Performance of five serological tests in the diagnosis of visceral and cryptic leishmaniasis: a comparative study

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Abstract

Introduction: Leishmaniasis is a major health problem and its diagnosis still represents a challenge. Since consistent evidence on the comparison of serological methods is lacking, our work aims to compare five serological tests for the diagnosis of visceral and asymptomatic leishmaniasis in southern France, a region where leishmaniasis is endemic.

Methodology: Serum samples from 75 patients living in Nice, France were retrospectively analyzed. They included patients affected by visceral leishmaniasis (VL; n = 25), asymptomatic carriers (AC; n = 25) and negative controls (n = 25). Each sample was tested using two immunochromatographic tests (ICT; IT LEISH® and TruQuick IgG/IgM®), an indirect fluorescent antibody test (IFAT) and two Western Blotting (WB; LDBio BIORAD® and an in-house method).

Results: Diagnosis of VL with IFAT and TruQuick® showed the highest diagnostic performance parameters. IFAT had 100% sensitivity and specificity, while TruQuick had 96% sensitivity and 100% specificity. Finally, the two tests showed high accuracy (100% for IFAT and 98% for TruQuick) for the AC group. WB LDBio® was the only method able to detect Leishmania latent infection, with a sensitivity of 92%, and a specificity of 100%, with a Negative Predictive Value (NPV) of 93%. This performance is reflected in the high accuracy of the test.

Conclusions: The data obtained with TruQuick® supports its application in the rapid diagnosis of leishmaniasis in endemic areas, a feature not shown by IFAT despite its high diagnostic performance. Regarding the diagnosis of asymptomatic leishmaniasis, the best results were obtained with WB LDBio®, confirming previous studies.

Key words: leishmaniasis; asymptomatic carriers; diagnosis; serology; immunochromatography; Western blot.


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Introduction

Leishmaniases are a group of diseases caused by the protozoan parasite Leishmania. There are three main types of leishmaniasis: i) visceral, often known as kala-azar and the most serious form of the disease (VL); ii) cutaneous, the most common clinical form (CL); and iii) mucocutaneous [1].

Leishmaniasis is a poverty-related disease which continues to be a major health problem in 3 eco-epidemiological regions of the world: the Americas, East Africa and North Africa, and West and South-East Asia [2].

As of September 2021, 55 VL-endemic countries (70%) and 56 CL-endemic countries (63%) reported data to the World Health Organization (WHO) Global Leishmaniasis program for 2020. About 87% of global VL cases were reported from eight countries: Brazil, Eritrea, Ethiopia, India, Kenya, Somalia, South Sudan and Sudan. In 2020, 7 countries reported more than 5000 CL cases: Afghanistan, Algeria, Brazil, Colombia, Iraq, Pakistan and the Syrian Arab Republic, which together account for 80% of global reported CL incidence. In 2020, there were 880 imported cases of CL and 99 imported cases of VL reported globally [1]. CL imported cases were mainly from Brazil, France and Lebanon while VL imported cases were mainly from Uganda [1].
In Europe, nine countries report cases of VL annually accounting for less than 2% of the global burden [3]. These cases are mostly confined to the Mediterranean countries, but a spread towards northern Europe is being reported as a result of many factors, related to vector, parasite migration and climate changes [4].

Leishmaniasis mainly affects poor people and is associated with malnutrition, population displacement, poor housing, weak immune system and lack of resources [5]. In addition to social, health and economic aspects, environmental variables have recently been highlighted as further risk factors [6]. VL is the most severe form of leishmaniasis and is potentially fatal in the absence of diagnosis and treatment. Mortality is very high (90%) in untreated cases [7]. In general, rapid diagnosis and early treatment reduce the risk of mortality. On the other hand, asymptomatic infection is common in endemic areas and affects apparently healthy individuals infected by Leishmania [8,9]. Diagnosis of leishmaniasis is established upon the identification of the parasite by microscopic examination, culture or polymerase chain reaction (PCR) on tissue samples. In addition, detection of specific anti-leishmania antibodies can aid diagnosis.

Several serological tests are currently available and broadly used: the immuno-chromatographic tests (ICT) based on the recombinant leishmania antigen, indirect fluorescent antibody test (IFAT), enzyme-linked immunosorbent assay (ELISA), western blot (WB), and direct agglutination test [10,11,12]. Unfortunately, there is fragmentary information on their efficacy in terms of sensitivity and specificity. In general, the antibody detection techniques have a high sensitivity for acute visceral disease but are not strictly specific for this disease stage. Antibodies wane slowly after treatment and can be detected in a large number of asymptomatically infected individuals [13]. Moreover, serology exhibits variable accuracy in diagnosis of VL depending on antigens and immune status of the human host. The human immunodeficiency virus (HIV) associated with Leishmania infection represents a further challenge due to minimal levels of antibody in this subgroup [14].

Serological diagnosis by ICT has been validated in regions with high VL burden, such as East Africa [15], the Indian subcontinent [16] and Brazil [17]. To date, only few large-sample size studies have carried out an evaluation of these techniques in the Mediterranean Basin area [18,19,20].

The aim of this study was to evaluate and compare the performance of five serological tests used in the diagnosis of autochthonous VL in southern France (Nice), a VL endemic region located in Southern Europe, by performing a retrospective analysis of sera collected from VL patients, asymptomatic carriers and negative controls.

**Methodology**

**Study design**

This study was designed as a retrospective comparative analysis. In November 2019, seventy-five serum samples from patients suffering from VL, asymptomatic *L. infantum* infected individuals and healthy controls were retrospectively collected at the Parasitology and Mycology Department of Nice University Hospital, France. The criteria for the definition of “VL case” were based on parasitological confirmation of *Leishmania* infection in bone marrow aspirate or positivity to molecular methods [real time PCR for the detection of kinetoplast DNA (kDNA-based qPCR Assay)], while asymptomatic *L. infantum* infection was detected only with kDNA qPCR, performed in whole blood in subjects without any symptoms of infection as previously described [21]. Negative samples came from immunocompetent patients living in the south of France who had no typical symptoms or history of VL and were previously negative for *Leishmania* kDNA qPCR Assay. Immunocompromised patients were formally excluded from the study because serology is not an appropriate diagnostic method in this subgroup [22]. The serum samples used in this study were anonymous and part of a registered collection. Thus, no formal ethical approval was deemed necessary; nonetheless a technical permission for this study has been given by our local Ethics Committee. Diagnostic tests were performed in blinded conditions. This work was carried out in accordance with the relevant guidelines and regulations and does not provide any information that may allow the identification of the enrolled patients.

**Serological tests**

For screening by ICT, samples were processed manually using the IT LEISH® (BIO-RAD, Hercules, USA) and TruQuick LEISH IgG/IgM® (Meridian Bioscience, Cincinnati, USA) tests according to the manufacturers’ instructions. For western blotting (WB), the LDBio® Leishmania IgG kit (LDBIO Diagnostics, Lyon, France) was used with the Autoblot 3000 (MedTEC Biolab, Durham, USA) apparatus according to the manufacturer’s instructions. Specific bands at 14 kDa or 16 kDa demonstrated the presence of anti-
Leishmania antibodies in the serum sample. An in-house WB (Lei.WB) and immunofluorescence antibody test (IFAT) were also included for comparison.

The same *L. infantum* promastigote preparation (zymodeme MON-1) was used for both IFAT and WB analyses. For IFAT, antibodies were revealed using fluorescein-conjugated goat anti-human IgG (Biomérieux, Marcy l’Etoile, France) with a cut-off point of 1/80. WB analysis was performed as previously described [23] and considered positive when the presence of antibodies, directed against 14 and/or 16 kDa *L. infantum* antigens, was detected [23,24]. The characteristics of each test are showed in Table 1. All samples included in the study were explored with each test.

**Results**

**Characteristics of the population**

We screened serum samples from patients living in Nice, France. Among the VL population (n = 25), the female to male sex ratio was 2:3, and age ranged from 18 to 93 years with a median of 43 years. Any demographic characteristic relating to gender and age could not be collected for patients belonging to the group of asymptomatic carriers (n = 25) and healthy controls (n = 25).

All patients in the three groups were immunocompetent. HIV patients, those undergoing transplantation, or taking immunosuppressive drugs were formally excluded.

**Diagnostic performance of different Leishmania spp. tests to detect VL**

We first examined the diagnostic performance of the different *Leishmania* spp. tests for VL diagnosis in terms of sensitivity, specificity, negative predictive value (NPV), positive predictive value (PPV), and accuracy. The results are summarized in Table 2. The IFAT and ICT test TruQuick Meridian® showed the highest diagnostic performance parameters. In particular, IFAT exhibited 100% sensitivity and specificity, while TruQuick reached 96% sensitivity and 100% specificity. Finally, the two tests demonstrated high accuracy (100% for IFAT and 98% for TruQuick®). Despite the high specificity found for IT LEISH®, Lei.WB, and WB LDBio®, their sensitivity was lower than 80% which was responsible for poor NPV with an accuracy below 90% (Table 2).

**Diagnostic performance of *Leishmania* spp. tests to detect asymptomatic carriers**

As a next step, the ability of the proposed *Leishmania* spp. tests was also evaluated in

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**Table 1. Characteristics of commercial kits and in house techniques for serological diagnosis of human visceral leishmaniasis.**

<table>
<thead>
<tr>
<th>Kit</th>
<th>Manufacturer</th>
<th>Method</th>
<th>Threshold</th>
<th>Sample type</th>
<th>Time</th>
<th>Antigen</th>
</tr>
</thead>
<tbody>
<tr>
<td>IT LEISH®</td>
<td>BIO-RAD Laboratories</td>
<td>ICT</td>
<td>Positive:</td>
<td>Serum/Blood</td>
<td>25 min</td>
<td>Recombinant k39 antigen</td>
</tr>
<tr>
<td>TruQuick® IgM/IgG</td>
<td>Meridian Bioscience</td>
<td>ICT</td>
<td>Positive:</td>
<td>Serum/Blood</td>
<td>15 min</td>
<td>Recombinant <em>L. donovani</em> antigen</td>
</tr>
<tr>
<td><em>Leishmania</em> Western Blot IgG®</td>
<td>LDBIO Diagnostic</td>
<td>WB</td>
<td>Positive: 14-KD and or 16-KD band:</td>
<td>Serum</td>
<td>3.5 h</td>
<td>Antigen from <em>L. infantum</em> promastigotes</td>
</tr>
<tr>
<td><em>Leishmania</em> Western Blot IgG</td>
<td>In house</td>
<td>WB</td>
<td>Positive: 14-KD and or 16-KD band:</td>
<td>Serum</td>
<td>3.5 h</td>
<td>Antigen from <em>L. infantum</em> promastigotes</td>
</tr>
<tr>
<td><em>Leishmania</em> immunofluorescence antibody test</td>
<td>In house</td>
<td>IFAT</td>
<td>Positive: cut-off point 1/80:</td>
<td>Serum</td>
<td>4 h</td>
<td>Antigen from <em>L. infantum</em> promastigotes</td>
</tr>
</tbody>
</table>

Min: minutes; h: hours; ICT: Immunochromatography; WB: Western Blot; IFAT: indirect fluorescent antibody test. Table adapted from: Lévêque et al. [18]
asymptomatic subjects. *Leishmania* spp. infection was confirmed in all asymptomatic subjects by qPCR. In addition, the cohort of negative subjects, served as controls. The diagnostic performance of the five tests in this population is summarized in Table 3. IT LEISH®, IFAT, Lei.WB and TruQuick® showed diagnostic performance with sensitivity equal to or lower than 20%. WB LDBio® test was the only test able to correctly detect *Leishmania* latent infection, with sensitivity of 92%, specificity of 100%, and NPV of 93%. This performance is reflected by the high accuracy of the test (Table 3, value [95% confidence interval]: 96.1 [86.5-99.5]) and also by comparing the ROC curves (Figure 1), where WB LDBio® showed a significantly higher AUC (Table 4) than all the other diagnostic tests (z-test, p < 0.001 for all comparisons).

**Discussion**

The gold standard for the diagnosis of VL is still the demonstration of the parasite by direct methods (e.g., culture, microscopy, PCR), although the need for invasive procedures and highly specialized personnel and equipment limit their use. On the other hand, indirect diagnostic methods, based on serology, are still widely applied due to their accessibility and relatively low costs. Several serological methods are available for VL diagnosis (IFAT, ELISA, ICT, WB, direct agglutination test, and latex agglutination test) [25]. However, comparative studies on these methods have yielded variable results.

**Table 2.** Diagnostic performance of the proposed tests for *Leishmania* spp., evaluated in a population of symptomatic positive subjects (n = 25) and negative controls (n = 25).

<table>
<thead>
<tr>
<th>Test</th>
<th>Sensitivity % (95% CI)</th>
<th>Specificity % (95% CI)</th>
<th>PPV % (95% CI)</th>
<th>NPV % (95% CI)</th>
<th>NLR (95% CI)</th>
<th>Accuracy % (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFAT</td>
<td>100.0 (86.3-100.0)</td>
<td>100.0 (86.8-100.0)</td>
<td>100.0</td>
<td>100.0</td>
<td>0.0</td>
<td>100.0 (93.0-100.0)</td>
</tr>
<tr>
<td>Lei.WB</td>
<td>40.0 (21.1-61.3)</td>
<td>100.0 (86.8-100.0)</td>
<td>100.0</td>
<td>63.4 (55.7-70.7)</td>
<td>0.60 (0.44-0.76)</td>
<td>76.5 (56.2-82.5)</td>
</tr>
<tr>
<td>WB LDBio®</td>
<td>64.0 (42.5-82.0)</td>
<td>100.0 (86.8-100.0)</td>
<td>100.0</td>
<td>74.3 (63.1-83.2)</td>
<td>0.36 (0.21-0.51)</td>
<td>82.4 (69.1-91.6)</td>
</tr>
<tr>
<td>IT LEISH®</td>
<td>76.0 (54.9-90.6)</td>
<td>100.0 (86.8-100.0)</td>
<td>100.0</td>
<td>81.3 (68.3-93.6)</td>
<td>0.24 (0.12-0.36)</td>
<td>88.2 (76.1-95.6)</td>
</tr>
<tr>
<td>TruQuick®</td>
<td>96.0 (79.7-99.9)</td>
<td>100.0 (86.8-100.0)</td>
<td>100.0</td>
<td>96.3 (79.2-100.0)</td>
<td>0.04 (0.01-0.07)</td>
<td>98.0 (89.6-99.9)</td>
</tr>
</tbody>
</table>

95% CI: 95% confidence interval; PPV: positive predictive value; NPV: negative predictive value; NLR: negative likelihood ratio; IFAT: indirect fluorescent antibody test; WB: Western Blot.

**Table 3.** Diagnostic performance of the proposed tests for *Leishmania* spp. in detecting infection in asymptomatic subjects (n = 25) and negative controls (n = 26).

<table>
<thead>
<tr>
<th>Test</th>
<th>Sensitivity % (95% CI)</th>
<th>Specificity % (95% CI)</th>
<th>PPV % (95% CI)</th>
<th>NPV % (95% CI)</th>
<th>NLR (95% CI)</th>
<th>Accuracy % (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFAT®</td>
<td>12.0 (2.6-31.2)</td>
<td>100.0 (86.8-100.0)</td>
<td>100.0</td>
<td>54.2 (30.6-69.6)</td>
<td>0.88 (0.76-0.99)</td>
<td>56.9 (42.3-70.7)</td>
</tr>
<tr>
<td>Lei.WB</td>
<td>16.0 (4.5-36.1)</td>
<td>100.0 (86.8-100.0)</td>
<td>100.0</td>
<td>55.3 (31.1-69.5)</td>
<td>0.84 (0.71-0.95)</td>
<td>58.8 (44.2-72.4)</td>
</tr>
<tr>
<td>WB</td>
<td>92.0 (73.9-99.9)</td>
<td>100.0 (86.8-100.0)</td>
<td>100.0</td>
<td>92.9 (77.5-99.5)</td>
<td>0.08 (0.02-0.14)</td>
<td>96.1 (86.5-99.5)</td>
</tr>
<tr>
<td>IT LEISH®</td>
<td>8.0 (0.9-26.0)</td>
<td>100.0 (86.8-100.0)</td>
<td>100.0</td>
<td>53.1 (30.2-75.1)</td>
<td>0.92 (0.82-0.99)</td>
<td>54.9 (40.3-68.87)</td>
</tr>
<tr>
<td>TruQuick®</td>
<td>20.0 (6.8-40.7)</td>
<td>100.0 (86.8-100.0)</td>
<td>100.0</td>
<td>56.5 (31.6-81.5)</td>
<td>0.8 (0.66-0.95)</td>
<td>60.8 (46.1-74.2)</td>
</tr>
</tbody>
</table>

95% CI: 95% confidence interval; PPV: positive predictive value; NPV: negative predictive value; NLR: negative likelihood ratio; IFAT: indirect fluorescent antibody test; WB: Western Blot.

**Serological methods and VL diagnosis**

In our study, the IFAT and the ICT test TruQuick Meridian® showed the highest diagnostic performance parameters for the diagnosis of VL caused by *Leishmania* spp. In particular, IFAT and TruQuick® showed a sensitivity of 100% and 96%, respectively, thus confirming that they can detect the actual disease.
in the tested population. The same was verified for specificity, which reached 100% for both tests confirming that they can correctly detect true negative subjects. Finally, the two tests proved highly accurate (accuracy: 100% for IFAT and 98% for TruQuick®), strengthening their ability to appropriately detect affected patients. With regard to IFAT, our results improve and expand what has been previously reported about IFAT indicating a sensitivity and specificity for VL diagnosis ranging from 78.8 to 100% in immunocompetent subjects [17,26]. However, data reported in literature are more heterogeneous in terms of ICT tests. These assays have been more frequently used in the diagnosis of anthroponotic rather than zoonotic leishmaniasis [27]. Furthermore, data on their diagnostic performance are highly variable depending on the geographical area, with 92-100% sensitivity in the Indian sub-continent, and dropping to 32-96% in East Africa and Latin America [28]. Several studies attest the 100% specificity of ICT for diagnosis of VL in immunocompetent subjects, a finding in agreement with our study [27].

However, the two ICT tests included in this study differ in sensitivity, 76% and 96% for IT LEISH® and TruQuick®, respectively. The lower sensitivity of IT LEISH® compared to TruQuick® was also demonstrated in another comparative study in a larger cohort living in the Mediterranean area, where the sensitivities of IT LEISH® and TruQuick® were 85% and 90%, respectively [18]. Furthermore, these data seem to subvert what is stated in the manufacturer’s instructions of the two tests, according to which IT LEISH® has a sensitivity of 99% and a specificity of 100% while TruQuick® has a sensitivity of 92.9% and a specificity of 98%. As disclosed for IT LEISH®, the results for the two WB assays (Lei.WB, and WB LDBio®) seem weak in providing VL diagnosis. In fact, despite a high specificity, their sensitivity is lower than 80% (Table 2), thus hampering their real diagnostic power. These results are quite surprising, since our data contrast with other studies which highlight the excellent results of this technique in the diagnosis of *L. infantum*-related VL with a reported sensitivity of 90-100% and specificity of 98-100% in immunocompetent patients [23,30,31]. Nevertheless, differences in antigen preparation protocols as well as the choice of reference strain and specific bands make the comparison of different studies difficult. Furthermore, the number of patients included in studies to evaluate these techniques was often too small (< 30 patients) to obtain reliable statistical data [27].

### Table 4. The area under the Receiver Operating Characteristic (ROC) curve for the examined tests.

<table>
<thead>
<tr>
<th>Test</th>
<th>AUC (95% CI)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFAT</td>
<td>0.56 (0.49-0.63)</td>
<td>0.070</td>
</tr>
<tr>
<td>Lei.WB</td>
<td>0.58 (0.51-0.65)</td>
<td>0.033</td>
</tr>
<tr>
<td>WB LDBio®</td>
<td>0.96 (0.91-1.01)</td>
<td>0.0001</td>
</tr>
<tr>
<td>IT LEISH®</td>
<td>0.54 (0.49-0.59)</td>
<td>0.149</td>
</tr>
<tr>
<td>TruQuick®</td>
<td>0.60 (0.52-0.68)</td>
<td>0.014</td>
</tr>
</tbody>
</table>

95% CI: 95% confidence interval; a * indicates a difference of the AUC value vs. WB LDBio®. IFAT: indirect fluorescent antibody test; WB: Western Blot.

### Serological methods and cryptic Leishmania infection

With regard to the diagnostic performance of *Leishmania* serological tests to detect asymptomatic carriers, the results obtained in our study are not surprising. Both ICT tests (IT LEISH® and TruQuick®), IFAT and Lei.WB disclosed a poor diagnostic performance, with sensitivity ≤ 20%, highlighting their inability to detect a latent infection of *Leishmania* spp. On the contrary, WB LDBio® was the only technique able to identify *Leishmania* latent infection, displaying a sensitivity of 92%, a specificity of 100% and a NPV of about 93%. This performance is reflected by the high accuracy of this test (Table 3, value [95% confidence interval]: 96.1 [86.5-99.5]), showing its ability to correctly classify the infected subjects in apparently healthy condition (Table 3, Figure 1). It is well known that in *L. infantum* endemic areas, asymptomatic *Leishmania* infection in humans is usually obtained by a positive Leishmanin skin test, PCR or serological test [13]. However, whilst the Leishmanin skin test and PCR have recognized roles in the diagnosis of cryptic leishmaniasis [32,33], solid data on the reliability of serological methods are lacking. Many of the serological methods available (ELISA, ICT, etc.), in fact, have been tested for asymptomatic infection in the animal reservoir (dog) rather than in humans [34]. The most studied serological method in the diagnosis of human asymptomatic leishmaniasis was WB, mainly employed in epidemiological studies with heterogeneous results [33,4,35].

### Conclusions

The discrepancies found between the different serological tests implemented in our study and previously published data may be due to the differences in antibody concentrations among the different geographic regions [28] or genetic diversity amongst *L. infantum* strains from different areas [27]. Based to our experience, ICT represents a good solution in terms of ease of use, field applicability, and performance. This applies to TruQuick® because its excellent sensitivity, specificity and diagnostic accuracy outperformed IT
LEISH® in diagnosing VL. However, users should be aware that ICT can be positive in asymptomatic carriers and for long time after treatment. Therefore, these assays cannot discriminate between VL relapse and other pathogens, like all the other serological assays [11]. With regard to IFAT, its results were also promising in the diagnosis of VL, although the need for specialized laboratory equipment and trained personnel, as well as the lack of standardized protocols and international standards restrict its wide application. Finally, our data confirm previous reports in the literature, where WB is the most widely used and reliable serologic test for epidemiological investigations of asymptomatic L. infantum infection carriers.

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Author's contributions
All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by MM. The first draft was written by MM and all authors amended/edited/commented previous versions of the manuscript. All authors read and approved the final manuscript.

References

Northeastern Italy: evaluation of seven serological tests. Microorganisms 8: 1847.


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**Conflict of interests:** No conflict of interests is declared.