AMERICAN SOCIETY FOR MICROBIOLOGY | Journal of Clinical Microbiology®

LETTER TO THE EDITOR

Concordance of Results by Three Chagas Disease Antibody Assays in U.S. Clinical Specimens

Matthew S. Moser,[a] Charles J. Fleischmann,[a] Emily A. Kelly,[b] Harry E. Prince,[c] Caryn Bern,[d] Jeffrey D. Whitman[a]

[a]Department of Laboratory Medicine, University of California, San Francisco, San Francisco, California, USA
[b]School of Medicine, University of California, San Francisco, San Francisco, California, USA
[c]Quest Diagnostics, San Juan Capistrano, California, USA
[d]Department of Epidemiology and Biostatistics, University of California, San Francisco, San Francisco, California, USA

Matthew S. Moser and Charles J. Fleischmann contributed equally. Author order was determined by the contribution to manuscript draft.

KEYWORDS Chagas disease, ELISA, serology, *Trypanosoma cruzi*, immunoassays

Confirmed diagnosis of chronic *Trypanosoma cruzi* infection requires positive results by two distinct serological tests (1, 2). Test performance varies by assay and geographic origin of the infection (3–5). Previously, we reported the performance of the four Chagas disease (CD) immunoassays cleared by the US Food and Drug Administration (FDA) (3). That evaluation was performed on plasma aliquots from U.S. blood donations, which are a different matrix from clinical serum specimens. Additionally, blood donors tend to be younger and healthier than clinical populations; both of these these factors could affect real-world test performance. Here, we present an updated evaluation of two previously assessed CD diagnostic assays and one novel CD assay in serum samples from a commercial reference laboratory that may better reflect clinical populations in the United States.

Deidentified remnants of serum samples were provided by Quest Diagnostics; the sera had previously undergone routine clinical screening by Chagatest Recombinante v3.0 (Wiener Lab Group, Rosario, Argentina). This research was approved by the University of California, San Francisco (UCSF) institutional review board (Human Research Protections Program). The study set was collected in 2021 (n = 144); no clinical data were associated with the specimens. Samples were frozen at −70°C and shipped to the UCSF for research testing. At UCSF, samples were stored at −20°C for 6 months prior to testing, thawed at room temperature (one freeze-thaw cycle), and run by three anti-*T. cruzi* serological assays, namely, Wiener Chagatest v.3.0 enzyme-linked immunosorbent assay (ELISA; Wv3), Chagas' kit ELISA (Hemagen Diagnostics, Columbia, MD), and the novel Chagas *Detect* Fast ELISA (CDF; InBios International, Seattle, WA), currently developed as a research use only kit (see companion submission, reference 6).

Testing was performed following manufacturer instructions using a PhD Ix automated ELISA system (Bio-Rad, Hercules, CA) for Hemagen and Wv3 kits, while a BioTek ELx50 auto strip washer (BioTek, Winooski, VT) and a Victor X4 multilabel plate reader (PerkinElmer, Waltham, MA) was used for the InBios CDF kit. Wv3 and Hemagen assays provided cutoff calculations from internal controls, while the InBios CDF assay cutoff was determined from a separate study (6). A seropositive concordance status (≥2 positive/reactive assays) was determined based on the algorithm in published recommendations (1, 2). Samples with indeterminant results (±10% of test cutoff) were counted as positive in analyses, because this result in clinical settings would prompt further testing. Data analysis was performed using STATA 14.2.

For each assay, we calculated positive and negative percent agreement with concordance status (Table 1). Agreement was highest for Wv3 and InBios CDF assays, with performance similar to that reported in U.S. blood donor specimens (3, 6). The Hemagen assay showed lower positive and negative agreement than the other assays, suggesting a difference in overall

Editor Bobbi S. Pritt, Mayo Clinic Minnesota

Address correspondence to Jeffrey D. Whitman, jeffrey.whitman@ucsf.edu.
The authors declare no conflict of interest.

For a companion article on this topic, see https://doi.org/10.1128/JCM.01762-22.

Published 28 February 2023

TABLE 1 Test performance based on concordance status of three assays[a]

Assay	No. of samples positive and concordant	Positive % agreement[b] (95% CI)	No. of samples negative and concordant	Negative % agreement[c] (95% CI)
Hemagen	57[d]	91.9 (82.1, 97.3)	79	96.3 (89.7, 99.2)
InBios CDF	60[d]	96.8 (88.8, 99.6)	81	98.8 (93.4, 100.0)
Wiener v3	60[d]	96.8 (88.8, 99.6)	80	97.6 (91.5, 99.7)

[a]A set of 144 samples was provided and analyzed using three CD diagnostic kits. Indeterminant results, which were ±10% of the associated cutoff for each assay, were included as positives. Binomial exact confidence intervals were used.
[b]A Total of 62 samples were positive by consensus of two or more assays with positive or indeterminant results.
[c]A total of 82 samples were negative by consensus of two or more assays with negative results.
[d]All three assays had one indeterminate result each, which were included as positives.

performance from that seen in our previous study; in that evaluation, the Hemagen assay showed lower sensitivity than other assays but had high specificity.

This study is limited by sample selection screening by Wv3 and the lack of a true gold standard for chronic CD diagnosis. Specimen selection using the Wv3 test could overestimate Wv3 test performance, while the lack of a gold standard diagnostic limits our analyses to "agreement" statistics between assays.

Currently, the Centers for Disease Control and Prevention (CDC) Division of Parasitic Diseases and Malaria laboratory is the main source of confirmatory CD testing for U.S. patients. As more CD tests become available, reference or hospital clinical laboratories will gain the ability to perform multistep confirmatory testing based on current recommendations (1, 2). Understanding individual assay performance is essential for designing effective multistep testing algorithms. An insensitive screening test will miss infections, while a test with low specificity will result in a high burden of false-positive results. This study complements our previous donor study with data from clinical specimens from a reference laboratory. Prospective clinical studies with adequate statistical power are needed to provide the best reflection of real-world performance of individual tests and multistep algorithms in U.S. populations at risk of Chagas disease.

ACKNOWLEDGMENTS

This study was supported by miscellaneous departmental funds from J.D.W.

We thank InBios International for the donation of Chagas Detect Fast test kits used in this study.

Only the authors had a role in study design, specimen collection, analysis and interpretation of the data, and the decision to submit for publication.

REFERENCES

1. Pan American Health Organization. 2019. Guidelines for the diagnosis and treatment of Chagas disease. https://iris.paho.org/bitstream/handle/10665.2/49653/9789275120439_eng.pdf?sequence=6&isAllowed=y. Accessed April 18, 2022.
2. Forsyth CJ, Manne-Goehler J, Bern C, Whitman J, Hochberg NS, Edwards M, Marcus R, Beatty NL, Castro-Sesquen YE, Coyle C, Stigler Granados P, Hamer D, Maguire JH, Gilman RH, Meymandi S. 2022. Recommendations for screening and diagnosis of Chagas disease in the United States. J Infect Dis 225:1601–1610. https://doi.org/10.1093/infdis/jiab513.
3. Whitman JD, Bulman CA, Gunderson EL, Irish AM, Townsend RL, Stramer SL, Sakanari JA, Bern C. 2019. Chagas disease serological test performance in U.S. blood donor specimens. J Clin Microbiol 57:e01217-19. https://doi.org/10.1128/JCM.01217-19.
4. Castro-Sesquen YE, Saldana A, Patino Nava D, Bayangos T, Paulette Evans D, DeToy K, Trevino A, Marcus R, Bern C, Gilman RH, Talaat KR, Chagas Working Group in Peru and the United States. 2021. Use of a latent class analysis in the diagnosis of chronic Chagas disease in the Washington Metropolitan Area. Clin Infect Dis 72:e303–e310. https://doi.org/10.1093/cid/ciaa1101.
5. Truyens C, Dumonteil E, Alger J, Cafferata ML, Ciganda A, Gibbons L, Herrera C, Sosa-Estani S, Buekens P. 2021. Geographic variations in test reactivity for the serological diagnosis of Trypanosoma cruzi infection. J Clin Microbiol 59:e0106221. https://doi.org/10.1128/JCM.01062-21.
6. Moser MS, Fleischmann CJ, Kelly EM, Townsend RL, Stramer SL, Bern C, Whitman JD. 2023. Evaluation of InBios Chagas Detect Fast, a novel enzyme-linked immunosorbent assay for the detection of anti-Trypanosoma cruzi antibodies. J Clin Microbiol https://doi.org/10.1128/jcm.01762-22.