.001\) | -                                       |
| **Stat-Pak**         |            |                 |                        |                                         |
| Sensitivity %, (n/N) | 89.7 (26/29) | 75.0 (9/12)\(^c\) | 100.0 (17/17)\(^b\) | 0.03                                    |
| Specificity %, (n/N) | 97.1 (340/350) | 96.2 (230/239) | 99.1 (110/111) | 0.13                                    |
| **Time-to-positive, minutes** |            |                 |                        |                                         |
| Seropositives, median (IQR) | 5.0 (5.0-5.0) | 5.0 (5.0-5.0) | 5.0 (5.0-5.0) | 0.49                                    |
| False positives, median (IQR) | 7.5 (5.0-15.0) | 5.0 (5.0-15.0) | 15.0 (15.0-15.0) | 0.25                                    |
| P-value (Difference between seropositive and false positives) | \(0.03\) | 0.29 | \(0.02\) | -                                       |
| **Band intensity, score** |            |                 |                        |                                         |
| Seropositives, median (IQR) | 4.0 (3.5-4.5) | 3.5 (2.5-4.0) | 4.0 (3.5-5.0) | \(<0.01\)                               |
| False positives, median (IQR) | 2.0 (1.5-2.0) | 2.0 (1.5-2.0) | 2.0 (2.0-2.0) | 0.85                                    |
| P-value (Difference between seropositive and false positives) | \(<0.001\) | \(0.005\) | 0.09 | -                                       |

CPD: Chagas Detect Plus, InBios International. IQR: Interquartile range. Bold values denote statistical significance.

a) Participants in this group were from: El Salvador (n=11), Guatemala (n=3), Honduras (n=1), and Mexico (n=1).

b) All participants in this group were from Bolivia.

c) Participants in this group were from: El Salvador (n=7), Guatemala (n=3), Honduras (n=1), and Mexico (n=1).
Table 2. Sensitivity, Specificity, and Youden Index of different cutoffs of band intensities observed with the two rapid tests (Chagas Detect Plus and Stat-Pak) in the two geographic areas.

| Cutoff* | Chagas Detect Plus | Stat-Pak | Stat-Pak |
|---------|-------------------|----------|----------|
|         | Sensitivity | Specificity | Youden Index | Sensitivity | Specificity | Youden Index |
| Both areas, n=335. | ROC: 0.94 (95% CI: 0.88-0.99) | ROC: 0.96 (95% CI: 0.91-1.00) |  |
| (≥1) | 0.92 | 0.82 | 0.74 | 0.93 | 0.93 | 0.87 |
| (≥2) | 0.89 | 0.89 | 0.78 | 0.93 | 0.96 | 0.89 |
| (≥3) | 0.86 | **0.96** | **0.82** | 0.79 | 1.00 | 0.79 |
| (≥4) | 0.69 | 0.99 | 0.68 | 0.52 | 1.00 | 0.51 |
| (≥5) | 0.53 | 1.00 | 0.52 | 0.17 | 1.00 | 0.17 |
| (≥6) | 0.33 | 1.00 | 0.33 | 0.10 | 1.00 | 0.10 |
| TcI-predominant areas, n=193 | ROC: 0.90 (95% CI: 0.80-1.00) | ROC: 0.90 (95% CI: 0.88-1.00) |  |
| (≥1) | 0.87 | 0.81 | 0.68 | 0.83 | 0.91 | 0.74 |
| (≥2) | 0.87 | **0.89** | **0.75** | 0.83 | **0.94** | **0.77** |
| (≥3) | 0.80 | **0.96** | **0.76** | 0.50 | 0.99 | 0.49 |
| (≥4) | 0.47 | 0.99 | 0.46 | 0.25 | 0.99 | 0.24 |
| (≥5) | 0.27 | 0.99 | 0.26 |  |
| (≥6) | 0.20 | 1.00 | 0.20 |  |
| TcII/V/VI-predominant areas, n=142 | ROC: 0.96 (95% CI: 0.90-1.00) | ROC: 1.00 (95% CI: 1.00-1.00) |  |
| (≥1) | 0.95 | 0.83 | 0.78 | 1.00 | 0.97 | 0.97 |
| (≥2) | 0.90 | 0.88 | 0.79 | 1.00 | **0.99** | **0.99** |

* Cutoffs: (≥1), (≥2), (≥3), (≥4), (≥5), (≥6)
|   |   |   |   |   |   |
|---|---|---|---|---|---|
|   | 0.90 | 0.95 | 0.86 | 1.00 | 1.00 |
| (≥3) |   |   |   |   |   |
| (≥4) | 0.86 | 0.99 | 0.85 | 0.71 | 1.00 |
| (≥5) | 0.71 | 1.00 | 0.71 | 0.29 | 1.00 |
| (≥6) | 0.43 | 1.00 | 0.43 | 0    | 1.00 |

ROC: Receiving Operating Characteristics.

95% CI: 95% Confidence Interval.

Bold values denote the cutoff point with the highest Youden index.

a) The cutoff values represent the score of band intensity in the test line using our band intensity card.