

# Diagnosing Neglected Tropical Diseases in HIV Coinfection



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**ABSTRACT:** Neglected tropical diseases (NTDs) and human immunodeficiency virus (HIV) coinfection are overlapping conditions around the world, mainly in tropical regions, affecting people living in absolute poverty (about US\$1.00 a day). Malaria, Chagas disease, and leishmaniasis are the main NTDs affected by HIV infection, in terms of clinical manifestation, diagnostics, and outcome after treatment. Unusual manifestation and reactivation of NTDs are more common in coinfecting patients. Traditional serological methods used in the diagnostics of NTDs show low sensitivity, and parasitological methods possess higher sensitivity. In this article, we discuss about the clinical presentation and laboratory diagnostics in the context of NTD and HIV coinfection.

**KEYWORDS:** neglected tropical disease, HIV infection, clinical presentation, diagnostic and laboratorial methods

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## Introduction

Neglected tropical diseases (NTDs) occur in some parts of the world, mainly in subtropical and tropical areas. Leishmaniasis, malaria, and Chagas disease are considered NTDs, occurring mainly in tropical regions and affecting people living under absolute poverty (on about US\$1.00 a day).<sup>1,2</sup> Additionally, HIV infections have increased in these regions, and naturally, overlapping between NTDs and HIV has taken place. Malaria, leishmaniasis, and Chagas disease are affected by coinfection with HIV, mainly in terms of clinical expression, therapeutic failure, and diagnosis.<sup>3</sup> Of note, diagnosis of NTDs in HIV coinfection has been observed to be particularly difficult, due to the decrease in sensitivity of traditional immunological methods and the unusual manifestations of leishmaniasis, malaria, and Chagas disease.<sup>3</sup> The gold standard diagnostic method for malaria, leishmaniasis, and acute Chagas disease is direct demonstration of the parasite by microscopy techniques.<sup>4–6</sup> This diagnostic method is inexpensive and can be conducted under field conditions, as it requires little structure. Once the parasite is seen, diagnosis can be made accurately. However, false-negative results are the inherent problem in the direct demonstration of parasites by microscopy. Causes of false-negative diagnosis may be low parasite load, lack of a well-trained expert to identify the parasites, and delayed diagnosis.<sup>7–9</sup> Another limitation of microscopic methods is the identification of species of the causal agent, which

is not possible for leishmaniasis and Chagas disease but possible for malaria, despite high error rates.<sup>10</sup> Other diagnostic methods have improved aggregate sensitivity and specificity relative to conventional techniques. In this scenario, the polymerase chain reaction (PCR) has contributed to improving the sensitivity and specificity of the diagnosis of these diseases throughout the world and, therefore, it has been increasingly used.<sup>11–14</sup> In this article, we discuss the clinical manifestations and laboratory diagnosis of NTDs (malaria, leishmaniasis, and Chagas disease) in relation to HIV infection.

## HIV Infection

The number of people who are newly infected with this virus continues to decline in most parts of the world. Yet, there were 2.1 million new HIV infections and 1.5 million acquired immune deficiency syndrome (AIDS)-related deaths in 2013, with the major proportion of these individuals living in sub-Saharan Africa.<sup>15</sup> A Joint United Nations Programme on HIV and AIDS (UNAIDS) report shows that 19 million of the 35 million people living with HIV today do not know that they have the virus.<sup>16</sup> The vast majority of people living with HIV are in low- and middle-income countries. According to the World Health Organization (WHO), sub-Saharan Africa is the most affected region, with 24.7 million people living with HIV in 2013.<sup>15</sup> Seventy-one percent of all people living with HIV in the world live in this region. Even today, despite



advances in our scientific understanding of HIV and its prevention and treatment, most people living with HIV or at risk for HIV do not have access to prevention, care, and treatment, and there is still no cure for the disease.<sup>16</sup> However, effective treatment with antiretroviral drugs can control the virus so that individuals with HIV can enjoy healthy lives and reduce the risk of transmitting the virus to others. The HIV epidemic not only affects the health of individuals, it also affects households, communities, and the development and economic growth of nations.<sup>15</sup> Many of the countries most affected by HIV also suffer from other infectious diseases such as NTDs. Due to the spread of HIV infection and overlap with NTD transmission, the burden of tropical diseases in these HIV-infected individuals is increasing around the world.

## Malaria

According to the 2014 World Malaria Report, 198 million cases of malaria occurred globally in 2013 and the disease led to 584,000 deaths.<sup>16</sup> As *Plasmodium falciparum* malaria and HIV have similar global distribution, with the majority of people affected living in sub-Saharan Africa, the Indian subcontinent, and Southeast Asia, interactions between the two diseases pose major public health problems.<sup>17</sup> Because the control of malaria parasitemia is mediated by the immune system, in theory, severely immunosuppressed HIV-infected patients should have more parasitemia episodes and clinical manifestations.<sup>18</sup> Furthermore, malaria infections have been shown to cause an increase in plasma HIV viral load and to be associated with a more rapid decline in cluster of differentiation 4 (CD4+) cells over time.<sup>19–21</sup> HIV disease progression and transmission are strongly associated with blood viral load. Therefore, high concentrations of HIV-1 RNA can be predictive of disease progression and correlated with the risk of blood-borne, vertical, and sexual transmission of the virus.<sup>19</sup> Although the five species of parasites of the genus *Plasmodium* can be involved in the coinfection, the interaction between HIV and *P. falciparum* is the most widely studied because of its predominance in Africa and the severity associated with this species.<sup>22</sup> The clinical pattern of severe malaria varies in different epidemiological settings. Clinical manifestations depend on the background level of the acquired protective immunity, which varies according to the pattern and the intensity of malaria transmission in the area of residence. In places where populations are continuously exposed, adolescents and adults are partially immune and most often present mild disease or asymptomatic parasitemia, while severe manifestation is acquired early in childhood. In areas of unstable or low transmission, individuals of all ages can have acute clinical malaria, with a high risk of progression to severe malaria, if untreated.<sup>23</sup> Pregnant women have increased parasite densities, are more susceptible to infection than other adults, and present risk of placental malaria and higher infant mortality.<sup>24</sup> Malaria caused by *P. vivax* has been usually considered benign; however, over the past years, there have

been increasingly frequent reports of severe malaria caused by *P. vivax* in different regions. The main signs of severity were severe thrombocytopenia, severe anemia, hepatic dysfunction, metabolic acidosis, and renal dysfunction. Mortality due to *P. vivax* infection is reported to be around 0.1%.<sup>25,26</sup>

The interplay between HIV infection and malaria varies according to this dynamics of malaria transmission. In theory, in areas with a high prevalence of HIV infection and a low occurrence of malaria, the effect of HIV infection on malaria is more noted, as there higher proportion of symptomatic cases is found among adults. In regions of unstable malaria, the concept that HIV infection could increase morbidity and mortality attributable to malaria is well established, especially in patients with severe immunosuppression.<sup>3,24</sup>

Some reports have shown that the occurrence of severity and mortality due to malaria in areas of stable transmission is also affected by HIV infection. A study conducted in Maputo, Mozambique, an endemic area for malaria, demonstrated an increase of severity and mortality due to malaria in coinfecting patients, compared to patients without HIV.<sup>17</sup> Another study carried out in Zambia, an area with high malaria transmission, found that HIV-1 infection was a highly significant risk factor for adults with severe malaria, compared to controls with uncomplicated malaria and asymptomatic controls.<sup>27</sup> Regarding women of childbearing age, in regions of stable malaria, the immunity developed by women over the years is impaired by pregnancy. In this period, there is a placental replication of parasites; however, local immune response increases during subsequent pregnancies.<sup>3,24</sup> Nevertheless, data from immunological studies indicate that HIV impairs this parity-specific immunity. HIV-infected pregnant women are at increased risk of parasitemia, clinical malaria, severe anemia, and placental malaria. Furthermore, malaria infection can also influence the dynamics of HIV transmission from mother to fetus.<sup>3,24,28</sup> Regardless of study population, it is important to note that most data describe the effect of HIV on malaria in heterogeneous groups, with respect to the use of antiretroviral treatment, prophylactic regimens, and other factors. Aspects of specific populations need to be further investigated, in addition to strategies to reduce the occurrence of parasitemia and clinical malaria in HIV-infected patients, such as the use of cotrimoxazole prophylaxis.<sup>29</sup> Laboratory diagnosis of malaria is traditionally made by demonstrating the presence of parasites in erythrocytes. On the other hand, the microscopic examination of thick and thin blood smears is considered the gold standard test due to its many advantages, such as accuracy, availability, low cost, and ability to quantify parasites and monitor parasite clearance.<sup>9,30</sup> Nevertheless, key quality recommendations are necessary for an efficient microscopic examination as it requires expertise, including a trained microscopist, proficiency/competency assessments, internal quality control, and standard slide sets. Besides microscopy, other techniques such as rapid diagnostic tests (RDTs), serological assays, and molecular assays have been used successfully



to diagnose malaria infections. RDTs have emerged as a promising alternative to microscopy for malaria diagnosis and have been listed as an acceptable means of diagnosis in recent WHO guidelines because of their simple concept and use, rapid results, and the fact that they can detect *P. falciparum* and other species.<sup>23</sup> Some disadvantages of these tests include their high cost, if used at the population level, being less sensitive than expert microscopy and molecular assays and not amenable for use in monitoring parasite clearance due to antigen persistence.<sup>9</sup> These immunochromatographic tests rely on the detection of parasite-specific antigens in blood samples with the use of monoclonal antibodies immobilized on test strip membranes using capillary and lateral flow technology. Malaria RDTs are currently used in some clinical settings and programs.<sup>5</sup> Serological assays for immune responses against *Plasmodium* spp. are used less often for acute disease diagnosis because the presence of antibody could refer to either a past or present infection and also because they do not discriminate between infection and disease. These assays can be useful for detecting specific humoral immune responses and for providing an estimate of past exposure or immune responses to a candidate vaccine.<sup>5,9</sup> Molecular tools offer advantages relative to the microscopic examination of blood smears. These molecular techniques overcome some limitations of conventional techniques; therefore, they have been increasingly used for the diagnosis of malaria. PCR assays have dramatically increased the analytical sensitivity of diagnosis while enabling the analysis of many malaria cases at the same time, monitoring of parasitemia, identification of the parasite species, and detection of mixed infections and *Plasmodium* infections with low parasite load.<sup>7,31–33</sup> PCR has contributed to the diagnosis of disease in pregnant women in countries where malaria and HIV are endemic. PCR has increased the detection of parasites in peripheral blood, placenta, and umbilical cord blood of pregnant women, when compared to detection by microscopy.<sup>34,35</sup> Increase in parasite detection in pregnant women with HIV allows the detection of subclinical infections and provides for remedial measures such as the use, during pregnancy, of drugs known to decrease parasite load of *Plasmodium* in the circulating blood, placenta, and umbilical cord blood, thus reducing the possibility of maternal–fetal transmission.<sup>35,36</sup> The reverse transcriptase quantitative PCR (RT-qPCR) approach has been used to quantify circulating gametocytes in the blood of human hosts, which contributes to the transmissibility of malaria, because the amount of gametocytes influences the transmission to the insect vector and, as a consequence, increases the chance of infecting new hosts. Assays using primers specific for male and female gametocytes and even species-specific sequences showed satisfactory levels of detection, even when gametocytes were circulating in small amounts in residents of areas of low transmission of the disease.<sup>37–39</sup> Loop-mediated isothermal amplification (LAMP) is a chemical method of DNA amplification that does not require a thermocycler, ultraviolet light, cooled stock

reagents, and highly trained professionals; therefore, it is called a field-friendly technique.<sup>40</sup> Many reports have assessed the performance of LAMP in malaria diagnosis, even in countries with intensive intervention to eliminate the disease. The LAMP assays commonly present diagnostic efficacy similar to the nested PCR assays, considered by many articles the gold standard.<sup>41–43</sup> These reports showed that LAMP assays enable detection of *P. falciparum* and *P. vivax* at the species level and diagnose asymptomatic infections or low parasitic loads.<sup>41,43,44</sup>

Molecular diagnosis may also facilitate the detection and identification of malaria cases in nonendemic countries, where individuals may become infected when traveling for business or tourism. Considering that HIV is widely distributed throughout the world, HIV-positive tourists can become infected. Molecular tools, which are not often part of routine health centers in nonendemic countries, can be very useful for the diagnosis of malaria. Once HIV carriers present more severe symptoms of malaria, which is a life-threatening situation, a rapid and specific diagnosis is very important for the rapid commencement of treatment.<sup>45,46</sup>

### Chagas Disease

Chagas disease occurs primarily in rural areas, and it is endemic in several regions of 21 countries in the American continent, from the south of the United States to Argentina. It has been estimated that 14 million people are infected with *Trypanosoma cruzi* and 60 million live in high-risk areas. The disease is caused by a protozoan called *T. cruzi* and it is transmitted mainly by the vectors' bite (triatomine insects).<sup>47</sup> However, transmission by blood transfusion can occur, mainly in nonendemic areas.<sup>47</sup> *T. cruzi* displays a relevant genetic variability shown by at least six discrete typing units (DTUs), from TcI to TcVI.<sup>48</sup> Studies showing the impact of this genetic variability on HIV coinfection are scarce. These studies<sup>49,50</sup> have suggested a differential tissue tropism of the infecting DTUs and have reported mixed infections in coinfecting patients, observing TcI and TcII—or TcI, TcV, and TcVI—in the blood, heart, and brain tissue of Chagas disease patients with HIV/AIDS coinfection with cardiomyopathy and encephalopathy. Among immunocompetent patients, Chagas disease can be present as an acute manifestation, but chronic forms are diagnosed more frequently. The main symptoms observed in acute form are fever, general malaise, inflammation of the inoculation site, periocular edema (Romaña sign), enlarged lymph nodes, and splenomegaly. About 5% of symptomatic cases die from meningoencephalitis, myocarditis, or both. After the initial phase, about 60%–70% of patients never develop symptoms (asymptomatic chronic phase). However, 40% can develop organ involvement a long time after the acute infection, characterized by cardiomyopathy, megaesophagus, and megacolon.<sup>51</sup> The prevalence of Chagas/HIV coinfection around the world is difficult to establish.<sup>52</sup> Almeida et al<sup>53</sup> published a study involving 716 patients with HIV treated in a University Hospital in Brazil. Of these, nine individuals (1.3%) tested positive on serologic



tests and were diagnosed with Chagas/HIV coinfection. *T. cruzi* infection behaves as an opportunistic parasite in individuals with HIV, but many questions related to coinfection, such as incidence, clinical and laboratory profile, treatment of Chagas disease, and better use of antiretroviral therapy, still need to be clarified.<sup>54</sup> In people living with HIV, Chagas disease can assume characteristics of an opportunistic infection, mainly in patients with severe immunosuppression.<sup>55</sup> The clinical manifestation of this population reflects the reactivation of previous chronic *T. cruzi*. Reactivation can involve the central nervous system (CNS) and heart.<sup>54</sup> Involvement of CNS is the most frequent manifestation, occurring in 75% of coinfecting patients that present reactivation.<sup>54,56</sup> The most common clinical symptoms are fever, headaches, vomiting, and altered consciousness; classically, coma, focal motor deficit, and convulsion can be observed as a meningoencephalitis manifestation.<sup>54</sup> The CNS image obtained by computed tomography is characterized by single or multiple lesions, similar to those in toxoplasmosis, predominantly located in the white matter of the brain lobes, with the occurrence of perilesional edema, deviation of midline shift, and compression of ventricles.<sup>55-57</sup> High mortality rate is associated in most studies. Cordova et al<sup>56</sup> performed a retrospective study with a total of 15 patients, in which the global mortality was 79%. Mortality rates depend on the degree of immunosuppression, antiretroviral therapy use, delay in diagnosis, and antiparasitic treatment efficacy.<sup>58</sup> The second most common manifestation of Chagas/HIV coinfection is acute myocarditis, and it is usually associated with CNS involvement. Clinical manifestations include arrhythmias, heart failure, pericardial effusions, and decompensation or accelerated progression of existing chronic heart disease.<sup>59,60</sup> The search for a disease progression predictor has been pursued for a long time. Serum levels of brain natriuretic peptide (BNP) are a reliable indicator of the presence of systolic left ventricular dysfunction in patients with Chagas disease.<sup>61</sup> High levels of BNP are also indicative of ventricular arrhythmia and diastolic dysfunction.<sup>62,63</sup> Moreover, BNP levels represent a strong predictor of the risk of stroke or death in longitudinal studies and might have a role in the clinical evaluation of patients with Chagas cardiomyopathy.<sup>64</sup> Levels of other biomarkers, such as cardiac muscle troponin T and several inflammatory cytokines (tumor necrosis factor and interferon- $\gamma$ ), correlate with the severity of cardiac disease and are candidate biomarkers to be used in clinical practice<sup>65,66</sup> However, in HIV-coinfecting patients, biomarkers have not been evaluated. Most probably, these patients present reactivation of chronic infection, so they develop severe clinical manifestations, due to low CD4+ T cell counts.

**Laboratory diagnosis of Chagas disease.** The main diagnosis method of acute Chagas disease is by direct search of the parasite and hemoculture, but it is rarely positive in chronic Chagas disease, except in HIV or immunosuppressed patients. Chronic Chagas disease can be detected by specific antibodies against *T. cruzi*, including use of techniques such as enzyme-linked immunosorbent assay (ELISA), indirect

immunofluorescence, or hemagglutination. Two positive results using different methodologies, or two different antigens in ELISA, are sufficient to confirm the diagnosis in most patients, especially in combination with epidemiological data.<sup>67</sup>

Inconclusive results of serological diagnosis in Chagas disease have an important impact on blood banks worldwide, reflected in the disposal of blood bags and an increased transmission by blood transfusion. Molecular techniques have been used for diagnosing and monitoring *T. cruzi* load in peripheral blood samples.<sup>68</sup> This promising perspective points to the possibility of detecting the parasite DNA in serum using the same samples collected for serological screening.

PCR has been increasingly used as an additional tool for the diagnosis of Chagas disease. During the acute phase of the disease, parasite loads are present in levels detectable by both conventional optical microscopy techniques and analysis of fresh buffy coat.<sup>69</sup> The onset of treatment during the acute phase has good resolution rates; therefore, PCR may help in the early diagnosis of Chagas disease.<sup>13,70</sup> A decrease in parasite load is observed in the chronic phase, and the diagnostic method indicated at this stage is serology. But depending on the serological assay used, cross-reactivity with other parasites, such as *Leishmania* spp., or false-negative results may occur. Thus, some authors indicate PCR as an ancillary diagnostic tool at this stage.<sup>6,71</sup> According to literature, the sensitivity of PCR in the chronic phase of Chagas disease is variable, and some studies have reported improvement in diagnostic sensitivity compared to serology and other diagnostic methods.<sup>72</sup> Regarding HIV patients, the use of PCR in the diagnosis of Chagas disease and monitoring of treatment efficacy is of paramount importance, as it is known that despite not completely eliminating the parasites, the treatment helps reduce the damage caused by the immune system in response to infection.<sup>73</sup> qPCR assay has been efficient in distinguishing between groups of patients coinfecting with HIV and Chagas who did or did not relapse.<sup>69</sup> Although some studies show low sensitivity for PCR in diagnosis of Chagas disease, HIV patients usually have higher parasite loads even in the acute phase, which facilitates diagnosis. qPCR has been able to show the reactivation of the disease in these patients and also show that the chronic phase may have parasitemia levels similar to those in the acute phase of the disease.<sup>55,71,72,74</sup> A major issue concerning the use of PCR in the diagnosis of Chagas disease is the identification of the DTU involved, which requires the association of some tests for the identification, thus making analysis time consuming and costly.<sup>49,75</sup>

### Leishmaniasis

Leishmaniasis comprises a group of diseases that cause tegumentary or visceral lesions. It is caused by a protozoan from the genus *Leishmania* and 21 species are responsible for tegumentary (TL) or visceral leishmaniasis (VL).<sup>12,76</sup> Between 1.5 and 2.0 million new cases of both TL and VL are reported yearly. Ninety percent of TL cases occur in six countries, whereas 90%



of VL cases occur in India, Nepal, Brazil, Sudan, Ethiopia, and Sudan.<sup>76</sup> The parasite is transmitted by the bite of the sandfly *Lutzomyia* in the New World and by *Phlebotomus* in the Old World, by inoculating the promastigote form of the parasite into the skin.<sup>52</sup> Species from the subgenus *Leishmania* can cause tegumentary or visceral lesions. *L. donovani* is the causal agent of VL in the Indian subcontinent, *L. infantum* in Europe and in some parts of Africa, and *L. infantum chagasi* in Latin America.<sup>76,77</sup> There are 15 dermatropic *Leishmania* species belonging to the subgenus *Leishmania* and *Viannia* occurring only in Latin America.<sup>12</sup> Depending on the *Leishmania* species and host immune response, different clinical forms are reported. Classically, VL is characterized by splenomegaly, hepatomegaly, fever, and pancytopenia.<sup>78,79</sup> Tegumentary leishmaniasis presents distinct forms and the main ones are as follows: 1) localized cutaneous leishmaniasis, characterized by a simple or multiple ulcer, can be caused by all dermatropic *Leishmania* species; 2) disseminated cutaneous leishmaniasis, characterized by multiple small ulcers; 3) diffuse cutaneous leishmaniasis is the anergic form of TL, characterized by multiple nodules or papules, generally presenting no ulceration; 4) mucosal leishmaniasis is mainly characterized by nasal involvement, presenting perforation or ulceration, which can also involve the palate and pharynx.<sup>12</sup> The spreading of HIV infection to rural areas and the urbanization of leishmaniasis has influenced the clinical progression and diagnosis of leishmaniasis. Concerning VL–HIV coinfection, the typical form, characterized by fever, splenomegaly, and hepatomegaly, is more common.<sup>80–83</sup> However, atypical manifestations, including the involvement of the gastrointestinal tract and kidneys, have been reported, whereas diarrhea and cough are more prevalent in HIV–VL coinfecting patients.<sup>84,85</sup> Regarding TL in HIV-infected patients, there are few reports in the literature. Clinical manifestations of TL in HIV-infected patients are diverse, and they depend on the immunological status of the patient. The reported typical lesions are similar to those observed in non-HIV-infected patients; however, unusual manifestations can occur due to severe immunosuppression.<sup>86</sup> Atypical manifestations are characterized by genital lesions and mucosal lesions associated with cutaneous lesions;<sup>87</sup> in addition, TL can be secondary to immune reconstitution inflammatory syndrome.<sup>88–90</sup>

**Laboratory diagnosis of TL.** Parasitological diagnosis of TL is based on the search for amastigotes using light microscopy to examine the biopsy specimen, scrapings, or impression smears subjected to Giemsa staining. Biopsy and aspirate samples can be further cultured in blood agar base (Novy, McNeal, and Nicolle medium) or injected into susceptible animals, such as hamsters, for parasite recovery.<sup>12</sup> The immunological diagnostic test, anti-*Leishmania* delayed-type hypersensitivity, reveals *Leishmania* infection, and therefore, it is used in epidemiological studies to determine the prevalence of infection. However, the test does not distinguish between present and past infection.<sup>91,92</sup> The most commonly used assays

for serodiagnosis in leishmaniasis are the indirect immunofluorescence assay and ELISA, which have shown a low sensitivity depending on the antigen preparation used.<sup>93–96</sup> Data from HIV/*Leishmania*-infected individuals using immunological tests and the observations collected in the Mediterranean area showed a relatively low sensitivity.<sup>82</sup> Nevertheless, in coinfecting patients in Brazil, sensitivity was not low, showing 77% positivity in serology.<sup>87</sup> Approaches for the detection of the etiological agent have relatively low sensitivity, and different methods do not identify the species of *Leishmania*.<sup>2</sup> Thus, recent efforts are aimed at developing assays to detect the parasite DNA. The use of PCR in the diagnosis of cutaneous leishmaniasis contributes to a sensitive detection of the parasite and also allows its identification. This is crucial as there are many species that can cause cutaneous leishmaniasis, and they are often endemic and present in the same area.<sup>96</sup> The differentiation of the species causing cutaneous leishmaniasis becomes even more important in the case of HIV-positive patients, because they have a higher chance of having the most severe clinical forms of the disease as well as its most unusual clinical forms.<sup>12</sup> Some targets widely used to detect *Leishmania* spp. are small subunit ribosomal DNA, microsatellite, internal transcribed spacer, mini-exon, and heat shock protein sequences.<sup>97–100</sup> Due to their sensitivity, these sequences enable distinction among the main species causing TL in South America, when associated with nested PCR, restriction fragment length polymorphism, or sequencing. Although present in lower copy numbers than kinetoplast DNA (kDNA), these targets have high sensitivity to detect parasites as well.<sup>64,101–103</sup>

**Laboratory diagnosis of VL.** Direct demonstration of *Leishmania* parasites in bone marrow aspirate or other biologic specimens is the most reliable diagnostic technique in the setting of VL–HIV coinfection. However, invasive procedures require trained physicians and the expertise and persistence of microscopists, factors of utmost importance for the final performance of the test. The bone marrow is the most commonly used biological material for further parasitological confirmation of *Leishmania* infection in the Americas, exhibiting high sensitivity.<sup>104,105</sup> Serological tests have a high diagnostic value for VL diagnosis in immunocompetent patients,<sup>106–108</sup> but their value is limited in HIV-infected patients.<sup>105–109</sup> According to data obtained from a study evaluating the accuracy of invasive and noninvasive tests for diagnosis of VL in a large series of HIV-infected patients at a reference center in Brazil, serological tests, such as indirect fluorescent antibody test and ELISA, showed lower sensitivity (<60%) when compared to the direct agglutination test (DAT), the sensitivity of which was 85%.<sup>105</sup> DAT is considered a highly sensitive and easy-to-use test and may be a good alternative for screening VL in HIV-infected patients. Molecular methods based on PCR have been evaluated as sensitive and specific methods to diagnose leishmaniasis both in non-HIV-infected and in HIV-infected patients. The parasite remains persistently in the peripheral blood and in the lesions, in VL and



TL, respectively, after specific treatment. In the context of HIV infection, PCR is a good method to measure relapse and reinfection. It has been used to monitor parasite load by real-time PCR (qPCR) in coinfecting VL–HIV patients to predict relapse after treatment. A parasite load  $>0.03$  parasites/mL in the third month after treatment represents about 100% sensitivity to predict relapse episodes. Similarly, a parasite load of 0.9% parasites/mL 12 months after treatment represents a high probability of relapse as well.<sup>110</sup> According to these data, Molina et al<sup>110</sup> consider that the treatment in coinfecting VL–HIV patients is efficient if PCR results are negative twice 6 months after its completion. Although PCR shows high sensitivity and specificity in the diagnosis of VL and TL, it is not able to discriminate between active disease and asymptomatic VL. Yet, PCR is an excellent method in clinical practice, as it can be used with different types of biological specimens, including noninvasive samples.<sup>111,112</sup>

## Conclusion

Although there is an overlap between HIV infection and NTDs, changes in the clinical presentation and an increase of lethality and relapse in the NTDs have been observed. In this scenario, malaria, Chagas disease, and leishmaniasis have been affected to a greater extent. In HIV-infected patients, clinical presentation of malaria is related mainly to the severity of malaria caused by *P. falciparum* and increased mother-to-fetus transmission. Clinical presentation of leishmaniasis can also be affected in HIV coinfection, as increase in lethality, relapse in VL–HIV coinfection, and atypical manifestations of VL have been reported. In addition, TL presents atypical manifestations, such as lesions in genital organs or manifestation of the immune reconstitution inflammatory syndrome. The same occurs in the case of Chagas disease, as the presence of lesions in the CNS and acute myocarditis has been described in HIV patients. Atypical manifestations or the increased lethality observed in this population coinfecting with HIV and NTDs is directly related to the severe immunosuppression mediated by low count of T CD4+ cells, which leads to an increase in replication of both HIV and the pathogens causing NTDs. Laboratory methods based on detection of antibodies and cellular immune response are affected as a result of their low sensitivity. However, parasitological methods possess high sensitivity, and methods based on DNA detection are equally good to detect the parasite, to monitor therapeutic response, and to identify the species of parasite involved in the lesion, in the same manner as they have been used for leishmaniasis.

## Author Contributions

Conceived the paper, took the lead in conception and design, and led the drafting of the paper: JALL. Contributed significantly to the writing of the paper: CMCG, ACSL, and MAC. All authors have read and approved the final version of the paper.

## REFERENCES

- Hotez PJ, Molyneux DH, Fenwick A, et al. Control of neglected tropical diseases. *N Engl J Med*. 2007;357:1018–1027.
- Lindoso JA, Lindoso AABP. Neglected tropical diseases in Brazil. *Rev Inst Med Trop Sao Paulo*. 2009;51(5):247–253.
- Karp CL, Auwaerter PG. Coinfection with HIV and tropical infectious diseases. I. Protozoal pathogens. *Clin Infect Dis*. 2007;45(9):1208–1213.
- Brasil. Ministério da Saúde. Secretaria de Vigilância em Saúde. Departamento de Vigilância Epidemiológica. Manual de vigilância e controle da leishmaniose visceral – 1. ed., 5. reimpr. – Brasília 2014. 120 p.: il.
- Centers for Disease Control and Prevention. Malaria gold standard. 2015. Available at: [www.cdc.gov/malaria/diagnosis\\_treatment/diagnosis.html](http://www.cdc.gov/malaria/diagnosis_treatment/diagnosis.html). [www.cdc.gov/malaria/diagnosis\\_treatment/diagnosis.html](http://www.cdc.gov/malaria/diagnosis_treatment/diagnosis.html)
- Gomes YM, Lorena VMB, Luquetti AO. Diagnosis of Chagas disease: what has been achieved? What remains to be done with regard to diagnosis and follow up studies? *Mem Inst Oswaldo Cruz*. 2009;104(suppl 1):115–121.
- Tahar R, Basco LK. Detection of *Plasmodium ovale* malaria parasites by species-specific 18S rRNA gene amplification. *J Clin Microbiol*. 1997;11(6):389–395.
- Ruiter CM, Van Der Veer C, Leeflang MMG, Deborggraeve S, Lucas C, Adams ER. Molecular tools for diagnosis of visceral leishmaniasis: systematic review and meta-analysis of diagnostic test accuracy. *J Clin Microbiol*. 2014;52(9):3147–3155.
- Murphy SC, Shott JP, Parikh S, Etter P, Prescott WR, Stewart VA. Review article: malaria diagnostics in clinical trials. *Am J Trop Med Hyg*. 2013;89(5):824–839.
- Snounou G, Viriyakosol S, Jarra W, Thaithong S, Neil Brown K. Identification of the four human malaria parasite species in field samples by the polymerase chain reaction and detection of a high prevalence of mixed infections. *Mol Biochem Parasitol*. 1993;58(2):283–292.
- Ciceron L, Jaureguiberry G, Gay F, Danis M. Development of a *Plasmodium* PCR for monitoring efficacy of antimalarial treatment. *J Clin Microbiol*. 1999;37(1):35–38.
- Goto H, Lindoso JA. Current diagnosis and treatment of cutaneous and mucocutaneous leishmaniasis. *Expert Rev Anti Infect Ther*. 2010;8(4):419–433.
- Cura CI, Lattes R, Nagel C, et al. Early molecular diagnosis of acute chagas disease after transplantation with organs from *Trypanosoma cruzi*-infected donors. *Am J Transplant*. 2013;13(12):3253–3261.
- Murphy SC, Prentice JL, Williamson K, et al. Real-time quantitative reverse transcription PCR for monitoring of blood-stage *Plasmodium falciparum* infections in malaria human challenge trials. *Am J Trop Med Hyg*. 2012;86(3):383–394.
- World Health Organization. HIV/AIDS Health Topic. Available at: <http://www.who.int/hiv/en>. Accessed April 29, 2015.
- USAIDS. Results and Data. Available at: <http://www.usaid.gov>. Accessed April 29, 2015.
- Berg A, Patel S, Aukrust P, et al. Increased severity and mortality in adults coinfecting with malaria and HIV in Maputo, Mozambique: a prospective cross-sectional study. *PLoS One*. 2014;9(2):e88257.
- Cohen C, Karstaedt A, Freen J, et al. Increased prevalence of severe malaria in HIV-infected adults in South Africa. *Clin Infect Dis*. 2005;41(11):1631–1637.
- Alemu A, Shiferaw Y, Addis Z, Mathewos B, Birhan W. Effect of malaria on HIV/AIDS transmission and progression. *Parasit Vectors*. 2013;6:18.
- Hochman S, Kim K. The impact of HIV and malaria coinfection: what is known and suggested venues for further study. *Interdiscip Perspect Infect Dis*. 2009;2009:617954.
- Brentlinger PE, Behrens CB, Kublin JG. Challenges in the prevention, diagnosis, and treatment of malaria in human immunodeficiency virus infected adults in sub-Saharan Africa. *Arch Intern Med*. 2007;167(17):1827–1836.
- Chang CC, Crane M, Zhou J, et al. HIV and co-infections. *Immunol Rev*. 2013;254(1):114–142.
- World Health Organization. World Malaria Report 2010. Available at: [www.who.int/malaria/world\\_malaria\\_report\\_2010/en/](http://www.who.int/malaria/world_malaria_report_2010/en/). Accessed April 21, 2015.
- Flateau C, Le Loup G, Pialoux G. Consequences of HIV infection on malaria and therapeutic implications: a systematic review. *Lancet Infect Dis*. 2011;11(7):541–556.
- Lacerda MV, Mourão MP, Alexandre MA, et al. Understanding the clinical spectrum of complicated *Plasmodium vivax* malaria: a systematic review on the contributions of the Brazilian literature. *Malar J*. 2012;11(1):12.
- Rahimi BA, Thakkestian A, White NJ, Sirivichayakul C, Dondorp AM, Chokejindachai W. Severe vivax malaria: a systematic review and meta-analysis of clinical studies since 1900. *Malar J*. 2014;13:1–10.
- Chalwe V, Van geertruyden JP, Mukwamataba D, et al. Increased risk for severe malaria in HIV-1-infected adults, Zambia. *Emerg Infect Dis*. 2009;15(5):749–755.
- Bulterys PL, Chao A, Dalai SC, et al. Placental malaria and mother-to-child transmission of human immunodeficiency virus-1 in rural Rwanda. *Am J Trop Med Hyg*. 2011;85(2):202–206.
- Kasiryre R, Baisley K, Munderi P, Grosskurth H. Effect of cotrimoxazole prophylaxis on malaria occurrence in HIV-infected patients on antiretroviral therapy in sub-Saharan Africa. *Trop Med Int Heal*. 2015;20(5):569–580.

30. World Health Organization. *Malaria Light Microscopy: Creating A Culture Of Quality*. Geneva: WHO; 2008.
31. Kamau E, Alemayehu S, Feghali KC, Saunders D, Ockenhouse CF. Multiplex qPCR for detection and absolute quantification of malaria. *PLoS One*. 2013;8(8):e71539.
32. Shahzadi S, Akhtar T, Hanif A, Sahar S, Niaz S, Bilal H. Molecular detection of malaria in South Punjab with higher proportion of mixed infections. *Iran J Parasitol*. 2014;9(1):37–43.
33. Speers DJ, Ryan S, Harnett G, Chidlow G. Diagnosis of malaria aided by polymerase chain reaction in two cases with low-level parasitaemia. *Intern Med J*. 2003;33(12):613–615.
34. Perrault SD, Hajek J, Zhong K, et al. Human immunodeficiency virus coinfection increases placental parasite density and transplacental malaria transmission in western Kenya. *Am J Trop Med Hyg*. 2009;80(1):119–125.
35. Noormahomed EV, Orlov M, do Rosario V, et al. A cross-sectional study of sub-clinical *Plasmodium falciparum* infection in HIV-1 infected and uninfected populations in Mozambique, South-Eastern Africa. *Malar J*. 2012;11(1):252.
36. Luntamo M, Rantala AM, Meshnick SR, et al. The effect of monthly sulfadoxine-pyrimethamine, alone or with azithromycin, on per-diagnosed malaria at delivery: a randomized controlled trial. *PLoS One*. 2012;7(7):e41123.
37. Kuamsab N, Putaporntip C, Pattanawong U, Jongwutiwes S. Simultaneous detection of *Plasmodium vivax* and *Plasmodium falciparum* gametocytes in clinical isolates by multiplex-nested RT-PCR. *Malar J*. 2012;11(1):190.
38. Schneider P, Reece SE, van Schaijk BC, et al. Quantification of female and male *Plasmodium falciparum* gametocytes by reverse transcriptase quantitative PCR. *Mol Biochem Parasitol*. 2015;199(1–2):29–33.
39. Waltmann A, Darcy AW, Harris I, et al. High rates of asymptomatic, sub-microscopic *Plasmodium vivax* infection and disappearing *Plasmodium falciparum* malaria in an area of low transmission in Solomon Islands. *PLoS Negl Trop Dis*. 2015;9:1–18.
40. Walker M, Basáñez M-G, Ouedraogo AL, Hermsen C, Bousema T, Churcher TS. Improving statistical inference on pathogen densities estimated by quantitative molecular methods: malaria gametocytaemia as a case study. *BMC Bioinformatics*. 2015;16(1):1–11.
41. Aydin-Schmidt B, Xu W, González JJ, et al. Loop mediated isothermal amplification (LAMP) accurately detects malaria DNA from filter paper blood samples of low density parasitaemias. *PLoS One*. 2014;9(8):1–9.
42. Sema M, Alemu A, Bayih AG, et al. Evaluation of non-instrumented nucleic acid amplification by loop-mediated isothermal amplification (NINA-LAMP) for the diagnosis of malaria in Northwest Ethiopia. *Malar J*. 2015;14(1):1–9.
43. Dinzouna-Boutamba SD, Yang HW, Joo SY, et al. The development of loop-mediated isothermal amplification targeting alpha-tubulin DNA for the rapid detection of *Plasmodium vivax*. *Malar J*. 2014;13(1):248.
44. Vallejo AF, Martínez NL, González JJ, Arévalo-Herrera M, Herrera S. Evaluation of the loop mediated isothermal DNA amplification (LAMP) kit for malaria diagnosis in *P. vivax* endemic settings of Colombia. *PLoS Negl Trop Dis*. 2015; 9(1):e3453.
45. Santos L, Pereira NR, Andrade P, et al. Prozone-like phenomenon in travellers with fatal malaria: report of two cases. *J Infect Dev Ctries*. 2015;9(03):1–4.
46. Ehrhardt J, Trein A, Krensner PG, Frank M. *Plasmodium knowlesi* and HIV co-infection in a German traveller to Thailand. *Malar J*. 2013;12(1):283.
47. Coura JR. Chagas disease: control, elimination and eradication. Is it possible? *Mem Inst Oswaldo Cruz*. 2013;108(8):962–967.
48. Zingales B, Miles MA, Campbell DA, et al. Infection, genetics and evolution the revised *Trypanosoma cruzi* subspecific nomenclature: rationale, epidemiological relevance and research applications. *Infect Genet Evol*. 2012;12(2):240–253.
49. Burgos JM, Begher SB, Freitas JM, et al. Molecular diagnosis and typing of *Trypanosoma cruzi* populations and lineages in cerebral chagas disease in a patient with AIDS. *Am J Trop Med Hyg*. 2005;73(6):1016–1018.
50. Hernández C, Cucunubá Z, Parra E, Toro G, Zambrano P, Ramírez JD. Chagas disease (*Trypanosoma cruzi*) and HIV co-infection in Colombia. *Int J Infect Dis*. 2014;26:146–148.
51. Coura JR, Borges-Pereira J. Chronic phase of Chagas disease: why should it be treated? A comprehensive review. *Mem Inst Oswaldo Cruz*. 2011;106(6):641–645.
52. De Almeida MC, Vilhena V, Barral A, Barral-Netto M. Leishmanial infection: analysis of its first steps. A Review. *Mem Inst Oswaldo Cruz*. 2003;98(7):861–870.
53. Almeida EA, Lima JN, Lages-Silva E, et al. Chagas' disease and HIV coinfection in patients without effective antiretroviral therapy: prevalence, clinical presentation and natural history. *Trans R Soc Trop Med Hyg*. 2010;104(7):447–452.
54. Almeida EA, Ramos Júnior AN, Correia D, Shikanai-Yasuda MA. Co-infection *Trypanosoma cruzi*/HIV: systematic review (1980–2010). *Rev Soc Bras Med Trop*. 2011;44(6):762–770.
55. Sartori AM, Ibrahim KY, Nunes Westphalen EV, et al. Manifestations of Chagas disease (American trypanosomiasis) in patients with HIV/AIDS. *Ann Trop Med Parasitol*. 2007;101(1):31–50.
56. Cordova E, Boschi A, Ambrosioni J, Cudos C, Corti M. Reactivation of Chagas disease with central nervous system involvement in HIV-infected patients in Argentina, 1992–2007. *Int J Infect Dis*. 2008;12(6):587–592.
57. DiazGranados CA, Saavedra-Trujillo CH, Mantilla M, Valderrama SL, Alquichire C, Franco-Paredes C. Chagasic encephalitis in HIV patients: common presentation of an evolving epidemiological and clinical association. *Lancet Infect Dis*. 2009;9(5):324–330.
58. Pérez-Molina JA, Rodríguez-Guardado A, Soriano A, et al; Chagas Study Group Of The SEMTSI (Sociedad Española de Medicina Tropical Y Salud Internacional [Spanish Society Of Tropical Medicine And International Health]). Guidelines on the treatment of chronic coinfection by *Trypanosoma cruzi* and HIV outside endemic areas. *HIV Clin Trials*. 2011;12(6):287–298.
59. Bern C. Chagas disease in the immunosuppressed host. *Curr Opin Infect Dis*. 2012; 25(4):450–457.
60. Vaidian AK, Weiss LM, Tanowitz HB. Chagas' disease and AIDS. *Kinetoplastid Biol Dis*. 2004;3:2.
61. Ribeiro AL, dos Reis AM, Barros MV, et al. Brain natriuretic peptide and left ventricular dysfunction in Chagas' disease. *Lancet*. 2002;360:461–462.
62. Talvani A, Rocha MO, Cogan J, et al. Brain natriuretic peptide and left ventricular dysfunction in chagasic cardiomyopathy. *Mem Inst Oswaldo Cruz*. 2004;99(6): 645–649.
63. Oliveira BMR, Botoni FA, Ribeiro ALP, et al. Correlation between BNP levels and doppler echocardiographic parameters of left ventricle filling pressure in patients with chagasic cardiomyopathy. *Echocardiography*. 2009;26(5):521–527.
64. Lima-Costa MF, Cesar CC, Peixoto SV, Ribeiro ALP. Plasma B-type natriuretic peptide as a predictor of mortality in community-dwelling older adults with Chagas disease: 10-year follow-up of the Bambuí Cohort Study of Aging. *Am J Epidemiol*. 2010;172(2):190–196.
65. Saravia SG, Haberland A, Bartel S, et al. Cardiac troponin T measured with a highly sensitive assay for diagnosis and monitoring of heart injury in chronic Chagas disease. *Arch Pathol Lab Med*. 2011;135(2):243–248.
66. Cunha-Neto E, Nogueira LG, Teixeira PC, et al. Immunological and non-immunological effects of cytokines and chemokines in the pathogenesis of chronic Chagas disease cardiomyopathy. *Mem Inst Oswaldo Cruz*. 2009;104(suppl 1): 252–258.
67. Ministério da Saúde. Secretaria de Vigilância em Saúde. Brazilian consensus on Chagas disease. *Rev Soc Bras Med Trop*. 2005;38:7–29.
68. Coura JR, Dias JCP. Epidemiology, control and surveillance of Chagas disease: 100 years after its discovery. *Mem Inst Oswaldo Cruz*. 2009;104:31–40.
69. de Freitas VL, da Silva SC, Sartori AM, et al. Real-time PCR in HIV/*Trypanosoma cruzi* coinfection with and without Chagas disease reactivation: association with HIV viral load and CD4+ level. *PLoS Negl Trop Dis*. 2011;5(8):e1277.
70. Urbina JA, Docampo R. Specific chemotherapy of Chagas disease: controversies and advances. *Trends Parasitol*. 2003;19(11):495–501.
71. Gilbert SR, Alban SM, Gobor L, De Oliveira Bescrevaine J, Myiazaki MI, Thomaz-Soccol V. Comparison of conventional serology and PCR methods for the routine diagnosis of *Trypanosoma cruzi* infection. *Rev Soc Bras Med Trop*. 2013; 46(3):310–315.
72. Duarte LF, Flórez O, Rincón G, González CI. Comparison of seven diagnostic tests to detect *Trypanosoma cruzi* infection in patients in chronic phase of Chagas disease. *Colomb Med*. 2014;45(2):61–66.
73. Viotti R, Vigliano C, Armenti H, Segura E. Treatment of chronic Chagas' disease with benznidazole: clinical and serologic evolution of patients with long-term follow-up. *Am Heart J*. 1994;127(1):151–162.
74. Perez-Ramirez L, Barnabé C, Sartori AM, et al. Clinical analysis and parasite genetic diversity in human immunodeficiency virus/Chagas' disease coinfections in Brazil. *Am J Trop Med Hyg*. 1999;61(2):198–206.
75. Schijman AG, Bisio M, Orellana L, et al. International study to evaluate PCR methods for detection of *Trypanosoma cruzi* DNA in blood samples from Chagas disease patients. *PLoS Negl Trop Dis*. 2011;5(1):e931.
76. Van Griensven J, Diro E. Visceral leishmaniasis. *Infect Dis Clin North Am*. 2012;26(2):309–322.
77. Silveira FT. Leishmania chagasi Cunha & Chagas, 1937: indigenous or introduced? A brief review. *Rev Pan-Amaz Saude*. 2010;1:143–147.
78. Gama MEA, Costa JML, Gomes CMC, Corbett CEP. Subclinical form of the American visceral leishmaniasis. *Mem Inst Oswaldo Cruz*. 2004;99(8):889–893.
79. Silveira FT, Lainson R, Crescente JA, et al. A prospective study on the dynamics of the clinical and immunological evolution of human Leishmania (L.) infantum chagasi infection in the Brazilian Amazon region. *Trans R Soc Trop Med Hyg*. 2010;104(8):529–535.
80. Lindoso JA, Cota GF, da Cruz AM, et al. Visceral leishmaniasis and HIV coinfection in Latin America. *PLoS Negl Trop Dis*. 2014;8(9):e3136.
81. Diro E, Lynen L, Ritmeijer K, Boelaert M, Hailu A, van Griensven J. Visceral leishmaniasis and HIV coinfection in East Africa. *PLoS Negl Trop Dis*. 2014;8(6): e2869.
82. Monge-Maillou B, Norman FF, Cruz I, Alvar J, López-Vélez R. Visceral Leishmaniasis and HIV Coinfection in the Mediterranean Region. *PLoS Negl Trop Dis*. 2014;8(8):e3021.
83. Burza S, Mahajan R, Sinha PK, et al. Visceral leishmaniasis and HIV coinfection in Bihar, India: long-term effectiveness and treatment outcomes with liposomal amphotericin B (AmBisome). *PLoS Negl Trop Dis*. 2014;8(8):e3053.



84. Alvar J, Cañavate C, Gutiérrez-Solar B, et al. Leishmania and human immunodeficiency virus coinfection: the first 10 years. *Clin Microbiol Rev.* 1997;10(2):298–319.
85. Alvar J, Aparicio P, Aseffa A, et al. The relationship between leishmaniasis and AIDS: the second 10 years. *Clin Microbiol Rev.* 2008;21(2):334–359.
86. Guerra JA, Coelho LI, Pereira FR, et al. American tegumentary leishmaniasis and HIV-AIDS association in a tertiary care center in the Brazilian Amazon. *Am J Trop Med Hyg.* 2011;85(3):524–527.
87. Lindoso JA, Barbosa RN, Posada-Vergara MP, et al. Unusual manifestations of tegumentary leishmaniasis in AIDS patients from the New World. *Br J Dermatol.* 2009;160(2):311–318.
88. Posada-Vergara MP, Lindoso JA, Tolezano JE, Pereira-Chioccola VL, Silva MV, Goto H. Tegumentary leishmaniasis as a manifestation of immune reconstitution inflammatory syndrome in 2 patients with AIDS. *J Infect Dis.* 2005;192(10):1819–1822.
89. Chrusciak-Talhari A, Ribeiro-Rodrigues R, Talhari C, et al. Case report: tegumentary leishmaniasis as the cause of immune reconstitution inflammatory syndrome in a patient co-infected with human immunodeficiency virus and Leishmania guyanensis. *Am J Trop Med Hyg.* 2009;81(4):559–564.
90. Gois L, Badaró R, Schooley R, Grassi MFR. Immune response to leishmania antigens in an AIDS patient with mucocutaneous leishmaniasis as a manifestation of immune reconstitution inflammatory syndrome (IRIS): a case report. *BMC Infect Dis.* 2015;15(1):1–7.
91. Sassi A, Louzir H, Ben Salah A, Mokni M, Ben Osman A, Dellagi K. Leishmanin skin test lymphoproliferative responses and cytokine production after symptomatic or asymptomatic leishmania major infection in Tunisia. *Clin Exp Immunol.* 1999;116(1):127–132.
92. Reed SG. Diagnosis of leishmaniasis. *Clin Dermatol.* 1996;14(5):471–478.
93. Guimarães MC, Celeste BJ, Franco EL. Diagnostic performance indices for immunofluorescent tests and enzyme immunoassays of leishmaniasis sera from northern and north-eastern Brazil. *Bull World Health Organ.* 1990;68(1):39–43.
94. Kar K. Serodiagnosis of leishmaniasis. *Crit Rev Microbiol.* 1995;21(2):123–152.
95. Barroso-Freitas AP, Passos SR, Mouta-Confort E, et al. Accuracy of an ELISA and indirect immunofluorescence for the laboratory diagnosis of American tegumentary leishmaniasis. *Trans R Soc Trop Med Hyg.* 2009;103(4):383–389.
96. R.L. Leishmania e leishmaniose, com particular referência à região Amazônica do Brasil. *Rev Para Med.* 1997;11:29–40.
97. Uliana SR, Nelson K, Beverley SM, Camargo EP, Floeter-Winter LM. Discrimination amongst Leishmania by polymerase chain reaction and hybridization with small subunit ribosomal DNA derived oligonucleotides. *J Eukaryot Microbiol.* 1994;41(4):324–330.
98. Katakura K, Kawazu SI, Sanjyoba C, et al. Leishmania mini-exon genes for molecular epidemiology of leishmaniasis in China and Ecuador. *Tokai J Exp Clin Med.* 1998;23(6):393–399.
99. Russell R, Pilar Iribar M, Lambson B, et al. Corrigendum to “Intra and inter-specific microsatellite variation in the Leishmania subgenus Viannia”. *Mol Biochem Parasitol.* 2000;107(2):331–331. [*Mol Biochem Parasitol.* 1999;103:71–77].
100. Graça GC, Volpini AC, Romero GA, et al. Development and validation of PCR-based assays for diagnosis of American cutaneous leishmaniasis and identification of the parasite species. *Mem Inst Oswaldo Cruz.* 2012;107:664–674.
101. Roelfsema JH, Nozari N, Herremans T, Kortbeek LM, Pinelli E. Evaluation and improvement of two PCR targets in molecular typing of clinical samples of Leishmania patients. *Exp Parasitol.* 2011;127(1):36–41.
102. Hammami-Ghorbel H, Ben Abda I, Badri T, et al. Mucosal leishmaniasis of the lip: an emerging clinical form in Tunisia. *J Eur Acad Dermatol Venereol.* 2015;29(6):1212–1215.
103. Montalvo AM, Fraga J, El Safi S, et al. Direct Leishmania species typing in old world clinical samples: evaluation of 3 sensitive methods based on the heat-shock protein 70 gene. *Diagn Microbiol Infect Dis.* 2014;80(1):35–39.
104. Lima IP, Müller MC, Holanda TA, Harhay M, Costa CHN, Costa DL. Human immunodeficiency virus/leishmania infantum in the first foci of urban American visceral leishmaniasis: clinical presentation from 1994 to 2010. *Rev Soc Bras Med Trop.* 2013;46(2):156–160.
105. Cota GF, de Sousa MR, de Freitas Nogueira BM, et al. Comparison of parasitological, serological, and molecular tests for visceral leishmaniasis in HIV-infected patients: a cross-sectional delayed-type study. *Am J Trop Med Hyg.* 2013;89(3):570–577.
106. Srivastava P, Dayama A, Mehrotra S, Sundar S. NIH public access. *Trans R Soc Trop Med Hyg.* 2011;105(1):1–6.
107. Chappuis F, Rijal S, Singh R, et al. Prospective evaluation and comparison of the direct agglutination test and an rK39-antigen-based dipstick test for the diagnosis of suspected kala-azar in Nepal. *Trop Med Int Heal.* 2003;8(3):277–285.
108. Maia Z, Lirio M, Mistro S, Mendes CMC, Mehta SR, Badaro R. Comparative study of rK39 Leishmania antigen for serodiagnosis of visceral leishmaniasis: systematic review with meta-analysis. *PLoS Negl Trop Dis.* 2012;6(1):e1484.
109. Cota GF, de Sousa MR, Demarqui FN, Rabello A. The diagnostic accuracy of serologic and molecular methods for detecting visceral leishmaniasis in HIV infected patients: meta-analysis. *PLoS Negl Trop Dis.* 2012;6(5):e1665.
110. Molina I, Fisa R, Riera C, et al. Ultrasensitive real-time pcr for the clinical management of visceral leishmaniasis in HIV-infected patients. *Am J Trop Med Hyg.* 2013;89(1):105–110.
111. Singh D, Pandey K, Das VNR, et al. Novel noninvasive method for diagnosis of visceral leishmaniasis by rK39 testing of sputum samples. *J Clin Microbiol.* 2009;47(8):2684–2685.
112. Das S, Halder A, Rabidas VN, Mandal A, Das P. Specific noninvasive detection of leishmania donovani in desquamated buccal cell swab samples from human visceral leishmaniasis-HIV coinfecting patients. *J Clin Microbiol.* 2014;52(4):1238–1241.