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A novel pan-*Leishmania* loop-mediated isothermal amplification (Loopamp) assay for diagnosis of cutaneous and visceral leishmaniasis: a systematic review and meta-analysis

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Abstract

Background There is an urgent need for accurate and robust point-of-care (PoC) assays for visceral and cutaneous leishmaniasis (VL and CL). The Loopamp™ *Leishmania* detection kit (Loopamp), a novel loop-mediated isothermal amplification (LAMP) assay, has shown promise for VL and CL diagnosis using Qiagen and simpler boil-and-spin (B&S) DNA extraction methods. But diagnostic performances were inconsistent across studies. This systematic review and meta-analysis aimed to evaluate the pooled sensitivity and specificity of Loopamp for CL and VL diagnosis.

Methods A comprehensive search of PubMed, Scopus, EMBASE, and Google Scholar was conducted to identify studies that evaluated the diagnostic performance of Loopamp for VL and CL suspects. Using the Quality Assessment of Diagnostic Accuracy Studies-2 (QUADAS-2), the methodological qualities of the included studies were evaluated. A bivariate random-effects meta-analysis was performed using R and Stata 14.2.

Results Ten studies comprising 18 datasets were included. Sensitivity among individual VL studies ranged from 92 to 100%, while specificity varied from 41 to 100%. For CL, sensitivity varied from 48 to 100% and specificity from 31 to 100%. Pooled sensitivity was 96% (95% CI, 94–98%) for VL and 93% (95% CI, 70–99%) for CL. Pooled specificity was 99% (95% CI, 94–100%) for VL and 87% (95% CI, 55–97%) for CL. Subgroup analysis revealed that whole-blood B&S-Loopamp for VL had similar sensitivity (96%, 95% CI: 93–98%) and specificity (99%, 95% CI: 89–100%) to Qiagen-Loopamp.

Conclusions Loopamp demonstrated robust diagnostic performance for VL in whole blood, meeting the 95% sensitivity and 99% specificity criteria outlined in the Target Product Profile (TPP). Similar to Loopamp-Qiagen, Loopamp-B&S performed excellently for VL diagnosis and is feasible to deploy in remote endemic areas. Loopamp showed high sensitivity and good specificity for CL diagnosis but fell short of the 95% sensitivity and 90% specificity required for CL PoC tests. Data on CL are limited, and its effectiveness in New World VL patients is unclear. Future research is needed to address this gap.

Trial registration CRD42023489463.

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Keywords Loop-mediated isothermal amplification, LAMP, Loopamp, Diagnosis, Cutaneous leishmaniasis, Visceral leishmaniasis, Systematic review, Meta-analysis

Background

Leishmaniasis is a vector-borne disease caused by *Leishmania* parasites through the bite of female sandflies [1]. It primarily manifests in two distinct clinical forms, visceral leishmaniasis (VL) and cutaneous leishmaniasis (CL) [2]. VL is a severe systemic infection primarily affecting the liver, spleen, bone marrow, and kidneys [3, 4]. It is mainly caused by *Leishmania (L.) infantum* in the Mediterranean region, the Middle East, and Latin America and *L. donovani* in Asia and Eastern Africa [5]. In contrast, CL is characterized by disfiguring skin lesions [6]. Over 20 *Leishmania* species can cause CL; the primary causative agents are *L. Viannia (V.) guyanensis*, *L. (V.) panamensis*, *L. (V.) braziliensis*, *L. amazonensis,* and *L. mexicana* in the New World (South and Central America), while *L. major*, *L. tropica*, *L. infantum,* and *L. aethiopica* are predominant in the Old World (Asia, South Europe, the Middle East, and Africa) [7, 8].

Clinically, VL is characterized by fever, weight loss, liver and spleen enlargement, and anemia [5]. On the other hand, CL can present with a range of symptoms such as localized CL (LCL), self-healing nodular or ulcerative lesions at the bite site; mucosal CL (MCL), destructive nasal, mouth, and throat mucosa; diffuse CL (DCL), multiple non-ulcerative nodules; and disseminated leishmaniasis (DL), multiple papules in two or more non-contiguous areas [9, 10]. Although not typically fatal, CL can cause severe skin disfigurement, potentially leading to social stigma and mental disorders [11]. While the annual worldwide estimate is 50,000–90,000 new cases of VL and 0.6–1 million new cases of CL [12], only 13,081 VL and 205,990 CL cases were reported to the World Health Organization (WHO) in 2022 [13]. This indicates severe underreporting of these diseases, primarily due to poor surveillance, limited healthcare access, and a lack of reliable diagnostic tools.

Diagnosis of CL and VL is still predicated on confirmation by microscopy using samples of biopsy, punch, or skin-slit from skin lesions and lymph nodes, bone marrow, or spleen aspirate, respectively [14]. This method is accurate for VL but requires invasive sampling [15, 16] and has limited sensitivity for detecting CL [17, 18]. The rK39 antigen-based rapid diagnostic tests (RDTs) are point-of-care tests for the diagnosis of VL. However, the RDT's sensitivity varies across the eco-epidemiological regions [19, 20] and lacks the ability to differentiate between recent, subclinical, or past infections [21]. Antibody-based tests were rarely used for the diagnosis of CL due to the inability to differentiate active from prior infections and the limited humoral response induced by CL patients [14]. Molecular assays, such as conventional polymerase chain reaction (PCR) and quantitative PCR (qPCR), are extremely sensitive and specific when combined with invasive [22–25] as well as less-invasive [16, 26–30] sample types. But PCRs are not available for the routine diagnosis of VL and CL in endemic areas, predominantly because the reagents are costly and require cold chain storage and sophisticated laboratory facilities and expertise [31].

In contrast, the Loopamp™ *Leishmania* detection kit (Loopamp: Eiken Chemicals, Tokyo, Japan) is a robust and novel pan-*Leishmania* loop-mediated isothermal amplification (LAMP) assay that does not require cold-chain storage. This diagnostic kit targets kinetoplast DNA (kDNA) and 18S ribosomal RNA (18S rRNA). This kit is a ready-to-use dried reagent comprising *Bacillus stearothermophilus* (*Bst*) DNA polymerase, calcein, and primers [32]. The kit uses a set of four to six primers that specifically recognize different regions of the target DNA. This enables DNA amplification within 40 min at 65 °C through a strand displacement catalyzed by *Bst* DNA polymerase. The Loopamp kit permits various methods for detecting amplified products. Initially, calcein within the reaction tube is bound to manganese ions, which quenches its fluorescence. As the amplification progresses, generated pyrophosphate ions bind to manganese ions, releasing calcein that emits a fluorescent light detectable by the naked eye as well as portable real-time fluorimeters [33]. Like other in-house LAMP methods, the Loopamp test requires only a thermoblock or incubator for DNA amplification. However, unlike in-house methods, Loopamp is a standardized, commercially available diagnostic kit [32]. This makes Loopamp a feasible assay for decentralized VL and CL patient diagnosis and care in endemic areas.

The Loopamp assay can amplify leishmanial DNA extracted using the commercial (Qiagen) and boil-and-spin (B&S) protocols [34]. The Qiagen method, a spin-column-based approach, yields high-purity DNA, promoting optimal target amplification. However, it requires high-speed multiple centrifugation with enhanced washing steps, limiting its field applicability [35, 36]. The B&S method involves lysis buffer, heating, and centrifugation to isolate a crude DNA-containing aqueous layer from cellular debris. This approach is quick and simple to use in basic healthcare [34].

Several studies have evaluated the Loopamp kit using commercial (Qiagen) and simpler boil-and-spin (B&S) DNA extraction methods for VL and CL patients infected with various *Leishmania* species across different endemic regions worldwide. But the diagnostic performances were inconsistent. Furthermore, no previous systematic review and meta-analysis had been conducted to assess the accuracy of this test. This systematic review and meta-analysis aimed to determine the pooled sensitivity and specificity of the Loopamp™ *Leishmania* detection kit for the diagnosis of VL and CL. The Loopamp kit has the potential to become a valuable diagnostic tool for VL and CL if it performs well.

Methods

Literature review protocol preparation

This systematic review and meta-analysis was registered on the international prospective register of systematic reviews (CRD42023489463). In addition, this study was carried out following the guidelines of the Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) [37] (Additional file).

Eligibility criteria

Studies were included if observational, cross-sectional or case–control diagnostic accuracy published in English language, VL and/or CL suspected patients involved, the Loopamp™ *Leishmania* detection kit performed, and numbers of true positive (TP), false positive (FP), true negative (TN), and false negative (FN) were directly or indirectly available. We excluded case reports, review articles, meta-analysis articles, studies with incomplete data, and duplicates.

Information sources and search strategy

Articles were gathered from PubMed, Scopus, EMBASE, and Google Scholar by searches using the key terms ((Loopamp™ *Leishmania* Detection Kit OR Loop-mediated isothermal amplification OR Loopamp OR LAMP kit OR LAMP OR molecular test) AND (Visceral leishmaniasis OR Black Fever OR Kala-Azar)) OR (Cutaneous leishmaniasis OR Leishmaniasis, American OR Leishmaniasis, New World OR Leishmaniasis, Old World OR Oriental Sore OR American tegumentary leishmaniasis OR diffuse cutaneous leishmaniasis OR mucosal leishmaniasis OR skin leishmaniasis))). Additional filter by English language was used. Other publications were recognized from references cited in important articles and manually hand-searched to identify further pertinent studies (Additional file 2).

Study selection

The retrieved articles were imported to EndNote X8 and duplicate articles were removed. Then articles were screened by their titles, abstracts, and full text according to the eligibility criteria by two reviewers (EG and BT) independently. Since there were no disagreements, no any article resolved with a third reviewer or by consensus.

Data extraction

Data extraction was performed by two reviewers (GC and BT). Variables extracted were the first author name, year of publication, country, geographic region, study design, index test sampling and DNA extraction method, study population, sample size, reference test, TP, FP, FN, and TN of the Loopamp.

Quality assessment

Two reviewers (HG, and BT) assessed the risks of bias and applicability concerns using the quality assessment of diagnostic accuracy studies 2 (QUADAS-2) tool [38]. Evaluation results were displayed in graphs using Review Manager 5.4 software.

Data synthesis and categorization

The primary accuracy measures assessed for Loopamp were sensitivity, the probability of a positive test result in a diseased individual, and specificity, the probability of a negative test result in a non-diseased individual. To assess these metrics, diagnostic 2× 2 tables were constructed against reference standards (microscopy, PCR, rK39-RDT), stratified by leishmaniasis type (VL or CL) and DNA extraction methods. Thus, for the same study, more than one dataset was extracted.

Statistical analysis

Data were extracted in Excel and then exported to Stata version 14.2 for analysis. A bivariate random-effects model was employed for meta-analysis using the Metadta package in Stata version 14.2 and the Mada package in R software. Results were presented in tabular format, forest plots, and/or summary receiver operating characteristic (SROC) plots. The degree of heterogeneity was quantified using I-Square (I^2) statistics by Zhou & Dendukuri

[39]. I^2 values above 25%, 50%, and 75% were assumed to be low, medium, and high heterogeneity, respectively. A sub-group analysis by DNA extraction method and reference test was performed. Deeks' funnel plot and Egger's statistics were done to detect publication bias. A *p*-value of ≤ 0.05 in Egger's test was considered evidence of statistically significant publication bias [40].

Results

Literature search

A total of 429 publications were retrieved. After removing duplicates, 241 studies were screened by title/abstract and 14 by full text. Studies were excluded at the title/abstract screening stage as diagnostic assays deviated from Loopamp and were incompatible with the target population (VL/CL). Four studies were excluded during the full-text screening stage because they tested Loopamp for animal or non-VL/CL leishmaniasis suspects [35, 41–43]. Finally, ten studies were included for the qualitative and quantitative analysis (Fig. 1).

Characteristics of original studies

Of the ten studies that were included, eight of them had two diagnostic test findings as they employed two different DNA extraction methods, reference tests, or disease types (CL and VL). This resulted in a total of eighteen observations (datasets) (Table 1). Of these eighteen datasets, eleven were for VL, and seven for CL. The included studies varied in sample size from 10 to 274 participants. This review comprises 2565 test results from 1729 individuals in total. Of the 2565 test results, 1504 and 1061 were for VL and CL, respectively. CL diagnosis employed diverse sample types (dental broach, swab, skin biopsy, and tape-disc), whereas nearly all VL samples analyzed with the Loopamp test were whole blood. Two studies used the Qiagen kit (QIA), two used the boil-and-spin (B&S) method, and three employed both QIA and B&S DNA extraction methods for VL samples. For CL, four studies utilized the QIA kit, while one employed the Maxwell LEV kit. Eight studies assessed Loopamp accuracy in patients from the Old World (Afghanistan, Ethiopia, Sudan, Bangladesh, and Spain), while two studies evaluated its efficacy in patients from the New World (Suriname and Colombia). Eight studies were classified as cross-sectional, whereas two studies employed a control group.

Methodological qualities of original studies

The results of the methodological quality assessment of the included studies were presented in Additional File 3, categorized by VL and CL diagnostic studies. The risk of applicability was low for all included studies. All CL and most VL studies demonstrated a low risk of bias in patient selection, index test, and flow and timing domains. However, most VL and all CL studies showed an uncertain risk of bias regarding the reference standard, as they employed a non-gold standard (PCR or rK39 RDT) for VL and no gold standard to classify CL [50].

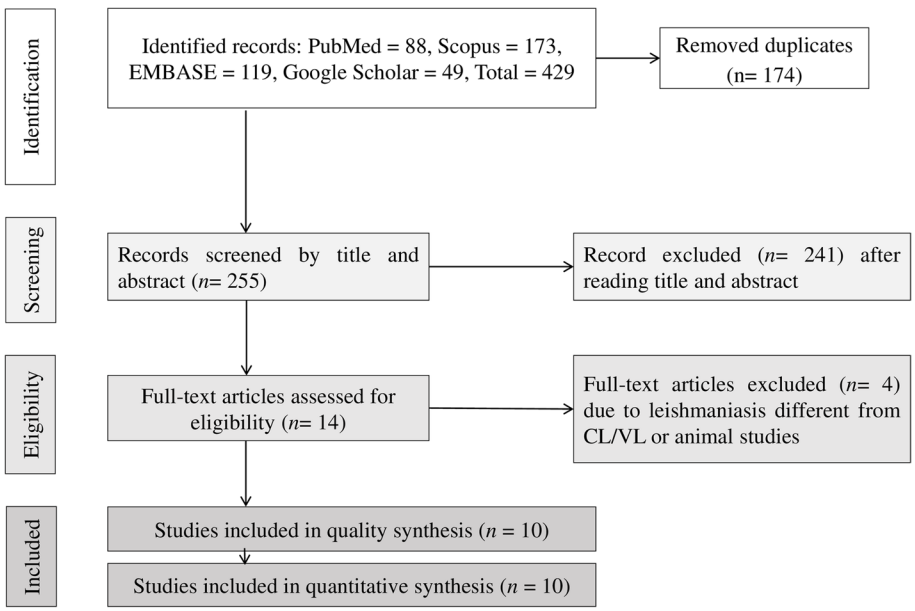

Fig. 1 PRISMA flow diagram of study selection for systematic review and meta-analysis of Loopamp for VL and CL diagnosis. CL: Cutaneous leishmaniasis, PRISMA: Preferred Reporting Items for Systematic Reviews and Meta-Analysis, n: number of articles, and VL: visceral leishmaniasis

Table 1 Characteristics of original studies

Author, Year	Country of Patients	Design	n	Sex[a] (%)	Mean age	*Leishmania* species	Case	Reference	Sampling	DNA Isolation	TP	FP	FN	TN
Adams, 2018 [32]	Ethiopia	C-s	50	UK	UK	*L. donovani*[c]	VL	Microscopy	WB	Qiagen	24	0	2	24
	Colombia	C-s	105	25.7	23	*L.(v.) panamensis*[b]	CL	Microscopy	SS	Qiagen	76	3	4	22
Mukhtar, 2018 [34]	Sudan	C-s	185	37.9	UK	*L. donovani*[c]	VL	Microscopy	WB	Qiagen	84	1	0	100
	Sudan	C-s	185	37.9	UK	*L. donovani*[c]	VL	Microscopy	WB	B&S	82	1	2	100
Ibarra-Meneses, 2018 [33]	Spain	C-s	10	UK	UK	*L. infantum*[c]	VL	PCR	WB, BM	Qiagen	6	0	0	4
	Spain	C-s	10	UK	UK	*L. infantum*[c]	VL	PCR	WB, BM	B&S	6	0	0	4
Vink, 2018 [44]	Afghanistan	C-s	274	52.2	20	*L. tropica*[b]	CL	Microscopy	SDB	Qiagen	186	48	18	22
	Afghanistan	C-s	274	52.2	20	*L. tropica*[b]	CL	PCR	SDB	Qiagen	237	1	15	21
Schallig, 2019 [45]	Suriname	C-s	93	7.5	33.6	*L. (v.) guyanensis*[b]	CL	Microscopy	SDB	Qiagen	67	8	12	6
	Suriname	C-s	93	7.5	33.6	*L. (v.) guyanensis*[b]	CL	PCR	SDB	Qiagen	74	1	7	11
Hagos, 2021 [46]	Ethiopia	C–c	114	4.9	23	*L. donovani*[c]	VL	Microscopy	WB	B&S	82	16	5	11
	Ethiopia	C–c	274	4.9	23	*L. donovani*[c]	VL	rk39-RTD	WB	B&S	113	5	7	149
Ibarra-Meneses, 2021 [47]	Spain	C-s	130	UK	UK	*L. infantum*[c]	VL	PCR	WB, BM	Qiagen	84	2	1	43
	Spain	C-s	100	UK	UK	*L. infantum*[c]	CL	PCR	SB	Qiagen	56	0	0	44
Hossain, 2021 [36]	Bangladesh	C–c	160	41.9	UK	*L. donovani*[c]	VL	rk39-RTD	WB	Qiagen	76	0	4	80
	Bangladesh	C–c	160	41.9	UK	*L. donovani*[c]	VL	rk39-RTD	WB	B&S	77	0	3	80
Hagos, 2024 [48]	Ethiopia	C-s	226	6.4	24	*L. donovani*[c]	VL	PCR	WB	B&S	135	2	8	81
Taye, 2024 [49]	Ethiopia	C-s	122	39.3	22	*L. aethiopica*[c]	CL	Microscopy	TD	Maxwell	31	7	33	51

[a] % of female patients, [b]predominant species confirmed by PCR, [c]species indicated, n: sample size, *TP* true positive, *FP* false positive, *TN* true negative, *FN* false negative, *B&S* boil-and-spin, *BM* bone marrow aspirates, *SB* skin biopsy, *SDB* skin-dental broach, *SS* skin-swab, *TD* Tape-disc, *WB* whole blood, *UK* unknown, *C–c* case–control, *C-s* cross-sectional, *PCR* polymerase chain reaction, *rk39-RDT* recombinant antigen 39 rapid diagnostic test, *CL* cutaneous leishmaniasis, *VL* visceral leishmaniasis

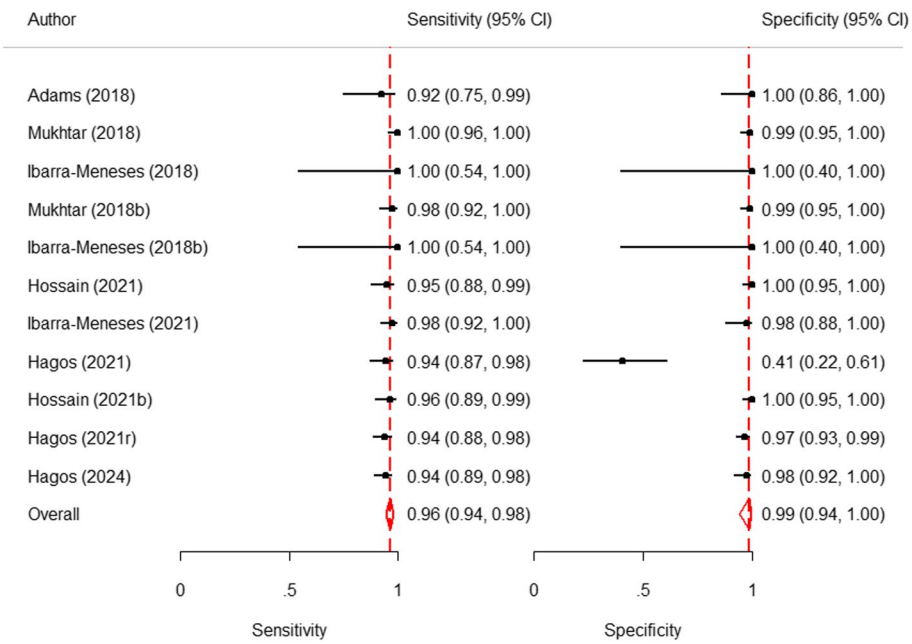

Fig. 2 Forest plot for pooled sensitivity and specificity of Loopamp for VL diagnosis. Value and pooled estimate (last rows per sensitivity and specificity analysis, red diamond)

Performance of Loopamp for VL diagnosis

Individual study sensitivities and specificities varied from 92 to 100% and 41% to 100%, respectively (Fig. 2). Loopamp demonstrated a 96% (95% CI: 94–98%) pooled sensitivity and 99% (95% CI: 94–100%) pooled specificity for VL diagnosis. There was low heterogeneity in both sensitivity ($I^2 = 3.04\%$) and specificity ($I^2 = 6.64\%$).

Performance of Loopamp for CL diagnosis

Sensitivities and specificities for each individual study ranged from 48 to 100% and 31% to 100%, respectively. For CL diagnosis, Loopamp showed 87% (95% CI: 55–97%) pooled specificity and 93% (95% CI: 70–99%) pooled sensitivity (Fig. 3). There was more

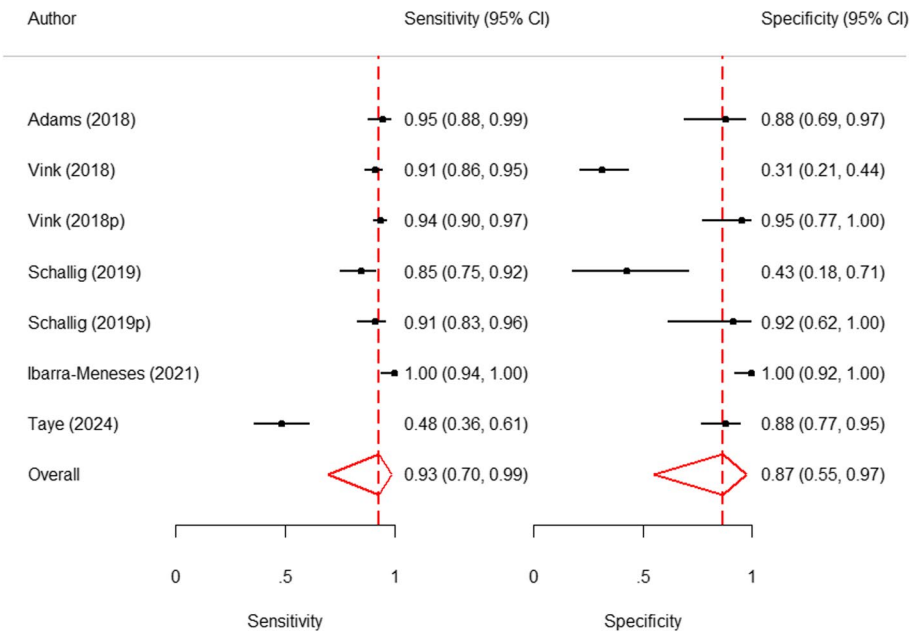

Fig. 3 Forest plot for pooled sensitivity and specificity of Loopamp for CL diagnosis. Value and pooled estimate (last rows per sensitivity and specificity analysis, red diamond)

heterogeneity in sensitivity ($I^2 = 74.75\%$) than in specificity ($I^2 = 62.00\%$).

Diagnostic accuracy of Loopamp for VL and CL diagnosis using SROC curve

The summary diagnostic accuracy of the Loopamp assay for VL and CL was presented by an SROC plot (Fig. 4). The individual study data points (arrows) were scattered around the summary point estimate (circle) on the SROC plot. The area under the curve (AUC) was 0.95 for VL and 0.93 for CL.

Subgroup analysis

A subgroup analysis was performed to explore the potential effects of DNA extraction methods and reference tests on the accuracy of Loopamp for VL diagnosis. The Qiagen Kit (QIA) extracted whole blood DNA from VL suspects, and testing by Loopamp showed 97% (95% CI: 93–99%) sensitivity and 99% (95% CI: 96–100%) specificity. Similar sensitivity (96%, 95% CI: 93–98%) and specificity (99%, 95% CI: 89–100%) were observed for the B&S-Loopamp approach (Fig. 5a). Heterogeneity analysis revealed low levels of variability for both the Qiagen kit ($I^2 = 10.56\%$ for sensitivity and 1.44% for specificity) and the B&S technique ($I^2 = 5.90\%$ and 11.15%, respectively).

Using microscopy, PCR, or rk39-RDT as reference tests, Loopamp pooled sensitivity was 97% (95% CI, 93–99%), 96% (95% CI: 92–98%), and 95% (95% CI: 91–97%), respectively. Compared to the corresponding reference standards, pooled specificity was 95% (95% CI: 47–100%), 98% (95% CI: 93–99%), and 99% (95% CI: 85–100%), respectively (Fig. 5b). There was low heterogeneity in sensitivity and specificity ($I^2 = 21.78\%$ and 28.44%) in the microscopy subgroup, $I^2 = 4.50\%$ and 25.58% in the rk39-RDT subgroup, and $I^2 = 0.00\%$ for both in the PCR subgroup.

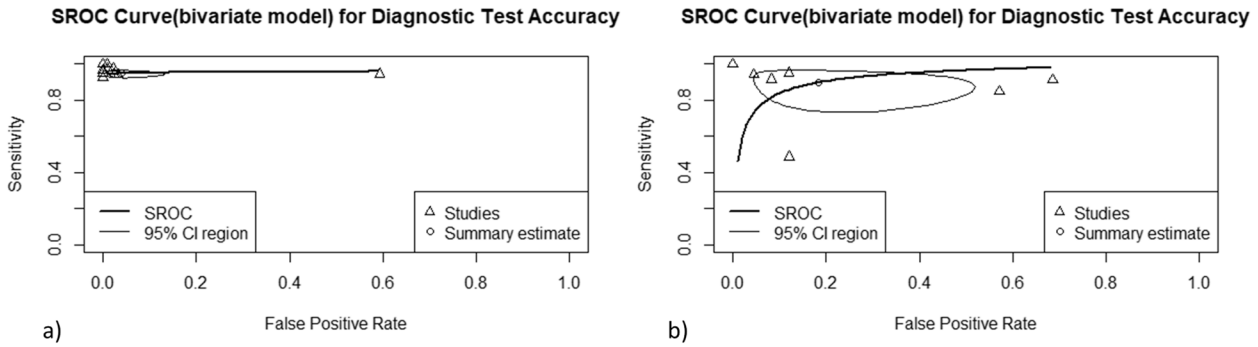

Fig. 4 SROC curve for Loopamp for VL (**a**) and CL (**b**) diagnosis. SROC: summary receiver operating characteristic. Arrows represent the single study data, and circles indicate summary estimates with 95% CI

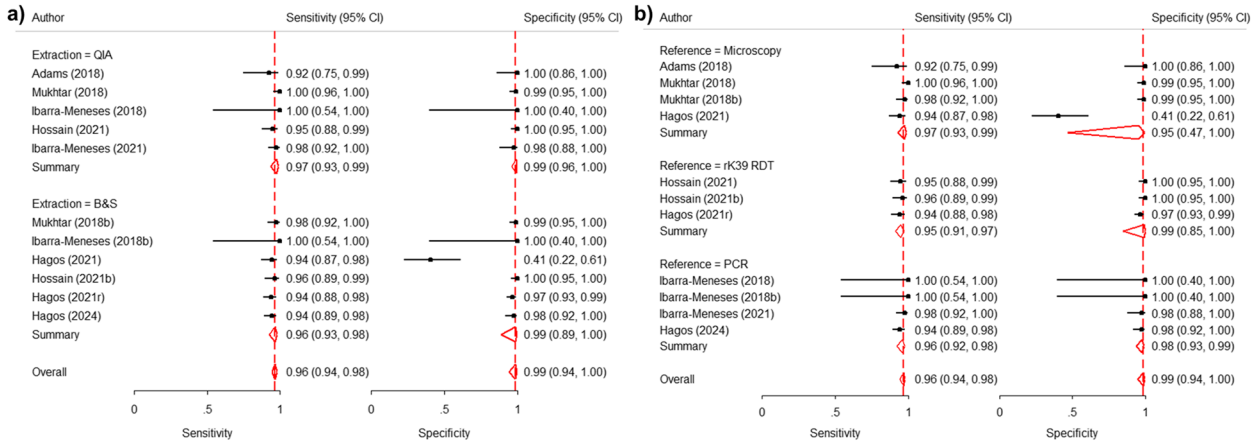

Fig. 5 Forest plot of subgroup analysis for pooled sensitivity and specificity of Loopamp for VL diagnosis. **a** shows results grouped by DNA extraction method (Qiagen kit [QIA] and Boil-&-Spin [B&S]). **b** shows results grouped by reference test (Microscopy, Polymerase Chain Reaction [PCR], and rk39-Rapid Diagnostic Test [rk39-RDT]). The red diamond in each plot represents the pooled estimate

Publication bias

The Deeks' funnel plots of the included studies in this systematic review and meta-analysis were almost symmetric, and the Egger weighted regression statistics showed a nonsignificant value (p-value = 0.33), indicating that there was no potential publication bias (Additional file 4).

Discussion

There is an urgent need for accurate and robust point-of-care (PoC) diagnostic assays for VL and CL, especially in resource-limited settings where many cases currently remain undetected [51, 52]. To address these needs, the Loopamp™ *Leishmania* detection kit, a molecular PoC diagnostic based on the LAMP assay, was recently developed for the diagnosis of VL and CL. To our knowledge, this is the first systematic review and meta-analysis to assess and compile the diagnostic accuracy of the Loopamp assay for VL and CL, using all relevant published literature.

In our meta-analysis, the Loopamp *Leishmania* detection kit demonstrated a robust pooled sensitivity (96%) for VL diagnosis. This meets the 95% sensitivity threshold outlined in a recent target product profile (TTP) for a PoC test to confirm VL disease [51]. With 99% pooled specificity, the Loopamp assay fulfills the TTP's 99% specificity standard for accurately ruling out other febrile illnesses [51]. Furthermore, this *Leishmania* LAMP kit achieved a robust ability to differentiate VL cases from non-cases (AUC = 0.95) [53, 54]. Overall, these diagnostic accuracy metrics align with the pooled estimates reported for PCR and rK39-RDT in VL diagnosis; however, the sensitivity and specificity of the Loopamp assay are slightly higher than those of PCR and rK39-RDT [19, 22].

The subgroup analysis found that the Loopamp assay exhibited high pooled sensitivity (≥ 95%) and specificity (≥ 95%) for VL diagnosis when compared to microscopy, rk39-RDT, or PCR. Additionally, both Qiagen and B&S methods for extracting whole-blood DNA from VL suspects yielded similar diagnostic performance when analyzed with Loopamp, achieving 99% specificity and comparable sensitivity (97% for Qiagen, 96% for B&S). In contrast to the more complex Qiagen kit, the B&S method for DNA extraction involves a simpler process of centrifuging whole blood after adding a lysis agent and heating [36, 55]. Moreover, the Loopamp-B&S approach is compatible with whole-blood samples, can be used in basic laboratory settings, and costs less than $7 USD per test, with a turnaround time of approximately 60 min [36]. Consequently, the Loopamp-B&S has the potential to become a valuable method for the decentralized diagnosis of VL in primary healthcare settings, significantly improving patient diagnosis rates.

The Loopamp assay for CL diagnosis demonstrated high pooled sensitivity (93%) in this meta-analysis. However, this is slightly below the 95% sensitivity outlined in the TPP for PoC testing for CL [52]. This diagnostic kit showed good specificity (87%) in ruling out other skin diseases but fell slightly below the TPP criterion of 90% specificity for PoC CL tests [52]. While the Loopamp demonstrated a strong ability to differentiate CL cases from non-cases with an AUC of 0.93, further optimization is needed to achieve an exceptional diagnostic accuracy (AUC ≥ 0.97) [53, 54]. Overall, these Loopamp diagnostic accuracy measures align with the pooled estimates reported for PCR in diagnosing CL, although its sensitivity and specificity are slightly lower than those of PCR [23].

The Loopamp could be a suitable diagnostic tool for CL, although its sensitivity and specificity remain below the 95% and 90% thresholds outlined in TPP for PoC CL tests [52]. This necessitates further optimization to meet these performance standards and work consistently across CL species. The Loopamp kit validation research shows that its primers can amplify not only kDNA, but also at least 18S rRNA of CL-causing *Leishmania* species [32]. However, the reduced sensitivity observed in Ethiopia, where *L. aethiopica* causes CL, is likely attributed to primer mismatches with *L. aethiopica* DNA [49]. To enhance the assay sensitivity in such settings, the development of novel primers specifically tailored for *L. aethiopica* is crucial. Furthermore, other species causing CL, such as *L. braziliensis*, *L. mexicana*, *L. major*, and *L. amazonensis,* were not included in this review due to a lack of available studies. This gap in the current research underscores the urgent need for further studies.

The strengths of this systematic review and meta-analysis are the employment of various searching strategies, critical appraisal of the methodological quality of included studies using the QUADAS-2 tool, and application of the PRISMA 2020 guideline. Additionally, most pooled estimates have minor heterogeneity. This review, however, has several limitations. Primarily, the risk of bias concerning the reference standard remains unclear for most VL and all CL studies. This stems from the lack of a gold standard for CL classification [50] and the substitution of tissue aspiration microscopy—the VL diagnostic gold standard—with PCR or RDT due to ethical considerations about bleeding risks from invasive sampling [36]. The lack of studies evaluating Loopamp accuracy in New World VL patients, which restricted their inclusion, underscores a crucial gap in current research.

For Loopamp VL diagnosis, all samples were treated as blood, even though 67 were bone marrow samples from the Ibarra-Meneses et al. studies [33, 47]. Nonetheless, this likely had a minimal effect on the overall estimate, as the vast majority of samples (968 out of 1035) were blood. The moderate heterogeneity observed in the pooled sensitivity and specificity for CL necessitates cautious interpretation. This variability could be attributed to several factors, such as variations in parasite species, sample types, and the reference tests employed across the included studies. Finally, the limited number of currently accessible studies restricts our capacity to perform meta-regression and further investigate the observed differences by evaluating variables like geographical regions and reference tests.

Conclusions

The Loopamp assay demonstrated robust diagnostic performance for VL, meeting the 95% sensitivity and 99% specificity criteria outlined in the Target Product Profile (TPP) for a point-of-care (PoC) VL test. Similar to Loopamp-Qiagen, Loopamp using B&S-extracted whole blood DNA demonstrated excellent diagnostic performance and is simple, rapid, and feasible for deployment in endemic areas. Therefore, we recommend the continued use of rK39-RDTs where needed and the replacement of microscopy with the rapid, robust, and feasible Loopamp-B&S method. The Loopamp assay showed high sensitivity and good specificity for CL diagnosis but fell short of the 95% sensitivity and 90% specificity thresholds outlined in the target product profile (TTP) for a point-of-care (PoC) CL test. However, the current data for CL diagnosis is limited, and its performance for New World VL patients remains unclear. Addressing these gaps through future research is critical.

Abbreviations

AUC	Area under the curve
B&S	Boil-&-spin
CL	Cutaneous leishmaniasis
CI	Confidence interval
FN:	False negative
FP	False positive
kDNA	Kinetoplast DNA
LAMP	Loop-mediated isothermal amplification
PCR	Polymerase chain reaction
PoC	Point-of-care
PRISMA	Preferred reporting items for systematic review and meta-analysis
QUADAS-2	Quality assessment of diagnostic accuracy studies-2
RDT	Rapid diagnostic test
rRNA	Ribosomal RNA
SROC	Summary receiver operating characteristic
TP	True positive
TN	True negative
TPP	Target product profile
VL	Visceral leishmaniasis
WHO	World health organization

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12879-025-11091-2.

Additional file 1: Preferred Reporting Items for Systematic Reviews and Meta-AnalysesChecklist

Additional file 2: Search strategy

Additional file 3: QUADAS-2 risk of bias and applicability concerns results for included studies

Additional file 4: Deeks' funnel plot for publication bias

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Not applicable.

Authors' contributions

BT: study conception and protocol, literature search, title/abstract and full-text screening, data extraction, quality assessment, statistical analyses, and interpretation of results. GC: data extraction and interpretation of results. HG: quality assessment and interpretation of results. EG: title/abstract and full-text screening, and interpretation of results. AB: interpretation of results. BT and EG prepared the original manuscript with considerable input from GC. All authors read and approved the final manuscript.

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Data availability

The datasets used for the current research are included within the article and its additional files.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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