

Diagnosis of leishmaniasis

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Abstract

Leishmaniasis is a clinically heterogeneous syndrome caused by intracellular protozoan parasites of the genus *Leishmania*. The clinical spectrum of leishmaniasis encompasses subclinical (not apparent), localized (skin lesion), and disseminated (cutaneous, mucocutaneous, and visceral) infection. This spectrum of manifestations depends on the immune status of the host, on the parasite, and on immunoinflammatory responses. Visceral leishmaniasis causes high morbidity and mortality in the developing world. Reliable laboratory methods become mandatory for accurate diagnosis, especially in immunocompromised patients such as those infected with HIV. In this article, we review the current state of the diagnostic tools for leishmaniasis, especially the serological test.

Key words: leishmaniasis; diagnosis; serology; HIV co-infection.

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Introduction

The term leishmaniasis refers to a group of vector-borne diseases caused by obligate intracellular protozoan parasites of the genus *Leishmania*, belonging to the family *Trypanosomatidae*, order *Kinetoplastida*. Natural transmission of the parasite occurs primarily via the bite of infected female sandflies of the genus *Phlebotomus* in the Old World and *Lutzomyia* in the New World. The disease can also be transmitted by shared syringes among intravenous drug abusers [1], by blood transfusion [2], by venereal infections [3,4], and congenitally from mother to infant [5]. However, these modes of transmission are very rare compared with vector-borne transmission. In a domestic environment, dogs are the most important reservoirs, maintaining the parasite endemic focus. Rodents also play a role as *Leishmania* reservoirs [6-8].

Leishmaniasis occurs in three clinical forms: (i) cutaneous leishmaniasis (CL), which is caused by *L. major*, *L. tropica*, *L. aethiopica* (Old World CL), *L. infantum*, *L. chagasi* (Mediterranean and Caspian Sea region CL), *L. amazonensis*, *L. mexicana*, *L. braziliensis*, *L. panamensis*, *L. peruviana*, and *L. guyanensis* (New World CL); (ii) mucocutaneous

leishmaniasis (MCL) or espundia, which is caused by *L. braziliensis*, *L. panamensis*, *L. guyanensis* in the New World and is occasionally encountered in the Old World, caused by *L. infantum* and *L. donovani*; (iii) visceral leishmaniasis (VL), which is caused by species of the *L. donovani* complex that consist mainly of *L. infantum*, *L. donovani*, and *L. chagasi*. LV is also known as kala-azar, black fever, and Dumdum fever. There is a fourth form, known as diffuse cutaneous leishmaniasis (DCL), which is caused by *L. amazonensis* and *L. aethiopica* [9].

CL is the most prevalent form and manifests as skin lesions that can heal spontaneously. VL is the most severe form of the disease and, left untreated, is usually fatal. Post-kala-azar dermal leishmaniasis (PKDL) is a complication of LV, characterized by a macular, maculo-papular, or nodular rash on the face, torso, or other part of the body. It occurs mainly in East Africa and the Indian subcontinent, behaving as the major reservoir of the parasite in areas of anthroponotic transmission [10]. The mucocutaneous form causes partial or total mutilation of mucous membranes in the nose, mouth, and throat.

Leishmania infections are worldwide in distribution; they have been reported in 98 countries

on all continents. The disease is endemic in tropical and subtropical regions. There are an estimated 1.3 million new cases worldwide annually, of which 1 million are CL or MCL and another 300,000 cases are VL, with approximately 200 million people at risk of infection [11]. Of the 1.3 million estimated cases, only 600,000 are actually reported [12].

VL occurs mainly (90% of cases) in Bangladesh, Brazil, Ethiopia, India, Nepal, South Sudan, and Sudan. The governments of these countries have committed to eliminate VL by 2015 and aim to reduce the incidence of VL to 1 per 10,000 population in endemic districts [13]. VL is an important opportunistic infection in AIDS patients; in countries where there is poor access to antiretroviral therapy, the prevalence of visceral disease is rising [14]. These alarming facts have been attributed in part to the absence of an effective VL vaccine [15].

CL is found primarily in Afghanistan, Algeria, Brazil, Colombia, the Islamic Republic of Iran, Pakistan, Peru, Saudi Arabia, the Syrian Arab Republic, and Tunisia. Almost 90% of MCL cases occur in Brazil, Peru, and Bolivia. The health burden of CL in these countries remains largely unknown, partly because those who are most affected live in remote areas and often do not seek medical attention [12,15].

Control of leishmaniasis requires a combination of intervention strategies; early diagnosis and treatment is an important aspect. In VL, diagnosis is made by combining clinical signs with parasitological or serological tests (rapid diagnostic tests and others). In addition, an accurate diagnostic test that can identify active VL versus asymptomatic disease remains a key component of measures that aim to control this serious disease [16]. In CL and MCL, serological tests have limited value. Table 1 presents a summary of all the serological and antigen detection techniques commonly used for leishmaniasis diagnosis.

Diagnosis of cutaneous and mucocutaneous leishmaniasis

In the laboratory, diagnosis is made microscopically by direct identification of amastigotes in Giemsa-stained lesion smears of biopsies, scrapings, or impression smears. Amastigotes are observed as round or oval bodies, 2–4 μm in diameter, with characteristic nuclei and kinetoplasts. The material from the ulcer base usually has the highest yield; however, other authors have not found significant differences in the diagnostic outcomes when smears or culture samples are taken from the center or the border

of the ulcer or from an incision made tangential from the ulcer [17,18]. Conventionally, three to five aspirates from different lesions or portions of lesions are dissected. The first samples should be used for microscopy and the last for culture to minimize the risk of contamination [19]. A combination of microscopy and culture increases diagnostic sensitivity to more than 85% [18,20].

Antileishmanial antibodies can be detected by serological tests; however, these are not the usual methods, because antibodies tend to be undetectable or present in low titers due to poor humoral response [21-23].

Culture and DNA detection by PCR are sensitive but are not currently practical in developing countries.

Diagnosis of visceral leishmaniasis

In developing countries where the disease is not prevalent, the existence of laboratory facilities enables an adequate and efficient follow-up of the disease. However, in developing countries with large numbers of patients in rural areas, simple diagnostic tools are necessary for field use [24]. Laboratory diagnosis of VL includes microscopic observation and culture from adequate samples, antigen detection, serological tests, and detection of parasite DNA.

Culture and microscopic observation

Definitive VL diagnosis is supported by direct demonstration of parasites in clinical specimens and specific molecular methods [25-27]. The commonly used samples are splenic or bone marrow aspirates. The presence of amastigotes can also be determined in other samples such as liver biopsies, lymph nodes, and buffy coats of peripheral blood. The sensitivity of the bone marrow stained with Giemsa is about 60% to 85%. In splenic aspirates, the sensitivity is higher (93%) [28], but sampling is associated with a risk of fatal hemorrhage in inexperienced hands. To increase the sensitivity, fluorescent dye-conjugated antibodies can be used [29]. The sensitivity in peripheral blood smears is low, especially in individuals with low parasitemia. In addition, results are dependent on technical expertise and the quality of prepared slides.

Culture of the parasite can improve diagnostic sensitivity, but is tedious, time-consuming, and expensive, and thus seldom used for clinical diagnosis. There are new culture methods that improve sensitivity, such as the micro-culture method (MCM); recent modifications of this method involve using the buffy coat and peripheral blood mononuclear cells [30,31].

Table 1. Serological and antigen detection techniques commonly used for leishmaniasis diagnosis

Assay or method	Sensitivity (%)	Specificity (%)	Advantages	Disadvantages	References
Antibody detection					
IFA test	87–100	77–100	Positive in the early stages of infection and undetectable six to nine months after cure.	Requires sophisticated laboratory. No application in the field.	41,42,43
ELISA			Can be used to screen a large scale of epidemiologic studies.	Sensitivity and specificity is greatly influenced by the antigen used. Requires skilled personnel, sophisticated equipment, and electricity.	
CSA as antigen	80–100	84–95			45,46,47
with fucose-mannose ligand	95–100	95			53
rK39 as antigen	75–98	79–89			58,59
Western blot	90–98	98–100	Provides detailed antibody responses to various leishmanial antigens.	Time consuming, technically cumbersome, and expensive.	69, 70, 71, 72, 73
DAT	85–100	91–100	Rapid test, applicable in the field.	Limited to use in regions of endemicity. Long incubation time is needed. Unavailability of commercial source of the positive antigen and fragility of is aqueous form.	74,75,76,77,79
IC test with rK39	90–100	93–100	Inexpensive, rapid, simple, and can be performed by untrained person.		84,85,86,88
Antigen detection					
Latex agglutination test in urine (KAtex)	79–100	60–100	Simple, easy performing. Useful in diagnosis of disease immunocompromised patients.	Difficult to distinguish weakly positive from negative results and the urine must be boiled to avoid false-positive reactions.	33,34,36,37

The culture media used may be biphasic and may include Novy-MacNeal-Nicolle medium and Tobie's medium (conversion of amastigotes to promastigotes and monophasic medium), Schneider's insect medium, M199, and Grace's insect medium (amplifying parasite number) [29].

Antigen detection in urine

Several studies have demonstrated leishmanial antigen in the urine of VL patients. In 1995, De Colmenares *et al.* reported two polypeptide fractions of 72–75 kDa and 123 kDa in the urine of kala-azar patients [32]. In 2002, Sarkari *et al.* described a urinary 5–20 kDa carbohydrate-based, heat stable antigen of VL patients [33]. A latex agglutination test

(KAtex, Kalon Biological, UK) for detection of this antigen in urine samples was evaluated using samples from confirmed cases and controls from endemic and non-endemic regions. This test showed good specificity (82% to 100%), but had low to moderate sensitivity that ranged from 47% to 95% [34-38]. Nowadays, this method is useful for the diagnosis of disease in cases with deficient antibody production. In this respect, this method has reported 100% sensitivity and 96% specificity in immunocompromised patients [39]. In another study, 87% specificity and 85% sensitivity were obtained for primary VL in HIV-co-infected patients, and the method had predictive capabilities in the follow-up of treatment and detection of subclinical infection in *Leishmania*/HIV co-infected

cases [40]. Another urinary leishmanial antigen, a low-molecular weight, heat-stable carbohydrate has been detected in the urine of VL patients by an agglutination test with 60% to 71% sensitivity and 79% to 94% specificity [41]. In summary, the latex agglutination test is simple, easy to perform, inexpensive, rapid, and can be used as a screening test. Efforts are being made to improve the performance of this technique, because it promises to be a test of cure in populations of developing areas [28].

Serological diagnosis

Specific serological diagnosis is based on the presence of a specific humoral response. Current serological tests are based on four formats: indirect fluorescent antibody (IFA), enzyme-linked immunosorbent assay (ELISA), western blot, and direct agglutination test (DAT). The sensitivity depends upon the assay and its methodology, but the specificity depends on the antigen rather than the serological format used.

It has been noted that all antibody detection tests share the same drawbacks; the antibodies remain positive for many months after the patient has been cured and do not differentiate between current and past infection. In endemic regions, asymptotically infected persons can also be positive in these tests.

Indirect fluorescent antibody (IFA) test

The IFA test shows acceptable sensitivity (87%–100%) and specificity (77%–100%) [41,42]. Promastigote forms should be the antigens of choice for diagnosis of visceral leishmaniasis by the IFA test because they minimize cross-reactivity with trypanosomal sera [43]. The antibody response is detectable very early in infection and becomes undetectable six to nine months after cure; hence, if the antibodies persist in low titers, it is a good indication of a probable relapse [44]. The need for a sophisticated laboratory with a fluorescence microscope restricts use of the IFA test to reference laboratories.

Enzyme-linked immunosorbent assay (ELISA)

ELISA is the preferred laboratory test for serodiagnosis of VL. The technique is highly sensitive, but its specificity depends upon the antigen used. Moreover, this assay can be performed easily and is adaptable for use with several antigenic molecules.

One of the antigens used in the ELISA test is a crude soluble promastigote antigen (CSA) that is obtained by freezing and thawing live promastigotes.

The sensitivity of ELISA using CSA ranges from 80% to 100%, and specificity ranges from 84% to 95% [45,46]. Cross-reactivity with sera from patients with tuberculosis, trypanosomiasis, and toxoplasmosis has been reported [47-51]. When selective antigenic molecules were used (with molecular weights of 116 kDa, 72 kDa, and 66 kDa), the specificity approached 100%, while the sensitivity was very low (37%) [31,52]. Other purified antigens used were a 36-kDa glycoprotein [53], metabolic antigens released by *L. donovani* [54], and A2 proteins implicated in the development of visceral disease [55-57].

A conserved portion of a kinesin-related protein recombinant antigen from a cloned protein of *L. chagasi*, called rK39, has been reported to be highly reactive to sera from human and canine VL cases when run in an ELISA format [58,59]. Using this recombinant antigen, 99% specificity and sensitivity were reported in immunocompetent patients with clinical VL. In India, this antigen was reported with a sensitivity of 98% and a specificity of 89% [60]; however, a report from Sudan and other countries revealed that this antigen showed low sensitivity (75%) and specificity (70%) [61]. In HIV-positive patients, rK39-ELISA showed higher sensitivity (82%) than the IFA test (54%), with higher predictive value for detecting VL [62]. With successful therapy, antibody titers declined steeply at the end of treatment and during follow-up; in contrast, patients who relapsed showed increased titers of antibodies to rK39. This suggests the possible application of rK39-ELISA in monitoring drug therapy and detecting relapse of VL [51].

In another study, two hydrophilic antigens of *Leishmania chagasi* were used (rk9 and rk26), leading to an increase in the list of available antigens for serodiagnosis of VL [63]. Another kinesin recombinant related protein used in ELISA assay is rKE16. The use of this antigen for VL diagnosis has been very sensitive and specific as rK39 when tested in patients from China, Pakistan, and Turkey [64].

Another new assay, based on the detection of the K28 fusion protein in studies performed in Sudan and Bangladesh with 96% sensitivity in Sudan and 98% in Bangladesh, has been developed [65]. Moreover, heat shock proteins HPS70 or histones proteins H2A, H2B, H3, and H4 may have potential use for serodiagnosis of VL [66,67]; furthermore, lipid-binding proteins (LBPs) as antigens have shown high levels of sensitivity and an absence of cross-reactions with the sera of patients with other diseases [69]. The ELISA test, due to the requirement of skilled personnel,

sophisticated equipment, and electricity, is not used in endemic regions for the diagnosis of VL.

Immunoblotting (western blot)

For this type of testing, promastigotes are cultured to log phase, lysed, and the proteins are separated on SDS-PAGE. The separated proteins are electrotransferred onto a nitrocellulose membrane and probed with serum from the patient. The western blot technique provides detailed antibody responses to various leishmanial antigens [69,70], and has been found to be more sensitive than the IFA test and ELISA, especially in co-infected HIV patients with VL [71-73], but the drawbacks of the technique (equipment and time requirements, cumbersomeness, and cost) limit its use to research laboratories.

Direct agglutination test (DAT)

The DAT is based on direct agglutination of *Leishmania* promastigotes that react specifically with anti-*Leishmania* antibodies in the serum specimen, resulting in agglutination of the promastigotes. Whole, trypsinized, coomassie-stained promastigotes can be used either as a suspension or in freeze-dried form that can be stored at room temperature for at least two years, facilitating its use in the field [74,75].

Chappuis *et al.* [76], in a meta-analysis that included thirty studies evaluating DAT, found that the DAT had sensitivity and specificity estimates of 94.8% (95% confidence intervals [CI], 92.7–96.4) and 97.1% (95% CI, 93.9–98.7), respectively. Moreover, in settings where parasitological confirmation is not feasible, the freeze-dried DAT together with classical clinical features of VL can be used for diagnosis at a cut-off for positive DAT, which is 1:12,800 as in endemic areas. The DAT is simpler than many other tests but presents severe problems in terms of reproducibility of results, which depends on antigen elaboration [77]. A new antigen elaboration method, the EasyDAT described in 2003, shows the same sensitivity, specificity, and durability as the traditional DAT antigen method but offers the additional advantages of cost reduction and standardization [78].

Although the DAT for the serodiagnosis of visceral leishmaniasis has high sensitivity and specificity, it still has some limitations; among these are the relative long incubation time (18 hours) and the serial dilutions of the samples that must be made. In order to circumvent these problems, Schoone *et al.* in 2001 [79] developed a fast agglutination screening test (FAST). The FAST utilizes only one serum dilution (qualitative result) and requires three hours of

incubation. This makes the test very suitable for the screening of large populations. The sensitivity and specificity of FAST were found to be 91.1%–95.4% and 70.5%–88.5%, respectively [80,81].

Anti-*Leishmania* antibodies may persist for years as a result of previous VL infection, so titers measured by DAT may remain positive for up to five years after recovery in > 50% of VL patients, which may limit the DAT's widespread applicability in regions of endemicity. Although the DAT is the first real field test, it remains the serological test of choice as well as the first antibody detection test for VL used in field settings, particularly in many developing countries and in *Leishmania*/HIV co-infections [73,82,83].

Immunochromatographic assay (IC)

The immunochromatographic test using rK39 antigen (39 amino-acid-repeat recombinant leishmanial antigen from *L. chagasi*) has become popular in recent years. It is a qualitative membrane-based immunoassay with nitrocellulose strips impregnated with recombinant K39 *Leishmania* antigen. A drop of blood or serum is smeared over the pad of the strips and dipped in a small amount of buffer; the results are ready within a few minutes.

In clinical cases of VL, the rK39 IC showed variation in the sensitivity and specificity among different populations. The rK39 IC showed 100% sensitivity and 93%–98% specificity in India [84,85], 90% sensitivity and 100% specificity in Brazil [86], and 100% sensitivity and specificity in the Mediterranean area [87]. In other reports in southern Europe, the rK39 IC test was positive in only 71.4% of the cases of VL [88]; in Sudan, rK39 IC showed a sensitivity of 67% [61]. These differences in sensitivity may be due to differences in the antibody responses observed in different ethnic groups. The rK39 IC assay has proven to be versatile in predicting acute infection, and it is the only available format for diagnosis of VL with acceptable sensitivity and specificity levels. It is also easy to use in the field, rapid (15–20 minutes), cheap, and gives reproducible results. Like the DAT assay, IC is positive in a significant proportion of healthy individuals in endemic regions and for long periods after cure; hence, this limits its usefulness in persons with a previous history of VL who present with recurrence of fever and splenomegaly, as these tests cannot discriminate between a case of VL relapse and other pathologies.

Latex agglutination test (LAT)

The LAT is one of the recently developed rapid diagnostic tests for the rapid detection of anti-*Leishmania* antibodies against the A2 antigen derived from the amastigote form as well as those against crude antigens derived from the promastigote form of an Iranian strain of *L. infantum*. In a comparative study with the DAT, the sensitivity of tested human sera from DAT-confirmed patients yielded 88.4% sensitivity, while the specificity was 93.5% on A2-LAT amastigote, with a higher degree of similarity in accuracy to the DAT [89,90].

Molecular methods: polymerase chain reaction (PCR)

Although different molecular methods have successively been evaluated for leishmaniasis diagnosis, PCR-based assays are the main molecular diagnostic tools, especially in immunosuppressed patients [91-94].

PCR protocols to detect *Leishmania* DNA in VL diagnosis have used a variety of samples, including spleen, lymph node, and bone marrow aspirates, whole blood, and buffy coat [92,95-101].

There are different target sequences used, which include ribosomal RNA genes, kinetoplast DNA (kDNA), miniexon-derived RNA (medRNA), and the β -tubulina gene region [103].

Many PCR-based methods for diagnosis of VL have been described with different specificities and sensitivities; PCR assay sensitivity depends on the sample used. Sensitivity is highest (near 100%) in spleen or bone marrow [93,97,102] samples. The ideal sample is peripheral blood due to its non-invasive character. Using peripheral blood, the sensitivity ranges described vary from 70% to 100% [93,102,103].

A comparative clinical study between conventional microbiologic techniques and a leishmania species-specific PCR assay in HIV-co-infected and HIV-uninfected patients has shown the sensitivity of the leishmania species-specific PCR to be 95.7% for bone marrow and 98.5% for peripheral blood samples; the sensitivity in HIV-co-infected and non-HIV-co-infected adults was 100% [94].

A PCR-ELISA was used to diagnose VL in HIV-negative patients; peripheral blood was used and yielded a high sensitivity [104]. A similar PCR-based technique was applied in the diagnosis of VL in HIV patients, with good results [28].

Recently, an evaluation of an oligochromatography-PCR for diagnosis of VL, cutaneous leishmaniasis (CL), and post kala-azar

dermal leishmaniasis (PKDL), showed a high sensitivity (> 95%) on lymph, blood, and bone marrow samples from confirmed VL patients [105].

Another interesting approach is a rapid fluorogenic PCR technique. Wortmann *et al.* used a fluorescent DNA probe for a conserved rRNA gene that is amplified using flanking primers; this technique using clinical samples showed great sensitivity and specificity [98]. The real-time PCR has the advantage of being quantitative, which could be useful in the follow-up of treatment, allowing for the assessment of the parasite burden [106,107].

However, PCR techniques remain complex and expensive, and in most VL-endemic countries, they are restricted to a few teaching hospitals and research centers.

Diagnosis of VL-HIV co-infection

According to the World Health Organization (WHO) [108], an estimated 35 million people worldwide are living with HIV. Immunosuppression may reactivate latent *Leishmania* infection in asymptomatic patients and among HIV/AIDS patients. It is known that *Leishmania* has emerged as an opportunistic disease among HIV patients in endemic areas [109-112]. Moreover, it has been noted that the risks of clinical VL in HIV patients increased by 100 to 1,000 [111]. Although cases of co-infection have so far been reported in 33 countries worldwide, most of the cases have been found in sub-Saharan African countries, especially in East Africa. In Humera (in northwest Ethiopia), the proportion of VL patients co-infected with HIV increased from 18.5% in 1998–99 to 40% in 2006 [113].

VL in HIV patients has atypical clinical presentation; only 75% of HIV-infected patients, as opposed to 95% of non-HIV-infected patients, exhibit the characteristic clinical pattern – namely, fever, splenomegaly, hepatomegaly, and gastrointestinal involvement [114-117]. The diagnostic principles remain the same as those for non-HIV-infected patients. The presence of *Leishmania* amastigotes in the bone marrow can often be demonstrated in buffy coat preparation and in unusual locations (stomach, colon, or lungs) [118], but it has lower sensitivity in VL-HIV patients.

For HIV patients, the sensitivity of antibody-based immunological tests such as the IFA test and ELISA is low. Serological tests have limited diagnostic value because over 40% of co-infected individuals have no detectable specific antibody levels against *Leishmania* [119]. In their meta-analysis, Cota *et al.* [73]

summarized the accuracy of different serological techniques used for diagnosing HIV-co-infected persons. The estimated sensitivities using random effect models and their respective 95% confidence intervals for the other tests were: IFA test, 51%; ELISA, 66% (40% to 88%); DAT, 81% (61% to 95%); and immunoblotting, 84% (75% to 91%). The estimated specificity using random effect models and their respective confidence intervals for the following tests were: immunoblotting, 82% (65% to 94%); ELISA, 90% (77% to 98%); IFA test, 93% (81% to 99%); and DAT, 90% (66% to 100%). Thus, due to the low sensitivity of the serological tests for VL diagnosis in HIV-infected patients, at least two different serological tests should be used for each patient to increase the sensitivity of antibody detection [120]. The detection of polypeptide fractions of 72–75 kDa and 123 kDa of *Leishmania* antigen in the urine of patients with VL was 96% sensitive and 100% specific; furthermore, these antigens were not detectable after three weeks of treatment, suggesting a good prognostic value [32]. In conclusion, serology should not be used to rule out a diagnosis of VL among HIV-infected patients; an additional specially recommended serological test and/or molecular or parasitological methods may be necessary if the results of serological tests are negative.

Conclusions

Leishmaniasis is a global health problem and a major killer in endemic countries. Recent years have witnessed extraordinary potential progress in diagnosing *Leishmania* infection. The main challenge in these trials has been to reach the gold standard test in order to establish effective strategic programs to control and eradicate the disease. There are several methods of laboratory diagnosis of leishmaniasis; these include parasite detection by microscopic examination, culture, or molecular biology-based assays for detecting parasite DNA (PCR). The molecular tests are more sensitive than microscopic examination and parasite culture, but they remain restricted to referral hospitals and research centers. It could be concluded from previous studies that serological diagnosis is an alternative tool to the invasive methods of parasitological diagnosis for large-scale and decentralized diagnosis of leishmaniasis, especially VL. These serodiagnostic techniques are, however, of limited use for CL and MCL because of low sensitivity and variable specificity. Serologic diagnosis of VL can be accomplished with many techniques, such as IFA,

ELISA, western blot, rapid strip testing for rK39 antigen (IC), DAT, or detection of *Leishmania* antigen in urine by latex agglutination (KAtex).

Selection of the serological test during diagnosis should be based on different parameters such as region, cost, sensitivity, specificity, feasibility, sustainability, and field applicability, especially in problematic endemic areas. Detection of antibody responses to parasite antigens has inherent limitations. First, high serum antibody levels are present in both asymptomatic and active VL. Second, serum anti-*Leishmania* antibodies remain present for several years and complicate the diagnosis of relapse. Third, the specificity of these tests in VL-endemic areas is variable because there are a number of individuals with no clinical VL who have antileishmanial antibodies, complicating this specificity. Additional challenges originate from *Leishmania*/HIV co-infection, as the co-infection may appear without the typical clinical signs. The immunosuppressive action of *Leishmania*/HIV co-infection makes the serological approach to diagnosis especially challenging. However, DAT, immunoblotting, and the latex agglutination test (KAtex) have been proven to be superior in diagnosis of these co-infection cases. Negative results of serology should not reliably exclude a diagnosis of VL among HIV-infected patients. An additional molecular or other serological or parasitological test may be necessary to reach an accurate diagnosis if the results of serological tests are negative.

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