

Performance of a rapid diagnostic test for the detection of visceral leishmaniasis in a large urban setting

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ABSTRACT

Introduction: Rapid diagnostic tests (RDTs) may improve the early detection of visceral leishmaniasis (VL), but their real-world performance requires additional study. Therefore, we evaluated the performance of an rK39-based RDT (Kalazar Detect™) for the detection of VL in an endemic, large urban area. **Methods:** Data were collected from a registry of rK39 RDT performed at 11 emergency care units in Belo Horizonte, Brazil, and from a national database of reportable communicable diseases of the *Sistema de Informação de Agravos de Notificação* (SINAN). **Results:** The rapid rK39 test was performed in 476 patients, with 114 (23.9%) positive results. The analysis of rK39 RDT performance was based on 381 (80%) cases reported to the SINAN database, of which 145 (38.1%) were confirmed cases. Estimates for sensitivity and specificity were 72.4% (95% CI: 64.6-79%) and 99.6% (95%CI: 97.6-99.9%), respectively. Positive and negative predictive values were estimated at 99.1% (95%CI: 94.9-99.8%) and 85.5% (95%CI: 80.8-89.1%), respectively. In addition, close agreement between the rK39 RDT and indirect immunofluorescence was observed. **Conclusions:** In summary, the rK39 RDT showed a high specificity but only moderate sensitivity. In endemic areas for VL, treatment may be considered in cases with clinical manifestations and a positive rK39 RDT, but those with a negative test should be subjected to further investigation.

Keywords: Visceral leishmaniasis. Serologic test. Sensitivity and specificity.

INTRODUCTION

Visceral leishmaniasis (VL) has a wide global distribution, with 200,000 to 400,000 estimated cases per year¹, and is the second largest cause of death among parasitic diseases worldwide². Approximately 90% of cases occur in six countries, including India, Bangladesh, Sudan, South Sudan, Brazil and Ethiopia, with 20,000 to 40,000 estimated deaths per year¹. In Brazil, VL is caused by *Leishmania (Leishmania) infantum* and has been geographically expanding in the past decade³. The mean annual incidence for the period 2001-2010 was 1.8/100,000 people, with a case fatality rate of 6.8%⁴. Belo Horizonte, a city of 2.4 million inhabitants located in the southeastern part of the country, reported a mean incidence of 5.2 cases/100,000 people during the same time period, with a case fatality rate of 13.4%⁵.

Early diagnosis and treatment of VL have been recommended by the Brazilian National VL Surveillance and Control Program to reduce mortality⁶. According to the Brazilian Ministry of Health, a diagnosis of VL requires the identification of the parasite in a smear or culture and/or positive serological testing in patients presenting with fever and spleen enlargement⁷. The

most widely available serological test for VL diagnosis in Brazil is indirect immunofluorescence (IFI)⁶; this diagnostic method is typically performed in referral laboratories around the country, and the results become available to the attending physician within a couple of days. As a delay in VL diagnosis may be associated with an increase in case fatality rates⁸, the use of rapid diagnostic tests (RDTs) can help reduce mortality.

The accuracy of immunochromatographic rapid tests based on the rK39 protein of *Leishmania infantum* has been assessed in studies from different regions^{2,9}. The sensitivity of the rK39 RDT has been reported to vary between 37 and 100%, and the specificity was estimated between 59 and 100% among immunocompetent patients in endemic regions⁹⁻¹⁶. In addition, a meta-analysis of 13 studies reported an overall sensitivity and specificity of 93.9% and 90.6%, respectively¹⁵. In Brazil, estimates of the sensitivity and specificity of RDTs vary between 85.7 and 100% and 82.0 and 100%, respectively¹⁷⁻²¹, but few studies have been conducted in real-world scenarios. For example, among immunocompromised patients, the sensitivity of RDTs seems to be lower, with one study reporting an estimation of 77.3%¹⁶. Moreover, in a previous Brazilian study, the performance of the rK39 RDT was inferior to the use of a parasitological method, real-time PCR in peripheral blood and a prototype kit for a direct agglutination test (DAT) among human immunodeficiency virus (HIV)-positive patients²².

In Belo Horizonte, the rK39 RDT was implemented at emergency care units in 2010 to foster the early treatment of suspected VL cases with a positive test, according to World Health Organization (WHO) recommendations²³. The objective of the present study was to assess rK39 RDT performance in widespread use in a large urban area with a high incidence of VL.

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METHODS

An RDT for VL was implemented in Belo Horizonte in May 2010 at 11 public emergency care units. This RDT provided by the Ministry of Health was marketed as Kalazar Detect™ (InBios International), an immunochromatographic test that provides the qualitative detection of antibodies against the recombinant K39 (rK39) protein of *Leishmania*.

The use of the rK39 RDT was recommended in Belo Horizonte for the following situations: patients of any age with I) fever of any duration with spleen enlargement or II) fever lasting 14 or more days associated with any cytopenia (anemia, leukopenia or low platelet counts). Serum samples were obtained immediately after consultation, and a trained health professional performed the RDT at the point-of-care. A sample of serum was sent to *Fundação Ezequiel Dias* (FUNED), a national referral laboratory, which blindly performed indirect immunofluorescence (IFI) assays for VL; this method is considered the standard serological test in Brazil. According to the recommendations from the Ministry of Health, the reporting of all suspected VL cases to a public health authority is mandatory, and data are recorded at a national database of reportable communicable diseases (SINAN). This study included all patients who received the rK39 RDT at one of the 11 public emergency care units of Belo Horizonte from May 2010 to July 2011 and had SINAN data available. Clinical and demographic information was obtained from the SINAN database. The RDT results were obtained from spreadsheets that were regularly filled out by the laboratory manager of each of the healthcare units, and the IFI results were obtained directly from the FUNED lab. Indirect immunofluorescence results were considered as reactive when the titers were equal or greater than 1:80.

The following Ministry of Health definition of a confirmed VL case was used to assess RDT performance: individuals from an area of occurrence of VL with fever and spleen enlargement I) in whom the parasite is identified in direct examination or culture and/or II) demonstrate a positive IFI reaction (titer equal or greater than 1:80) or, although not showing laboratory confirmation, III) demonstrate a favorable treatment response. Notably, all confirmed cases were followed by the city health department's epidemiological bureau to assess patient's outcomes, particularly their response to treatment.

To assess the performance of the RDT among HIV patients, information on HIV serology was obtained from the SINAN database.

Statistical analyses were conducted using OpenEpi (Open Source Epidemiologic Statistics for Public Health, Version 2.3.1).

Ethical considerations

The study was approved by the ethics committee at the Belo Horizonte Health Department and was performed in accordance with the ethical standards of the Declaration of Helsinki.

RESULTS

The rapid diagnostic test for VL was performed in 476 patients throughout the study period. Among these patients, 381 (80%) had SINAN data available and were included in the study. There was a predominance of male patients (68.8%), with a mean age of 28 years (7-88 years) (**Table 1**). According to the Ministry of Health case definition, 145 (38.1%) patients had confirmed VL, with 28 (19.3%) cases meeting the parasitological criteria, 112 (77.2%) meeting the serological (IFI) criteria

TABLE 1 - Demographic and clinical characteristics of 381 patients that performed rK39 rapid diagnostic test for diagnosis of visceral leishmaniasis.

Characteristics	Number	Percentage
Male (%)	262	68.8
Age group (years)		
< 1	32	8.4
1-9	100	26.25
10-19	33	8.66
20-49	133	34.91
50-79	74	19.42
≥ 80	9	2.36
Criteria for confirmation of visceral leishmaniasis* (n=145)		
parasitological	28	19.3
serological (IFI)	112	77.0
response to therapy	5	3.4

*Confirmation criteria was ranked in the following order: parasitological, serological and response to therapy. IFI: indirect immunofluorescence for detection of *Leishmania* antibodies; rK39: recombinant K39.

and 5 (3.4%) demonstrating a favorable treatment response without positive results in parasitological or serological tests.

Among the 145 confirmed VL cases, 105 had a positive RDT, and among the 236 non-confirmed cases, 235 had had a negative RDT. The sensitivity and specificity of rK39 RDT were estimated at 72.4% (95%CI: 64.6-79%) and 99.6% (95%CI: 97.6-99.9%), respectively (**Table 2**). Estimates for the positive (PPV) and negative (NPV) predictive values were 99.1% (95%CI: 94.8-99.8%) and 85.5% (95%CI: 80.8-89.1%), respectively. Because information on the final diagnosis was not available for 95 of the 476 patients who received the RDT, we conducted a sensitivity analysis including all patients for whom we had RDT results available. For this analysis, we considered two hypothetical extreme situations to address the missing data for a final diagnosis: I) all 95 patients with missing data had visceral leishmaniasis or II) all of these patients did not have VL. Considering the first scenario, the estimates for sensitivity, specificity, PPV and NPV were 47.1% (95%CI: 40.9-50.4%), 99.6% (95%CI: 97.6-99.9%), 99.1% (95%CI: 95.2-99.8%) and 64.9% (95%CI: 59.9-69.6%), respectively. For the latter situation, the estimates for the same parameters were 72.4% (95%CI: 64.3-79%), 97.3% (95%CI: 94.9-98.6%), 92.1% (95%CI: 85.7-95.8%) and 88.9% (95%CI: 85.3-91.8%), respectively.

Among the 28 patients with amastigotes detected in bone marrow aspirates, the rK39 RDT was positive in 17 (60.7%) cases. Of note, 14 (50%) of these 28 patients were HIV-positive.

Information on HIV co-infection was available for 224 of the 381 included patients, and 93 of these patients were found to be HIV-positive. Among HIV-infected patients, the sensitivity and specificity of the RDT were estimated at 60% (95%CI: 40.7-76.6%) and 100% (95%CI: 94.7-100%), respectively. The positive and negative predictive values for this subset of patients were estimated at 100% (95% CI: 79.6-100%) and 87.2% (95% CI: 77.9-92.9%), respectively.

Information on the results of indirect immunofluorescence was available for 296 of the 381 included patients. A reactive IFI result was observed in 63 (86.3%) of the 73 samples that had previously been shown to be positive in the rK39 RDT, and 200 (89.6%) of the 223 samples from patients with a negative result on the RDT were also negative for IFI (**Table 3**).

TABLE 2 - Evaluation of rK39 rapid diagnostic test performance for diagnosis of visceral leishmaniasis.

RDT	Cases		Total
	confirmed ^a	not confirmed	
Positive	105	1	106
Negative	40	235	275
Total	145	236	381

RDT: rapid diagnostic test; ^aconfirmed according to Brazilian Ministry of Health criteria; rK39: recombinant K39.

TABLE 3 - Analysis of agreement between rK39 rapid diagnostic test and indirect immunofluorescence for the diagnosis of visceral leishmaniasis.

RDT	Indirect immunofluorescence		
	≥ 1:80	< 1:80	total
Positive	63	10	73
Negative	23	200	223
Total	86	210	296

RDT: rapid diagnostic test. Kappa 0.71 (CI95% 0.60-0.83). rK39: recombinant K39; CI: confidence interval.

DISCUSSION

Rapid diagnostic tests (RDTs) based on rK39 showed good accuracy for VL diagnosis when widely implemented in a large urban area. Moreover, the high PPV estimates support the World Health Organization²³ and the Brazilian Ministry of Health⁷ recommendations for the use of RDTs to confirm a VL diagnosis in suspected cases. However, due to the moderate estimates for NPV, our findings suggest that patients with negative RDT results should undergo further investigation with additional serological tests and examination of bone marrow aspirates. Polymerase chain reaction for the detection of *Leishmania* deoxyribonucleic acid (DNA) in bone marrow or peripheral blood specimens might also be useful to confirm or rule out VL diagnosis in challenging situations²³⁻²⁶.

An evaluation of rK39 RDT performance revealed better results for studies conducted in the Indian subcontinent as compared to those from East Africa or Brazil⁹. Differences in study design, the species of *Leishmania* responsible for VL cases and antibody concentrations might explain this variation in RDT performance. An initial evaluation of the performance of the rK39 RDT in India estimated its sensitivity and specificity at 100% and 98%, respectively¹¹. Of note, this previous study considered confirmed VL cases to be only those with parasitological confirmation. A controlled study subsequently conducted in India by the same research group, which sought to validate another type of rK39 RDT (One Step InsureTM), reported a sensitivity of 100% and a specificity of 93% using the same case criteria as their previous study²⁷.

In Uganda, Kalazar DetectTM was used for the diagnosis of VL among 250 inpatients admitted with a clinical presentation compatible with VL²⁸, and this RDT demonstrated good accuracy, with a sensitivity and specificity estimated at 82% and 99%, respectively. It is interesting to note that this Uganda study used confirmation criteria that included the detection of the parasite in a spleen aspirate smear, a positive direct agglutination test (i.e., titers greater than 1:12,800) or a good treatment response.

In a study conducted in Sudan using a different rK39 RDT (IT-LEISHTM) but also including a serological test (direct agglutination test) among the confirmation criteria for VL cases,

similar results were found for sensitivity, specificity, PPV and NPV (81%, 97%, 98% and 71%, respectively)¹⁰.

In Brazil, validation studies of the IT-LEISHTM have reported a sensitivity varying from 93% to 100% and a specificity between 94% and 96%^{18,20}, considering confirmed cases only as those with a parasitological diagnosis. In addition, the PPV and NPV of 98% and 89%, respectively, were similar to those found in our study. Evaluations of the performance of Kalazar DetectTM have shown sensitivity values between 85.7% and 90% and a specificity between 82% and 100%^{18,19}.

In a multicentric study, four commercially available RDTs were compared, including Kalazar DetectTM. Both at Brazilian and African centers, Kalazar DetectTM presented a lower sensitivity when compared to IT-LEISHTM, which must be taken into account when using these tests in clinical practice⁹.

Our study also found that the sensitivity of the RDT seemed to be lower among HIV-infected patients compared to the general population. However, we had no information regarding the cluster of differentiation 4 (CD4)-T cell counts or HIV staging of these patients with which to establish the impact of immunosuppression on test performance. In addition, we were unable to find any studies in the literature designed specifically to address rK39 RDT accuracy among HIV-infected patients. A study in Ethiopia, a country with a high HIV prevalence, reported an RDT sensitivity of 77% for HIV-positive patients, which was lower than that observed for HIV-negative patients (87%), but this difference was not statistically significant¹⁵. In addition, a recent meta-analysis of diagnostics tests for VL in HIV-infected patients did not include the rK39 RDT due to the lack of previously published studies²⁹.

Finally, we found a close agreement between the RDT and the frequently used IFI test. A similar finding was observed in a study comparing an RDT with the direct agglutination test (DAT)³⁰. Nonetheless, a Brazilian study showed that IT-LEISHTM, but not Kalazar DetectTM, performed significantly better than IFI with greater specificity and PPV²¹. This finding suggests that RDTs may be an effective substitute for IFI in most clinical situations, considering their rapid results and ease of use.

One limitation of our study involves the criteria used to confirm VL cases. We chose to use the Brazilian Ministry of Health criteria and therefore considered confirmed cases as not only those with parasitological diagnosis but also those with a reactive IFI and/or good treatment response. In our view, the use of the Ministry of Health criteria better reflects the real-world scenario of clinical practice. In addition, because we did not have information on the final diagnosis of the non-confirmed cases, interpretations of the estimates regarding specificity should consider this limitation. Finally, the clinical and demographic data were obtained from the SINAN database, and although reporting is mandatory, the attending physicians do not always report suspected cases. As a result, we were unable to obtain these data for approximately 20% of the cases. To address this gap, we performed a sensitivity analysis considering two extreme situations, and these results indicated that this lack of data did not change our main finding of the low sensitivity and good specificity of the rK39 RDT.

In summary, the rK39 RDT showed good accuracy for VL diagnosis and close agreement with IFI and may therefore be useful for the timely identification of cases in point-of-care treatment. However, this test is not sufficient to rule out VL, and further testing is warranted in suspected cases with negative RDT results.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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