**Assessment of the direct agglutination test, fast agglutination screening test, and rK$_{39}$ dipstick test for the sero-diagnosis of visceral leishmaniasis in Syria**

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**ABSTRACT**

**Objective:** To evaluate the performances of 3 serological assays (direct agglutination test [DAT], fast agglutination screening test [FAST], recombinant protein [rK$_{39}$] dipstick) test for use in primary care, for the diagnosis visceral leishmaniasis (VL) in Syria.

**Methods:** We utilized 267 serum samples obtained during 2007 from patient groups (confirmed and suspected VL, confirmed cutaneous leishmaniasis, toxoplasmosis) from endemic areas in Syria and control samples, and applied the 3 serological tests in the Damascus University, Damascus and Health laboratories at the same time, on these samples.

**Results:** Our data show that the tests were very sensitive, where the DAT was the most specific followed by FAST, then rK$_{39}$ dipstick.

**Conclusion:** Our study confirmed that all the tests performed well, and proved to be very important sero-diagnosis tools for visceral leishmaniasis.

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Leishmaniasis is caused by an intracellular protozoan parasite belonging to the genus *Leishmania*. This disease ranges from self-healing cutaneous lesions to fatal systemic disease, depending on the parasite and the host immune response. A common estimate of the worldwide annual incidence is 600,000 newly reported clinical cases. The overall prevalence of Leishmaniasis was 12 million cases in the world, and the estimated population at risk is approximately 350 million.

Visceral leishmaniasis (VL) caused by *Leishmania donovani* (*L. donovani*), *Leishmania infantum*, and *Leishmania chagasi*, is a serious illness that can give rise to an epidemic and provoke high mortality rate, if left untreated.

The clinical symptoms of VL are similar throughout the world. Large numbers of amastigote infected mononuclear phagocytes in the liver and spleen, result in progressive hypertrophy. In sub-acute or chronic cases, symptoms are splenomegaly, prolonged irregular fever and weakness, loss of appetite, weight loss, pallor, anemia, and diarrhea. At present, routine diagnosis of VL is carried out by direct microscopic examination of patient material, or by culture. However, sample retrieval is inconvenient for the patients, and parasite isolation by culture is time consuming, expensive and
Methods. We utilized serum samples (267 samples) from patient groups from endemic areas, and control samples from other regions in Syria during 2007. A questionnaire with clinical and epidemiological data (age, gender, address, symptomatology, and so forth) was filled out for each patient. The patients were notified on all the procedures and signed informed consent was obtained. The Ethics Committee of the Damascus University Science Faculty, Damascus, Syria approved the study. The serum samples used were obtained from different regions in Syria, where a total of 193 VL human cases were reported by the Department of Disease Control during the last 8 years (2000-2007). There were 98 males (50.8%), 95 females (49.2%) that harbored the disease. By age, children under 6 years old constituted 78.8% (152 cases) of the reported cases. By provinces, the highest number of reported cases was 129 from Aleppo and Idlep, 52 from Lattakia and Tartous, and 8 from Daraa governorates over the period of 8 years.

Results. The results of DAT, FAST and rK39 testing of positive and negative controls, and of the serum samples of patients with other confirmed infectious diseases are presented in Table 1. The sensitivity of the
DAT, FAST, and rK$_{39}$ in the present study was calculated to be 100%. The calculated specificity of the DAT was 98.7%, FAST was 94.7% and rK$_{39}$ was 88.2% on the basis of the results obtained. The results of DAT, FAST, and rK$_{39}$ testing of serum samples from confirmed and suspected patients (115), are summarized in Tables 2-4, in order to evaluate the efficacy of these tests in detecting the VL antibodies especially in the suspected persons. A high degree of agreement (97%; $p=0.0001$) between the DAT and FAST was observed (Table 2). In addition, important agreement between DAT and rK$_{39}$ (95%; $p=0.0001$, Table 3), or FAST and rK$_{39}$ (97%; $p=0.0001$, Table 4) was also observed.

**Discussion.** In view of the public health importance of VL and the inherent difficulties of conventional

### Table 1 - Comparison results between DAT, FAST, and rK$_{39}$ dipstick using serum from confirmed and suspected VL patients, healthy endemic and non-endemic controls, and samples from patients with other confirmed diseases.

<table>
<thead>
<tr>
<th>Tests</th>
<th>DAT*</th>
<th>DAT-</th>
<th>FAST+</th>
<th>FAST-</th>
<th>rK$_{39}$*</th>
<th>rK$_{39}$-</th>
</tr>
</thead>
<tbody>
<tr>
<td>Confirmed VL patient sera (n=30)</td>
<td>30 (100)</td>
<td>0 (0)</td>
<td>30 (100)</td>
<td>0 (0)</td>
<td>30 (100)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Suspected VL patient sera (n=85)</td>
<td>61 (72)</td>
<td>24 (28)</td>
<td>63 (75)</td>
<td>22 (26)</td>
<td>67 (79)</td>
<td>18 (21)</td>
</tr>
<tr>
<td>Confirmed CL patient sera (n=110)</td>
<td>2 (2)</td>
<td>108 (98)</td>
<td>6 (5.4)</td>
<td>104 (94)</td>
<td>13 (11.8)</td>
<td>97 (88)</td>
</tr>
<tr>
<td>Confirmed toxoplasmosis sera (n=7)</td>
<td>0 (0)</td>
<td>7 (100)</td>
<td>0 (0)</td>
<td>7 (100)</td>
<td>0 (0)</td>
<td>7 (100)</td>
</tr>
<tr>
<td>Healthy non-endemic controls (n=10)</td>
<td>0 (0)</td>
<td>10 (100)</td>
<td>0 (0)</td>
<td>10 (100)</td>
<td>0 (0)</td>
<td>10 (100)</td>
</tr>
<tr>
<td>Healthy endemic controls (n=25)</td>
<td>0 (0)</td>
<td>25 (100)</td>
<td>2 (8)</td>
<td>23 (92)</td>
<td>5 (20)</td>
<td>20 (80)</td>
</tr>
</tbody>
</table>

DAT - direct agglutination test, FAST - fast agglutination screening test, rK$_{39}$ - recombinant protein, VL - visceral leishmaniasis, CL - cutaneous leishmaniasis.

DAT* = 34.8%; DAT- = 65.2%, FAST* = 37.8%, FAST- = 62.2%, rK$_{39}$* = 43.1%; rK$_{39}$- = 56.9% at 95% confidence interval.

### Table 2 - Comparison results between DAT and FAST dipstick using sera from suspected visceral leishmaniasis patients.

<table>
<thead>
<tr>
<th>Tests</th>
<th>FAST+</th>
<th>FAST-</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>DAT*</td>
<td>93 (35)</td>
<td>0 (0)</td>
<td>93 (35)</td>
</tr>
<tr>
<td>DAT-</td>
<td>8 (3)</td>
<td>166 (62)</td>
<td>174 (65)</td>
</tr>
<tr>
<td>Total</td>
<td>101 (38)</td>
<td>166 (62)</td>
<td>267 (100)</td>
</tr>
</tbody>
</table>

DAT - direct agglutination test, FAST - fast agglutination screening test, FAST* = 37.8%, FAST- = 62.2% at 95% confidence interval.

### Table 3 - Comparison results between DAT and rK$_{39}$ dipstick using sera from suspected visceral leishmaniasis patients.

<table>
<thead>
<tr>
<th>Tests</th>
<th>rK$_{39}$*</th>
<th>rK$_{39}$-</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>DAT*</td>
<td>91 (79)</td>
<td>0 (0)</td>
<td>91 (79)</td>
</tr>
<tr>
<td>DAT-</td>
<td>6 (5)</td>
<td>18 (16)</td>
<td>24 (21)</td>
</tr>
<tr>
<td>Total</td>
<td>97 (84)</td>
<td>18 (16)</td>
<td>115 (100)</td>
</tr>
</tbody>
</table>

DAT - direct agglutination test, rK$_{39}$ - recombinant protein, rK$_{39}$* = 84.4%, rK$_{39}$- = 15.6%; at 95% confidence interval.

### Table 4 - Comparison results between FAST and rK$_{39}$ dipstick using sera from suspected visceral leishmaniasis patients.

<table>
<thead>
<tr>
<th>Tests</th>
<th>rK$_{39}$*</th>
<th>rK$_{39}$-</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>FAST*</td>
<td>93 (81)</td>
<td>0 (0)</td>
<td>93 (81)</td>
</tr>
<tr>
<td>FAST-</td>
<td>4 (3)</td>
<td>18 (16)</td>
<td>22 (19)</td>
</tr>
<tr>
<td>Total</td>
<td>97 (84)</td>
<td>18 (16)</td>
<td>115 (100)</td>
</tr>
</tbody>
</table>

FAST - fast agglutination screening test, rK$_{39}$ - recombinant protein, rK$_{39}$* = 84.4%, rK$_{39}$- = 15.6% at 95% confidence interval.
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diagnosis techniques in Syria, we tried in this study to evaluate the performance of sero-diagnostic tests. The 3 assays displayed a very high sensitivity (100%), whereas the specificity was 98.7% (DAT), 94.7% (FAST), and 88.2% (rK₃₉ dipstick). The specificity of these tests was assessed by using sera from healthy endemic and non endemic controls, and from patients with other confirmed infectious diseases. Several samples of healthy endemic controls and confirmed human cutaneous leishmaniasis (CL), tested positive with FAST and rK₃₉ dipstick, whereas, only 2 positive sample was obtained with DAT. These results corroborate with many previous studies.9,17-23 It is noted that the sensitivity and specificity of these tests, observed in the present study were determined on a limited number of healthy controls, patients with 2 other infectious diseases, and patients with confirmed VL. Therefore, 100% sensitivity is not claimed. However, there was a good agreement between the performances of the 3 tests with regard to the sero-diagnosis of VL. In contrast, both tests, FAST and rK₃₉ dipstick, found only a very limited number of CL sero-positive. In conclusion, the findings further confirmed earlier reports that DAT, FAST, and rK₃₉ dipstick are suitable tools for the sero-diagnosis of VL. The 3 tests are easy to interpret, and are sensitive and specific, as well. The rK₃₉ dipstick is a rapid and simple test not requiring extensive training of the operator. However, this test requires cold storage of the chase buffer, and the test strips cannot be stored at high ambient temperature. The DAT, using the freeze-dried Leishmania antigen, is very practical under field or rural conditions, as no equipment is required nor cold storage is necessary for reagents. A limitation of the DAT is the relatively long incubation period (18 hours), and the serial dilutions of the samples that must be made. Finally, the FAST is also practical under field conditions, and it can be used for screening large populations as well, as it requires only one serum dilution, and the results can be read in 3 hours. Therefore, if only validity criteria are considered for the 3 tests, then they are all applicable, however, the simplest is the dipstick test. It is necessary to mention that the serological tests are an aid in the diagnosis of VL. The results of rK₃₉ dipstick, or FAST, or DAT should always be correlated with clinical, epidemiological, and other diagnostic test data.

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References

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