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REVIEW ARTICLES

Immunochromatographic tests for human and canine leishmaniosis diagnosis: a critical review and future prospects

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Highlights

- A critical review was performed comparing results by continent, countries, clinical form and antigen used
- ICTs with low performance are being marketed and used in different countries
- The protein rK39 as antigen showed an average 88% sensitivity in Africa, 92% in Asia
- Discrepancies in relation to sensitivity and specificity results make it difficult to choose the most effective methods

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KEYWORDS
Antigen;
Immunochromatographic test;
Neglected diseases;
Rapid test;
Recombinant protein.

Abstract: Leishmaniosis has two main clinical forms: cutaneous and visceral leishmaniosis, which can leave serious sequelae and even cause death if not properly diagnosed and treated. Diagnostic methods include parasitological, immunological, or DNA amplification. Rapid immunochromatographic tests (ICTs) are considered the best option for an initial diagnosis of leishmaniosis at the point of care and disease control. This is because they are easy to use, interpret, and are low-cost. This paper reviews the diagnostic performance of commercial ICTs evaluated and published by various researchers. A total of 78 articles were found, 50 of which correspond to human leishmaniosis, and the other 28 address canine leishmaniosis. Many of these kits do not present high sensitivity and specificity, and their use as screening methods in endemic areas is problematic. Recombinant proteins rK39 and rK28 were used as antigens in the commercial test for diagnosing both human visceral leishmaniosis (VL) and canine visceral leishmaniosis (CVL), and they showed similar performance. However, a lot of discrepancies among authors related to sensitivity and specificity results make it difficult to choose or recommend any of these commercial ICTs for an effective diagnosis of leishmaniosis. Only the ICT with recombinant protein rKE16 showed good performance for VL diagnosis in Asia. In contrast, the CL Detect™ Rapid Test, which uses peroxidoxin as an antigen, is the only approved test to diagnose cutaneous leishmaniosis (CL), but it showed low sensitivity on the three continents (Africa, Asia, and South America) where CL is endemic. The review conducted in this study indicates the need to consider the *Leishmania* species that are infecting the endemic area in the development of the antigen used in ICTs, together with the development of platforms and/or new techniques that increase the efficiency of ICTs.

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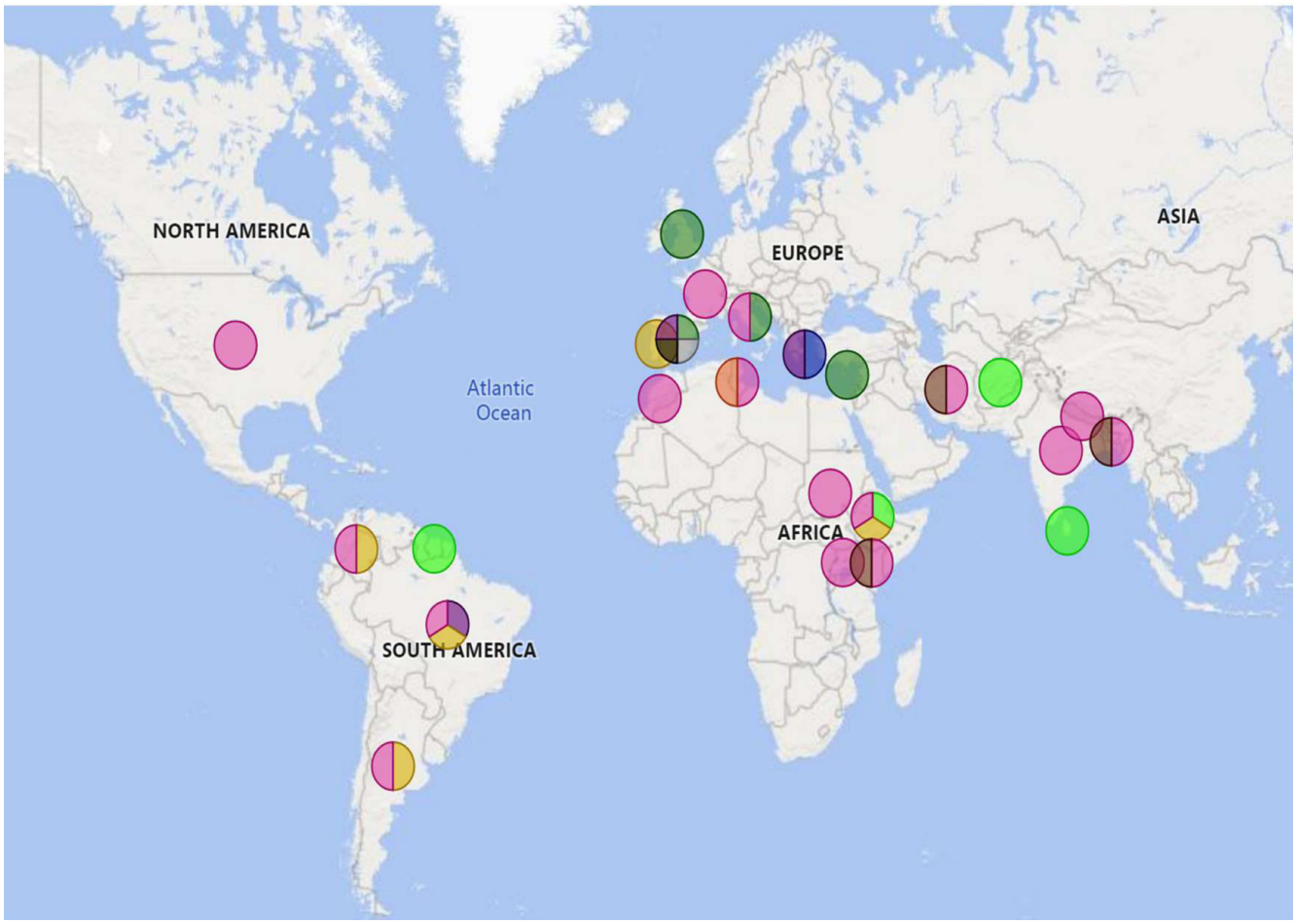
Graphical Abstract



Introduction

Leishmaniosis is caused by several protozoan species of the order Kinetoplastida, family Trypanosomatidae, and genus *Leishmania*, affecting both humans and animals (Anversa et al., 2018; Gedda et al., 2021; Kammona & Tsanaktsidou, 2021; Schallig et al., 2019). More than 50 species of *Leishmania* have been identified worldwide, of which at least 21 have significant medical importance (Anversa et al., 2018). The infection is mainly transmitted by the bite of infected female sandflies, which are the only arthropods adapted to biologically transmit *Leishmania*, belonging to the genus *Phlebotomus* in the Old World or to *Lutzomyia* in the New World (Anversa et al., 2018; Basurco et al., 2020; Herrera et al., 2019; Solano-Gallego et al., 2014). Leishmanioses are endemic in most of the world (at least 98 countries), affecting nearly 12 million people, with 0.6 to two million new clinical cases reported annually and notable mortality and morbidity (Anversa et al., 2018; Dantas-Torres et al., 2019; Gedda et al., 2021). The disease is more common in the countryside than in the urban environment. However, deforestation, urbanization, population migration, and climate change have led to the colonization of urban areas by *Leishmania*-transmitting vectors, resulting in the disease's expansion into new regions. Leishmaniosis is considered the second most prevalent pathogenic disease and a major public health problem (Anversa et al., 2018; Kammona & Tsanaktsidou, 2021).

There are two main clinical forms of *Leishmania*: cutaneous leishmaniosis (CL) and visceral leishmaniosis (VL) (Schallig et al., 2019). More than 90% of the worldwide cases of VL occur in six countries: India, Bangladesh, Sudan, South Sudan, Ethiopia, and Brazil; and CL cases occur in the Americas, the Mediterranean basin, and western Asia (mainly in Afghanistan, Algeria, Colombia, Brazil, Iran, Syria, Ethiopia, North Sudan, Costa Rica, Peru, Saudi Arabia, Pakistan and Tunisia) (Anversa et al., 2018; OPAS, 2020; World Health Organization, 2015). The most life-threatening form of the disease is VL, affecting 0.5 to 0.9 million people annually and proving fatal if not treated timely (Kammona & Tsanaktsidou, 2021; Lévêque et al., 2020). VL is typically caused by *Leishmania donovani* and *Leishmania infantum*, such that the latter is considered predominantly zoonotic and domestic dogs are its major reservoir (Humbert et al., 2019). In dogs, the infection is difficult to diagnose due to non-specific clinical presentations and variable laboratory findings. The clinical presentations of canine leishmaniosis can range from subclinical/asymptomatic to full-blown disease, and the variation depends on the host's immune response (Proverbio et al., 2013).

In humans, the infection can be asymptomatic or lead to an acute form characterized by irregular fever, anorexia, cachexia, night sweat, splenomegaly, hepatomegaly, anemia, or pancytopenia (Lévêque et al., 2020). In contrast, CL is the most common form of the disease, causing skin infection, scarring, disfigurement, stigmatization, and affecting 0.6 to 1 million people annually. Deaths due to CL are rare; however, the skin lesions can produce psychological, social and economic harm (Silva et al., 2017; Kammona & Tsanaktsidou, 2021; Van Henten et al., 2022).

There are different methods for diagnosing leishmaniosis, and they are performed based on the clinical form of the disease. To diagnose CL, microscopic examination of Giemsa-stained skin scrapings or fine needle aspirates are commonly used to observe the *Leishmania* parasites amastigote form; however, this method reportedly presents low sensitivity (Schallig et al., 2019; Van Henten et al., 2022). The isolation of parasites in cultures from infected tissues is also used as a diagnostic method, although it is not suitable for rapid diagnosis (Solano-Gallego et al., 2014). The leishmanin skin test (LST), also known as the Montenegro skin test, determines the type-IV hypersensitivity and does not diagnose the acute disease (Gedda et al., 2021). This test can show sensitivity rates ranging from 84 to 100% (Schubach et al., 2005). Currently, only Iran produces antigens for this method. However, since there are more than 20 species of *Leishmania* that can infect humans, and the sensitivity of the test depends on the parasite species present in the region, it is necessary to produce the test locally according to the endemic parasites (Gedda et al., 2021). The most useful diagnostic approaches for investigating infection include the detection of specific anti-leishmanial serum antibodies using quantitative serological techniques and the detection of parasitic DNA in tissues through molecular techniques. Despite this, the presence of low antibody levels does not necessarily indicate the presence of the disease and needs to be confirmed by other diagnostic methods (Solano-Gallego et al., 2014).

The gold standard method to diagnose VL is observation of the parasite in bone marrow aspirates under a microscope, which is a cheap method still used in endemic areas. However, this technique presents low sensitivity, requires invasive procedures, and needs trained personnel capable of recognizing the parasites (Gedda et al., 2021; Lévêque et al., 2020). Culturing parasites isolated from patients to confirm microscopy results may improve sensitivity; however, this method is highly susceptible to contamination (Basurco et al., 2020; Lévêque et al., 2020). Serological methods based on the detection of specific antibodies, e.g., the enzyme-linked immunosorbent assay (ELISA), the indirect immunofluorescence antibody test (IFAT), the direct agglutination test, Western blot, and ICT offer more uniform procedures for antigen preparation and provide standardized experimental conditions. However, these tests can present low sensitivity and specificity, as they may cross-react with other pathogens like *Trypanosoma*, *Mycobacterium* and *Toxoplasma* (Farahmand et al., 2015; Humbert et al., 2019; Basurco et al., 2020; Lévêque et al., 2020).

In order to improve the accuracy of the immunochromatographic test, various antigens have been produced and tested. One such antigen is the recombinant protein rK39, a member of the kinesin protein family derived from 39 repetitive amino acids encoded by the *L. chagasi* gene, and it has also been reported in *L. donovani* and *L. infantum* (Chappuis et al., 2005, Bezuneh et al., 2014, Rezaei et al., 2022), though reports on its performance vary from country to country (Souza Filho et al., 2016). Another antigen is the recombinant protein rk28, which is a synthetic polyprotein or multiepitope chimeric protein generated by the fusion of the rK39 and rK26 proteins from *L. infantum* and rA2 protein from *L. donovani* (Grimaldi Junior et al., 2012). In addition, the recombinant protein rKE16, an antigen developed from an Indian strain of *L. donovani*, has been used (Mbui et al., 2013). Inactivated antigens from *L. infantum* and a complex of recombinant peptides have also been used to diagnose CVL.

In order to control leishmaniosis and mitigate its consequences through the implementation of effective control measures, it is crucial to employ efficient diagnostic methods that align with the ASSURED criteria (Affordable, Sensitive, Specific, User-friendly, Rapid and Robust, Equipment-free, and Deliverable) and can be readily used at the point of care (Fujisawa et al., 2021). Given that ICTs were developed based on the ASSURED criteria, we have conducted a review of ICTs available in endemic countries for diagnosing both visceral and cutaneous leishmaniosis.

Here, we analyze and discuss the sensitivity and specificity performance of these tests across different countries and continents. Our aim is to gain insight into the effectiveness of the antigens used in these commercial kits for detecting VL, CL, and CVL caused by various genera of *Leishmania*, and across different ethnic groups. The outcomes obtained from this critical review of ICTs should contribute to enhancing the effectiveness of these tests and potentially developing novel diagnostic methods tailored to particular countries or ethnic groups.

Results and discussion

Search strategy and eligibility

The search was conducted using the internet-based search engines PubMed (https://pubmed.ncbi.nlm.nih.gov/) and Google Scholar. On June 29, 2022, the search was initially conducted in the PubMed search engine by entering the following keywords: (commer* OR immunochromat* OR point of care OR rapid OR dipstick* OR sero*) AND test* AND *Leishmania** AND Diagnos*, with no time scale. As a result, 1699 articles published from 1945 to 2022 were retrieved. After the selection phase, only 77 articles were chosen. The following considerations were taken into account for selection: evaluation of commercial ITCs, statistical analysis with 95% confidence intervals, sensitivity and specificity values. Second, the Google Scholar search engine was used to search articles with the same keywords; 5326 articles published from 1995 to 2022 were retrieved. After the selection phase, 78 articles were chosen, but 77 were repeats of those found using the PubMed search engine and only one article was different.

In summary, 78 articles published from 2002 to 2022 addressed the evaluation of commercial ICTs, of which 50 used ICTs for diagnosing human leishmaniosis, and the other 28 for diagnosing canine leishmaniosis. The flowchart of the search strategy is shown in Figure 1.

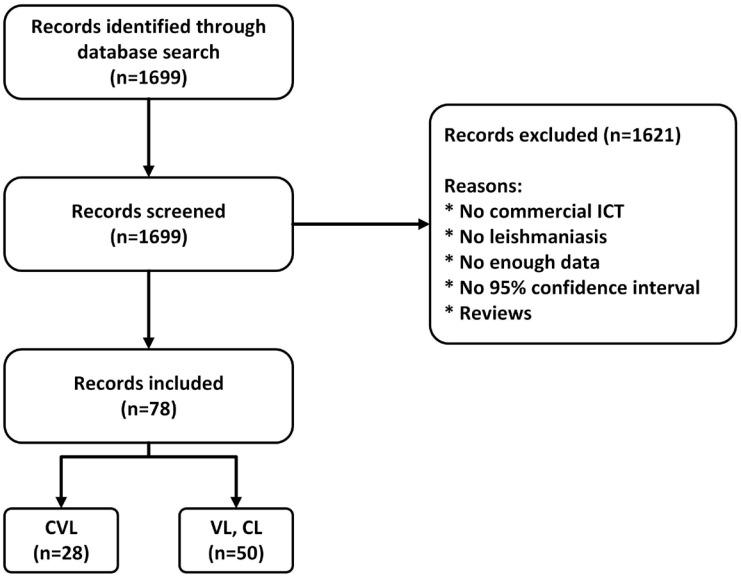


Figure 1. Flowchart of the works identified, screened, and included in this review.

General data

The ICT test was developed to provide a more rapid and user-friendly diagnosis, and for its potential valuable in mass screening (Proverbio et al., 2013). The commercial ICT tests evaluated and published in 78 articles are summarized in Tables 1 and 2. These tables include the commercial name, the laboratory where it is produced, the test reading time, the antigen used, the number of samples used, the country where they were tested, and the corresponding reference. All samples used to evaluate these ICTs were previously classified using PCR, enzyme-linked immunosorbent assay (ELISA), indirect immunofluorescence test (IFAT), and/or direct agglutination test (DAT). The results were statistically evaluated by all reference authors at a 95% confidence interval. The differences among these tests are discussed below, taking into consideration the *Leishmania* species present on each continent, the antigens used in the tests, and the results of sensitivity and specificity analyses (Table 3).

Human immunochromatographic test

A total of 26 ICTs with published diagnostic performance were found for the diagnosis of human leishmaniosis. Of these, 25 were used for qualitative diagnosis of VL, and the remaining one was used for qualitative diagnosis of CL. Table 1 summarizes the commercial ICT for VL and CL. All ICTs showed specificity higher than 90%, whereas the sensitivity results were highly variable (see Table 3 for details).

In Africa and Asia, where VL is caused by *L. donovani*, ICTs with the recombinant protein rK39 as antigen showed an average sensitivity of 88.12% in Africa and of 92.76% in Asia. ICTs with the recombinant protein rK28 as antigen was only tested in Africa and showed an average sensitivity of 95.69%. ICTs with the recombinant protein rKE16 as antigen showed an average sensitivity of 77.10% in Africa and 98.63% in Asia. However, in Africa, the rKE16 protein was tested using splenic aspirates, unlike the other continents where it was tested in serum samples. In Europe and America, where VL is caused by *L. infantum*, ICTs with the recombinant protein rK39 as antigen showed an average sensitivity of 75.01% in Europe, 90% in North America, and 86.16% in South America. Moreover, in Europe, an ICT was tested with a recombinant protein derived from *L. donovani* as antigen and showed sensitivity of 90.1%.

There were differences observed in the performance of the same commercial ICT used in different countries by different authors. For instance, InBios Kalazar Detect™ used in Ethiopia showed sensitivity of 75.4% and specificity of 70% in the study conducted by Boelaert et al. (2008), while in the study conducted by Cañavate et al. (2011), sensitivity was 90.5% and specificity was 90.7%. Furthermore, the same ICT showed variability in sensitivity when used in Brazil, ranging from 46.2% in the study conducted by Cota et al. (2013) to more than 80% in studies conducted by other authors.

The use of ICTs with rk39 recombinant protein as an antigen for diagnosing VL in Asia shows variable specificity values, both among countries and among authors. This excludes its use for diagnosing VL on this continent. However, ICTs with rK16 as an antigen showed sensitivity and specificity greater than 90%, making it the best option for diagnosing VL in Asia. On the other hand, in Africa, all the ICTs showed variable sensitivity and specificity values. Nevertheless, in the case of InBios Kalazar Detect™ that used rk39 as antigen, it showed sensitivity and specificity values greater than 90% in all the African countries where it was tested in studies with more than 100 samples. The studies with less than 100 samples showed specificity values of 27.8% (62 serum samples) and 70% (38 serum samples) in Ethiopia. Furthermore, only one ICT with rK16 as antigen has been tested in Africa using splenic aspirates as samples, and showed low sensitivity values. Due to the limited information available and discrepancies among the results, it is not possible to provide a recommendation for an appropriate ICT for diagnostic of VL in Africa. It is important to test ICTs with rK16 as antigen using blood or serum samples to compare their results with other tests.

Table 1. Commercial immunochromatographic rapid test for human leishmaniasis diagnosis.

Immunochromatographic Rapid Test	Laboratory	Read test time	Antigen	Clinical form	Quantity of used samples	Sensitivity (%)	Specificity (%)	Study country	Reference
InBios Kalazar Detect™	InBios International Inc.	15 min	Recombinant K39 protein	VL	243 serums	82.00	99.00	Uganda	Chappuis et al., 2005
				VL	508 serums	99.00	89.00	India	Sundar et al. 2006
				VL	142 bone marrow	89.00	90.00	Nepal	Chappuis et al., 2006
				VL	94 serums	90.00	100.00	USA	Welch et al., 2008
				VL	38 serums	75.40	70.00	Ethiopia	Boelaert et al., 2008
				VL	308 serums	84.70	89.90	Kenya	Boelaert et al., 2008
				VL	294 serums	77.90	91.80	Sudan	Boelaert et al., 2008
				VL	352 serums	99.60	90.00	India	Boelaert et al., 2008
				VL	158 serums	96.50	90.90	Nepal	Boelaert et al., 2008
				VL	929 serums	87.10	94.40	Tunisia	Saghrouni et al., 2009
				VL	455 serums	98.00	89.00	India	Singh et al., 2010
				VL	439 serums	100.00	92.40	India	Chakravarty et al., 2011
				VL	439 urine	96.40	66.70	India	Chakravarty et al., 2011
				VL	246 serums	90.50	90.70	Ethiopia	Cañavate et al., 2011
				VL	278 serums	88.10	90.60	Brazil	Peruhype-Magalhães et al., 2012
				VL	599 serums	99.50	96.90	India	Vaish et al., 2012
				VL	476 serums	72.40	99.60	Brazil	Moura et al., 2013
				VL	113 serums	46.20	98.40	Brazil	Cota et al., 2013
				VL	206 serums	92.60	98.20	Ethiopia	Bezuneh et al., 2014
				VL	95 serums	96.77	96.88	Bangladesh	Ghosh et al., 2015
				VL	62 serums	84.10	27.80	Ethiopia	Kiros & Regassa, 2017
				VL	116 blood	81.33	97.50	Brazil	Silva et al., 2018
				VL	49 serums	95.80	100.00	Morocco	Mniouil et al., 2018
				VL	743 serums	78.00	100.00	Spain	Bangert et al., 2018
				VL	156 serums	91.50	89.20	Colombia	Herrera et al., 2019
				VL	236 serums	92.50	94.90	Brazil	Freire et al., 2019
				VL	383 serums	90.00	100.00	Iran	Rezaei et al., 2019
				VL	91 serums	89.00	92.50	Ethiopia	Kassa et al., 2020
				VL	128 serums	89.80	96.30	Brazil	Sanchez et al., 2020
				VL	415 serums	77.50	98.60	Iran	Rezaei et al., 2021
				VL	355 serums	71.00	99.20	Iran	Rezaei et al., 2022
InBios Kalazar Detect™ plus	InBios International Inc.	15 min	Recombinant K28 protein	VL	206 serums	97.90	82.90	Ethiopia	Bezuneh et al., 2014
Insure Rapid Test for Visceral Leishmaniasis®	InBios International Inc.	10 min	Recombinant K39 protein	VL	205 blood	100.00	100.00	Nepal	Bern et al., 2000
				VL	184 serums	97.00	71.00	Nepal	Chappuis et al., 2003
Leishmania Cartridge Rapid test	DiaSys Europe Ltd	10 min	Recombinant K39 protein	VL	264 serums	100.00	100.00	Italy	Monno et al., 2009
OrangeLife®	OrangeLife	NI	Recombinant K39 protein	VL	116 blood	80.26	97.50	Brazil	Silva et al., 2018
rK39 Amrad ICT	Amrad ICT, Australia	NI	Recombinant K39 protein	VL	77 serums	93.00	70.00	Sudan	Veeken et al., 2003
Chembio Dual Path Platform rK28	Chembio Diagnostic Systems	NI	Recombinant K28 protein	VL	206 serums	95.80	94.60	Ethiopia	Bezuneh et al., 2014
EASE-Medtrend Dynamic Flow (rK28)	EASE-Medtrend Biotech Ltd.	NI	Recombinant K28 protein	VL	206 serums	98.90	80.20	Ethiopia	Bezuneh et al., 2014
SD *Leishmania* Ab	Standars Diagnostics, INC.	10-15 min	Recombinant K39 protein	VL	861 serums	100.00	100.00	Bangladesh	Banu et al., 2016
Ad-bio *Leishmania* IgG/IgM Combo	CTK Biotech, Inc.	15 min	Recombinant K39 protein	VL	156 serums	91.50	93.20	Colombia	Herrera et al., 2019

NI = Not information

Table 1. Continued...

Immunochromatographic Rapid Test	Laboratory	Read test time	Antigen	Clinical form	Quantity of used samples	Sensitivity (%)	Specificity (%)	Study country	Reference
IT-Leish®	BIO-RAD Laboratories, Inc.	25 min	Recombinant K39 protein	VL	332 serums	93.00	97.00	Brazil	de Assis et al., 2011
				VL	278 serums	93.30	96.50	Brazil	Peruhype-Magalhães et al., 2012
				VL	183 serums	81.10	98.70	Sudan	Abass et al., 2013
				VL	236 serums	96.30	96.20	Brazil	Freire et al., 2019
				VL	91 serums	91.20	100.00	Ethiopia	Kassa et al., 2020
				VL	128 serums	94.40	97.20	Brazil	Sanchez et al., 2020
				VL	319 serums	85.10	99.30	France, Morocco, Tunisia	Lévêque et al., 2020
				VL	520 serums	90.00	94.00	Brazil	Figueiredo et al., 2021
				VL	355 serums	81.00	98.82	Iran	Rezaei et al., 2022
DUAL IT L/M	DiaMed AG	20 min	Recombinant K39 protein	VL	243 serums	97.00	97.00	Uganda	Chappuis et al., 2005
DiaMed IT-Leish®	DiaMed AG	20 min	Recombinant K39 protein	VL	334 serums	89.60	99.00	Sudan	Ritmeijer et al., 2006
				VL	332 blood	93.00	97.00	Brazil	Assis et al., 2008
				VL	246 serums	89.00	91.00	Ethiopia	Cañavate et al., 2011
				VL	404 serums	94.00	100.00	Brazil	Machado de Assis et al., 2012
				VL	219 splenic	89.30	89.80	Kenya	Mbui et al., 2013
				VL	250 serums	96.80	98.20	Sudan	Bezuneh et al., 2014
TruQuick™ LEISH IgG/IgM	Meridian Bioscience	15 min	Recombinant *L. donovani* protein	VL	319 serums	90.10	95.70	France	Lévêque et al. 2020
Signal*KA	Span diagnostic Ltd.	10 min	Recombinant KE16 protein	VL	599 serums	95.50	100.00	India	Vaish et al., 2012
				VL	219 splenic	77.10	95.50	Kenya	Mbui et al., 2013
				VL	95 serums	100.00	98.44	Bangladesh	Ghosh et al., 2015
Crystal*KA	Span diagnostic Ltd.	30 min	Recombinant KE16 protein	VL	599 serums	99.00	98.50	India	Vaish et al., 2012
				VL	95 serums	100.00	98.44	Bangladesh	Ghosh et al., 2015
RapydTest	Intersep, UK	5 - 8 min	Recombinant K39 protein	VL	175 serums	72.00	61.00	Brazil	Reithinger et al., 2002
				VL	143 serums	100.00	100.00	Italy	Brandonisio et al., 2002
rK39 ICT RapydTest	Diagnostic International Distribution S.p.A	NI	Recombinant K39 protein	VL	94 serums	52.00	96.00	Italy	Varani et al., 2017
Onsite *Leishmania* Ab (Rev A) rapid test	CTK Biotech, Inc.	15 min	Recombinant K39 protein	VL	201 serums	94.00	96.00	Bangladesh	Khan et al., 2011
				VL	95 serums	100.00	95.31	Bangladesh	Ghosh et al., 2015
Onsite *Leishmania* Ab (Rev B) rapid test	CTK Biotech, Inc.	15 min	Recombinant K39 protein	VL	95 serums	100.00	31.25	Bangladesh	Ghosh et al., 2015
Onsite *Leishmania* rk39-plus	CTK Biotech, Inc.	15 min	Recombinant K28 protein	VL	285 blood	92.50	100.00	Sudan	Mukhtar et al., 2015
				VL	285 serums	94.50	97.60	Sudan	Mukhtar et al., 2015
Onsite *Leishmania* Ab Combo rapid test	CTK Biotech, Inc.	10 min	Recombinant K28 protein	VL	91 serums	92.20	77.50	Ethiopia	Kassa et al., 2020
				VL	206 serums	98.00	94.40	Ethiopia	Bezuneh et al., 2014
OnSite™ *Leishmania* IgG/IgM Combo test	CTK Biotech, Inc.	15 min	Recombinant K39 protein	VL	186 serums	91.20	94.50	Brazil	Freire et al., 2018
				VL	77 serums	63.00	98.00	Italy	Ortalli et al., 2020
Leishmania Dipstick Rapydtest	Apacor	NI	Recombinant K39 protein	VL	77 serums	70.00	96.00	Italy	Ortalli et al., 2020
Leishmania Strip quick test	Cypress Diagnostic	15 min	Recombinant K39 protein	VL	77 serums	52.00	100.00	Italy	Ortalli et al., 2020
				VL	355 serums	70.00	97.70	Iran	Rezaei et al., 2022
CL Detect™Rapid Test	InBios International Inc.	30 min	peroxidoxin	CL	74 skin	36.00	100.00	Sri Lanka	Silva et al., 2017
				CL	274 skin	65.40	100.00	Afghanistan	Vink et al., 2018
				CL	219 skin	68.00	94.00	Morocco	Bennis et al., 2018
				CL	93 skin	65.40	100.00	Afghanistan	Schallig et al., 2019
				CL	93 skin	33.30	85.70	Suriname	Schallig et al., 2019
				CL	165 skin	22.70	96.20	Ethiopia	Van Henten et al., 2022

NI = Not information

Table 2. Commercial immunochromatographic rapid test for canine visceral leishmaniasis diagnosis.

Immunochromatographic Rapid Test	Laboratory	Read test time	Antigen	Number of used samples	Sensitivity	Specificity	Study country	Reference
Kalazar Canine Rapid Test	Immunospec Corporation Canoga Park	10 min	Recombinant K39 protein	89 serums	92.59	100.00	Italy	Proverbio et al., 2013
rK39-RDT: Kalazar Detect™ Canine	InBios International Inc.	10 min	Recombinant K39 protein	109 serums	83.00	100.00	Brazil	Lemos et al., 2008
				322 serums 54 dogs	86.70	98.70	Brazil	Quinnell et al., 2013
				126 serums	82.90	92.60	Colombia	Herrera et al., 2019
				189 serums	79.60	95.70	Brazil	Ribeiro et al., 2019
				431 serums	76.90	98.60	Argentina	Salomón et al., 2020
DiaMed Vet IT-Leish®	Diamed AG	20 min	Recombinant K39 protein	123 serums	96.70	94.00	Switzerland	Mettler et al., 2005
rK39 dipstick test	Cypress Diagnostic	14 min	Recombinant KE39 protein	268 serums	70.90	84.90	Iran	Mohebali et al., 2004
rKE16 dipstick rapid test	Crystal KA, Co.	15 min	Recombinant KE16 protein	350 serums	32.40	92.90	Iran	Farahmand et al., 2015
Anigen Rapid *Leishmania* Ab Test	Isomedic S.r.L.	20 min	Recombinant k28 protein	66 serums	89.74	100.00	Brazil	Proverbio et al., 2016
Alere™ ICT	Alere S.A	20 min	Recombinant K28 protein	158 serums	91.00	100.00	Brazil	de Souza Filho et al., 2016
				189 serums	73.60	97.80	Brazil	Ribeiro et al., 2019
Snap Canine *Leishmania* Antibody Test	IDEXX Laboratories Inc.	6 min	purified antigens of *L. infantum* promastigote	60 serums	66.00	100.00	Spain	Rodríguez-Cortés et al., 2013
				109 serums	89.23	100.00	Greece	Athanasiou et al., 2014
				541 serums	94.70	93.60	Brazil	Souza et al., 2019
ImmunoRun Antibody Detection kit	Biogal Galed Labs	10 min	gold-conjugated *L. infantum* antigen	109 serums	86.15	100.00	Greece	Athanasiou et al., 2014
FASTest LEISH® test	MEGACOR Diagnostik GmbH	15 min	*L. infantum* recombinant peptides	244 serums	100.00	99.10	Spain	Villanueva -Saz et al., 2019
				215 serums	99.38	98.40	Spain	Basurco et al., 2020
				150 serums	100.00	98.00	Spain	Villanueva-Saz et al., 2022
Speed Leish K®	BVT Groupe Virbac	15 min	Complex of recombinant kinesin	250 serums	96.30	100.00	Italy	Ferroglio et al., 2013
				203 serums	63.60	100.00	Italy, UK, Cyprus and Spain	Solano-Gallego et al., 2014
				150 serums	97.00	98.00	Spain	Villanueva -Saz et al., 2022
DFV Test *Leishmania*	Divasa	10 min	*L. infantum* inactivated antigen	150 serums	59.00	100.00	Spain	Villanueva -Saz et al., 2022
Uranotest *Leishmania*	UranoVet	15 min	NI	150 serums	96.00	100.00	Spain	Villanueva -Saz et al., 2022
Uranotest *Leishmania* 2.0	UranoVet	15 min	NI	150 serums	97.00	98.00	Spain	Villanueva -Saz et al., 2022
WITNESS® *Leishmania*	Zoetis	10 min	NI	60 serums	58.00	100.00	Spain	Rodríguez-Cortés et al., 2013
				150 serums	84.00	95.00	Spain	Villanueva -Saz et al., 2022
INGEZIM® LEISHMACROM	INGENASA	10 min	*L. infantum* inactivated antigen	60 serums	75.00	100.00	Spain	Rodríguez-Cortés et al., 2013
RapydTest	DiaSys Europe Ltd	10 min	Recombinant K39 protein	108 serums	97.00	100.00	Italy	Otranto et al., 2004
DPP® kit	Fiocruz Bio-Manguinhos unit	15 min	Recombinant K28 protein	190 serums	98.00	96.00	Brazil	Grimaldi Junior et al., 2012
				431 serums	93.70	95.90	Argentina	Salomón et al., 2020
				144 serums	93.00	92.00	Brazil	Silva et al., 2013
				154 serums	88.30	97.50	Brazil	Fraga et al., 2014
				428 whole blood	87.50	73.30	Brazil	Schubach et al., 2014
				428 serums	88.00	69.20	Brazil	Schubach et al., 2014
				184 serums	90.60	95.10	Brazil	Laurenti et al., 2014
				116 serums	92.00	99.00	Brazil	Ribeiro et al., 2015
				780 serums	86.00	94.00	Brazil	Fraga et al., 2016
				1446 serums	89.00	70.00	Brazil	Figueiredo et al., 2018
				189 serums	97.90	93.60	Brazil	Ribeiro et al., 2019
				126 serums	85.70	92.60	Colombia	Herrera et al., 2019
				541 serums	95.80	100.00	Brazil	Souza et al., 2019
				194 serums	96.60	99.10	Portugal	Maia et al., 2022
				184 serums	85.70	90.70	Brazil	Maia et al., 2022

NI = Not information

Table 3. Average sensitivity and specificity percentage of commercial antigens used for the different types of leishmaniasis and continent of study.

Continent of study	Type of Leishmaniasis	Antigen	Average by antigen		Average by type of Leishmaniasis		Average by Country	
			Sensitivity (%)	Specificity (%)	Sensitivity (%)	Specificity (%)	Sensitivity (%)	Specificity (%)
Africa	CL	peroxidoxin	45.35%	95.10%	45.35	95.10	86.56	89.68
	VL	Recombinant K28 protein	95.69	89.60	89.73	89.26		
		Recombinant K39 protein	88.12	88.78				
		Recombinant KE16 protein	77.10	95.50				
Asia	CL	peroxidoxin	55.60	100.00	55.60	100.00	86.89	91.82
	CVL	Recombinant K39 protein	70,90	84,90	51.65	88.90		
		Recombinant KE16 protein	51.65	88.90				
	VL	Recombinant K39 protein	92.76	89.48	93.74	91.04		
		Recombinant KE16 protein	98.63	98.85				
Europe	CVL	Complex of recombinant kinesin	74.62	99.67	83.65	99.07	81.63	98.86
		gold-conjugated *L. infantum* antigen	86.15	100.00				
		L. infantum inactivated antigen	67.00	100.00				
		L. infantum recombinant peptides	99.79	98.50				
		no information	83.75	98.25				
		purified antigens of *L. infantum* promastigote	77.62	100.00				
		Recombinant K28 protein	96.60	99.10				
		Recombinant K39 protein	95.43	98.00				
	VL	Recombinant K39 protein	75.01	98.66	76.69	98.33		
		Recombinant *L. donovani* protein	90.10	95.70				
North America	VL	Recombinant K39 protein	90.00	100.00	90.00	100.00	90.00	100.00
South America	CL	peroxidoxin	33.30	85.70	33.30	85.70	86.03	93.15
	CVL	purified antigens *of L. infantum* promastigote	94.70	93.60	88.23	92.87		
		Recombinant K28 protein	89.74	91.57				
		Recombinant K39 protein	81.82	97.12				
	VL	Recombinant K39 protein	86.16	93.92	86.16	93.92		

In Europe, ICTs using rK39 as antigen for diagnosing VL have been evaluated in Italy, France, Switzerland, and Spain. On average, they demonstrate low sensitivity values (75.01%). Sensitivity outcomes in Italy varied among different researchers, with some reporting values as low as 52%. Given the limited available information and the variability in sensitivity results, ICTs with rK39 as antigen cannot be considered the optimal choice for VL diagnosis in Europe. It is imperative to acquire consistent results (with comparable statistical evaluations) regarding the performance of ICTs with rK39 as antigen, tested across numerous European countries where VL is endemic. Only through such cohesive findings can these ICTs be considered a reliable diagnostic option for VL in Europe. Similarly, in America, only ICTs with rk39 as antigen were tested, showing variable sensitivity and specificity values among researchers. However, DiaMed IT-Leish® is one of the ICTs validated and approved by the National Health Surveillance Agency (ANVISA) in Brazil (Peruhype-Magalhães et al., 2012), since this test showed sensitivity and specificity values greater than 90% and can thus be considered the best option for the diagnosis of VL in Brazil.

It is important to mention that the recombinant protein rK39 has been tested on all four continents where leishmaniosis is endemic. The recombinant proteins rK28, rKE16, and peroxidoxin have only been tested on three continents, while other antigens have been tested on two or less continents where leishmaniosis is endemic. Figure 2 shows the map of the commercial antigens used in different countries of study.

A comprehensive global comparison of ethnic groups could not be conducted based on the information provided in the articles. Many researchers employed sample collections from several regions within a state or country, making direct comparisons challenging. Nonetheless, some researchers (listed below) focused on specific demographic criteria, such as age ranges, sex, or HIV patient co-infection, in their investigations on LV diagnosis.

Rezaei et al. (2019, 2021, 2022) exclusively used samples from Iranian children (<18 years old). Employing InBios Kalazar Detect™, the article where samples from 1 to 16-year-old children were used presents results of 90% sensitivity and 100% specificity. In contrast, the other two articles using samples from children under one-year old yielded sensitivities of 71 to 77.5% and specificities of 98.60 to 99.20%.

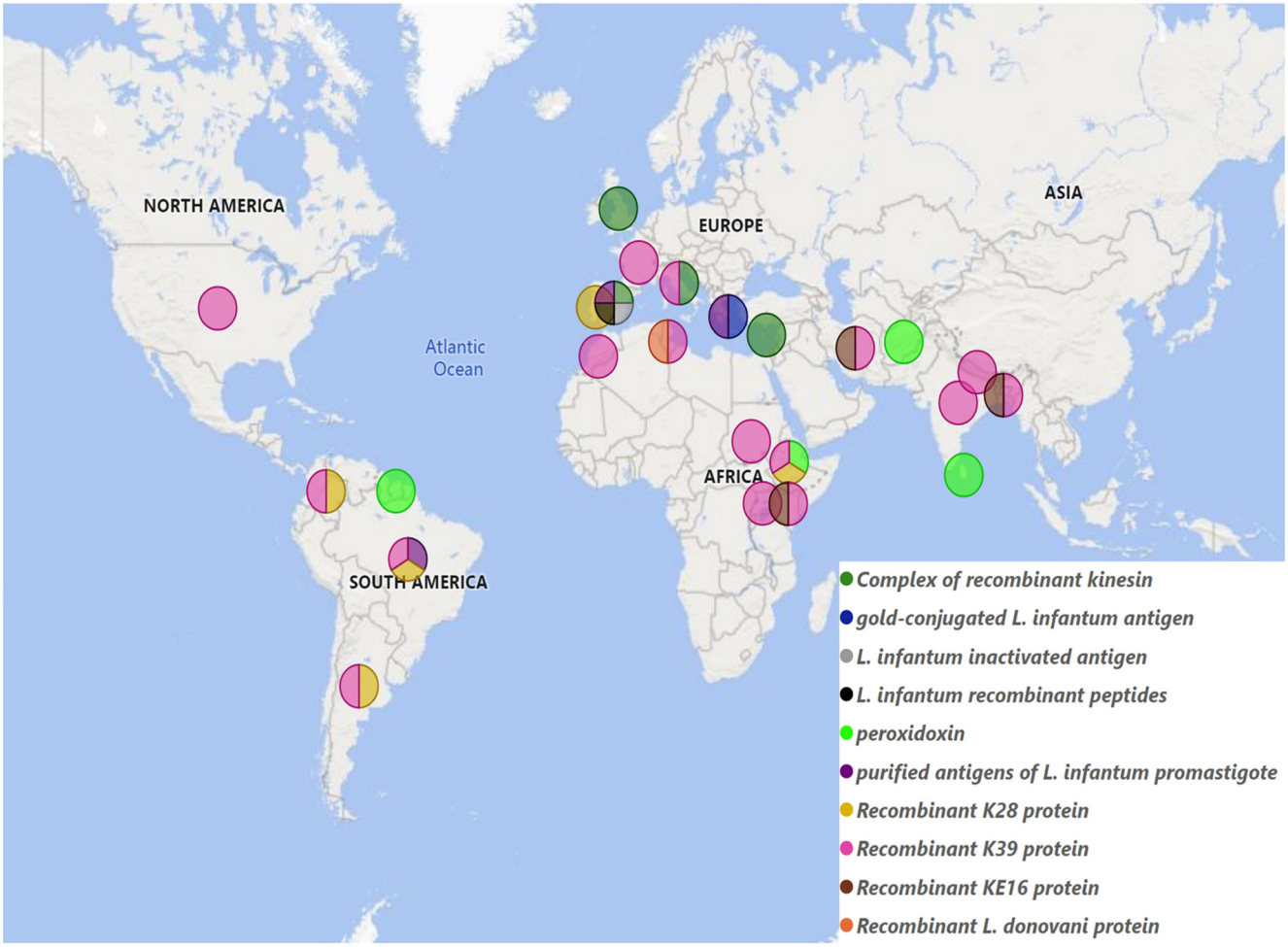


Figure 2. The map shows the commercial antigens used on different clinical forms of leishmaniosis and on different continents. The most widely distributed antigen in the world is the recombinant rK39 protein. It is used in North and South America, Europe, Africa and Asia. The rK28 protein is used alone or in combination in South America and Africa. Perodoxin is used in South America and Asia.

Notably, these articles did not specify that lower sensitivity results were derived from samples of children under one year old. On a different note, Moura et al. (2013) exclusively used samples from male Brazilian patients, yielding a performance of 72.40% sensitivity and 99.60% specificity. This sensitivity level contrasts with other authors' results using the same ICT on samples from Brazilian patients with no gender distinction. Lastly, Bezuneh et al. (2014) and Kassa et al. (2020) included samples from HIV-positive Ethiopian patients, obtaining sensitivities of 92.6% and 89%, and specificities of 98.2% and 92.5%, respectively. Cota et al. (2013), Silva et al. (2018), and Freire et al. (2019) conducted research on Brazilian patients, including those with HIV, obtaining sensitivities of 46.25, 81.33, and 92.5%, respectively, alongside specificities of 98.4%, 97.5%, and 94.9%. All the authors obtained results using the ICT InBios Kalazar Detect™. These variations in test performance in the same country could potentially stem from differences in sample origin and storage time.

The variability in performance from different tests may be related to co-infection with another *Leishmania* species and intraspecies diversity (Castillo-Castañeda et al., 2022; Herrera et al., 2019). For example, there is genetic variation in k39 among different strains of *L. donovani*. An Iranian *L. infantum* showed a k39 locus that is heterozygous, with a sequence like *L. infantum* from Brazil but differing by 6% in amino acid sequencing (Ortalli et al., 2020; Rezaei et al., 2019). This could explain why recombinant proteins present contrasting performances on different continents and in different countries.

Peroxidoxin antigen, used in the CL ICT Detect™ Rapid Test, was approved by the U.S. Food and Drug Administration (FDA) after being tested in endemic areas for CL caused by *L. major* (Van Henten et al., 2022). Peroxidoxin antigen is the only antigen used in ICTs for diagnosing CL, but it shows low sensitivity in Africa, Asia, and South America. In Suriname, an area endemic for *L. guyanensis*, the commercial test showed sensitivity of only 33.3%. In Afghanistan, an area endemic for *L. tropica*, the test showed sensitivity of 65.4%. In Sri Lanka, an area endemic for *L. donovani*, the test showed sensitivity of 36%. In Ethiopia, an area endemic for *L. aethiopica*, the test showed sensitivity of 22.7%. Finally, in Morocco, where CL is caused by *L. major* in the southeast region of the country, *L. tropica* in the north region, and other sporadic cases due to *L. infantum*, the test showed sensitivity of 68%, demonstrating the limited capacity of peroxidoxin in diagnosing CL.

The review conducted in this study demonstrates that peroxidoxin cannot be used in areas endemic for CL caused by other *Leishmania* species, since the sensitivity values are poor (22.7% to 68%). Other antigens must be developed and evaluated for the other *Leishmania* species that cause the same clinical form of the disease. Until then, another option to improve the diagnosis of CL with peroxidoxin is producing the antigen according to the endemic parasites. Figure 3 shows the global average sensitivity and specificity of all tests for the two clinical forms of leishmaniosis. The ICTs for diagnosing VL and CVL showed an average sensitivity of 88.64 and 84.53%, respectively, whereas the ICTs for diagnosing CVL show an average sensitivity of 48.47%.

Canine immunochromatographic test

Since dogs are the primary reservoir for *L. infantum*, immunochromatographic tests for dogs are designed to detect canine visceral leishmaniosis (CVL) caused by this species. Table 2 summarizes the 18 ICTs with diagnostic performance identified for CVL.

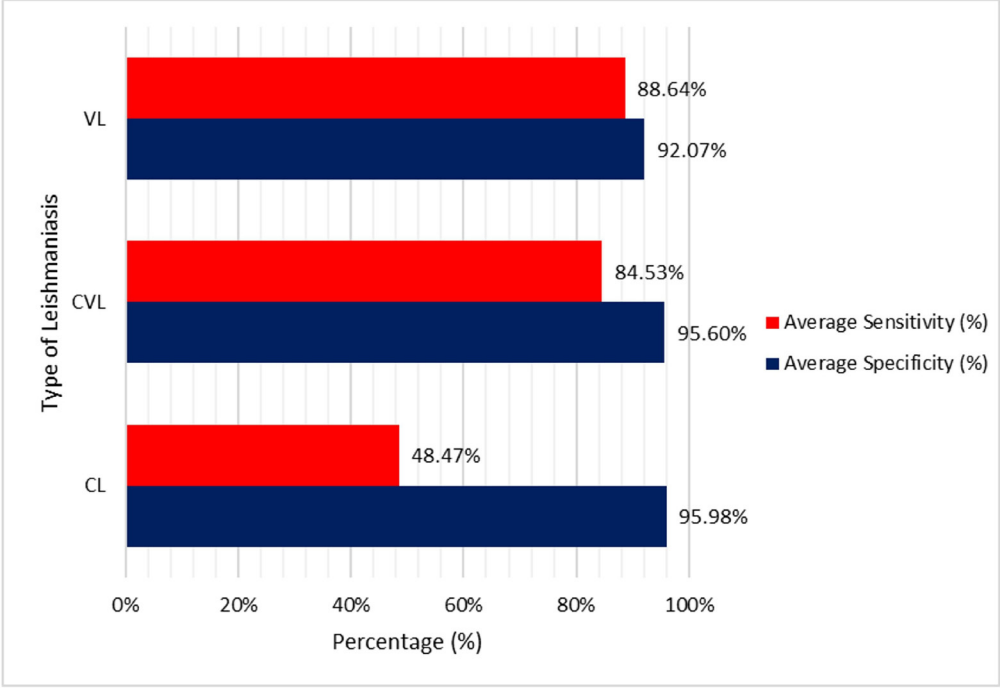


Figure 3. Overall average of the sensitivity and specificity of all immunochromatographic tests evaluated by infection for *Leishmania* diagnoses. For visceral leishmaniosis, sensitivity is higher, whereas for cutaneous leishmaniosis, it is less than 50%.

CVL is present in Europe, Central America, South America, Asia, and Africa; however, almost all the ICTs were tested in either Europe or South America. Only the ICT using recombinant protein rKE16 as antigen was tested in Asia, showing an average sensitivity of 51.65%. In the case of the recombinant protein rK39, the average sensitivity in South America was lower (81.82%) than the average sensitivity in Europe (95.43%). ICTs using recombinant peptides and purified promastigotes of *L. infantum* as antigen were also tested and showed an average sensitivity of 94.7% in South America and 81% in Europe. These results are not promising, considering that *L. infantum* is the causative agent of VL on both continents. In contrast, ICTs using the recombinant protein rK28 showed sensitivity of 89.74% in South America and 96.60% in Europe. The performance of the recombinant protein rK28 as antigen in ICTs for CVL diagnosis is similar to the recombinant protein rK39.

Table 3 indicates that the recombinant protein rk39 used as antigen in ICTs for CVL diagnosis differed greatly to those used for VL diagnosis in Europe, with sensitivity of 95.43% and 75.01%, respectively. Meanwhile, in South America, the performance of rk39 is similar when diagnosing CVL and VL.

Similarly, for human VL, some commercial ICTs have shown discrepant performances when used by different authors in the same country. For example, WITNESS® *Leishmania* used in Spain showed sensitivity of 58% in an investigation conducted by Rodríguez-Cortés et al. (2013) and sensitivity of 84% in an investigation conducted by Villanueva-Saz et al. (2022).

In summary, there is no information available on ICTs tested in Africa. The ICTs tested in Asia showed low sensitivity values and specificity greater than 80%. However, in Europe and America, all the ICTs showed specificity greater than 90% (99.07% and 92.87% correspondingly), while the sensitivity value varied from 67% to 99.79% in Europe and from 81.82% to 94.7% in South America. ICTs with rK39 and rK28 as antigens presented sensitivity values greater than 80%, making them the best options for diagnosing CVL in Europe and America. The effective diagnosis of CVL is crucial for the control of leishmaniosis in endemic areas; therefore, greater efforts should be made to improve ICTs that can detect leishmaniosis in asymptomatic dogs, reduce false negatives in dogs vaccinated against the disease, and in those who have received treatment. Additionally, an ICT with rk28 as antigen that was only tested in Portugal showed sensitivity of 96.6% and specificity of 99.1%.

The review conducted in this study demonstrates that the results of commercial ICTs varied across different authors and among the countries where they were tested. This is due to many of them using IFAT as a standard diagnostic test, which produces subjective results that depend on operator experience (Villanueva-Saz et al., 2019). Others have compared ICTs with several quantitative serological tests, such as DAT (Direct Agglutination Test) or ELISA, but the performance of these serological tests was poor in comparison with ICTs. Factors like the number of samples, study location, infectious species and statistical analysis contribute to greater variability in the results, making adequate comparison difficult. However, by analyzing results by continent and endemic *Leishmania* species, we can identify appropriate antigens for use in ICTs for diagnosing leishmaniosis in a certain region, as well as determine necessary steps to improve the ICT.

Other factors that may contribute to the variability in test performance include the use of different solid phases that can affect the antigen conformation (Rezaei et al., 2019), and the format of the test, which can impact sensitivity. For instance, dipsticks require mixing the serum and conjugate, while in other ICT strips, the conjugate is already incorporated (Rezaei et al., 2022). Ongoing investigations are aimed at improving ICTs by developing new, affordable, user-friendly, and effective diagnostic tests that include both the antigen and the platform.

Future prospects

Laser direct-write (LDW) is a technology that has shown versatility in the manufacturing of diagnostic devices in porous materials (Humbert et al., 2019). It does not need special laboratories and materials to create devices and uses low-cost equipment. LDW was used to create a new lateral flow device made on low-cost paper with a double geometrical canal to perform rapid ICTs for VL diagnosis. This new lateral flow device contains two recombinant proteins of *Leishmania* (ß-tubulin and LiHyp1) as antigens. The sensitivity and specificity levels of the new device were 90.9 and 98.7%, respectively. This test showed reactivity to VL, but not to other forms of leishmaniosis (Humbert et al., 2019). This technology was exclusively tested on blood samples from Brazilian patients (Minas Gerais); hence, the available information is limited. To gain a more comprehensive understanding of the performance of this new technology and the antigens employed, it is imperative to assess this prototype using samples from diverse regions and countries. Exploring the incorporation of alternative antigens in the LDW and evaluating their effectiveness could also be considered.

Nanotechnology offers new options for medical diagnosis due to the properties of nanoparticles (NPs) to generate high sensitivity and specificity. The physicochemical properties of NPs could contribute to new advances in the diagnosis of leishmaniosis as they improve the deficiencies of traditional diagnostic methods (Gedda et al., 2021). Metal NPs and carbon inks have been used to develop biosensors with advantageous characteristics, including low toxicity, biocompatibility, chemical inactivity, water solubility, abundance, and low-cost resources. Gold NPS have been extensively used to develop diagnostic sensors since they have excellent properties that enhance the sensitivity of diagnostic tools (Gedda et al., 2021; Kammona & Tsanaktsidou, 2021). For example, gold NPs were probed in a lateral flow device with a recombinant chimeric antigen and showed sensitivity of 98.4% and specificity of 98.9% for the rapid diagnosis of VL. Gold NPs were also probed in immunoelectrochemistry and showed specificity of 100%, while also avoiding cross-reactions (Kammona & Tsanaktsidou, 2021). All of these assays have solely undergone testing using samples from a particular region. As with LDW, it is essential to conduct tests using samples from diverse countries and regions to assess the nanoparticles' performance for detecting all clinical forms of leishmaniosis caused by numerous *Leishmania* species.

It is important to acknowledge that the various tests involving nanoparticles have shown an enhancement in the activity of the linked molecule. Consequently, a thorough evaluation and optimization are necessary for both the nanoparticle and the molecule it will be affixed to.

To date, molecular techniques for detecting parasitic nucleic acids are considered the most reliable due to their higher sensitivity and specificity. For this reason, serological tests are often combined with molecular tests in routine practices. The polymerase chain reaction (PCR) and real-time quantitative PCR (qPCR) are the main techniques (Basurco et al., 2020; Gedda et al., 2021; Lévêque et al., 2020). The sensitivity of these techniques varies between 90 to 100%, while the specificity is 98%. The advantages of these techniques include high detection capacity (less than one parasite per milliliter of blood), discrimination between the clinical forms of *Leishmania*, and high, uniform gene expression. On the other hand, the disadvantages include a lack of information regarding parasite viability, the probability of an early false positive, no distinction among *Leishmania* species, and the requirement of qualified personnel, equipment, and high-cost materials for their development (Brito et al., 2020).

With the aim of enhancing molecular techniques and rendering them more user-friendly, several studies have been undertaken, yielding significant outcomes.

Loop-mediated isothermal amplification (LAMP) is a new DNA amplification method that demonstrated high specificity and sensitivity for VL and CL diagnosis, with the capacity to detect DNA from different species of *Leishmania*, thus reducing the occurrence of false-positive results. In addition, it does not need high technology equipment to visualize the result, and it is considered a low-cost method (Brito et al., 2020). To diagnose human leishmaniosis, sensitivities of 80 to 100% and specificities of 94 to 100% have been reported for LAMP (Nzelu et al., 2019). Despite this, LAMP presents usability challenges, requiring skilled personnel to conduct the assays, and its application is limited to well-equipped laboratories rather than point-of-care settings. To address these limitations, innovative technologies are needed to mitigate the drawbacks associated with LAMP.

Clustered regularly interspaced short palindromic repeats (CRISPRs) systems and their associated Cas proteins have been included in DNA-detection methods and their applicability on DNA detection scheme for the diagnosis of VL has been demonstrated. Bengtson et al. (2022) developed a three-step method without the use of equipment using CRISPR-dCas9 based detection that can be read visually, to identify VL from blood and urine samples. First, they performed DNA extraction from blood and urine samples using mammalian extraction buffer and chitosan-functionalized paper discs, respectively. Second, an isothermal-amplification was performed by recombinase polymerase amplification (RPA) with a biotinylated primer, which is capable of identifying amounts as small as 10 target DNA copies. Finally, the amplificated DNA target was bounded by dCas9/sgRNA labelled with a DNA oligomer, initiating a rolling circle amplification (RCA). The RCA generates tandem repeats of G-quadruplexes that pick up a heme group, obtaining peroxidase activity. The final product characteristics, enable the generation of a colorimetric reaction that can be observed by the naked eye. This DNA detection scheme can be adapted to detect any DNA with minimal means and can be performed at room temperature, which facilitates its application in non-technical situations, such as point-of-care diagnosis in resource limited settings (Bengtson et al., 2022). In contrast to LAMP, research involving CRISPR-Cas9 for leishmaniosis diagnosis highlights the potential for deploying this emerging technology directly at the point of care. Nonetheless, proficient personnel are still necessary to conduct the tests using this method. Consideration could be given to conducting studies aimed at assessing the feasibility of user-friendly kits. Among all the novel technologies and inquiries highlighted in this section, the use of gold nanoparticles in immunoelectrochemistry stands out as presenting superior specificity outcomes, effectively mitigating cross-reactions. This outcome is especially significant since it addresses a common drawback associated with ICTs, false positives. Immunoelectrochemistry has been shown to be easy to handle and has great potential for point-of-care application. However, thus far, only prototypes for disease diagnosis have been explored and assessed, with no translation to practical point-of-care use. The optimization of these biosensors is imperative, since they have displayed the most exceptional performance thus far and have even surpassed expectations.

Conclusion

In conclusion, the study shows that low performance ICTs are being marketed and used in different countries where leishmaniosis is endemic. This puts the lives of both people and dogs at risk, since false negatives delay timely treatment of the disease, and false positives expose the patient to toxic substances due to unnecessary treatment, which could be harmful to a healthy person or dog.

The discrepancies between the sensitivity and specificity results of the different researchers demonstrate the lack of methods and statistical formulas to establish reference patterns and standardize the analysis of results for serological diagnostic methods.

It is crucial to evaluate all antigens used in commercial tests with sera from different parts of the world to determine which present the best performance, for use worldwide or in specific countries. Understanding the performance of these antigens within different ethnic groups could shed some light on improving or creating new chimeric proteins.

The new projects currently under investigation are good alternatives to improve the diagnostic performance of ICTs, including nanotechnology and new lateral flow platforms, which so far have shown high efficacy in the diagnosis of visceral leishmaniosis.

Conflict of interests

The authors declare no competing interests.

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