The Diagnostic Accuracy of Direct Agglutination Test for Serodiagnosis of Human Visceral Leishmaniasis: A Systematic Review with Meta-analysis

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Abstract

**Background:** Direct agglutination test (DAT) as simple, accurate and non-expensive tool that has been used widely for serodiagnosis of visceral leishmaniasis (VL) during the last three decades. We conducted a systematic review and meta-analysis to evaluate the diagnostic accuracy of DAT for serodiagnosis of human VL.

**Methods:** Electronic databases, including MEDLINE (via PubMed), SCOPUS, Web of Science, SID and Mag Iran (two Persian scientific search engines) were searched from December 2004 to April 2019. The study quality was evaluated using the QUADAS checklist. We determined the sensitivities and specificities across studies, calculated positive and negative likelihood ratios (LR+ and LR−), and constructed summary receiver operating characteristic (ROC) curves parameters.

**Results:** Of the 2928 records identified in the mentioned electronic databases and through articles' reference lists, 25 articles met inclusion criteria and enrolled into the systematic review and among them 22 records were qualified for meta-analysis. The pooled sensitivity and specificity of DAT was 96% [(95% CI, 93–98)] and 95% [(95% CI, 88–98)], respectively. The likelihood ratio of a positive test (LR+) was found to be 19.8 [CI95%, 7.6–51.8] and the likelihood ratio of a negative test (LR−) was found to be 0.04 [CI95%, 0.02–0.08]. The combined estimate of the diagnostic odds ratio for DAT was high [454136-1561]. We found that the summary receiver operating characteristic curve (SROC) is positioned near the upper left corner of the curve and the area under curve (AUC) was 0.98 (95% CI, 0.97 to 0.99).

**Conclusion:** Based on our analysis, we find DAT can be considered as valuable tool for the serodiagnosis and seroprevalence of human VL with high sensitivity and specificity rates. As DAT is simple, accurate, non-invasive and efficient serological test, it can be used for serodiagnosis of human VL particularly in endemic areas of the disease.

**Background**

Visceral leishmaniasis (VL) is one of the most important neglected tropical diseases that is caused by *Leishmania donovani* and *L. infantum/chagasi* in both humans and canines (1-3). The morbidity and mortality due to VL are estimated at 200,000 to 400,000 new cases a year and approximately 20,000 to 40,000 deaths occur annually (4). More than 90% of all VL cases occur in 6 countries including India, Bangladesh, Brazil, Ethiopia, Sudan, and South Sudan (5).

Since the fatality rate of VL is high and it can reach 100% when not treated properly, a diagnostic test with both high validity and reproducibility rates is required (1,6). Treatment of VL cases should be recommended after confirmation of the disease. The first line regimen for primary VL treatment is pentavalent antimonial compounds such as meglumine antimoniate (Glucantime®) and Sodium stibogluconate(Pentostam®) which can be used as a monotherapy (administered as intramuscular injections 20 mg/ kg/day for 28-30 days) or combination with cryotherapy or other drugs such as paramomycin (1-3). Other drugs such as amphotericin B deoxycholate, Liposomal amphotericin B (Ambisome®) and miltefosine (administred orally) may be used for the treatment of VL particularly in patients with clinical or laboratory resistance to pentavalent drugs as well as contraindications caused by these drugs (1).

Ambisome® in VL is the first line treatment in pregnancy, for patients whose condition is severe and HIV co-infected patients (1). A follow–up visit six months after the end of treatment is recommended to make sure the treatment was successful. The gold standard for diagnosing VL is mainly parasitological examinations including the demonstration of parasites by microscopic examination of splenic or bone-marrow aspiration (4, 6).

Sample preparation for parasitological examinations of VL is highly invasive. In spite of the high specificity of parasitological examinations, the sensitivity of these methods depends on the type and preparation of samples (7). Moreover, the accuracy and precision of parasitological examinations is also subject to the experience of the laboratory microscopist. Molecular methods including polymerase chain reaction (PCR)-based methods have been developed and assessed for human and canine VL using various target genes and different clinical specimens (8). The pooled sensitivity and specificity of PCR for detection of infections caused by *L. infantum/chagasi* on human peripheral blood samples were
reported as 93.1% and 95.6%, respectively (7, 8). The main limitation of PCR assays is the lack of standardization due to the large numbers of different administrative protocols (7). In addition, the specificity of the molecular methods varied significantly in different studies in the literature due to the fact that in some studies parasitological methods are not able to identify true-positive patients (7). Moreover, these methods require specific and expensive equipment and materials and also the performance of molecular methods particularly in remote areas has some limitations.

Several serological tests are used for serodiagnosis of VL, including the indirect fluorescent antibody test (IFAT), the enzyme linked immunosorbent assay (ELISA), the latex agglutination test (LAT), immunoblotting, the direct agglutination test (DAT) and the rK39 rapid diagnostic test. Among which, DAT and rK39 are simple and do not require sophisticated equipment; thus, they are usually used in field studies as well as in laboratories.

One of the major drawbacks of serological tests is that they are not able to detect relapses among VL cases because anti-Leishmania antibodies remain in the body for a long time after clinical cure (9, 10) and many people who live in endemic areas have anti-Leishmania antibody titers due to high exposure to Leishmania parasites. In the previous meta-analysis of the diagnostic performance of the DAT for VL from January 1986 to December 2004, the pooled sensitivity and specificity rates were calculated as 94.8% and 86%, respectively (11). This study aimed to update the diagnostic accuracy of the DAT for human VL diagnosis from December 2004 to April 2019.

**Methods**

**Study design**

This study has systematically searched all the studies related to diagnostic performance of DAT during 2004-2019.

Selection was made independently by two reviewers (SSH and AB) and discrepancies were solved by consensus after discussion.

This systematic review with meta-analysis was conducted as per PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analysis) guidelines (12).

**Search strategy**

Electronic databases, including MEDLINE (via PubMed), SCOPUS, Web of Science and two Persian scientific search engines “Scientific Information Database” (www.sid.ir) and "Mag Iran" (www.magiran.com) were searched systematically with various combinations of the following scientific keywords: “Visceral leishmaniasis”, “Leishmania infantum”, “Leishmania donovani” “DAT”, “Direct Agglutination Test”, “Parasitology”, “Microscopy”, “Specificity” and “Sensitivity” using “OR” and/or “AND”. The reference lists of selected articles were also screened manually for possibly relevant articles. Abstracts of articles which published in congresses were not explored.

**Case definition**

Patients in VL-endemic countries with pathognomonic signs and symptoms such as fever >2 weeks, hepatosplenomegaly, progress weakness, anaemia or pancytopenia, and lymphadenopathy with confirmation of following tests particularly in VL endemic areas were considered as VL cases.

1-Parasitological diagnosis

(a)

Microscopic examination of bone marrow aspirate, lymph node aspirate, and/or spleen aspirate using direct semi-quantified microscopic examination of Giemsa-stained smears for detection of amastigote form of Leishmania spp.
Culture of bone marrow, lymph node, and/or spleen aspirations in Novy–MacNeal–Nicolle (NNN) culture media. Demonstration of at least one amastigote upon microscopic examination of tissue smears or one promastigote in culture is sufficient for the diagnosis.

2- Serological tests

DAT was performed on each sample in standardized conditions, and each test was read by two readers who were blind to other test results.

Study selection and data extraction

Titles and abstracts of all articles were screened by one reviewer, and eligibility of the screened articles was assessed by two independent investigators using the following criteria.

Inclusion criteria were: a) have a full description of accuracy of DAT as a diagnostic test for the detection of VL in patients. b) Articles were published from December 2004 to April 2019. c) include both DAT test and parasitological examinations (direct demonstration, culture) as the confirmatory diagnostic method for VL. c) Cases with no previous history of VL were included.

Exclusion criteria were: a) insufficient primary and/or secondary data information of (lack information about sensitivity and specificity), b) unavailable full text article, and c) written in a language other than English or Persian.

Eligibility of all explored papers was assessed by three reviewers. The discrepancies among studies were obviated by discussion and consensus. Afterward, data of interest were gathered using a pre-designed data extraction form containing all the descriptive variables and test results. The following information was extracted: authors, year and country in which the study was carried out, diagnostic methods applied, reference test used, cut off, characteristics of the participants, quality of the study and sample size.

Assessment of Study Quality

We assessed the quality of studies using the Quality Assessment of Studies of Diagnostic Accuracy Approach-QUADAS (13).

Data Synthesis and Statistical Analysis

All meta-analysis methods were performed using STATA (Release 12. statistical software. College Station, Texas: STATA Corp LP). Sensitivity and specificity were calculated when available data were presented. To calculate sensitivity and specificity values for the tests, we cross-tabulated each result against the reference standard. Whenever possible, we extracted raw data from primary studies to fill in the four cell values of a diagnostic 2×2 table: true positives, false positives, true negatives, and false negatives.

In many articles the numbers of true positive, false negative, true negative, and false positive observations were available. If not, we derived the numbers from the marginal totals and the reported sensitivity and specificity.

Meta-analyses were accomplished by using random-effects inverse-variance weights. Results of the meta-analysis were illustrated by a forest plot diagram, which demonstrated the accuracy estimates and their relevant 95% confidence interval (CI).

The Cochran’s $Q$ and $I^2$ statistics were used for assessment of the between-study inconsistency and heterogeneity, respectively. The $I^2$ values of 25%, 50%, and 75% were representatives of low, moderate and high heterogeneity, respectively. Publication bias was evaluated through the Deeks’ funnel plots (14).
Results

From the literature searches, we identified 2928 primary citations from electronic databases and through articles’ reference lists. Study selection flow is shown in Figure 1.

Among these records, 24 articles met inclusion criteria and enrolled into the systematic review. The characteristics of the included studies are summarized in Table 1.

The reference method for diagnosis of VL in all studies was a positive result on microscopic examination and/or culture of lymph node, bone marrow, or spleen aspirates (15-17).

Some studies performed DAT using both freeze-dried (FD) and aqueous (AQ) antigen (18, 19). No significant difference in the sensitivity of the DAT (FD and AQ) was found. In one study (18), relatively higher sensitivity (99%) was recorded for the LQ-DAT than for the FD-DAT (96%) that might be due to the use of the endemic autochthonous *Leishmania donovani* isolate as the antigen.

In overall, studies had a wide geographical distribution and were carried out in different countries including Brazil (20-22), Ethiopia (16, 23), India (19, 24-28), Iraq (29), Iran (30-32), Saudi Arabia (15), Sudan (18, 33-36), Turkey (17) and Venezuela (37).

The study quality analysis as assessed by QUADAS tool showed that 22 out of 24 studies (92%) met more than seven criteria (Table 1).

Among 24 studies, 20 records were qualified for meta-analysis. All studies except 2 reports (one cohort (28) and one case-control (15) were cross sectional studies evaluating the diagnostic accuracy of DAT and meta-analysis was performed on all cross sectional studies evaluating the diagnostic accuracy of DAT. Three studies were excluded because there was zero cell and sensitivity and/or specificity cannot be calculated. Cut off dilution for a positive test on DAT reported to vary between 1:800 to 1:3200 (Table2).

Figures 1 shows the results of individual and combined sensitivity and specificity estimates of the DAT test for diagnosis of VL. The pooled sensitivity of the included studies evaluating the DAT was 96% [CI95% 92–98] and the pooled specificity was 95% [CI95% 86–99] (Figure 2). The likelihood ratio of a positive test (LR+) was found to be 21 [CI95% 6.6–66.5] and the likelihood ratio of a negative test (LR−) was found to be 0.04 [CI95% –0.02–0.08] (Figure 3). Moreover, the combined estimate of the diagnostic odds ratio for DAT was high (467 [114-1912]).

Among studies which considered DAT cut off value of 1:800, the pooled sensitivity and specificity was 95% [CI95% 66–100] and 99% [CI95% 93-100], respectively. With considering this cut off value, the LR+ was found to be 107 [CI95% 12.7-901] that was more higher than LR+ obtained from analysis of studies with cut off value of 1:1600 and 1:3200. Although the pooled sensitivity of DAT with cut off value of 1:1600 and 1:3200 was remained stable (97% and 96%, respectively), the pooled specificity was declined to 87% [CI95%83-90] with cut off value of 1:1600 and 93% [CI95% 73-99] with cut off value of 1:3200, respectively.

The pooled sensitivity and specificity of DAT for serodiagnosis of *L. donovani* was 96% [CI95% 92–98] and 98% [CI95% 93-99], respectively; while it was 92% [CI95% 83–96] and 98% [CI95% 15-100] for serodiagnosis of *L. infantum*, respectively (Table2).

We found that the summary receiver operating characteristic curve (SROC) is positioned near the upper left corner of the curve and the area under curve (AUC) was 0.98 (95 % CI, 0.97 to 0.99) (Figure 4).

The Deeks’ funnel plots for publication bias also showed no asymmetry (Figure 5). The evaluation of publication bias showed no potential for publication bias (p=0.69).
Discussion

Until 1990, VL diagnosis was in need of parasitological confirmation including microscopy or culture of the spleen, bone-marrow, lymph nodes and sometimes peripheral blood specimens. The invasiveness and sometimes fatal complications particularly associated with splenic aspiration brought about the development of simple and accurate serological tests such as DAT (38). Although the IFAT and ELISA are two important serological methods for diagnosis of human VL they require specific materials and equipment (1, 33). The rK39-based tests are easy to perform, quick, cheap and give reproducible results and can therefore be used for early diagnosis of VL at both peripheral and central levels of public health centers. Studies comparing the rK39 strip test and DAT found a similar sensitivity (94%) and specificity (89%); however, the DAT showed a slightly higher specificity (39).

On the other hand, rK39 strip tests have certain limitations, as with other serologic tests, anti-Leishmania antibodies in patients can persist for months after cure of VL, moreover, it has low specificity in sub-clinical and asymptomatic forms of L. donovani or L. infantum/chagasi infections, particularly among the Sudanese population (18, 33). A newly developed assay based on the detection of antibodies to the rk28 fusion protein reported a very promising sensitivity and specificity (96% and 98%, respectively) of ELISA to detect anti-Leishmania antibodies in sera among VL patients. The rK26, A2-ELISA and rKE16 dipstick recombinant antigens from amastigote forms of L. infantum or L. donovani was prepared and laboratory assessments were performed with desirable results (40-42). Among the available serological tests for the diagnosis of VL, DAT is a simple, highly specific and sensitive, reliable and cost-effective test that can be used in field as well as laboratory studies (43-47). DAT is a semi-quantitative serological test and it has been used for the serodiagnosis and seroepidemiological studies of VL in humans and animal reservoir hosts during the last 4 decades. According to our knowledge, this is the second systematic review and meta-analysis about the diagnostic accuracy of DAT for diagnosis of VL. Chappuis et al. included 30 relevant studies that evaluated DAT from January 1986 to December 2004 (11) and reported the pooled sensitivities and specificities of 94.8% and 86%, respectively. In the present study, 24 eligible studies from April 2004 to December 2019 were evaluated using DAT for the diagnosis of VL in immunocompetent patients by the systematic review. Our meta-analysis showed DAT is still a validated serodiagnostic test with high pooled sensitivity of 95% [CI95%,66–100], 97% [CI95%,94–99] and 96% [CI95%, 93–97], respectively while 1:800, 1:1600, and1:3200 cut-off titer were considered. Higher pooled specificity 99% (95% CI, 93–100) was found at a 1:800 cut-off titer.

Specific Leishmania antibodies at a titer of 1:800 showed VL infection (33) whereas Leishmania antibodies at a titer of 1:3200 with pathognomonic clinical signs such as hepatosplenomegaly, dromedary fever, anemia and progress weakness reflected active VL (43).

DAT can be performed on serum, plasma or even urine samples (11, 48) with high diagnostic accuracy.

According to the results of meta-analysis that compared DAT with rK39 strip test, almost 1% more sensitivity and 2% more specificity than rK39 strip test was found.

Although we did not have sufficient information regarding human immunodeficiency viruses status of the cases, it has been reported that DAT has an acceptable sensitivity in the diagnosis of VL in HIV-positive patients (49). Although sensitivity of DAT in the majority of studies was more than 90% some heterogeneity in the sensitivity of the tests might be related to the geographical location of the study, differences in antibody concentrations, and immune or nutritional status of the patient (50). In the current study, it was not possible to evaluate the test performance according to these factors.

Early diagnosis and treatment has a potential role for control and elimination of the anthropoontic form of VL (i.e. Indian and African forms of VL), but control of zoonotic VL is highly difficult and the current control strategies for zoonotic VL rely on animal reservoir hosts and phlebotomine vectors (1), the use of insecticide-impregnated materials to prevent insect bites, and active case detection with appropriate treatment to decrease the mortality rate of VL (45).
VL is highly fatal in the absence of appropriate anti-Leishmania drugs. Pentavalent antimonial compounds are the drugs of choice for the treatment of VL but intramuscular or painful intravenous injections with high toxicity and high price are necessary for a long period of time. Other anti-leishmanial drugs also have significant toxicity and are usually expensive. As the clinical manifestations of VL are not completely pathognomonic and have low specificity, confirmatory tests are required to recognize the VL patients needing treatment. These tests must not only be sensitive, but they also need to be specific because the current anti-Leishmania drugs prescribed to treat VL are highly toxic (7).

The most important limitation for the evaluation of serological tests is the absence of an appropriate gold standard test. Confirmation of VL depends on the finding of Leishman bodies of Leishmania sp. in samples prepared from bone marrow or spleen, lymph nodes and liver. This procedure is highly invasive, and thus it can be done only in suspected cases of VL. Moreover, the sensitivity rate of this method is varied (47).

In some VL-endemic areas, DAT is prepared and used routinely for the diagnosis and sero-epidemiological studies of VL because it is simple and highly sensitive (33, 44).

The performance of DAT is neither Leishmania species-specific nor region dependent (6, 43).

Major shortcomings of DAT are its long incubation period for getting the results back, batch to batch variability of the antigen and cross-reactivity with Trypanosoma cruzi infection (43, 44). DAT titers decline over time falling below the cut off (1:800) in the ninth month of cure but still remain positive for a relatively long time (up to 5 years in more than 50% of VL cases) after the cure. Thus, the test cannot be used for follow up of treatment or for the diagnosis of disease relapse (49). To overcome the problem of long incubation time, a fast agglutination screening test (FAST) has been introduced, which uses only one serum dilution and requires only three hours of incubation (51). The validity of FAST for the detection of L. infantum infection in the field was compared with the conventional DAT in Iran and the results showed a sensitivity of 95.4% and specificity of 88.5% for fast DAT in comparison with conventional DAT (51).

Our study has some limitations. First, we compared pooled estimates between different study populations. So, the possibility of confounding should not be ignored. Second, the study used the data provided by published literature, and some data including sex and age for the included patients was unavailable and made subgroup analysis difficult.

**Conclusion**

Based on our analysis, the use of DAT is the best choice for early detection of acute and chronic clinical forms of VL as well as the asymptomatic form of the infections in immunocompenent individuals. Further research on DAT antigen standardization in the laboratories located in endemic areas of VL is recommended.

**Abbreviations**

DAT: direct agglutination test  
VL: visceral leishmaniasis  
FAST: fast agglutination screening test  
PCR: Polymerase chain reaction  
IFAT: indirect fluorescent antibody test  
ELISA: enzyme linked immunosorbent assay  
FD: freeze-dried
AQ: aqueous antigen
SROC: summary receiver operating characteristic curve
AUC: area under curve

Declarations

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Authors' contributions

MM: involved in designing, interpretations and writing of the manuscript. HK, SM and GH: participated in interpretation of the DAT results. SS, BA and AB: involved in gathering and grouping the articles regarding DAT for visceral leishmaniasis and drafted the manuscript. SM: involved in data analysis and writing of the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

All data obtained

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Tables

Table 1. The characteristics of the included studies
| Country   | Year     | Sample size | Diagnostic method                          | Cut off | Prasite | DAT+ Parasitology+ | DAT+ Parasitology- | DAT- Parasitology+ | DAT- Parasitology- | QUADAS score |
|-----------|----------|-------------|-------------------------------------------|---------|---------|---------------------|---------------------|--------------------|--------------------|---------------|
| India     | 2005     | 799         | Bone marrow and spleen aspiration          | ≥1:800  | L. donovani | 99                | 0 | 9                          | 691                | 12            |
| Brazil    | 2005     | 16          | Bone marrow aspiration                     | ≥1:800  | L. donovani | 16               | 0 | 0                          | 0                   | 11            |
| India     | 2006     | 329         | Spleen aspiration                          | ≥1:3200 | ND       | 227               | 3 | 5                          | 94                  | 13            |
| India     | 2006     | 508         | Spleen aspiration                          | ≥1:1600 | ND       | 144               | 54 | 6                          | 304                 | 13            |
| India     | 2006     | 508         | Spleen aspiration                          | ≥1:1600 | ND       | 146               | 45 | 4                          | 313                 | 13            |
| India     | 2006     | 548         | Bone marrow and spleen aspiration          | ≥1:800  | L. donovani | 63               | 4 | 17                         | 464                 | 10            |
| Sudan     | 2004-05  | 302         | Lymph nodes, bone marrow or spleen        | ≥1:3200 | L. donovani | 92               | 16 | 7                          | 187                 | 13            |
| Iran      | 2007     | 12          | Bone marrow aspiration                     | ≥1:3200 | L. infantum | 12               | 0 | 0                          | 0                   | 9             |
| Sudan     | 2004-06  | 174         | Lymph node aspirate                        | ≥1:3200 | L. donovani | 24               | 1 | 1                          | 148                 | 11            |
| Venezuela | 2007     | 54          | Bone marrow or spleen aspiration           | >1:1:600 | L. donovani | 26               | 0 | 0                          | 28                  | 10            |
| Iran      | 2004-06  | 15          | Bone marrow aspiration                     | ≥1:3200 | L. infantum | 12               | 3 | 0                          | 0                   | 12            |
| India     | 2008     | 18          | Spleen or bone marrow aspiration           | ≥1:800  | L. donovani | 6                | 2 | 0                          | 10                  | 11            |
| India     | 2008     | 20          | Spleen or bone marrow aspiration           | ≥1:800  | L. donovani | 10               | 0 | 0                          | 10                  | 11            |
| Turkey    | 2008     | 59          | Bone marrow aspiration - culture           | ≥1:1600 | L. donovani | 24               | 4 | 0                          | 31                  | 12            |
| India     | 2005-06  | 57          | Spleen and bone marrow aspiration         | ≥1:800  | L. donovani | 16               | 1 | 0                          | 40                  | 10            |
| Iran      | 2005-09  | 90          | Bone marrow or spleen aspiration          | ≥1:3200 | L. infantum | 27               | 0 | 2                          | 61                  | 12            |
| Ethiopia  | 2006     | 137         | Spleen or lymph node aspiration           | ≥1:1600 | ND       | 41               | 20 | 43                         | 33                  | 10            |
| Sudan     | 2004-05  | 322         | Lymph node aspirate                        | ≥1:3200 | L. donovani | 114              | 17 | 11                         | 180                 | 11            |
| Ethiopia  | 2006-07  | 699         | Spleen aspiration                          | ≥1:3200 | L. donovani | 144              | 338 | 9                         | 208                 | 10            |
| Iraq      | 2004-06  | 57          | Bone marrow aspiration                     | ≥1:800  | ND       | 10               | 0 | 47                         | 0                   | 11            |
| India     | 2005     | 355         | Bone marrow or spleen aspiration           | ≥1:800  | L. donovani | 5                | 34 | 0                          | 316                 | 12            |
| Sudan     | 2011     | 183         | Lymph node aspiration                      | ≥1:3200 | L. donovani | 100              | 6 | 0                          | 77                  | 11            |
| Country  | Year   | No. | Diagnostic Test | Cut off      | Leishmania species | No. of Positive | Sensitivity | Specificity | Positive Likelihood Ratio | Negative Likelihood Ratio | Diagnostic Odds Ratio |
|----------|--------|-----|-----------------|--------------|-------------------|------------------|-------------|-------------|--------------------------|--------------------------|------------------------|
| Saudi Arabia | 2004-2006 | 33  | Bone marrow aspiration - culture | ≥1:1600 | L. donovani | 9 | 1 | 1 | 22 | 12 |
| Saudi Arabia | 2004-2006 | 65  | Bone marrow aspiration - culture | ≥1:1600 | L. donovani | 24 | 2 | 1 | 38 | 12 |
| Brazil | 2004-2007 | 356 | Bone marrow smear | ≥1:3200 | L. infantum | 192 | 41 | 21 | 102 | 8 |
| Brazil | 2015 | 7   | Bone marrow aspiration | ≥1:3200 | L. infantum | 3 | 4 | 0 | 0 | 10 |
| Sudan | 2016 | 135 | Lymph node aspirate | ≥1:3200 | L. donovani | 92 | 0 | 1 | 42 | 11 |
| Sudan | 2016 | 141 | Lymph node aspirate | ≥1:3200 | L. donovani | 95 | 0 | 4 | 42 | 11 |

*Coinfection of VL in HIV+ patients  
** VL in immunocompetent patients  
†Sudanese VL patients  
‡ Asian VL patients  
¶ Freezed Dried DAT (FD) antigen  
¶¶ Aqueous DAT antigen (AQ)  
U Freezed Dried (FD) DAT antigen  
UU Liquid (LQ) DAT antigen

**Table 2.** Diagnostic accuracy of DAT for diagnosis of VL based on different cut off values and *Leishmania* species
