

Systematic Review

Global Landscape of Molecular and Immunological Diagnostic Tests for Human Leishmaniasis: A Systematic Review and Meta-Analysis

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Abstract

Background: Leishmaniasis is a serious global public health challenge. Its accurate diagnosis is essential for guiding treatment but remains complex, often requiring a combination of tests. **Methods:** We conducted a systematic review and meta-analysis to evaluate the applicability and diagnostic accuracy of molecular and immunological tests for human tegumentary (TL) and visceral leishmaniasis (VL), searching PubMed for studies published between 1990–2021. **Results:** Among 165 included studies, PCR-based tests showed high accuracy, with area under the curve values (AUC_{FPR}) of 0.919 for TL and 0.965 for VL. For TL, serological tests exhibited median sensitivities of 83.3–88.1% and specificities of 82.0–92.4%. For VL, the direct agglutination test (DAT) and ELISA demonstrated higher sensitivity (93.0–93.8%) than rapid tests (89.1%) or immunofluorescence (82.0%), all with high specificity (95.5–96.9%). DAT performance (AUC_{FPR} = 0.966) was comparable to molecular tests. **Conclusions:** Molecular tests are highly accurate for diagnosing leishmaniasis. However, their routine implementation in endemic areas is limited by the need for specialized expertise and infrastructure. We advocate for developing diagnostic procedures tailored to each endemic setting, where molecular methods could facilitate early and accurate diagnosis.

Keywords: leishmaniasis; diagnostic tests; diagnostic accuracy; systematic review; meta-analysis; sensitivity; specificity

1. Introduction

Leishmaniasis is a neglected vector-borne infectious disease caused by protozoan parasites belonging to the genus *Leishmania* (Trypanosomatida: Trypanosomatidae), which encompasses over 20 species pathogenic to humans [1]. The transmission occurs through the bite of infected female phlebotomine sandflies of the genus *Phlebotomus* in the Old World and *Lutzomyia* in the New World [2]. Being endemic in nearly 100 countries globally, leishmaniasis poses a health risk to approximately 350 million people [3]. According to the World Health Organization (WHO), an estimated 700,000 to 1 million new cases and 20,000 to 30,000 deaths due to leishmaniasis occur annually [4,5]. Populations with limited resources are primarily affected by leishmaniasis, resulting in substantial financial losses. Factors such as global warming, globalization, and war/conflict are significant contributors to the spread of insect vector species to new areas, furthering the emergence of leishmaniasis [6,7]. These elements collectively establish leishmaniasis as a major global public health concern.

Leishmania infections can present as either asymptomatic or symptomatic. When the infection becomes symptomatic, it can result in diverse clinical manifestations of variable severity, depending on the infecting *Leishmania* species, host factors (largely immune responses), and vector biology [8]. Tegumentary leishmaniasis (TL) refers to a collection of skin conditions, including localized cutaneous, disseminated cutaneous, diffuse cutaneous, and mucocutaneous leishmaniasis, and post-kala-azar dermal leishmaniasis (a skin sequela of visceral leishmaniasis). The most prevalent form, localized cutaneous leishmaniasis (CL), comprises painless skin sores and ulcers on exposed body parts, such as the face, arms, and legs, with a low risk of life-threatening complications but leaving lifelong scars that result in stigma [7,9]. Mucocutaneous leishmaniasis (MCL) extends to mucous membranes of the nose, mouth, or throat, causing destructive ulcers that can lead to severe disfigurement [9]. Visceral leishmaniasis (VL), also known as kala-azar, represents the most severe form of the disease, affecting internal organs, with symptoms including prolonged fever, weight loss, hepatomegaly, splenomegaly, anemia, and compromised immunity; if left untreated, VL can be fatal [7]. Common histopathological findings in all clinical forms of leishmaniasis include chronic granulomatous inflammation defined by the presence of macrophages harboring *Leishmania* amastigotes, plasma cells, and T lymphocytes [10].

While efforts to develop effective vaccines against human leishmaniasis are underway [11], early and accurate diagnosis and timely chemotherapy remain the major tools in the fight against the different forms of the disease. In turn, the effectiveness of these tools is influenced by the varied host–parasite interactions, immune responses, and immunopathology that underlie the different clinical manifestations of human leishmaniasis [8].

Due to the wide clinical spectrum of leishmaniasis, which often overlaps and resembles other diseases of infectious or non-infectious origin [12], the clinical management of leishmaniasis is challenging and requires specific expertise plus diagnostic tools. Its diagnosis encompasses clinical evaluation, epidemiological context, and laboratory tests. The choice of diagnostic tools is based on the clinical presentation as well as available diagnostic tests, resources, infrastructure, and technical expertise [13,14]. Traditional diagnostic tools include microscopic examination of stained smears and culture [13]; although being highly specific, both methods suffer from insufficient sensitivity [15–17]. Immunoassays for serological diagnosis show variable sensitivity and specificity, depending on the antigen used and clinical presentation [18–23]. Serological tests are of limited utility in TL diagnosis because of the typical low to moderate antibody titers, with the exception being diffuse CL that is associated with a high humoral response [24]. By contrast, in VL, characterized by a high parasite load in internal organs, serological testing is of clinical utility since VL patients typically present high titers of anti-*Leishmania* antibodies [25]. The leishmanin

(Montenegro) skin test, which measures the delayed-type hypersensitivity response to *Leishmania* antigens, has been used to detect exposure to TL- and VL-causing *Leishmania* species and the development of cell-mediated immunity [26]. A range of T cell-mediated immune responses characterizes the clinico-immunological spectrum of TL [24]. At one end of the spectrum, an exaggerated cell-mediated immune response mediates immunopathology in patients with mucosal leishmaniasis, despite the ability to control the parasite load (i.e., low parasite numbers within the lesions) [24]. At the other end of the spectrum, patients with diffuse CL have a lack of T cell-mediated immunity, and their lesions present a high parasite load [24]. Immunohistochemistry has also proven useful as a complementary tool for the diagnosis of cutaneous and mucosal leishmaniasis [27]. Parasite loads in cutaneous lesions correlate inversely with disease stage (i.e., chronic lesions harbor fewer amastigotes), which has an impact on the sensitivity of diagnostic tests [17,28].

Polymerase chain reaction (PCR)-based techniques have become important molecular tools in leishmaniasis diagnosis, as they provide high sensitivity and specificity for both detecting and identifying the infecting parasite species and are directly applied to clinical specimens [29]. Molecular tools are especially valuable to help diagnose atypical and difficult-to-diagnose cases [30]. Currently, the main shortcoming is that these modern diagnostic tools are mainly restricted to reference centers and research laboratories due to the requirement of infrastructure, resources, and trained personnel. Quantitative evidence underscores this gap: a systematic review revealed that in low-resource settings, PCR-based diagnostics for CL are implemented in below 10% of suspected cases, with most diagnoses relying on microscopy or clinical presentation alone [31]. For visceral leishmaniasis VL in East Africa, access is similarly constrained, with fewer than 15% of health facilities in endemic areas possessing the capacity for molecular tests [32]. Moreover, a study in Kenya and Ethiopia found that only 12% of VL patients were diagnosed using molecular methods, with the majority relying on serological tests like rK39, which have known limitations in sensitivity and specificity [33]. Hence, low-resource health centers in leishmaniasis endemic areas mostly can rely only on microscopy, as they have limited access (if any) to molecular tools. This may result in delayed diagnosis, in turn impacting the timely initiation of appropriate treatment for patients. By recognizing the limitations in the confirmatory diagnosis of leishmaniasis, advances in research efforts continue to be made to develop easy-to-use diagnostic tools, which are much needed to enhance the accessibility of diagnosis and support leishmaniasis control efforts [34,35]. Promising avenues include the use of portable diagnostic devices, such as handheld PCR machines [36], and the development of isothermal nucleic acid amplification tests, such as LAMP and RPA, either alone or in combination with CRISPR-Cas technology [34].

Here, we conducted a comprehensive systematic review and meta-analysis spanning 31 years of published biomedical literature to summarize the evidence on the diagnostic accuracy of molecular and immunological tests available for diagnosing human leishmaniasis. We hope that this work provides valuable insights to researchers, healthcare workers, and public health practitioners involved in leishmaniasis case management and control.

2. Materials and Methods

2.1. Study Protocol

This systematic review was conducted following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Table S1) [37]. The protocol for this systematic review was registered on the International Platform of Registered Systematic Review and Meta-analysis Protocols (INPLASY) website (registration number INPLASY2023110066) and is available in full at [inplasy.com \(https://inplasy.com/inplasy-2023-11-0066/; accessed on 16 November 2023\)](https://inplasy.com/inplasy-2023-11-0066/).

2.2. Information Sources and Search Strategy

We used the Medical Subject Headings (MeSH) term “*leishmaniasis*” to find terms related to the diagnosis of leishmaniasis in the biomedical literature. The results were plotted in a network diagram of the co-occurrence of MeSH terms in the VOSviewer software (version 1.6.18) [38]. To select terms related to leishmaniasis diagnostic tests, we examined clusters in the network map. Additionally, a second round of searches was performed by associating each MeSH term found in the cluster analysis with the MeSH terms “*sensitivity and specificity*,” which are commonly considered indicators for evaluating the diagnostic performance of a test [39], and the MeSH term “*leishmaniasis*”. Records were retrieved from the bibliographic database PubMed (<https://pubmed.ncbi.nlm.nih.gov/>, last accessed on 21 December 2021) for the period 1990–2021.

2.3. Selection Criteria and Data Extraction

Three separate stages were engaged in the selection of studies for this review. In the initial identification phase, only studies on human patients published from 1990 to 2021 were considered. Duplicate articles, non-English publications, review papers, and meta-analyses were excluded. The subsequent screening phase involved checking titles and abstracts of the identified articles. In the eligibility/qualification phase, full-text studies highly relevant to the research question, specifically focusing on diagnostic tests for leishmaniasis, were retrieved. Data extraction from each selected study included information on the diagnostic test, total sample size, number of leishmaniasis patients, clinical characteristics of the patients, clinical form of leishmaniasis, sample type, and controls. Only studies assessing diagnostic accuracy with sensitivity and specificity measures were included, while those with limited, incomplete, or conflicting information were excluded. Traditional diagnostic tests such as microscopy, culture, and histopathology were not included because we decided to focus on molecular and immunological testing due to their increasing application in the detection of early *Leishmania* infections in clinical and research laboratories [14]. Data extraction was conducted by B.M.R.-P. and independently validated by M.A.C.-P., with any discrepancies resolved through discussion and consultation with M.A.C.-F.

2.4. Statistical Analysis

Results were entered into a Microsoft Excel (version 19.0, Microsoft Corporation, Redmond, WA, USA) spreadsheet and analyzed in the R programming environment (version 4.2.3) using the “*mada*” package (version 0.5.11), available at: <https://cran.r-project.org/web/packages/mada/index.html> (accessed on 21 December 2021). Mada, which stands for meta-analysis of diagnostic accuracy, applies a statistical method used to combine and analyze multiple studies evaluating the accuracy of a diagnostic test or procedure. This statistical package takes into account variability between studies, examines possible sources of heterogeneity, and may incorporate methods such as subgroup analysis or meta-regression to explore factors affecting test performance [40,41]. Initially, the number of true negatives (TN), false negatives (FN), true positives (TP), and false positives (FP) was analyzed separately for each diagnostic test, while the assessment of sensitivity and specificity was used to determine the diagnostic performance. Sensitivity was defined as the true positive rate, i.e., the probability that a positive test result will be obtained for a subject who has the disease, and calculated as $TP/(TP + FN)$. Specificity was defined as the true negative rate, i.e., the probability that a negative test result will be obtained for a subject who does not have a disease or condition, and calculated as $TN/(TN + FP)$. Also, the following ratios were calculated: the positive likelihood ratio (LR+), the negative likelihood ratio (LR−), and the diagnostic odds ratio (DOR). The LR+ was defined as the true positivity rate divided by the false positivity rate. The LR− was defined as the

ratio between the false negative rate and the true negative rate. The lower the LR[−] value, the larger the decrease in the odds of having a condition when the test result is negative, indicating better diagnostic accuracy. The DOR of a test is the ratio of the odds of a positive test in those with disease relative to the odds of a positive test in those without disease. The DOR combines both the positive and negative likelihood ratios and provides a comprehensive measure of diagnostic accuracy. A DOR greater than 1 indicates that the test result is associated with an increased likelihood of having the condition, while a DOR less than 1 indicates a decreased likelihood [42]. The summary receiver operating characteristic (sROC) curve was fitted according to the “Reitsma” model parameters of the “mada” package and used to compare the diagnostic accuracy of the tests [43]. The sROC curve combines sensitivity and specificity data from individual studies to create a summary plot that represents the relationship between the true positive rate (sensitivity) and false positive rate (1-specificity) across a range of diagnostic thresholds. The sROC curve provides a visual summary of the trade-off between sensitivity and specificity of a diagnostic test, and the area under the curve (AUC) is a quantitative measure of overall test performance that also serves to identify sources of heterogeneity by visually analyzing the dispersion of individual study points around the curve, where extensive scattering indicates high heterogeneity [44]. Unlike the conventional AUC, which integrates the entire ROC curve and therefore includes stretches where no study actually provided data, the AUC restricted to the observed false-positive rate (AUC_{FPR}) measures test performance only across the FPR values that were truly documented. By cutting away the extrapolated portions, the AUC_{FPR} keeps the estimate grounded in evidence and prevents inflation by speculation. This makes it especially valuable when false positives are costly, as in low-prevalence diseases where even a small FPR can generate many misdiagnoses and unnecessary expenses [45]. The confidence level for all calculations was set at 95%, using a continuity correction of 0.5 if relevant.

3. Results

3.1. Data Sources and Study Selection

A flowchart of the study strategy is given in Figure 1. A search for the MeSH term “leishmaniasis” was performed in PubMed, and a MeSH term co-occurrence network map was developed. The search yielded 1084 scientific articles from 1990 to 2021. The minimum number of keyword occurrences was set to five, resulting in a network graph containing 3632 keywords (Figure 2). Analysis of the network map revealed the formation of five main clusters. The cluster related to serological diagnostic tests (yellow color) included terms such as “enzyme-linked immunosorbent assay”, “fluorescent antibody technique”, “direct agglutination test”, and “Western blot”. In the cluster related to molecular diagnostic tests (purple color), terms such as “polymerase chain reaction”, “real-time polymerase chain reaction”, and “nucleic acid amplification techniques” were found. Furthermore, terms such as “leishmaniasis, visceral”, “leishmaniasis”, “leishmaniasis, cutaneous”, “antiprotozoal agents”, “Leishmania infantum”, “Brazil”, and “Leishmania donovani” were common (Figure 2). The terms discovered in the initial analysis were used in a second search in the PubMed database. The new search strings were created by combining the new terms with “leishmaniasis” and “sensitivity and specificity,” as delineated in File S1.

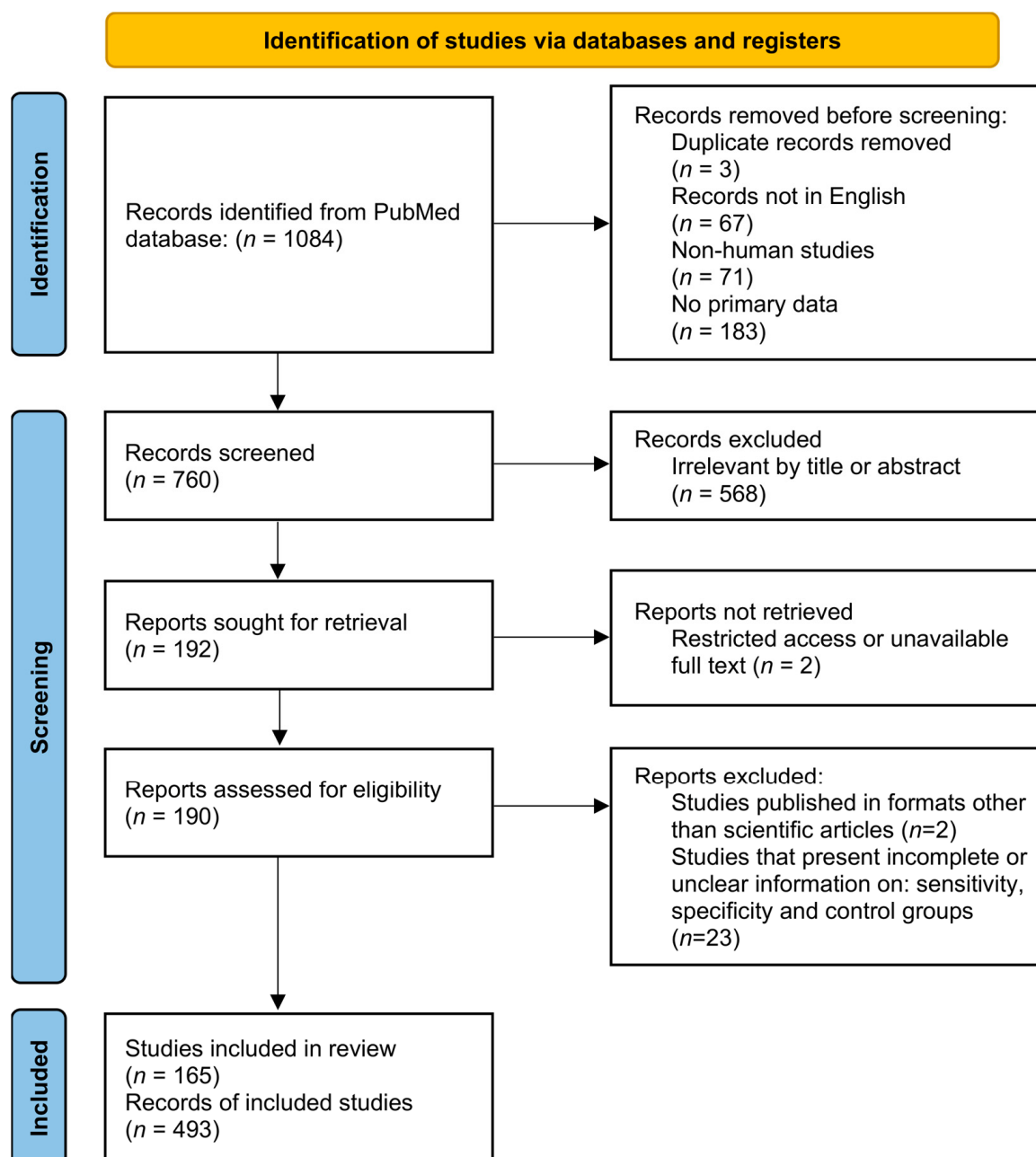


Figure 1. A flowchart of the systematic review and meta-analysis of the study selection process.

Among the retrieved studies on the performance of immunological diagnostic tests for leishmaniasis, there were 5 studies for LST, 277 for ELISA, 89 for IFAT, 10 for IHA, 78 for RDT, 138 for DAT, and 33 for WB. The retrieved studies on molecular diagnostic tests for leishmaniasis comprised 389 for PCR, 64 for qPCR, and 3 for LAMP. The three-step selection criteria we followed resulted in the exclusion of 324 articles during the identification phase, 568 articles during the screening phase, and 27 articles during the eligibility phase. As a result, 165 articles were selected for the present systematic review and meta-analysis. Some of these studies reported the evaluation of more than one diagnostic index test, resulting in a total of 493 records included in the present review. The PRISMA flow diagram summarizes record search and selection and reasons for exclusion (Figure 1).

urine, peripheral blood, and bone marrow aspirates. Controls were rigorously selected to include healthy individuals from endemic and non-endemic areas, as well as patients with diseases known to cause serological cross-reactivity (e.g., malaria, tuberculosis, and Chagas disease), thereby enabling robust specificity assessments. Study designs were predominantly case–control (70%), with prospective and comparative cohorts also well-represented, underscoring a global effort to validate diagnostic accuracy under varied epidemiological conditions.

The index tests most frequently evaluated among all included studies were ELISA, DAT, PCR, and RDT (Figure 3A). Concerning the geographical region of the studies, Brazil, India, and Sudan were the countries for which we found the highest number of records related to diagnostic tests for leishmaniasis (Figure 3B).

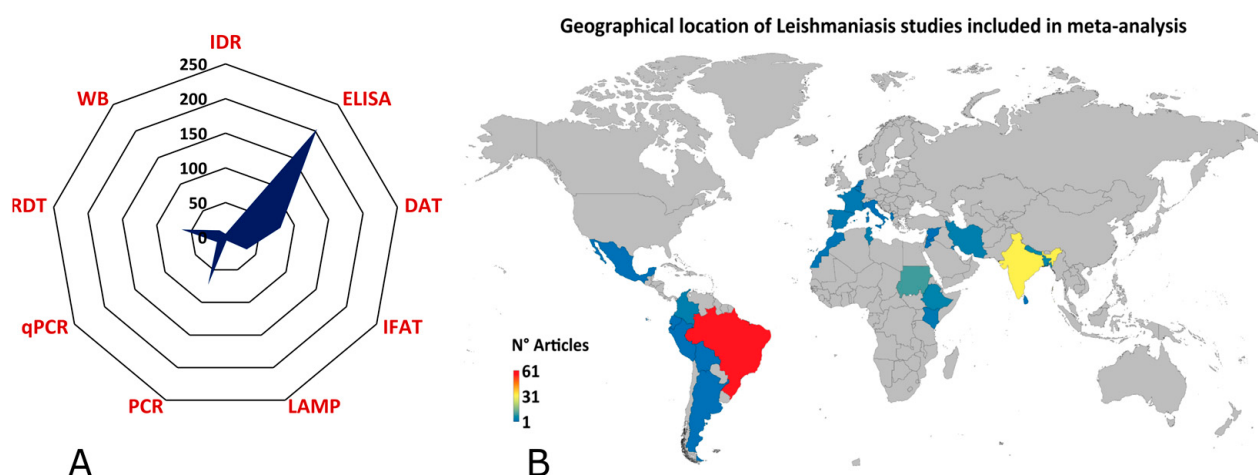


Figure 3. The geographical location of leishmaniasis studies. (A) The radial graph shows the type of diagnostic tests used in the leishmaniasis studies included in the meta-analysis. (B) Demographic representation of global leishmaniasis studies included in the meta-analysis (lower-blue to upper-red numbers).

3.3. Meta-Analysis of Diagnostic Performance of Laboratory Tests for Leishmaniasis

3.3.1. Tegumentary Leishmaniasis

Analyzed Tests

In the context of diagnostic tests for TL, sixty-one primary reports met the eligibility criteria. There were 5 studies for LST [46–50], 20 for ELISA [47,51–69], 6 for IFAT [46,49,53,60,70,71], 3 for WB [53,61,72], 23 for PCR [46–49,73–91], and 4 for qPCR [86,92–94]. Median diagnostic performance is summarized in Table 1. LST and conventional PCR yielded the greatest sensitivities (91.7% and 90.6%, respectively). The highest specificities were observed for WB (92.4%), PCR (95.1%), and qPCR (98.3%). A negative correlation between sensitivity and false-positive rate was noted for ELISA and qPCR, indicating that a greater sensitivity in these assays coincided with fewer false positives. ELISA and IFAT demonstrated DOR > 1 but remained inferior to molecular tests in discriminative ability.

Table 1. Meta-analysis of diagnostic tests for tegumentary leishmaniasis.

Parameters	Diagnostic Techniques					
	LST	ELISA	IFAT	WB	PCR	qPCR
Number of scientific articles	5	20	6	3	23	4
Number of records	5	60	16	12	36	8
Number of subjects studied	735	3938	920	571	3380	332

Table 1. Cont.

Parameters		Diagnostic Techniques					
		LST	ELISA	IFAT	WB	PCR	qPCR
Sensitivity	Range (%)	88.0–99.1	40.3–99.3	56.6–97.5	76.6–98.7	8.30–99.2	37.5–99.0
	Median (%)	91.7	83.3	88.1	86.3	90.6	73.8
	95% CI	79.0–98.1	71.5–91.6	74.7–94.1	75.2–92.8	76.1–94.6	58.0–85.2
	χ^2	9.71	620.85	115.23	52.62	401.61	59.07
	df	4	59	15	11	35	7
	<i>p</i> -value	0.04	2.00×10^{-16}	2.00×10^{-16}	2.10×10^{-7}	2.00×10^{-16}	2.32×10^{-10}
Specificity	Range (%)	63.2–95.0	13.6–99.7	22.7–96.4	59.1–99.4	41.7–99.6	67.6–98.6
	Median (%)	83.4	83.3	82.0	92.4	95.1	98.3
	95% CI	65.5–87.7	70.9–90.1	60.7–88.7	66.2–97.9	78.2–99.3	85.9–99.9
	χ^2	18.39	688.59	143.33	38.48	334.26	36.37
	df	4	59	15	11	35	7
	<i>p</i> -value	0.001	2.00×10^{-16}	2.00×10^{-16}	6.49×10^{-5}	2.00×10^{-16}	6.18×10^{-6}
Correlation between sensitivities and false positive rates	<i>r</i>	0.284	−0.284	0.043	0.111	0.194	−0.557
	95%CI	−0.798–0.933	−0.502–0.032	−0.462–0.528	−0.494–0.644	−0.144–0.491	−0.906–0.242
LR+	Median (%)	5.31	5.00	4.95	11.42	15.45	47.72
	95% CI	1.97–7.22	2.72–9.17	1.48–7.93	1.91–47.07	2.22–136.88	3.04–749.93
LR−	Median (%)	0.09	0.20	0.21	0.15	0.13	0.27
	95% CI	0.03–0.25	0.11–0.49	0.11–0.46	0.08–0.28	0.06–0.34	0.16–0.46
DOR	Median (%)	192.07	41.18	32.97	98.20	113.38	159.84
	95% CI	11.26–3276.10	8.50–154.71	5.63–130.97	15.15–913.66	9.89–2119.91	9.80–2829.78
Supplementary Figure		Figures S1 and S2	Figures S3 and S4	Figures S5 and S6	Figures S7 and S8	Figures S9 and S10	Figures S11 and S12

CI, Confidence interval; χ^2 , chi-square; df, degrees of freedom; *r*, Pearson's correlation coefficient.

Other Diagnostic Tests

Two studies on the performance of RDT [93,95] and two studies on DAT [96,97] for TL diagnosis were selected. According to the established criteria, at least 5 studies were needed for the analysis with a *p*-value < 0.05. Therefore, no analysis could be performed regarding these diagnostic tests.

Summary ROC Curves (sROC)

Data comparison of diagnostic tests for TL (LST, ELISA, IFAT, WB, PCR, and qPCR) was carried out using a sROC curve analysis (Figure 4). The AUC calculated for each diagnostic test showed that WB, PCR, and qPCR offered the best diagnostic performance (Figure 4). Furthermore, PCR performed robustly for TL when the AUC was restricted to the observed false positive rate ($AUC_{FPR} = 0.919$, Figure 4).

3.3.2. Visceral Leishmaniasis

Analyzed Tests

Concerning diagnostic tests for VL, there were 23 included studies using DAT [52,98–141], 58 for ELISA [52,61,69,116–119,121–123,132,135,136,142–186], 12 for IFAT [52,98,116,117,125,128,132,171,187–190], 33 for RDT [98,102,116,117,122,125,127–130,132–135,137,140,141,146,158,180,184–189,191–197], 3 for LAMP [142,193,196], 22 for PCR [77,85,125,159,179,193,196–211], and 5 for qPCR [142,166,189,210,212]. A detailed examination of the diagnostic performance of these tests in the included studies is shown in Table 2. DAT showed the most favorable accuracy profile, with a median sensitivity of 93.8%, a median specificity of 96.9%, and the highest AUC_{FPR} (0.966). ELISA and qPCR offered comparable sensitivities (92.9%) but lower specificities than DAT. LAMP, PCR, and qPCR provided the greatest specificities (>96%). Lastly, DAT, ELISA, IFAT, and RDT displayed an inverse relation between sensitivity and false-positive rate.

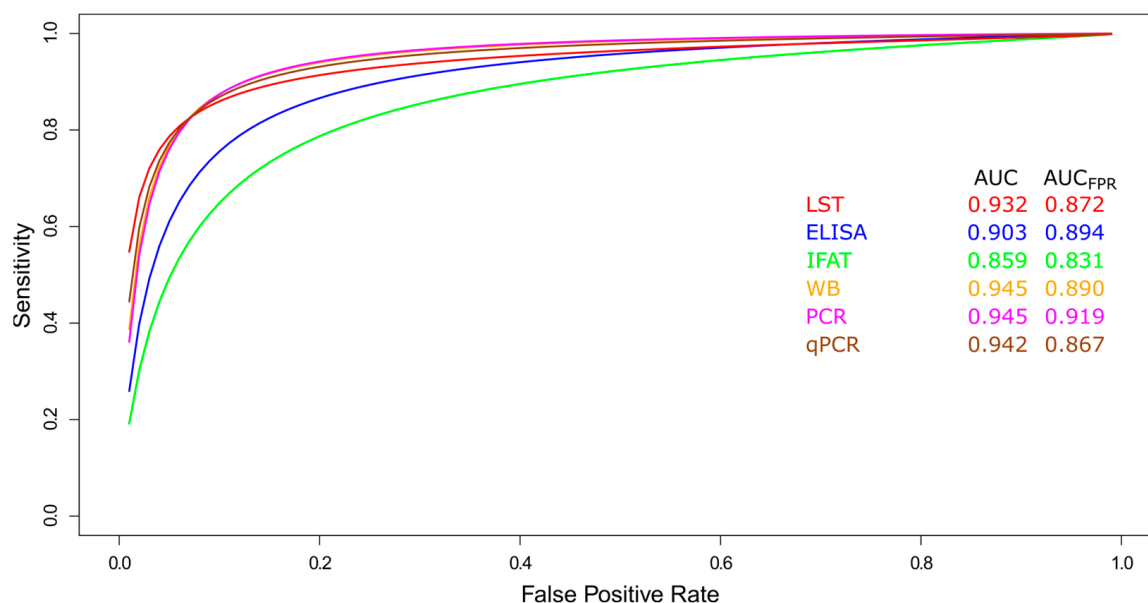


Figure 4. Meta-analysis of diagnostic test accuracy studies for tegumentary leishmaniasis. Summary receiver operating characteristic (sROC) curve of sensitivity (true positive rate) against false positive rate (1-specificity). Comparison between LST, ELISA, IFAT, WB, PCR, and qPCR methods used in the diagnosis of tegumentary leishmaniasis.

Table 2. Meta-analysis of diagnostic tests for visceral leishmaniasis.

Parameters		Diagnostic Techniques						
		DAT	ELISA	IFAT	RDT	LAMP	PCR	qPCR
Number of scientific articles		23	58	12	33	3	22	5
Number of records		77	143	19	61	5	37	6
Number of subjects studied		10,561	15,142	4010	9633	536	4839	808
Sensitivity	Range (%)	47.8–99.8	32.2–99.6	30.2–98.9	19.6–99.9	84.6–95.7	17.6–99.0	72.2–98.8
	Median (%)	93.8	93.0	82.0	89.1	90.1	89.8	92.9
	95% CI	85.7–97.5	83.1–97.0	71.8–88.3	81.4–94.0	81.4–95.0	78.9–95.1	85.2–97.0
	χ ²	947.23	2218.86	517.19	2182.96	7.80	839.36	35.52
	df	76	142	18	60	4	36	5
	p-value	2.00 × 10 ^{−16}	2.00 × 10 ^{−16}	2.00 × 10 ^{−16}	2.00 × 10 ^{−16}	0.10	2.00 × 10 ^{−16}	1.18 × 10 ^{−16}
Specificity	Range (%)	43.0–99.9	26.8–99.7	63.3–99.7	40.7–99.9	99.4–99.5	11.9–99.7	89.0–99.7
	Median (%)	96.9	95.5	95.6	95.6	99.4	98.4	99.4
	95% CI	89.1–99.0	83.3–98.4	79.1–98.4	86.4–98.9	94.4–99.9	89.3–99.7	94.4–99.9
	χ ²	1802.87	2497.12	237.41	592.85	0.03	905.85	25.07
	df	76	142	18	60	4	36	5
	p-value	2.00 × 10 ^{−16}	2.00 × 10 ^{−16}	2.00 × 10 ^{−16}	2.00 × 10 ^{−16}	1.00	2.00 × 10 ^{−16}	1.35 × 10 ^{−4}
Correlation between sensitivities and false positive rates	r	−0.177	−0.297	−0.468	−0.023	0.263	0.164	0.392
	95%CI	−0.386–0.049	−0.440–−0.140	−0.760–−0.017	−0.273–0.230	−0.806–0.930	−0.169–0.463	−0.616–0.913
LR+	Median (%)	31.50	16.75	17.92	19.70	155.00	48.62	134.00
	95% CI	6.15–107.95	4.30–53.83	3.60–50.23	5.40–73.09	9.78–2457.87	4.68–440.52	8.44–2127.86
LR−	Median (%)	0.07	0.08	0.18	0.11	0.10	0.12	0.07
	95% CI	0.03–0.16	0.03–0.25	0.12–0.34	0.07–0.20	0.05–0.20	0.05–0.23	0.03–0.19
DOR	Median (%)	440.13	372.04	79.31	215.20	1854.07	510.21	1409.21
	95% CI	71.04–4196.90	32.35–2779.15	22.19–1081.73	39.86–1549.43	104.18–32,998.12	46.97–6424.00	85.50–26,179.10
Supplementary Figure		Figures S13 and S14	Figures S15 and S16	Figures S17 and S18	Figures S19 and S20	Figures S21 and S22	Figures S23 and S24	Figures S25 and S26

CI, Confidence interval; χ^2 , chi-square; df, degrees of freedom; r, Pearson's correlation coefficient.

Other Diagnostic Tests

Two studies, one concerning WB [61] and one on IHA [187], were selected for VL diagnosis. According to the established criteria, at least 5 studies with a p -value < 0.05 were required for the analysis. As a result, no analysis of these diagnostic tests could be conducted.

Summary ROC Curves (sROC)

Data for VL diagnostic tests (DAT, ELISA, IFAT, RDT, LAMP, PCR, and qPCR) were compared using a sROC curve analysis (Figure 5). All diagnostic tests showed an overall good performance ($AUC > 0.9$, Figure 5), with LAMP standing out ($AUC = 0.990$, Figure 5). Yet, when the AUC was limited to the observed false positive rate, LAMP showed lower accuracy ($AUC_{FPR} = 0.499$, Figure 5) than the other tests (AUC_{FPR} range between 0.899 and 0.966, Figure 5). This discrepancy arises because the AUC_{FPR} isolates the test's ability to minimize false positives, revealing a potential weakness of LAMP in distinguishing between diseased and non-diseased individuals, despite overall good performance. On the other hand, DAT performed properly for VL ($AUC = 0.975$ and $AUC_{FPR} = 0.966$; Figure 5), and its results were comparable to those of PCR tests ($AUC = 0.967$ and $AUC_{FPR} = 0.965$; Figure 5).

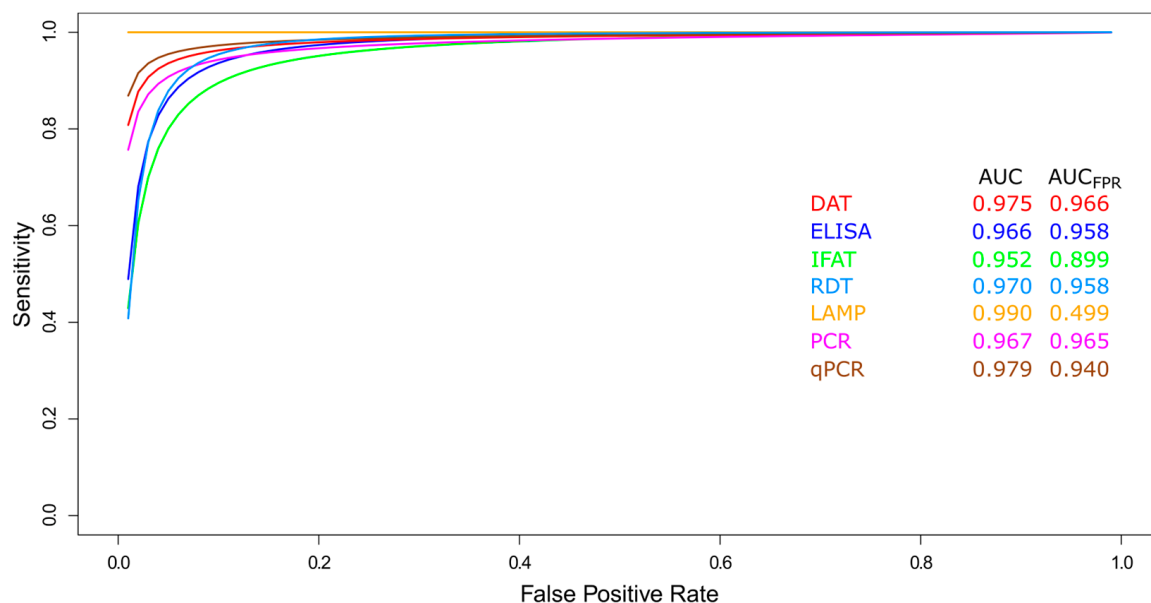


Figure 5. Meta-analysis of diagnostic test accuracy studies for visceral leishmaniasis. Summary receiver operating characteristic (sROC) curve of sensitivity (true positive rate) against false positive rate (1-specificity). Comparison between DAT, ELISA, IFAT, LAMP, PCR, qPCR, and RDT methods used in the diagnosis of visceral leishmaniasis.

4. Discussion

According to the WHO, early diagnosis and prompt treatment for leishmaniasis reduce disease prevalence, prevent disabilities and death, help lower transmission, and facilitate the estimation of disease burden and the development of interventions for disease control [5]. In this context, the WHO Roadmap for Neglected Tropical Diseases 2021–2030 highlights diagnosis as a key pillar in the efforts to control infectious diseases such as leishmaniasis, emphasizing the need to strengthen diagnostic capacity to achieve the proposed objectives [213]. To this end, the implementation of accurate and accessible molecular and/or immunological diagnostic tests is crucial for improving the early detection and clinical management of leishmaniasis cases, thereby contributing to global efforts towards the effective control and eventual elimination of leishmaniasis as a public health problem.

4.1. Summary of Main Findings

This study constitutes a comprehensive systematic review and meta-analysis that evaluates the global landscape of molecular and immunological diagnostic tests for human leishmaniasis. The findings reveal a complex diagnostic scenario, characterized by

significant geographical and methodological heterogeneity, as well as clear trends in test performance depending on the clinical form of the disease.

For TL, the results demonstrate that molecular tests, particularly conventional PCR and qPCR, exhibit the best diagnostic performance profile. PCR demonstrated exceptional median sensitivity (90.6%) and high specificity (95.1%), as supported by an AUC in the sROC analysis, confirming its robustness. Although evaluated in a smaller number of studies, qPCR showed the highest reported specificity (98.3%), positioning it as an ideal confirmatory tool in contexts where specificity is paramount, such as in areas with co-endemic diseases [214]. In contrast, serological tests such as ELISA and IFAT, although widely used, have shown inferior discriminatory ability (DOR) compared to molecular techniques, suggesting their utility may be more limited to initial screening studies or in resource-limited settings where PCR is not available.

In the case of VL, the findings are different. Serological tests demonstrated outstanding performance. DAT emerged as the most accurate test, with median sensitivity and specificity of 93.8% and 96.9%, respectively, and an AUC_{FPR} of 0.966, indicating an excellent ability to minimize false positives. ELISA and RDT also demonstrated high sensitivities (~93%), reinforcing the central role of serology in VL diagnosis, particularly in the field where rapid results are required. Although molecular techniques like LAMP showed a near-perfect overall AUC (0.990), its AUC_{FPR} was significantly lower (0.499). This discrepancy suggests that, despite its high potential, the LAMP technique may present challenges in terms of specificity in real-world practice, as observed in the included studies.

4.2. Interpretation of Findings in the Context of Existing Literature

The diagnostic performance of molecular and immunological tests for leishmaniasis has been a subject of intense research in biomedical literature. Various studies have highlighted that in immunocompromised patients, the sensitivity of serological tests tends to be reduced due to the inability of these individuals to mount an effective immune response against *Leishmania* parasites [215]. According to the statistical definition, sensitivity is a property of the test that reflects its ability to correctly identify individuals with the disease [216]. In this clinical context, the low antibody production frequently leads to false-negative results, especially in the early stages of the disease or in cases with low parasite loads. By contrast, PCR has been recognized as a more reliable test, capable of directly detecting the parasite's genetic material, even in low-level infections, in human host tissue samples [14]. However, the applicability of PCR in resource-limited settings remains a challenge due to its requirement for specialized equipment and trained personnel, which limits its use in rural areas or countries with underdeveloped healthcare infrastructures.

4.3. Strengths and Limitations

This review's main strength is the thorough examination of the published literature, which made it possible to assess the accuracy of leishmaniasis diagnostic tests across various endemic geographic regions. The use of PRISMA guidelines for conducting the systematic review ensured transparency and reproducibility of the procedures, reinforcing the reliability of the findings [217]. Additionally, prospectively registering the protocol in INPLASY allowed for a standardized methodology, ensuring the review maintains its relevance over time [218]. The inclusion of diverse diagnostic techniques—both serological and molecular tests—enriched the discussion, offering a broad perspective on the diagnostic performance of available tests across different populations and settings. This strengthens the validity of the conclusions and their applicability to real-world diagnostic challenges in leishmaniasis.

There are important limitations of this review. The inherent heterogeneity among the included studies—evidenced by differences in population study designs (acute/chronic stages of disease, New/Old World clinical forms), sampling methods, controls used, composite reference standards, and reference tests (microscopy/PCR/culture) used—hindered direct comparisons and prevented the assessment of diagnostic test performance according to specific clinical-epidemiological contexts. A second limitation relates to the issue of possible publication bias. For the selection of studies included in this review, we considered journal articles published in English as an eligibility criterion. In addition, there is potential underrepresentation of studies with negative findings. Under-reporting is also possible since we found a scarcity of data from endemic regions with a weak publication record (e.g., sub-Saharan Africa, rural areas of Asia). While we did not formally test for publication bias, we believe that the overall estimation of the diagnostic test accuracy itself was not directly affected. However, we issue a warning regarding the conclusions of this review's transregional generalizability.

4.4. Recommendations for Standardization and Future Research

To improve the accuracy of leishmaniasis diagnostic tests, it is essential to standardize methods and result interpretation criteria. Multicenter prospective studies are recommended, including a variety of *Leishmania* species and different biological samples to provide a more comprehensive assessment of test performance [219]. Furthermore, the development of standardized reference panels would allow for objective comparisons between diagnostic tests. When addressing the different symptoms and signals for each type of leishmaniasis, it is crucial to apply appropriate analytical methods tailored to the clinical context. This includes using stratified analysis based on *Leishmania* species or biological sample types, and multivariate techniques to identify factors like patient age, geographic location, or co-infections, which can impact diagnostic accuracy. A clear methodology that accounts for these variables will enhance the interpretation of symptoms and improve the comparison of diagnostic tests across different settings.

4.5. Health Policy Perspectives and Technological Development

The findings of this review could influence public health policy decisions, guiding investments in research and the development of new diagnostic technologies. Global health policies, such as those proposed by the WHO, should promote the integration of rapid and accessible diagnostic tests into leishmaniasis control programs, particularly in rural areas and countries with limited infrastructure. Investment in research on new tests, such as low-cost PCR-based methods, could be key to improving the diagnosis and treatment of this neglected disease.

5. Conclusions

The results showed that while serological assays (ELISA, IFAT) represent an accessible alternative in resource-limited settings, their variable sensitivity compromises their reliability for the early diagnosis of leishmaniasis. In contrast, molecular methods (PCR) demonstrate superior sensitivity (>90%) and a critical capacity to detect low parasite loads, establishing themselves as indispensable pillars for timely clinical management. It is crucial to consider that in recent years, the SARS-CoV-2 pandemic has driven the global acquisition of equipment and infrastructure for molecular testing, such as PCR, an advance that could mitigate historical operational barriers in remote endemic areas. Nevertheless, major challenges persist, such as methodological heterogeneity and the lack of global standardization. These findings reinforce the public health imperative to develop integrated strategies—combining serology for screening and PCR for confirmation—alongside policies

that capitalize on this new diagnostic capacity, drive accessible technological innovation, and prioritize equity. Only then can the WHO's goal of controlling this neglected disease be realized, transforming technical precision into better human outcomes for the most vulnerable communities.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/pathogens14111123/s1>, Figure S1: Study data and paired forest plot of the sensitivity and specificity of the leishmanin skin test (LST) in tegumentary leishmaniasis diagnosis [46–50]; Figure S2: Study data and paired forest plot of the positive likelihood ratio, negative likelihood ratio, and diagnostic odds ratio of the leishmanin skin test (LST) in the diagnosis of tegumentary leishmaniasis [46–50]; Figure S3: Study data and paired forest plot of the sensitivity and specificity of enzyme-linked immunosorbent assay (ELISA) in tegumentary leishmaniasis diagnosis [47,51–69]; Figure S4: Study data and paired forest plot of the positive likelihood ratio, negative likelihood ratio, and diagnostic odds ratio of enzyme-linked immunosorbent assay (ELISA) in the diagnosis of tegumentary leishmaniasis [47,51–69]; Figure S5: Study data and paired forest plot of the sensitivity and specificity of indirect fluorescent antibody test (IFAT) in tegumentary leishmaniasis diagnosis [46,49,53,60,70,71]; Figure S6: Study data and paired forest plot of the positive likelihood ratio, negative likelihood ratio, and diagnostic odds ratio of indirect fluorescent antibody test (IFAT) in the diagnosis of tegumentary leishmaniasis [46,49,53,60,70,71]; Figure S7: Study data and paired forest plot of the sensitivity and specificity of Western blot (WB) in tegumentary leishmaniasis diagnosis [53,61,72]; Figure S8: Study data and paired forest plot of the positive likelihood ratio, negative likelihood ratio, and diagnostic odds ratio of Western blot (WB) in the diagnosis of tegumentary leishmaniasis [53,61,72]; Figure S9: Study data and paired forest plot of the sensitivity and specificity of polymerase chain reaction (PCR) in tegumentary leishmaniasis diagnosis [46–49,73–91]; Figure S10: Study data and paired forest plot of the positive likelihood ratio, negative likelihood ratio, and diagnostic odds ratio of polymerase chain reaction (PCR) in the diagnosis of tegumentary leishmaniasis [46–49,73–91]; Figure S11: Study data and paired forest plot of the sensitivity and specificity of real-time polymerase chain reaction (qPCR) in tegumentary leishmaniasis diagnosis [86,92–94]; Figure S12: Study data and paired forest plot of the positive likelihood ratio, negative likelihood ratio, and diagnostic odds ratio of real-time polymerase chain reaction (qPCR) in the diagnosis of tegumentary leishmaniasis [86,92–94]; Figure S13: Study data and paired forest plot of the sensitivity and specificity of the direct agglutination test (DAT) in visceral leishmaniasis diagnosis [52,98–141]; Figure S14: Study data and paired forest plot of the positive likelihood ratio, negative likelihood ratio, and diagnostic odds ratio of the direct agglutination test (DAT) in the diagnosis of visceral leishmaniasis [52,98–141]; Figure S15: Study data and paired forest plot of the sensitivity and specificity of enzyme-linked immunosorbent assay (ELISA) in visceral leishmaniasis diagnosis [52,61,69,116–119,121–123,132,135,136,142–186]; Figure S16: Study data and paired forest plot of the positive likelihood ratio, negative likelihood ratio, and diagnostic odds ratio of enzyme-linked immunosorbent assay (ELISA) in the diagnosis of visceral leishmaniasis [52,61,69,116–119,121–123,132,135,136,142–186]; Figure S17: Study data and paired forest plot of the sensitivity and specificity of indirect fluorescent antibody test (IFAT) in visceral leishmaniasis diagnosis [52,98,116,117,125,128,132,171,187–190]; Figure S18: Study data and paired forest plots of the positive likelihood ratio, negative likelihood ratio, and diagnostic odds ratio of indirect fluorescent antibody test (IFAT) in the diagnosis of visceral leishmaniasis [52,98,116,117,125,128,132,171,187–190]; Figure S19: Study data and paired forest plot of the sensitivity and specificity of loop-mediated isothermal amplification (LAMP) in visceral leishmaniasis diagnosis [142,193,196]; Figure S20: Study data and paired forest plot of the positive likelihood ratio, negative likelihood ratio, and diagnostic odds ratio of loop-mediated isothermal amplification (LAMP) in the diagnosis of visceral leishmaniasis [142,193,196]; Figure S21: Study data and paired forest plot of the sensitivity and specificity of polymerase chain reaction (PCR) in visceral leishmaniasis diagnosis [77,85,125,159,179,193,196–211]; Figure S22: Study data and paired forest plot of the positive likelihood ratio, negative likelihood ratio, and diagnostic odds ratio of polymerase chain reaction (PCR) in the diagnosis of visceral leishmaniasis.

sis [77,85,125,159,179,193,196–211]; Figure S23: Study data and paired forest plot of the sensitivity and specificity of real-time polymerase chain reaction (qPCR) in visceral leishmaniasis diagnosis [142,166,189,210,212]; Figure S24: Study data and paired forest plot of the positive likelihood ratio, negative likelihood ratio, and diagnostic odds ratio of real-time polymerase chain reaction (qPCR) in the diagnosis of visceral leishmaniasis [142,166,189,210,212]; Figure S25: Study data and paired forest plots of the sensitivity and specificity of rapid diagnostic tests (RDT) in visceral leishmaniasis diagnosis [98,102,116,117,122,125,127–130,132–135,137,140,141,146,158,180,184–189,191–197]; Figure S26: Study data and paired forest plots of the positive likelihood ratio, negative likelihood ratio, and diagnostic odds ratio of rapid diagnostic tests (RDT) in the diagnosis of visceral leishmaniasis [98,102,116,117,122,125,127–130,132–135,137,140,141,146,158,180,184–189,191–197]; Table S1: PRISMA 2020 Checklist; Table S2: Main methodological aspects of studies on tegumentary leishmaniasis; Table S3: Main methodological aspects of studies on visceral leishmaniasis.

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Abbreviations

The following abbreviations are used in this manuscript:

AUC	Area under the curve
AUC _{FPR}	Area under the curve restricted to the false positive rates
CI	Confidence interval
CRISPR	Clustered regularly interspaced short palindromic repeats
DBEI	Dot blot enzyme immunoassay
DFAT	Direct fluorescent antibody test
DOR	Diagnostic likelihood ratio
DRIHT	Direct rapid immunohistochemical test
ELISA	Enzyme-linked immunosorbent assay
FAVNT	Fluorescent antibody virus neutralization test
FN	False negatives
FP	False positives
ICA	Immunochromatographic assay
INPLASY	International Platform of Registered Systematic Review and Meta-analysis Protocols
IHT	Immunohistochemical tests
IIFT	Indirect immunofluorescence test
IPIA	Immunoperoxidase inhibition assay
IPT	Immunoperoxidase tests
LAT	Latex agglutination test
LF	Lateral flow
LR−	Negative likelihood ratio
LR+	Positive likelihood ratio
MeSH	Medical subject headings
MIT	Mouse inoculation test

NCBI	National Center for Biotechnology Information
NGS	Next generation sequencing
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
RTCIT	Rabies tissue culture infection test
RFFIT	Rapid fluorescent focus inhibition test
RIA	Rapid immunodiagnostic assay
RIT	Rapid immunochromatographic tests
RNA	Ribonucleic Acid
RNAT	Rapid neutralizing antibody test
RT-LAMP	Loop-mediated isothermal amplification
RT-PCR	Reverse transcription polymerase chain reaction
RT-qPCR	Reverse transcription real-time polymerase chain reaction
RT-RPA	Reverse transcription recombinase polymerase amplification
Se	Sensibility
Sp	Specificity
sROC	Summary receiver operating characteristics
TN	True negatives
TP	True positives
WHO	World Health Organization

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