TRANSFUSION

Assessment of Liaison XL Murex Chagas diagnostic performance in blood screening for Chagas disease using a reference array of chimeric antigens

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Abstract

Background: Chagas disease (CD) serological screening at blood banks is usually performed by a single highly sensitive serological assay, with chemiluminescent immunoassays (CLIAs) being the method of choice. CLIAs employ recombinant, fusion peptides and/or chimeric antigens that selectively capture anti-*Trypanosoma cruzi* antibodies. However, despite high sensitivity, the ability of these tests to identify CD-positive cases should be evaluated against *T. cruzi* strains circulating in specific locales. Herein, we used a latent class analysis (LCA) approach employing an array of four chimeric antigens to assess the diagnostic performance of the Liaison XL Murex Chagas CLIA for the detection of anti-*T. cruzi* IgG in serum samples.

Study Design and Methods: The study included a panel of 5014 serum samples collected from volunteer blood donors at the Hematology and Hemotherapy Foundation of the State of Bahia, submitted to anti-*T. cruzi* antibody detection using Liaison Chagas CLIA and LCA as a reference test in the absence of a gold standard.

Results: LCA classified 4993 samples as negative, while positivity for *T. cruzi* antibodies was predicted in 21 samples. Compared with LCA, CLIA demonstrated sensitivity and specificity of 76.2% and 99.5%, respectively, providing an overall accuracy of 99.4%.

Discussion: In blood banks lacking a de facto highly sensitive screening immunoassay, the low sensitivity offered by Liaison Chagas CLIA renders it unsuitable for standalone use in serological screening procedures for CD. Moreover, blood banks are encouraged to carefully assess the ability of diagnostic methods to identify local *T. cruzi* strains in circulation.

KEYWORDS
Chagas disease, chemiluminescence, immunoassay, latent class analysis, serological screening

1 | INTRODUCTION

American trypanosomiasis or Chagas disease (CD) is a neglected infectious anthropozoonosis caused by the parasitic hemoflagellate *Trypanosoma cruzi*. This parasite can be transmitted through various routes such as by means of an insect vector, consumption of contaminated food and/or beverages, from mother-to-child, sexually, and through blood transfusion or the use of blood products. According to the World Health Organization, approximately 6–7 million individuals are infected with *T. cruzi*, mainly in South America,[1] resulting in an annual loss of over 800,000 disability-adjusted life-years.[2] Moreover, due to population mobility patterns, climate change, accelerated urbanization, and deforestation, the epidemiological profile of this disease has shifted. In recent decades, CD has spread to more previously non-endemic regions in comparison to its evolutionary path over the last 9000 years.[3] In non-endemic settings, the primary routes of transmission are the transfusion of blood or blood products, vertical transmission from mother-to-child, and tissue/organ transplantation.[1]

A peculiar characteristic of this parasite is its extensive genetic and phenotypic intraspecific diversity. Over 6000 different *T. cruzi* strains, categorized into seven genetic lineages or discrete typing units denominated TcI-TcVI and TcBat, are heterogeneously distributed around the world.[4,5] As a result of its wide genetic and antigenic diversity, substantial diversity among parasite lineages has led to the variable diagnostic performance of immunoassays.[6,7] Consequently, two different serological assays are recommended to achieve a definitive diagnosis of this particular disease.[8] However, WHO recommendations for blood donation screening,[9] and technical regulations for hemotherapeutic procedures issued by the Brazilian Ministry of Health (Ordinance No. 158/2016), both require blood banks to execute a single highly sensitive in vitro diagnostic (IVD) test for CD screening. Importantly, neither of these protocols define a sensitivity threshold, which leaves recommendations of "highly sensitive" test capabilities open to interpretation.[10]

Immunoassay diagnostic parameters can be enhanced by increasing epitope diversity, for example, by adding different antigens or utilizing chimeric antigens.[11,12] Chimeric antigens, proteins composed of various antigenic sequences, offer higher epitope diversity, thus leading to improved diagnostic sensitivity. Indeed, the use of chimeric antigens has greatly contributed to the performance of serological IVD testing regardless of sample geographic origin.[13,14] Chimeric antigens have already been successfully implemented in serological IVD platforms, such as DiaSorin's Liaison XL Murex Chagas chemiluminescent immunoassay (CLIA). This system utilizes a fusion protein comprised of two distinct antigens, shed acute-phase antigen (SAPA) and TcF, bound to magnetic particles. SAPA is widely used in different assays, as this antigen is known to elicit an early and specific humoral response, whereas TcF is a branched tetrapeptide synthetic protein comprised of an assembly of immunodominant repetitive epitopes of four distinct antigens: TcLo1.2, TcE, TcD, and PEP-2.[15] Chemiluminescent and electrochemiluminescent assays offer extremely high diagnostic and analytical sensitivity due to enhanced signal detection, as evidenced by elevated signal-to-noise ratios. The Liaison XL Murex system uses a monoclonal anti-human secondary antibody, which is known to provide a lower signal-to-noise ratio compared to indirect polyclonal immunoassays. By contrast, polyclonal secondary antibodies can address this issue; an Abbott Diagnostics achieved improved analytical sensitivity in the Architect chemiluminescent microparticle immunoassay (CMIA) in comparison to the company's older Prism CMIA platform.

In addition to TcF, other proteins comprise the repertoire of chimeric antigens, such as newer IBMP (IBMP-8.1, IBMP-8.2, IBMP-8.3 and IBMP-8.4) chimeric antigens. These antigens consist of linear immunodominant repetitive epitopes of structural, surface, and cytosolic *T. cruzi* antigens.[16] IBMP sequences were compared with other peptide sequences deposited in the GenBank (NCBI–NIH) database via basic local alignment search tool to rule out possible similarities between epitopes of other known pathogens, especially *Leishmania* spp.[16] Additionally, the diagnostic potential of these four IBMP antigens has been extensively evaluated in both endemic and non-endemic settings in South America,[12,14] as well as in Barcelona (Spain),[13] with high performance noted despite variable sample geographical origin. Furthermore, these antigens performed remarkably well when utilized for the serodiagnosis of canine chronic CD in naturally and experimentally infected dog populations across different Brazilian states.[17]

The present study endeavored to evaluate, using four chimeric IBMP antigens combined with a latent class analysis (LCA) statistical approach, the performance of the Liaison XL Murex Chagas CLIA to detect anti-*T. cruzi* IgG in serum samples at a blood bank in Salvador, Bahia-Brazil.

2 | MATERIAL AND METHODS

2.1 | Ethical considerations

Approval was granted by the Institutional Review Board (IRB) for Human Research at the Gonçalo Moniz Institute of the Oswaldo Cruz Foundation (IGM-FIOCRUZ), Salvador, Bahia-Brazil (CAAE: 67809417.0.0000.0040). To protect blood donor privacy, the IRB required that all

sample and donor identification be coded to avoid blood donor identification, thereby avoiding the need to obtain verbal or written consent.

2.2 | Volunteer blood donors

The study included a panel of 5014 serum samples collected from volunteer blood donors at the Hematology and Hemotherapy Foundation of the State of Bahia (HEMOBA Foundation) between December 2018 and August 2019 (Figure 1). Routinely, prior to donation, all volunteers are interviewed by a physician; those who disclose a recent history of infections, fever, flu-like symptoms, malaise, or fatigue are exempted. Routine screening for CD at HEMOBA is performed solely via Liaison XL Murex CLIA. No other assay is used, and samples are retested only when the resulting reactivity index (RI) is equal to or above 0.75. When reactivity remains equal to or above 0.75 upon retesting, the donation is discarded and the donor is called to revisit HEMOBA to provide a new blood sample. When reactivity continues to return results equal to or above 0.75, the sample is then forwarded to LACEN-BA (the central laboratory of the state of Bahia-Brazil) for diagnostic confirmation, and the donor is referred to a reference center for clinical evaluation and treatment. Conversely, when reactivity returned below 0.75, the blood sample is deemed suitable for donation. Most volunteer blood donors resided in the state of Bahia (98.4%), an endemic region for chronic CD. After routine screening testing, samples were frozen, stored, and finally transported to the institution's laboratory (LASP-IGM-FIOCRUZ) for serological analysis.

2.3 | Study design

All sera were initially tested for *T. cruzi* on a Liaison XL Murex Chagas (DiaSorin, Saluggia, Italy) system at HEMOBA. Subsequently, all sera were retested using four ELISAs employing one of the four IBMP chimeric recombinant proteins. Details on the obtainment of IBMP proteins and ELISA protocols were previously described by Santos et al.[16] and Santos et al.,[12] respectively. These results were then employed in a LCA, which was used as a reference test due to the lack of a gold standard for *T. cruzi* diagnosis. Researchers were blinded to the serological findings produced by CLIA until the results from LCA were compiled and CLIA performance parameters were calculated. The flowchart depicted in Figure 1 illustrates the study design in accordance with the Standards for the Reporting of Diagnostic accuracy studies guidelines.[18]

2.4 | Latent class analysis

As no gold standard exists for the diagnosis of chronic CD, LCA was used as a reference test and carried out in accordance with a previously described and validated
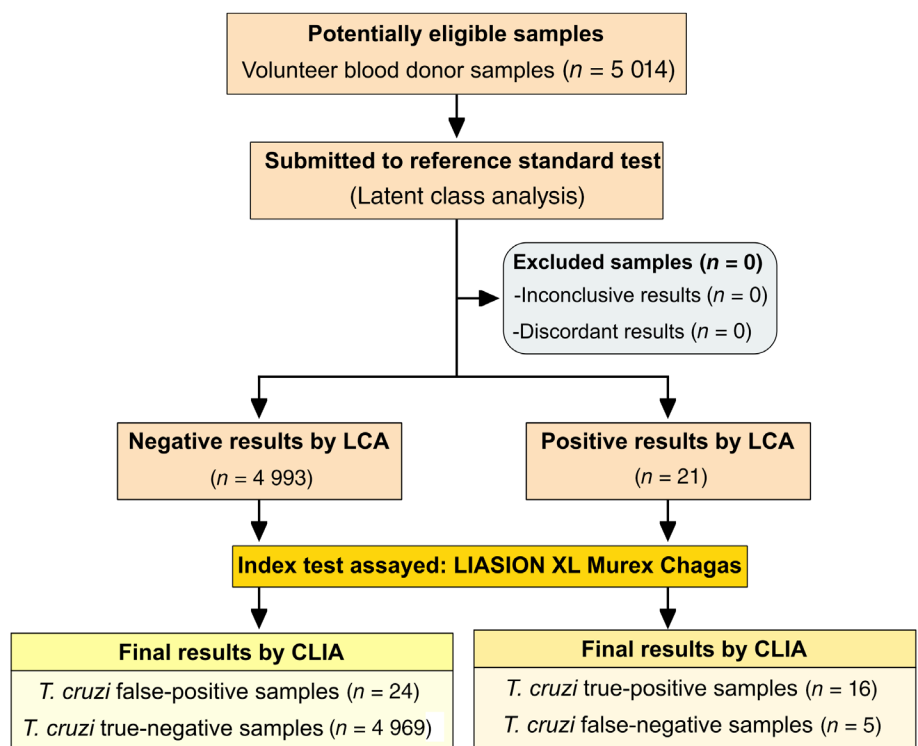

FIGURE 1 Study workflow design assessing Liaison XL Murex Chagas chemiluminescent assay, conducted in accordance with the Standards for the Reporting of Diagnostic accuracy studies guidelines. CLIA, chemiluminescence immunoassay [Color figure can be viewed at wileyonlinelibrary.com]

statistical model.[19] To define the latent variable capable of accurately identifying *T. cruzi* infection, four indicators representing IBMP-8.1, IBMP-8.2, IBMP-8.3, and IBMP-8.4 chimeric antigens were established. The potential of these antigens for chronic CD diagnosis has already been extensively studied.[12–14,16,20–23] Sera were grouped into two categories: "negative" and "positive." Latent classes were characterized based on response patterns from negative/positive results derived from these four chimeric proteins (Figure 2) and conditional probabilities, that is, the probability of obtaining a particular result (negative/positive) using one of four chimeric proteins in an individual diagnostic scenario (negative/positive). According to Akaike information criteria and Bayesian information criteria, lower results indicate better performance, whereas for entropy values closer to one imply better classification quality. Conditional independence was verified using bivariate residuals. All analyses were conducted using Mplus v5.2 software (Muthén & Muthén, Los Angeles, CA).

LCA defined a given sample as *T. cruzi*-negative when negative results were returned from at least three out of the four chimeric antigens (posteriori probability ≤ 0.8%). Contrarily, when positivity was observed in at least two different chimeric antigen-based assays, a given sample was considered as positive for *T. cruzi* (posteriori probability ≥ 88%). The results from each IBMP ELISA were expressed in index format, then plotted to represent the ratio between a given sample's OD and the cutoff (CO) optical density (OD) corresponding to each microplate. Index values are referred to as RI; all results <1.00 were considered negative.

2.5 | Chemiluminescence assay

Chemiluminescent assays, performed in accordance with the manufacturer's instructions, were conducted on a Liaison XL Murex Chagas (DiaSorin, Saluggia, Italy) system, a fully automated indirect chemiluminescent immunoassay platform. This process is completely automated, with sample registration and sample rack insertion as the only manual operations required to perform analysis. This system employs a fusion protein immobilized onto magnetic particles as a capture antigen, while mouse isoluminol-conjugated monoclonal anti-human IgG is used as the secondary antibody. Briefly, after insertion, samples are automatically diluted and incubated with antigen-coated magnetic beads in a reaction vessel. When anti-*T. cruzi* IgG is present in a sample, it binds to the antigen immobilized on the magnetic beads during the initial incubation, creating an antigen–antibody complex. In the second incubation step, the conjugated secondary antibody binds to the human IgG bound to the magnetic bead. A wash cycle follows each incubation to remove any unbound material. Following the final wash, a starter reagent, responsible for the chemiluminescent reaction, is added to the reaction vessel, initiating a flash chemiluminescence reaction, which is then amplified and detected by a photomultiplier tube. The resulting signal is quantified in terms of relative light units (RLU). RLU and RI values are provided by the system, which also calculates each microplate's CO values. All specimens with RI ≥1.00 (positive) are then retested for verification. In accordance with established protocols, negative samples with RI < 0.75 are not retested, and samples are only retested if errors occur during analysis or when resulting RI values reach ≥0.75 (for safe purposes).

2.6 | Statistical analysis

Data were encoded, analyzed, and represented by scatterplots using graphing software (GraphPad Prism version 8, San Diego, CA). Descriptive statistics are presented as geometric means ±95% confidence interval (95% confidence interval [CI]). All analyses were two-tailed, with *p* values less than 5% considered significant (*p* value < .05). The area (AUC) under the receiver-

ELISA	P1	P2				P3						P4				P5
IBMP-8.1	-	+	-	-	-	+	+	+	-	-	-	+	+	+	-	+
IBMP-8.2	-	-	+	-	-	+	-	-	+	+	-	+	+	-	+	+
IBMP-8.3	-	-	-	+	-	-	+	-	+	-	+	+	-	+	+	+
IBMP-8.4	-	-	-	-	+	-	-	+	-	+	+	-	+	+	+	+
LCS	N	N	N	N	N	P	P	P	P	P	P	P	P	P	P	P
PP %	0	0.3	0.1	0.3	0.8	90	97	99	88	96	99	100	100	100	100	100
S	4.991	0	0	1	1	0	0	0	0	1	2	0	0	0	0	18

FIGURE 2 Response patterns of the chimeric antigens used in latent class analysis. LCS, latent class status; N, negative; P, positive; P1, P2, P3, P4, and P5, response patterns; S, number of samples [Color figure can be viewed at wileyonlinelibrary.com]

operator curve was calculated to evaluate the global accuracy of the chemiluminescence assay, which was classified as low (0.51–0.61), moderate (0.62–0.81), elevated (0.82–0.99), or outstanding (1.0).[24] Results were expressed as RI values representative of the ratio between a sample's RLU and the CO value established for its corresponding microplate; results ≥1.00 were considered positive. Samples were deemed inconclusive when RI values were 1.0 ± 10%. Chemiluminescence assay performance parameters were assessed using a dichotomous approach, comparing sensitivity (Sen), specificity (Spe), accuracy (Acc), likelihood ratios, diagnostic odds ratio (DOR), and pretest and posttest probability. Fagan's nomogram illustrates how pretest probability and likelihood ratios were used to determine subsequent posttest positive and negative probability for CLIA.[25] The strength of agreement between LCA and chemiluminescence assay results was evaluated by Cohen's kappa (κ) coefficient, and interpreted as follows: κ = 0 (poor agreement), 0.20 ≤ κ ≥ 0 (slight agreement), 0.40 ≤ κ ≥ 0.21 (fair agreement), 0.60 ≤ κ ≥ 0.41 (moderate agreement), 0.80 ≤ κ ≥ 0.61 (substantial agreement), and 1.0 ≤ κ ≥ 0.81 (almost perfect agreement).[26]

3 | RESULTS

Sera from 5014 volunteer blood donors were tested for *T. cruzi* infection using LCA, which was employed to assess the diagnostic performance of a chemiluminescent assay (Liaison XL Murex Chagas, DiaSorin, Saluggia, Italy). LCA classified 4993 (99.6%) samples as negative, while positivity for *T. cruzi* antibodies was predicted for 21 (0.4%) samples. By contrast, CLIA classified 4974 (99.2%) samples as negative and 40 (0.8%) as positive. In total, discordant test results were identified in 29 (0.6%) samples. CLIA identified 5 of 29 (17.2%) samples as negative, which were predicted to be positive by LCA. In addition, 24 of 29 (82.7%) samples were considered negative by LCA, yet tested positive by CLIA. Table 1 describes all individual data points for the 29 samples identified as false-negatives or false-positives by CLIA in comparison to LCA. The probability that all five (5/29) false-negative samples were positive under LCA was ≥99%; only one sample (# 3028) did not present positivity under all IBMP antigens. In regard to the false-positive samples (24/29), most samples (96%) were negative under all IBMP antigens, yielding a near zero probability of CD positivity. Only one sample (# 5619) was characterized as positive by the IBMP-8.3 antigen; however, its predicted positivity value was extremely low (<0.3%).

Most samples with a low CLIA RI were also classified as negative by all four chimeric antigens under ELISA. Only 2 of 4.993 samples tested positive using just one of

TABLE 1 Individual data points for false-negative and false-positive samples

Sample	ELISA-IBMP				LCA	CLIA	
ID	8.1	8.2	8.3	8.4	PP (%)	RI	Res
3028	0.61	0.58	2.12	1.42	99	0.50	FN
3295	1.46	1.38	1.22	1.93	100	0.35	FN
5231	1.56	2.82	2.69	2.38	100	0.58	FN
5617	1.59	2.78	2.21	2.20	100	0.74	FN
5936	1.69	1.29	1.14	1.81	100	0.25	FN
3809	0.23	0.24	0.26	0.32	0	1.00	FP
4847	0.18	0.34	0.17	0.26	0	1.00	FP
6036	0.18	0.54	0.17	0.18	0	1.00	FP
6040	0.12	0.43	0.10	0.10	0	1.00	FP
6041	0.14	0.17	0.08	0.10	0	1.00	FP
6043	0.23	0.60	0.18	0.22	0	1.00	FP
6044	0.13	0.15	0.09	0.08	0	1.00	FP
6995	0.06	0.12	0.86	0.09	0	1.00	FP
7076	0.09	0.11	0.13	0.11	0	1.00	FP
4101	0.24	0.16	0.30	0.19	0	1.10	FP
5250	0.34	0.25	0.11	0.27	0	1.10	FP
5892	0.13	0.17	0.21	0.13	0	1.10	FP
6812	0.31	0.05	0.19	0.09	0	1.10	FP
5607	0.01	0.12	0.04	0.10	0	1.14	FP
4159	0.38	0.16	0.12	0.20	0	1.20	FP
6979	0.11	0.17	0.13	0.32	0	1.20	FP
5223	0.21	0.27	0.18	0.35	0	1.40	FP
5619	0.59	0.02	1.83	0.32	0.3	1.40	FP
6872	0.07	0.12	0.10	0.05	0	1.40	FP
5924	0.29	0.21	0.16	0.17	0	1.60	FP
5240	0.14	0.30	0.31	0.30	0	1.70	FP
7086	0.16	0.09	0.16	0.14	0	2.40	FP
7017	0.14	0.12	0.97	0.13	0	4.00	FP
5620	0.24	0.18	0.10	0.17	0	8.10	FP

Note: Cutoff = 1.00.
Abbreviations: CLIA, chemiluminescence immunoassay; FN, false-negative; FP, false-positive; ID, identification; LCA, latent class analysis; PP, posteriori probability; Res, result; RI, reactivity index.

these four molecules, one for IBMP-8.3 and another one for IBMP-8.4. The a posteriori probability of these samples being positive is lower than 0.8%, suggesting that they were correctly classified as negative (Figure 2). Regarding the samples classified as positive by LCA, 18 of 21 samples tested positive under all IBMP antigens, while 3 of 21 samples presented positivity for two sets of antigens: one for IBMP-8.2 + IBMP-8.4 and two for IBMP-8.3 + IBMP-8.4. The probability of these samples
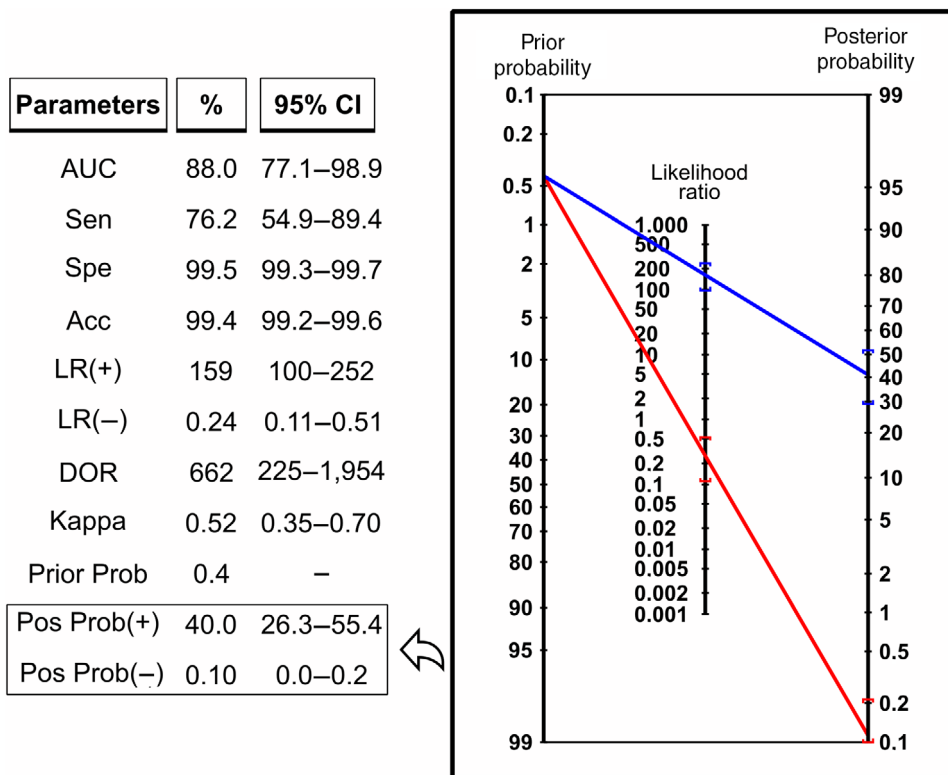
Parameters	%	95% CI
AUC	88.0	77.1–98.9
Sen	76.2	54.9–89.4
Spe	99.5	99.3–99.7
Acc	99.4	99.2–99.6
LR(+)	159	100–252
LR(−)	0.24	0.11–0.51
DOR	662	225–1,954
Kappa	0.52	0.35–0.70
Prior Prob	0.4	–
Pos Prob(+)	40.0	26.3–55.4
Pos Prob(−)	0.10	0.0–0.2

FIGURE 3 Performance parameters of Liaison XL Murex Chagas chemiluminescent assay, obtained using serum samples from *Trypanosoma. cruzi*-positive and negative volunteer blood donors screened at the Hematology and Hemotherapy Foundation of the State of Bahia (HEMOBA) between December 2018 and August 2019. Blue and red lines represent the Pos Prob(+) and Pos Prob(−), respectively. Acc, accuracy; AUC, area under curve; CI, confidence interval; DOR, diagnostic odds ratio; LR, likelihood ratio; Pos, posterior; Prob, probability; Sen, sensitivity; Spe, specificity [Color figure can be viewed at wileyonlinelibrary.com]

being positive is greater than 96% and 99%, respectively, suggesting that they are very likely correctly classified as positive.

Next, the performance parameters of Liaison XL Murex Chagas were compared with the reference results established by LCA (Figure 3). The AUC analysis revealed a value of 0.88, indicating elevated overall capacity of CLIA to correctly identify positivity and negativity in serum samples. The sensitivity and specificity rates of the chemiluminescence assay were 76.2% and 99.5%, respectively. As shown in Figure 3, CLIA test accuracy was estimated at 99.4%. The DOR value, established based on the obtained likelihood ratios, was 622. The pretest probability, reflecting the prevalence of chronic CD in the population studied, was estimated at 0.4%. Moreover, the estimated positive and negative posttest probability values were calculated at 40% and 0.10%, respectively (Figure 3).

4 | DISCUSSION

The present study represents an initial attempt to evaluate the diagnostic performance of the CLIA method to detect anti-*T. cruzi* IgG in a large sample of sera, using a reference array of chimeric *T. cruzi* antigens under LCA as a proxy in the absence of a gold standard.[19] LCA offered more precise diagnostic precision, as

samples deemed inconclusive by CLIA were definitively classified by LCA. The overall rates of false-positive and false-negative results in the 5014 samples screened by chemiluminescence were 0.5% and 23.8%, respectively. Respective sensitivity and specificity values of 76.2% and 99.5% were found. Our results stand in contrast to the performance parameters specified by the manufacturer of the Liaison XL Murex Chagas assay in Latin American blood donors: 99.1% (95% CI: 98.2%–99.7%) and 99.5% (95% CI: 98.5%–99.9%), respectively. Likewise, in a recent study that evaluated prevalence and risk factors for CD in Latin American immigrants residing in Italy, the authors found a sensitivity of 96.8% (95% CI: 82.2%–100%); importantly, this study was limited to 263 samples not obtained from blood donors, of which only 31 were positive.[27]

Another recent study stringently evaluated the performance parameters of seven commonly utilized platforms for CD diagnosis, including Roche's Elecsys Chagas electrochemiluminescence assay, DiaSorin's Liaison XL Murex Chagas and Abbott's Prism Chagas and Architect Chagas CLIAs, among other assays, including four commercially available ELISAs.[28] Interestingly, these authors evaluated the analytical sensitivity of all platforms utilizing two WHO reference sera (NIBSC 09/188: TcI; NIBSC 09/186: TcII), observing that DiaSorin's Liaison XL offered detection sensitivity rates similar to those of some of the ELISAs evaluated. Indeed, the Liaison XL Murex

Chagas system was only capable of recognizing sample positivity at dilutions ranging between 1:2 and 1:4, whereas the Elecsys Chagas system still returned positive results in samples diluted at up to 1:512; a dilution range between 1:16 and 1:32 was reported for Abbott's Architect Chagas system. Surprisingly, these authors found that the analytical sensitivity offered by most of the evaluated ELISA kits surpassed the analytical sensitivity of Diasorin's Liaison XL Murex Chagas.[28] We speculate that the lower sensitivity identified in this assay could be related to the monoclonal nature of secondary antibody, considering that the monoclonal secondary antibodies employed have a lower antibody avidity, since only a single epitope is targeted. By contrast, polyclonal antibodies recognize multiple epitopes and are therefore less susceptible to antigenic determinant conformational changes.[29] This could serve to explain the lower analytical sensitivity seen in DiaSorin's Liaison XL Murex Chagas compared with the Abbott Architect Chagas CLIA system, Wiener Lab ELISA Chagatest, Ortho T. cruzi ELISA, NovaTech NovaLisa Chagas, and Biokit Bioelisa Chagas (for NIBSC 09/186; TcII), which all utilize polyclonal secondary antibodies. Interestingly, despite the fact that Abbott's Prism system uses monoclonal secondary antibodies, it nonetheless outperformed Liaison XL Murex Chagas by detecting positive samples in dilutions ranging from 1:4 to 1:16. This performance could be influenced by the antigens employed in Abbott's Prism and Architect systems (FP3, FP6, FP10, and TcF), which could provide greater epitope diversity than those used by Liaison Murex Chagas (SAPA-TcF). Conversely, the Elecsys system, which uses a double-antigen sandwich design, that is, a conjugated antigen acts as the antigen–antibody interaction reporter, negating the need for a secondary antibody. Furthermore, the electrochemiluminescent design of the Elecsys system provides significantly stronger signal amplification, thus providing higher analytical sensitivity.

The lack of a gold standard test to diagnose chronic CD presents challenges for serodiagnosis and effective screening strategies at blood banks. The WHO currently recommends the use of two distinct immunoassays to diagnose chronic CD.[8] Due to the vast amount of blood samples routinely analyzed in this context, this recommendation could be considered an impractical approach by health authorities. In Brazil, screening is performed using a single highly sensitivity testing method.[10] Most blood banks have opted to use CLIA for donor screening purposes, as these systems offer high sensitivity and are automated.[30,31] In fact, due to this high sensitivity, false-positive results are often reported,[30] increasing costs associated with sample reprocessing and diagnostic

confirmation. False-negative results may also occur, for instance when antigens are unable to detect antibodies arising from different T. cruzi strains.[31] Despite being highly efficient, some CLIA systems do not achieve 100% sensitivity, resulting in a portion of the screened population being misdiagnosed as negative, leading to T. cruzi transmission via transfusion.

The present study employed IBMP chimeric T. cruzi antigens due to previously demonstrated exceptionally high diagnostic performance, regardless of sample geographic origin. Several descriptions in the literature have shown these antigens to successfully distinguish between CD-positive and negative samples. Chimeric antigens have also been successfully employed as capture antigens in other systems, including indirect ELISA, double-antigen ELISA, multiplex liquid microarray, and lateral flow immunochromatographic assays, without any significant sacrifice in performance.[12–14,16,19–23] As reactivity was observed under all four IBMP chimeras in each of the five of the samples identified as false-negative by Liaison XL Murex Chagas, the possibility of common epitopes shared by the TcF-SAPA antigens used in Liaison XL Murex Chagas and the IBMP antigens lacks relevance. Therefore, the occurrence of pre-analytical errors must be considered, for example, incorrect tube type, hemolyzed and icteric samples, undetected fibrin aspiration, system errors, or incorrect sample registration could serve to explain why the system failed to correctly detect 5 out of the 21 positive samples.

The main limitation of the present study was the low number of positive samples, which compromised our sensitivity assessments. As data regarding the donor's serological profile were unknown until the completion of LCA, it was not possible to control the size of positive and negative sample groups. Furthermore, it is possible that many CD-affected donors could have been dismissed after the initial screening interview, which could explain the low proportion of positive samples (21/4993 or 0.42%), which is substantially below the reported CD prevalence of 3.5% in Salvador.[32] However, a recent study revealed a similar prevalence (0.62%) of CD among donors in Bahia deemed positive for T. cruzi infection following initial serological screening.[33]

5 | CONCLUSION

Our results indicate that the Liaison XL Murex Chagas system failed to detect 5 out of 21 CD-positive samples, which suggests low (76.2%) sensitivity. This finding may be attributable to the use of a monoclonal secondary antibody, the inability of the TcF-SAPA fusion antigen to

identify antibodies arising from diverse *T. cruzi* lineages, or pre-analytical errors. In blood banks, CD screening systems that do not provide a de facto highly sensitive assay (approaching 100%) should not be utilized in isolation for serological screening procedures.

CONFLICT OF INTEREST

The authors have disclosed no conflicts of interest.

DATA AVAILABILITY STATEMENT

All data supporting the conclusions reached in this article are provided within the article. The datasets generated and analyzed during the elaboration of current study are available upon reasonable request.

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