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Diagnostic accuracy of Enzyme-Linked Immunosorbent Assays to detect anti-*Leishmania* antibodies in patients with American Tegumentary Leishmaniasis: a systematic review

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ABSTRACT

American Tegumentary leishmaniasis (ATL) is an infectious disease caused by several species of *Leishmania*. Even though the direct detection of parasites has low sensitivity, it is still the gold standard for the laboratory diagnosis of ATL. Recent studies have shown promising results of Enzyme-Linked Immunosorbent Assays (ELISAs) using recombinant antigens. The aim of this study is to compare the accuracy of ELISAs using novel antigens with the standard ELISA based on soluble antigens of *Leishmania* (SLA) to diagnose ATL. Studies that analyzed patients with ATL and studies that evaluated the diagnostic accuracy of ELISAs using novel antigens and SLA were included. The Fourteen studies from PubMed, Regional Portal of the Virtual Health Library (BVS), Brazilian Society of Dermatology, Virtual Health Library (IBECS), Literature in the Health Sciences in Latin America and the Caribbean (LILACS), Medical Literature Analysis and Retrieval System Online (Medline), Elsevier Embase, Cochrane Library, The National Institute for Health and Care Excellence (NICE), and Cumulative Index to Nursing and Allied Health Literature (CINAHL) were included. The novel ELISA antigens showed a high sensitivity (93.8%-100%) and specificity (82.5-100%), a better diagnostic performance than SLA-based ELISAs (1-97.4% and 57.5-100%, respectively). Only 10 studies analyzed cross-reactions in serum samples from patients with Chagas disease, and only two studies reported a percentage of cross-reactivity. In this systematic review, the novel ELISA antigens showed better sensitivity and specificity with respect to SLA-based ELISAs. However, a meta-analysis should be performed to confirm this finding.

KEYWORDS: American Tegumentary Leishmaniasis. ELISA. Accuracy. Diagnosis. Serology. Recombinant antigens. Novel antigens. Leishmaniasis.

INTRODUCTION

American Tegumentary Leishmaniasis (ATL) is an infectious disease that affects the skin and mucous membranes, showing distinct clinical manifestations determined by an equilibrium between parasitic factors (tropisms, virulence, resistance and species) and the host immune response[1-3]. The main clinical forms are cutaneous leishmaniasis (CL), muco-cutaneous leishmaniasis (MCL) and disseminated cutaneous leishmaniasis (DCL). The cutaneous leishmaniasis (CL) is caused by *L. (V.) braziliensis*, *L. (V.) guyanensis*, *L. (L.) amazonensis* and *L. (V.) naiffi*. The muco-cutaneous leishmaniasis (MCL) is caused by *L. (V.) braziliensis* and the disseminated cutaneous leishmaniasis (DCL) is caused by *L. (L.) amazonensis* and *L. (L.) mexicana*[4].

According to the World Health Organization (WHO), leishmaniasis is one of the most important neglected tropical diseases due to its high detection coefficient and capacity to produce deformities. This disease affects mainly individuals of low socioeconomic level, and the number of cases in endemic areas have increased significantly in recent years[5-8]. It is estimated that 350 million people are at risk of contracting the infection, and the annual incidence is estimated at about two million new cases[9].

In Brazil, about 20 thousand cases/year are recorded, with an incidence coefficient of 10.3 cases/100,000 inhabitants in the last five years. In 2015, the Brazilian North region had the highest incidence rate (51.1 cases/100,000 inhabitants), followed by the Midwest (19.0 cases/100,000 inhabitants) and the Northeast (9.1 cases/100,000 inhabitants)[10].

The diagnosis of leishmaniasis is based on epidemiological data, clinical characteristics and laboratory test results, including parasitological examination, serological and molecular methods as well as the Montenegro skin test[4].

Clinical practice guidelines recommend multiple testing of active cutaneous lesion samples. Thus, the parasitological diagnosis is based on the evidence of the parasite through direct microscopy of smears and/or culture, requiring a trained laboratory staff[11,12]. In addition, these tests require invasive procedures of sample collection, which limit their use[13]. Even though the direct detection of parasites has low sensitivity, it is still the gold standard for the laboratory diagnosis of ATL. The Montenegro skin test (MST), with a sensitivity rate of 86.4-100%[14], is the main diagnostic test in primary care. In the presence of a suspicious cutaneous lesion, MST supports the diagnosis of *Leishmania* infections. However, MST results may be influenced by the clinical presentation and the disease duration[14].

The association of MST with molecular biology methods, especially in reference treatment centers, offers higher diagnostic accuracy as well as good sensitivity and specificity. Therefore, a combination of diagnostic methods is usually required to obtain accurate results[15,16]. There is a need to develop faster, more effective and simpler assays for the diagnosis of this disease[17]. Among these tests, serology has been the most widely used method in epidemiological studies, providing additional support for disease control measures. However, since most patients have low antibody titers against *Leishmania* species, this method may be associated with a relatively high proportion of false negative results. In addition, different species of *Leishmania* overlap the distribution of *Trypanosoma cruzi* in many geographic regions, increasing the risk of cross-reactions in these endemic areas[18,19].

ELISAs are useful diagnostic tools for ATL, being fast and relatively affordable. However, their accuracy is variable[20,21]. Several studies have selected proteins common to *Leishmania* species to develop an assay capable of detecting the disease irrespective of the etiological agent species. Among the proteins used, cytochrome c oxidase VII (CcOx), IgE-dependent histamine-releasing factor (HRF) and histones have been used[21,22].

Recently, several recombinant proteins have been successfully developed and used for the serological diagnosis of ATL, showing better sensitivity and specificity. In ELISAs, the recombinant antigens rK108, rLbHyM, rLHHs, rHSPH83, RA2, rLb6H, and rLb8E were used[23-27].

Promising results in several studies on ELISAs using recombinant antigens have been reported. The successful implementation of these methods reflects their good performance. Therefore, this study provides a brief systematic review of studies on the accuracy of available serological tests, aiming to evaluate the accuracy of these tests for the detection of antibodies against *Leishmania* species.

MATERIALS AND METHODS

The protocol of this systematic review was published in the International Prospective Record of Systematic Reviews (PROSPERO 2018: CRD42018114275) before its implementation and is described in the Complementary Materials. The protocol and the final report were developed based on the Cochrane Handbook for Systematic Reviews of Diagnostic Test Accuracy[28].

Review question

What is the diagnostic accuracy of ELISAs with novel antigens compared to the standard serological test based on soluble antigens of *Leishmania* species (SLA) for the serological diagnosis of American Tegumentary Leishmaniasis (ATL)?

Inclusion and exclusion criteria

This review considered studies that included patients with ATL that were diagnosed by at least one of the parasitological gold standards (Montenegro skin test, direct microscopy of smears and/or culture) or by the Polymerase Chain Reaction (PCR). In addition, we included studies that evaluated the diagnostic accuracy of ELISAs based on novel antigens for the serological diagnosis of ATL, as well as those using soluble *Leishmania* antigens (SLA) in order to detect antibodies against species of *Leishmania* that cause

ATL. Studies that analyzed the accuracy of ELISAs but did not define the endemic controls were excluded.

Types of study

Cross-sectional studies based on diagnosis by serological tests providing detailed measurements of sensitivity, specificity, positive and negative predictive values, likelihood ratio and area under the curve (AUC), were included.

Search strategy

An initial search limited to MEDLINE was performed by using the MeSH index terms and related keywords. This search was performed followed by the analysis of words along the text that were also mentioned in the titles and summaries and terms of the index used to describe the studies. A second search using all the identified keywords and index terms was performed on all included databases. In a third search, the reference list of all dissertation theses with clearly detailed accuracy values were evaluated. Considering that ATL is a disease that affects the American continent, the search was limited to English, Spanish and Portuguese. The search for primary literature was not limited to the initial date and was completed in October 2018.

The search for studies used the following databases: PubMed, Regional Portal of the Virtual Health Library (BVS), Brazilian Society of Dermatology, Virtual Health Library (IBECS), Literature in the Health Sciences in Latin America and the Caribbean (LILACS), Medical Literature Analysis and Retrieval System Online (Medline), Elsevier Embase, Cochrane Library, The National Institute for Health and Care Excellence (NICE), and Cumulative Index to Nursing and Allied Health Literature (CINAHL). The MeSH Index Terms searched were: Leishmaniasis, cutaneous Leishmaniasis, Mucocutaneous; ELISA; Serological, Test; Diagnosis; *Leishmania*; Serology; Data Accuracy. The keywords used were: "American Tegumentary Leishmaniasis, Serodiagnosis, Specificity and Sensitivity. The terms were combined via the boolean operators "AND" and/or "OR" to compose the search strings.

Methodological quality assessment

The articles selected for data collection were read by two independent reviewers to evaluate the methodological validity of each text before their inclusion in this review. The standardized critical evaluation instrument QUADAS 2 was used, which was released in 2011 after the revision of the original QUADAS (Quality Assessment of Diagnostic Accuracy Studies)[29,30]. All disagreements between reviewers were resolved through discussion or by a third reviewer.

Data extraction

Quantitative data were extracted from the texts included in this review using the STARD (Standards for Reporting Studies of Diagnostic Accuracy) checklist[31]. All disagreements between both reviewers were resolved through discussion or by a third reviewer.

Data synthesis

The results are shown in three groups: 1) Novel-based ELISA (novel antigens); 2) SLA-based ELISA (soluble antigens of *Leishmania*) and 3) immunofluorescence assays (IFA). The development of a meta-analysis to analyze the general performance of the tests was not performed as there was only one study for each ELISA based on a given novel antigen and also due to a great heterogeneity in the standard ELISA based on SLA. Data found in this study will be shown only in a narrative and qualitative way.

RESULTS

Our research resulted in 315 manuscripts related to the serology of leishmaniasis by the combined application of descriptors in the databases described above. Only 14 studies remained (Table 1)[20,22,24,25,27,32-40] after applying the eligibility criteria (duplicate texts, articles related to other topics, texts excluded due to review or quality method criteria). The studies evaluated the diagnostic accuracy of different specific and novel antigens. The function of some of these antigens are not known, for instance conserved hypothetical proteins such as rLiHyS, rLbHyM, LiHypA and LbrM.30.3350. Other antigens are heat shock proteins (HSP),rLb8E, rLb6H and HSP83.1, histamine-releasing factor (HRF), cytochrome C oxidase VII (CcOx), tryparedoxin peroxidase, eukaryotic initiation factor 5a, enolase, and γ-Tubulin. These assays were compared to standard ELISAs using soluble antigens from different *Leishmania* species (SLA), enriched membrane fraction and indirect immunofluorescence (IFA) assays. The results of our search strategy are shown in a PRISMA flowchart (Figure 1).

The methodological quality assessment revealed that the studies included in this systematic review had a "low risk of bias" in the domains of patient selection, index test, reference standard as well as flow and timing. Regarding

Table 1 - A summary of the included studies.

Journal	Year	Author	Samples		EC	CD
			CL	ML		
Parasitol Int.	2018	Dias DS	0	23	35	235
Parasitol Res.	2017	Lima MP	20	25	25	10
Clin Microbiol.	2017	Sato CM	219		68	91
Cell Immunol.	2017	Carvalho AMRS	27	30	40	15
Vet Parasitol.	2016	Coelho EA	12	12	20	8
Clin Vaccine Immunol.	2015	Duarte MC	23	20	30	10
Braz J Infect Dis.	2015	Soares KA	98		80	24
Clin Vaccine Immunol.	2014	Menezes-Souza D	45	20	50	20
J Clin Lab Anal.	2010	Cataldo JI	76		76	0
Braz J Infect Dis.	2009	Szargiki R	87		13	10
J Clin Lab Anal.	2009	Nascimento LD	189		189	0
Trans R Soc Trop Med Hyg.	2009	Barroso-Freitas APT	74	20	92	0
Acta Trop.	2008	Vidigal C de P	48		48	30
Bol Malariol Salud Ambient.	2007	Añez N	87		104	0

EC = Endemic control; CD = Serum samples from Chagas Disease patients; NI = Not Included; CL = Cutaneous Leishmaniasis; ML = Mucosal Leishmaniasis.

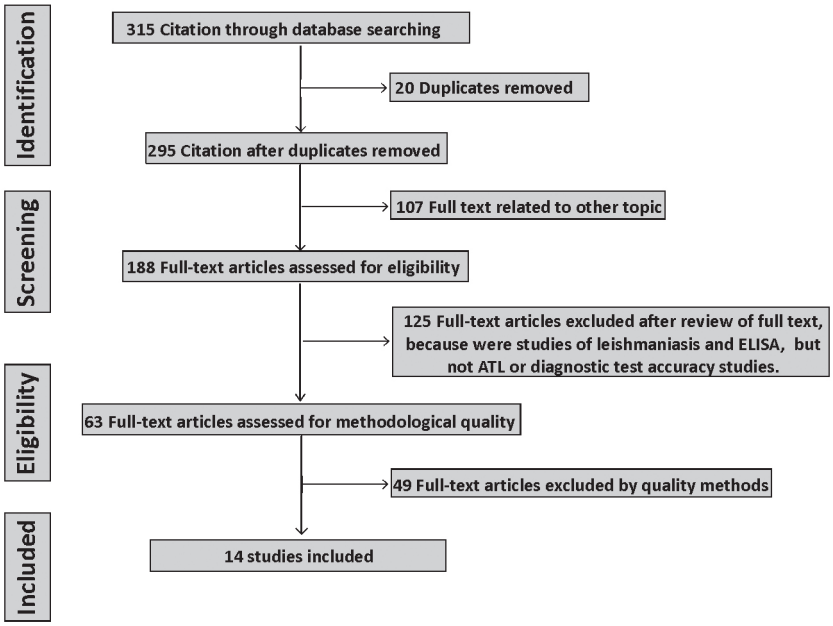

Figure 1 - A flowchart of the steps performed in the systematic review.

the "applicability concerns," the selected studies showed a low concern in the domains of patient selection, index test and reference standard (Figure 2). Data extracted from the final selection are shown in Table 2.

In the 14 studies included in this systematic review, 2,478 ELISAs were distributed among 1,155 (46.6%) samples from patients with ATL, 870 (35.1%) were endemic controls and 453 (18.3%) analyses were evaluated cross-reactions with serum samples from patients with Chagas disease. Only seven studies distributed the samples of patients with ATL according to the clinical form of the disease into the categories cutaneous leishmaniasis (CL) and muco-cutaneous leishmaniasis (MCL) (Table 1). Regarding the comparisons with IFA, 417 tests were performed in only two studies. It is important to mention that cross-reactions with other dermatological diseases were also analyzed in

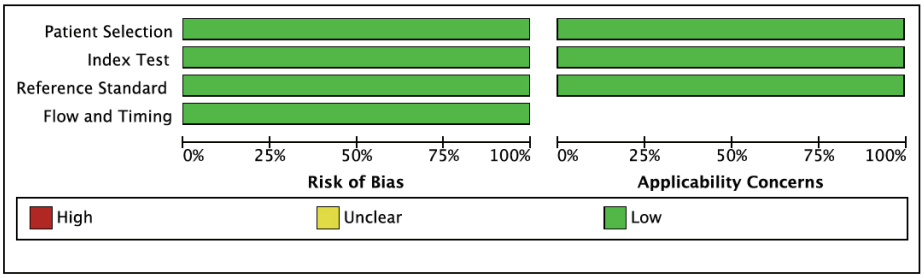

Figure 2 - Assessment of methodological quality domains in all the studies. Proportions of studies rated as "high," "unclear," and "low" are presented.

Table 2 - PRISMA checklist.

Section/topic	#	Checklist item	Reported on page #
		TITLE	
Title	1	Identify the report as a systematic review, meta-analysis, or both.	Title
		ABSTRACT	
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	Abstract
		INTRODUCTION	
Rationale	3	Describe the rationale for the review in the context of what is already known.	Introduction
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes and study design (PICOS).	Introduction and Methods: Review Question.
		METHODS	
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and if available, provide registration information including registration number.	Methods
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	Methods: Inclusion Criteria
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	Methods: Search Strategy
Search	8	Present full electronic search strategy for at least one database, including any limits used, so that that it could be repeated.	Methods: Search Strategy
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review and, if applicable, included in the meta-analysis).	Methods: Study Strategy
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming the data obtained by investigators.	Methods: Data extraction
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	Methods: Data extraction/ Quality assessment
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was performed in the study or outcome level), and how this information is to be used in any data synthesis.	NA
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	NA
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	NA
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	NA

Table 2 - PRISMA checklist.

Section/topic	#	Checklist item	Reported on page #
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	Methods: Data Synthesis
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	Results (Figure 1)
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	Results (Table 1)
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	NA
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	Results: Figure 2 and 3
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	NA
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	NA
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	Results
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	Discussion
Limitations	25	Discuss limitations at study and outcome levels (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	Discussion
Conclusions	26	Provide a general interpretation of the results in the context of other evidence and implications for future research.	Conclusion
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	NA

NA = Not Applicable. Data collected on Prisma[42]

a few of the included studies (histoplasmosis, malaria, paracoccidioidomycosis, toxoplasmosis, tuberculosis, and leprosy). Regarding the geographic distribution, 89.6% of the samples were collected in Brazil and only 10.4% in Venezuela.

Synthesized measurements of accuracy

A global estimate of the accuracy of ELISAs used as serological methods in the diagnosis of ATL was summarized.

Based on recombinant antigens and other known proteins, novel antigens (HSP, rLb8E, rLb6H, HRF, LiHypA, CcOx, trypidoxin peroxidase, eukaryotic initiation factor 5, enolase and γ-tubulin) and standard SLA-based ELISAs were compared (Table 3).

In novel-based ELISAs, only four studies calculated the positive and negative predictive values (PPV and NPV), two calculated the likelihood ratio (LR), three calculated the percentage of accuracy and three the area under the curve (AUC). Regarding SLA-based ELISA tests, in nine studies, the positive and negative predictive values were calculated, the likelihood ratio by two, the percentage of accuracy by three and the AUC by two. Finally, in the two studies that compared IFA methods, the PPV and NPV were calculated (Table 3).

The novel-based ELISAs showed a sensitivity between 93.8-100% and a specificity between 82.5-100%, with equally high confidence intervals (CI) of 95% (Figure 3). The PPV showed values between 98.3-100% and NPV was 100% in the five studies that calculated these data. The accuracy percentage was between 90.4-100%, and the AUC values were between 0.989 -1. The LR values reported in two studies were 50 and 55 (Table 3).

The SLA-based ELISAs showed an inconsistent and poor performance, with a range of sensitivity between

Table 3 - Accuracy of ELISAs and IFA for diagnosis of ATL using different *Leishmania* antigens. Summary overall of accuracy for each *Leishmania* antigen used in the ELISAs.

Author, year	Antigen		Country	Accuracy Study											
				Sensitivity	CI 95%	Specificity	CI 95%	PPV	CI 95%	NPV	CI 95%	LR+	Accuracy	AUC	CI 95%
Dias DS, 2018	Novels Antigens	rLiHyS	Brazil	100%	94.7 - 100	100%	93.8 - 100	100%	94.7 - 100	100%	93.8 - 100	N.C	N.C	N.C	N.C
Lima MP, 2017	Novels Antigens	rLbHyM	Brazil	100%	89.4 - 99.9	98.0%	89.4 - 99.9	N.C.	N.C.	N.C.	N.C.	50	N.C.	N.C	N.C
Sato CM, 2017	Novels Antigens	rLB8E	Brazil	100%	94.7 - 100	98.5%	92.1 - 100	N.C.	N.C.	N.C.	N.C.	N.C.	99.2%	N.C	N.C
		rLb6H	Brazil	100%	94.7. 100	98.5%	92.1 - 100	N.C.	N.C.	N.C.	N.C.	N.C.	9.2%	N.C	N.C
Carvalho AMRS, 2017	Novels Antigens	LiHypA	Brazil	100%	93.7 - 100	98.2%	90.3 - 100	98.3%	90.8 - 100	100%	93.4 - 100	55	N.C.	1	N.C
Coelho EA, 2016	Novels Antigens	CcOx	Brazil	100%	85.8 - 100	100%	85.8 - 100	100%	N.C	100%	N.C	N.C	N.C	N.C	N.C
		HRF	Brazil	100%	85.8 - 100	100%	85.8 - 100	100%	N.C	100%	N.C	N.C	N.C	N.C	N.C
Duarte MC, 2015	Novels Antigens	Tryparedoxin peroxidase	Brazil	100%	91.8 - 100	100%	91.2 - 100	N.C	N.C	N.C	N.C	N.C	100%	1	1.0 - 1.0
		Eukaryotic initiation factor 5a	Brazil	100%	91.8 - 100	92.5%	79.6 - 98.4	N.C	N.C	N.C	N.C	N.C	96.4%	1	0.9 - 1.0
		Enolase	Brazil	100%	91.8 - 100	85.0%	70.2 - 94.3	N.C	N.C	N.C	N.C	N.C	92.8%	0,9	0.9 - 1.0
		β-Tubulin	Brazil	100%	91.8 - 100	82.5%	67.2 - 92.7	N.C	N.C	N.C	N.C	N.C	91.6%	0,9	0.9- 1.0
		Hypothetical protein (LbrM.30.3350)	Brazil	95.4%	91.0 - 100	85.0%	70.2 - 94.3	N.C	N.C	N.C	N.C	N.C	90.4%	1	0.9 - 1.0
Menezes-Souza D, 2014	Novels Antigens	HSP83.1	Brazil	93.8%	84.9 - 98.3	95.7%	87.9 - 99.1	95.3%	N.C	94.3	N.C	N.C	94.8%	0,989	0.979 - 1.00
Dias DS, 2018	SLA	*L. braziliensis*	Brazil	1.0%	0.4 - 7.9	100%	93.8 - 100	100%	2.5 - 100	46.6%	37.4 - 55.5	N.C.	N.C.	N.C.	N.C.
		L. infantum	Brazil	62.0%	46.4 - 75.5	73.0%	60.9 - 82.4	59.0%	44.2 - 73.0	75.0%	62.9 - 84.2	N.C.	N.C.	N.C.	N.C.
Lima MP, 2017	SLA	*L. braziliensis*	Brazil	75.6%	60.5 - 87.1	98.0%	89.4 - 99.9	N.C.	N.C.	N.C.	N.C.	37,5	N.C.	N.C.	N.C.
Sato CM, 2017	SLA	*L. major*-Like	Brazil	91.2%	81.8 - 96.7	95.6%	87.6 - 99.1	N.C.	N.C.	N.C.	N.C.	N.C.	91.1%	N.C.	N.C.
Coelho EA, 2016	SLA	*L. braziliensis*	Brazil	77.4%	58.9 -	100%	83.9 -	100	N.C.	75.0%	N.C.	N.C.	N.C.	N.C.	N.C.
Duarte MC, 2015	SLA	*L. braziliensis*	Brazil	65.1%	49.1 - 79.0	57.5%	40.9 - 73.0	N.C.	N.C.	N.C.	N.C.	N.C.	61.4%	0,6	0.5 - 0.7
Soares KA, 2015	SLA	*L. mexicana*	Brazil	90.8%	83.5 - 95.1	80.3%	73.9 - 85.4	71.2%	62.7 - 78.4	94.2%	89.4 - 96.9	N.C.	83.9%	N.C.	N.C.
Cataldo JI, 2010	SLA	*L. braziliensis*	Brazil	89.5%	80.6 - 95.3	89.5%	80.3 - 95.3	89.5%	81.7 - 94.4	89.5%	81.8 - 94.2	8,5	N.C.	0,918	80.6 - 95.3
Szargiki R, 2009	SLA	*L. Amazonensis*	Brazil	71.7%	N.C.	84.6%	N.C.	95.5%	N.C.	39.3%	N.C.	N.C.	N.C.	N.C.	N.C.
		L. braziliensis	Brazil	95.0%	N.C.	92.3%	N.C.	98.0%	N.C.	80.0%	N.C.	N.C.	N.C.	N.C.	N.C.
Nascimento LD, 2009	SLA	*L. braziliensis*	Brazil	97.4%	93.9 - 99.1	93.7%	89.2 - 96.7	93.9%	89.3 - 96.9	97.3%	93.4 - 98.9	N.C.	N.C.	N.C.	N.C.
Barroso-Freitas APT, 2009	SLA	*L. braziliensis*	Brazil	95.7%	89.5 - 98.8	100%	97.7 - 100	100%	95.9 - 100	100%	93.8 - 99.3	N.C.	N.C.	N.C.	N.C.
		L. major-Like	Brazil	78.7%	69.1 - 86.5	82.8%	76.0 - 88.3	73.3%	63.5 - 81.6	86.6%	80.2 - 91.7	N.C.	N.C.	N.C.	N.C.
Vidigal CdeP, 2008	SLA	*L. braziliensis*	Brazil	85.4%	73.3 - 93.4	89.6%	78.4 - 96.1	80.4%	67.8 - 89.5	93.7%	87.9 - 97.2	N.C.	N.C.	N.C.	N.C.
Añez N, 2007	SLA	N.S.	Venezuela	53.0%	43.0 - 61.0	83.0%	73.0 - 89.0	79.0%	68.0 - 86.0	59.0%	50 - 66	N.C.	N.C.	N.C.	N.C.
Cataldo JI, 2010	Enriched membrane fraction	*L. braziliensis*	Brazil	89.5%	80.3 - 95.3	93.4%	85.3 - 97.8	93.2%	85.8 - 96.9	89.9%	82.4 - 94.4	13,6	N.C.	0,934	88.2 - 96.8
Barroso-Freitas APT, 2009	IFA	*L. braziliensis*	Brazil	81.5%	70.0 - 90.1	86.2%	79.0 - 91.6	73.4%	62.3 - 82.7	94.7%	88.9 - 98.0	N.C.	N.C.	N.C.	N.C.
		L. major-Like	Brazil	95.4%	87.1 - 99.0	77.7%	69.6 - 84.5	63.5%	53.1 - 73.1	96.9%	91.2 - 99.4	N.C.	N.C.	N.C.	N.C.
		Bio-Manguinhos kit	Brazil	75.4%	63.1 - 85.2	89.2%	82.6 - 94.0	79.6%	66.5 - 89.4	84.8%	77.8 - 90.4	N.C.	N.C.	N.C.	N.C.
Añez N, 2007	IFA	N.S.	Venezuela	23.0%	16.0 - 31.0	87.0%	78.0 - 92.0	67.0%	51 .0 - 80.0	59.0%	50.0 - 66.0	N.C.	N.C.	N.C.	N.C.

CI 95% = Confidence Inverval 95%; PPV = Positive Predictive Value; NPV = Negative Predictive Value; LR = Likelihodd Ratio; AUC = Area Under Curve; SLA = Soluble Antigens of Leishmania; IFA =Indirect Immunofluorescence Assay; N.C. = Not Calculated; N.S. = Not Specified.

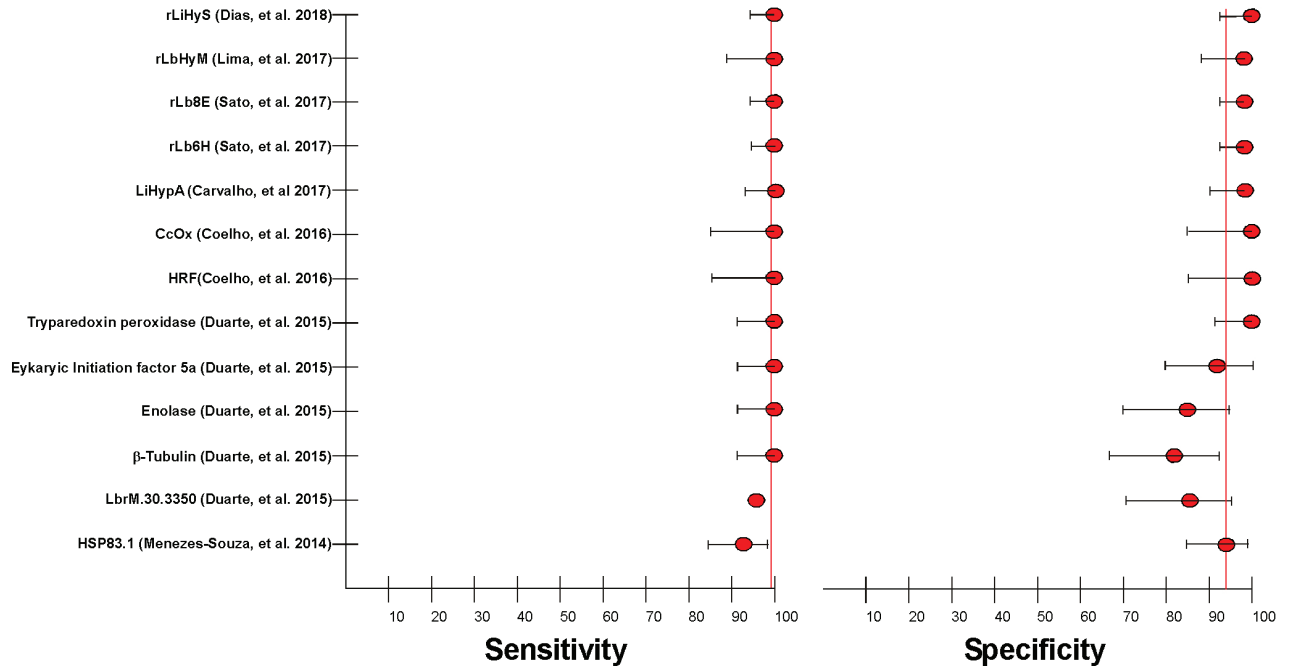

Figure 3 - Summary of sensitivity and specificity values of the novel-based ELISAs reported for each antigen.

1-97.4%, and a specificity between 57.5-100%, with equally heterogeneous CI 95% (Figure 4). The PPV values ranged from 59- 100%and the NPV values ranged from 39.3-100%. The accuracy percentage ranged from 61.4-91.1% and the two AUC measured were 0.6 and 0.989. The reported LR values were 8.5 and 37.5% (Table 3).

A single study used Enriched Membrane Fraction and reported sensitivity and specificity of 89.5% and 93.4%, respectively. In addition, PPV (93.2%), NPV (89.9%), LR (13.6) and AUC (0.934) were also reported (Table 3).

Regarding the accuracy of SLA-based ELISAs by leishmania species, *L. (V.) braziliensis* antigens were the

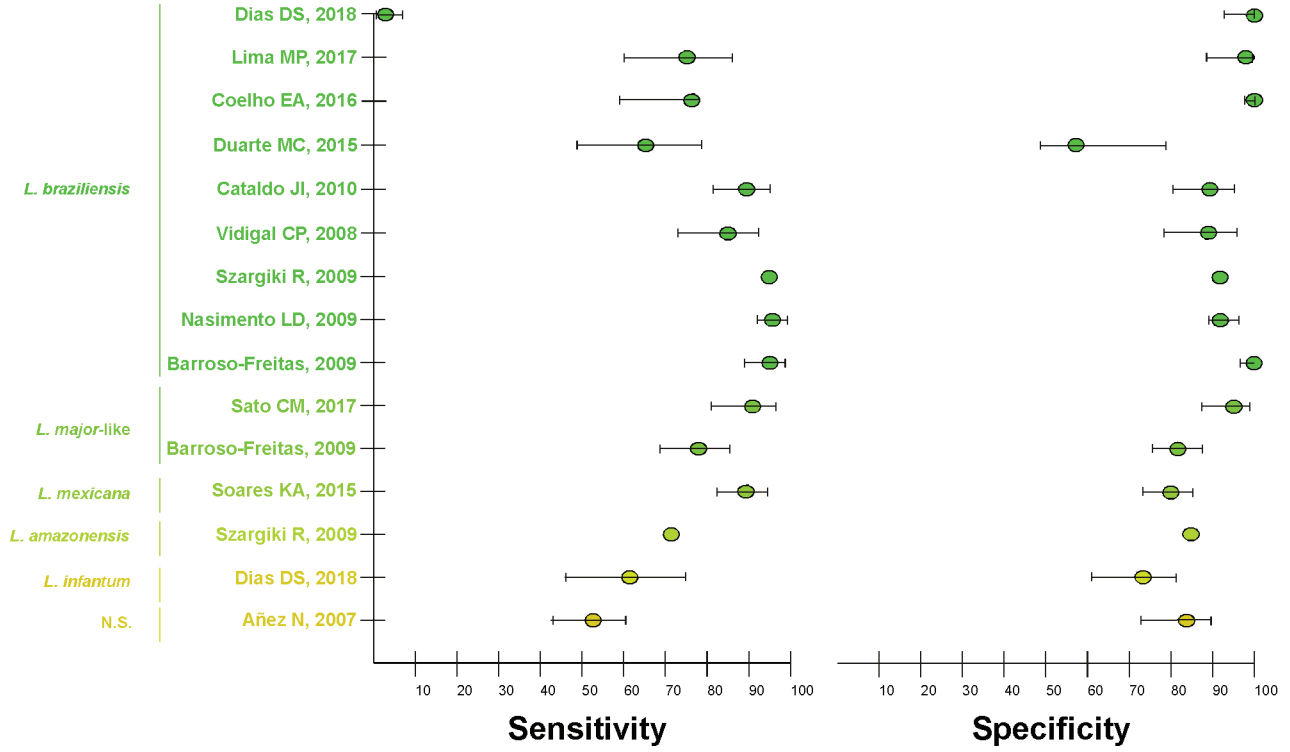

Figure 4 - Summary of sensitivity and specificity values of standard ELISA assays based on soluble antigen of specific *Leishmania* species. Observation: the author did not specify the *Leishmania* specie used.

most used, showing values from 1- 95.7% of sensitivity and 57.5- 100% of specificity. Two studies used *L. (L.) major*-like and reported values of 85.4- 91.2% of sensitivity and 82.8- 95.6% of specificity. *L. (L.) mexicana* 90.8-80.3%, *L. (L.) amazonensis* 71.7- 84.6% and *L. (L.) infantum* 62-73%, respectively.

The IFA tests were performed using different species of *Leishmania* (*L. braziliensis, L. major*-Like, Bio-Manguinhos commercial antigen and another that was not specified). Regarding the diagnostic performance of IFAs. These tests showed a sensitivity between 23- 95.4% and a specificity between 77.7- 89.2%. The PPV values ranged from 63.- 79.6% and the NPV values ranged from 69- 96.9% (Table 3).

Cross-reactivity with Chagas disease

Ten studies performed comparisons of cross-reactions with serum samples from patients with Chagas disease, and only two reported reactivity (lack of specificity). Among the studies analyzing ELISAs with novel antigens, only one reported 17% (95% CI, 12-27.9) of reactivity in ELISAs based on the recombinant rLb6H antigen[27]. As for standard ELISAs based on SLA, several percentages of cross-reactivity were reported according to the species used: *L. major*-Like 69% (95% CI, 66.1 - 83.5)[27], *L. mexicana* 66.7%[35], *L. amazonensis* 10% and *L. braziliensis* 20%[36].

DISCUSSION

Our research shows data that point to a problem regarding the choice of serological methods for the diagnosis of ATL. Several ELISAs using different types of antigens, have been proposed as sensitive tools for the diagnosis of this disease. From the available literature, we analyzed and summarized data on diagnostic accuracy studies of ELISAs based on novel antigens, some of them recombinants. They were compared with standard ELISAs based on soluble *Leishmania* antigens (SLA). Due to the existence of only one study per novel antigen, a meta-analysis was not conducted in this review as at least four studies are necessary to perform a meta-analysis.

Our research question was restricted to the Americas aiming to produce useful evidence for ATL control programs in the region. Since 92.2% of ELISAs included in this systematic review were developed in Brazil and the remaining 7.8% in Venezuela, our results reflect the state of serological diagnostic tests for ATL in Brazil.

Several indicators of diagnostic performance were proposed, such as the percentage of sensitivity and specificity. Novel ELISA antigens showed a high sensitivity and specificity, proving a better diagnostic performance than standard ELISAs (SLA), which showed heterogeneous values. However, the use of paired indicators (sensitivity and specificity) can create a bias when the performance of competing tests is performed, especially if one test does not outperform the other with respect to both indicators. Through a meta-analysis, the diagnostic odds ratio (DOR), which is the only indicator of diagnostic performance, would help to evaluate the diagnostic test performance in the studies objectively[41].

Regarding SLA, the soluble antigens of *L. (V.) braziliensis* showed a better performance than the soluble antigens of the other species used in the serological diagnosis of LTA. In IFA tests, sensitivity and specificity values were similar to those observed in standard ELISAs (SLA). This could be explained by the fact that both, SLA-based ELISAs and IFAs were developed using the same *Leishmania* species.

From the total of 14 studies included in this systematic review, ten made comparisons on cross-reactions with serum samples from patients with Chagas disease, and only two reported the occurrence of cross-reactivity. The only novel-based ELISA that reported cross-reactivity was developed using rLb6H[27]. This recombinant antigen had a reactivity of 17%, which is lower when compared to the 69% observed in the SLA-based ELISA of *L. major*-like and 33% with *L. (L.) mexicana*. SLA-based ELISAs of *L. amazonensis* and *L. braziliensis* showed a lower or equal reactivity to rLb6H[20].

The percentage of cross-reactivity to serum samples from patients with Chagas disease, in the serological methods used for the diagnosis of ATL, should be reported to estimate or at least to be aware of the probability of a cross-reactivity. For this reason, when using tests that show a high percentage of cross-reactivity for the diagnosis of ATL, the possibility of a coinfection with *T. cruzi* cannot be ruled out. Any suspicion of coinfection should be investigated using specific Chagas disease tests.

Using the genome sequencing, we can calculate the percentage of similarity of a target protein sequence among species of *Leishmania* and *Trypanosoma cruzi* before they are used as antigen in ELISAs to save time and resources.

There are some limitations in our study. First, the novel antigens as well as soluble antigens of different species of *Leishamnia* are different, resulting in different cut-off values. Second, although the specificity was almost 100%, only a few authors included groups of control patients with diseases other than Chagas, such as tuberculosis, histoplasmosis, malaria and paracoccidioidomycosis. Finally, some studies included in this systematic review did not analyze positive and negative predictive values, and only a few showed values of likelihood ratio, area under the curve and accuracy.

The lack of these data limited the comparison between the methods analyzed with regard to indicators such as sensitivity and specificity. Using paired indicators can be a disadvantage to compare the performance of competing tests, especially if one test does not outperform the other with respect to both indicators[41]. Both, sensitivity and specificity provide information about the probability of obtaining an accurate result (positive or negative) depending on the true condition of the patient with respect to the disease. However, PPV and NPV are useful in clinical practice informing the probability of the patient to be really sick or healthy according to a positive or negative result.

In this systematic review, the novel ELISAs antigens showed an advantage in sensitivity (95.4-100%) and specificity (82.5-100%) compared to standard ELISAs based on SLA (1%-97.4% and 57.5%-100%, respectively). These ELISAs based on SLA, together with IFAs showed heterogeneous accuracy values.

The development of new serological diagnostic tools, with higher accuracy and reliability in the diagnosis of ATL and capable of reducing the risk of cross-reactivity with Chagas disease or other infectious diseases is of great importance for laboratory diagnosis, analysis of treatment efficacy, epidemiology and disease control.

AUTHORS' CONTRIBUTIONS

AZ and CMS contributed with the analysis and interpretation of study data, in addition to the preparation of preliminary versions of this manuscript. FGLP contributed to the strategy of searching and analyzing data. SMBF contributed with data analysis and content review. OAE contributed with the design and delineation of the study, revision of the content and the approval of the final version of the manuscript. The authors approve the final version of the manuscript and are responsible for all its aspects.

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