

## A meta-analysis of the diagnostic performance of the direct agglutination test and rK39 dipstick for visceral leishmaniasis

François Chappuis, Suman Rijal, Alonso Soto, Joris Menten, Marleen Boelaert

### Abstract

**Objective** To compare the performance of the direct agglutination test and rK39 dipstick for the diagnosis of visceral leishmaniasis.

**Data sources** Medline, citation tracking, January 1986 to December 2004.

**Selection criteria** Original studies evaluating the direct agglutination test or the rK39 dipstick with clinical visceral leishmaniasis as target condition; adequate reference classification; and absolute numbers of true positive, true negative, false positive, and false negative observations available or derivable from the data presented.

**Results** 30 studies evaluating the direct agglutination test and 13 studies evaluating the rK39 dipstick met the inclusion criteria. The combined sensitivity estimates of the direct agglutination test and the rK39 dipstick were 94.8% (95% confidence interval 92.7% to 96.4%) and 93.9% (87.7% to 97.1%), respectively. Sensitivity seemed higher and more homogenous in the studies carried out in South Asia. Specificity estimates were influenced by the type of controls. In phase III studies carried out on patients with clinically suspected disease, the estimated specificity of the direct agglutination test was 85.9% (72.3% to 93.4%) and of the rK39 dipstick was 90.6% (66.8% to 97.9%).

**Conclusion** The diagnostic performance of the direct agglutination test and the rK39 dipstick for visceral leishmaniasis is good to excellent and seem comparable.

### Introduction

Visceral leishmaniasis affects up to 500 000 people yearly, mostly in poor rural areas of east Africa, South Asia, and Brazil.<sup>1</sup> Standard treatment with pentavalent antimonials carries a significant toxicity and is expensive as branded formulations. Microscopical examination of spleen aspirates is sensitive and specific but requires expertise. Examination of bone marrow or lymph node aspirates is equally specific but less sensitive.<sup>w1</sup>

Serological tests have been developed to replace parasitological methods for diagnosing visceral leishmaniasis in the field. The direct agglutination test consists of microplates with wells in which increasing

dilutions of patient's serum or blood is mixed with stained killed promastigotes of *Leishmania donovani*. If antibodies to the protozoan are present, agglutination is visible with the naked eye. The test has been validated in several endemic areas and is being used in countries such as Sudan.<sup>w1</sup> A serological test against a recombinant antigen of *Leishmania chagasi* (rK39) has also been developed as a dipstick. Patient's serum or blood is added to a strip and migrates towards the fixed rK39 antigen. A reagent reveals the presence of specific antibodies on the rK39 antigen line. The results are available within 20 minutes. We carried out a meta-analysis of the diagnostic performance of the direct agglutination test and the rK39 dipstick.

### Materials and methods

We searched Medline for articles published from January 1986 to December 2004 that reported on the diagnostic accuracy of the direct agglutination test and the rK39 (see [bmj.com](http://bmj.com) for search terms). We obtained additional articles by citation tracking of reviews and original articles.

We included original studies only. Other inclusion criteria were current clinical visceral leishmaniasis as the target condition; human participants; the absolute numbers of true positive, false negative, true negative, and false positive observations available or derivable from the data presented; and the reference classification judged adequate to correctly classify the target condition. We included such studies, independent of quality and reporting. We excluded studies that evaluated the tests in patients coinfecting with HIV and studies of the rapid version of the direct agglutination test.<sup>2</sup>

We extracted data on study phase, *Leishmania* species, country in which the study was carried out, participants, study design, and test results. The number and type of participants was recorded and categorised as confirmed cases or controls (healthy non-endemic,

Editorial by  
Lockwood and  
Sundar

Geneva University  
Hospitals, Travel  
and Migration  
Medicine Unit, rue  
Micheli-du-Crest  
24, 1211 Geneva  
14, Switzerland

François Chappuis  
lecturer

BP Koirala Institute  
of Health Sciences,  
Dharan, Nepal  
Suman Rijal  
professor

Hospital Nacional  
Hipolito Unanue,  
Universidad  
Peruana Cayetano  
Heredia, Lima, Peru  
Alonso Soto  
investigator

Institute of Tropical  
Medicine, Antwerp,  
Belgium

Joris Menten  
statistician  
Marleen Boelaert  
professor

Correspondence to:  
F Chappuis  
[francois.chappuis@hcuge.ch](mailto:francois.chappuis@hcuge.ch)

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Additional tables and references w1-w38 are on [bmj.com](http://bmj.com)



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**Table 1** Sensitivity and specificity of rK39 dipsticks in various subgroups

Subgroups	No of studies	Sensitivity (95% CI)	No of studies	Specificity (95% CI)
All studies	13	93.9 (87.7 to 97.1)	13	95.3 (88.8 to 98.1)
Trial phase:				
I	5	86.0 (67.1 to 94.9)	5	96.9 (86.3 to 99.4)
II	4	96.5 (86.0 to 99.2)	4	96.8 (90.7 to 98.9)
III	4	94.8 (87.6 to 97.9)	4	91.2 (66.8 to 98.2)
Region:				
South Asia	7	97.1 (91.7 to 99.0)	7	95.3 (87.3 to 98.3)
East Africa	2	79.0 (46.7 to 94.2)	2	85.2 (28.2 to 98.8)
Elsewhere	4	88.8 (83.7 to 92.4)	4	97.0 (79.4 to 99.6)
<i>Leishmania</i> species:				
<i>L. donovani</i>	10	94.6 (86.1 to 98.0)	10	95.2 (87.3 to 98.3)
Other	3	90.0 (84.9 to 93.5)	3	95.7 (62.7 to 99.7)
Brand:				
Kalazar Detect* (InBios International)	7	95.1 (89.5 to 97.8)	7	93.1 (84.1 to 97.2)
Other	6	91.2 (74.3 to 97.3)	6	97.2 (79.1 to 99.7)
Sample size:				
Cases or controls ≤100	8	89.6 (77.4 to 95.6)	6	89.0 (71.5 to 96.3)
Cases or controls >100	5	95.6 (88.2 to 98.5)	7	97.2 (94.1 to 98.7)
Quality assessment of studies of diagnostic accuracy:				
Score ≤7	4	92.7 (80.6 to 97.5)	4	93.9 (67.8 to 99.1)
Score >7	9	94.2 (85.6 to 97.8)	9	95.9 (89.2 to 98.5)
Health states of controls†:				
Healthy non-endemic	—	NA	0	NA
Healthy endemic	—	NA	10	95.9 (90.6 to 98.3)
Cross reacting diseases	—	NA	7	97.1 (88.5 to 99.3)
Clinically suspected disease	—	NA	7	93.0 (77.5 to 98.1)
Clinically suspected disease in phase III studies	—	NA	4	90.6 (66.8 to 97.9)

NA=not applicable.

\*Previously known as Insure Rapid Test for Visceral Leishmaniasis.

†Several studies included more than one type of control.

healthy endemic, controls with potentially cross reacting diseases, and controls with similar clinical syndrome, see [bmj.com](http://bmj.com) for descriptions). If the numbers of true positive, false negative, true negative, and false positive observations were not available, we derived the numbers from the marginal totals and the reported sensitivity and specificity.

Two independent readers assessed the papers according to the quality assessment of studies of diagnostic accuracy approach<sup>3</sup> (see [bmj.com](http://bmj.com) for a description of the tool and the included criteria).

### Data analysis

We used standard formulas to calculate sensitivity, specificity, and diagnostic odds ratio for each study. Wilson's score method was used to calculate confidence intervals for sensitivities and specificities,<sup>4</sup> and the normal approximation with continuity correction for the log of the odds ratio for confidence intervals for the diagnostic odds ratio.<sup>5</sup>

We explored the relation between sensitivity and specificity by plotting the log odds of a positive test result in cases against the log odds of a negative test result in controls. Publication bias was assessed.

Meta-analyses for sensitivity and specificity were carried out using logistic regression models accounting for over-dispersion.<sup>6</sup> The meta-analysis of the diagnostic odds ratio was carried out using the DerSimonian and Laird random effects model, with studies weighted using the Mantel-Haenszel method.<sup>7</sup> To assess the heterogeneity of studies we carried out separate meta-analyses in subgroups stratified by study

phase, sample size, study quality, geographical region, species of *Leishmania*, type of direct agglutination test antigen, brand of dipstick, and type of controls.

We used the generalised linear models module to fit over-dispersed logistic regression models and the R meta-analysis library to fit the DerSimonian and Laird random effects model.

## Results

Thirteen studies evaluating the rK39 dipstick (1119 patients with visceral leishmaniasis, 2676 controls) and 30 evaluating the direct agglutination test (1698 patients, 3876 controls) were included in the meta-analysis.<sup>w1-w38</sup> The ideal method for diagnosis of visceral leishmaniasis in all studies was a positive result on microscopical examination of lymph node, bone marrow, or spleen aspirate. Diagnosis was also confirmed by detection of parasites in culture (n=4 studies) or by polymerase chain reaction (n=1) or a good clinical response to antileishmanial drugs in patients with clinically suspected disease who had positive serology results (n=4). Results of the quality assessment of studies of diagnostic accuracy evaluation are on [bmj.com](http://bmj.com). No evidence of publication bias was observed. Owing to the lack of correlation between the observed sensitivities and specificities (data not shown), the individual sensitivities and specificities for diagnostic accuracy were determined.

### Sensitivity, specificity, and diagnostic odds ratio

The estimated sensitivity of both tests was relatively consistent across studies. The estimated sensitivity for the rK39 dipstick was 93.9% (95% confidence interval 87.7% to 97.1%; see [bmj.com](http://bmj.com)). Only one study seemed inconsistent with this estimate (67.2%, 54.1% to 78.2%).<sup>w20</sup> The estimated sensitivity for the direct agglutination test was 94.8% (92.7% to 96.4%). This estimate and confidence intervals were generally consistent with the individual study estimates.

Some heterogeneity in sensitivity of the tests seemed to be related to the geographical location of the study (tables 1 and 2). Sensitivity seemed higher and more homogenous in studies carried out in South Asia.

The estimated specificity of both tests was heterogeneous across studies (see [bmj.com](http://bmj.com) and tables 1 and 2).<sup>8</sup> Specificity estimates were highly influenced by the type of controls (see [bmj.com](http://bmj.com)). Studies using healthy controls showed high specificities. The estimated specificity in participants with clinically suspected disease in phase III studies of the rK39 dipstick was 90.6% (95% confidence interval, 66.8% to 97.9%) and of the direct agglutination test was 85.9% (72.3% to 93.4%).

For most studies the diagnostic odds ratio exceeded 100; the ratio was generally lower in participants with clinically suspected disease. In participants with clinically suspected disease from phase III studies, the combined estimate of the diagnostic odds ratio for the rK39 dipstick was 128 (26 to 629) and for the direct agglutination test was 76 (13 to 433).

## Discussion

This meta-analysis showed good to excellent diagnostic performance of the rK39 dipstick and the direct

agglutination test for visceral leishmaniasis in populations not known to be infected with HIV. The overall sensitivity of both tests was excellent but was lower in studies from East Africa than from South Asia. For reasons that remain unclear, Sudanese patients seem to develop lower titres of antibodies against rK39 than do Indian patients (S G Reed, personal communication, 2206).

The estimated specificity of the rK39 dipstick and the direct agglutination test was too heterogeneous across studies to enable a reliable measurement of a single estimate. We thus stratified the meta-analysis by subgroups, showing that the type of controls significantly influenced the estimates for specificity. Studies that included healthy controls showed very high specificity. The estimate of the specificity of both tests most relevant for clinical practice is obtained in phase III studies. Our meta-analysis includes only four such studies for both tests, resulting in specificity estimates with wide confidence intervals.<sup>w16 w19 w25 w26 w34</sup> Some degree of underestimation of these specificity estimates might have occurred, as spleen puncture was not systematically carried out among patients with suspected disease in most of these phase III studies.<sup>w1 w16 w19 w25 w34</sup> Serological tests with these high sensitivity and “acceptable” specificity levels have been shown to be better alternatives for case detection algorithms than tests with insufficient sensitivity.<sup>9</sup>

By focusing the meta-analysis on sensitivities and specificities, we did not take into account the effect of heterogeneity from variations in diagnostic thresholds. These studies may have used different thresholds to define positive and negative test results. The thresholds might also differ owing to factors such as differences in titration cut off points for the direct agglutination test or to unknown factors such as differing reactivity of antigen batches or variations between observers.<sup>10</sup> We did not, however, detect any correlation between sensitivity and specificity, indicating that variations due to diagnostic thresholds were insignificant compared with other sources of heterogeneity between the studies.

As the performance of the tests is comparable, the choice of test should be made on the basis of other

**Table 2** Sensitivity and specificity of direct agglutination test in various subgroups

Subgroups	No of studies	Sensitivity (95% CI)	No of studies	Specificity (95% CI)
All studies (n=30)	29	94.8 (92.7 to 96.4)	27	97.1 (93.9 to 98.7)
Trial phase:				
I	20	94.3 (91.5 to 96.2)	17	98.1 (94.2 to 99.4)
II	5	97.7 (87.4 to 99.6)	5	97.2 (92.5 to 99.0)
III	4	94.3 (87.9 to 97.4)	5	90.9 (75.9 to 96.9)
Region:				
South Asia	11	97.1 (94.9 to 98.4)	10	95.7 (88.1 to 98.5)
East Africa	11	93.2 (89.1 to 95.8)	10	96.1 (89.2 to 98.6)
Elsewhere	7	92.8 (86.8 to 96.2)	7	99.8 (97.5 to 100)
<i>Leishmania</i> species:				
<i>L. donovani</i>	23	95.1 (92.7 to 96.7)	21	96.4 (92.5 to 98.4)
Other	6	93.0 (85.1 to 96.9)	6	99.7 (94.6 to 100)
Type of antigen:				
Freeze dried	4	89.0 (84.1 to 92.5)	4	99.1 (74.4 to 100)
Aqueous	25	96.2 (94.2 to 97.5)	23	96.7 (93.0 to 98.5)
Sample size:				
Cases or controls ≤100	26	95.7 (93.3 to 97.3)	12	91.3 (81.7 to 96.1)
Cases or controls >100	3	92.8 (85.6 to 96.5)	15	98.2 (96.4 to 99.1)
Quality assessment of studies of diagnostic accuracy:				
Score ≤7	19	96.0 (92.5 to 97.9)	17	98.6 (96.7 to 99.4)
Score >7	10	93.7 (90.7 to 95.8)	10	92.6 (83.7 to 96.9)
Health state of controls*:				
Healthy non-endemic		NA	8	100.0 (98.2 to 100)
Healthy endemic		NA	20	98.7 (97.1 to 99.5)
Cross reacting diseases		NA	16	98.8 (95.6 to 99.7)
Clinically suspected disease		NA	8	82.6 (70.4 to 90.4)
Clinically suspected disease in phase III studies		NA	4	85.9 (72.3 to 93.4)

NA=not applicable.

\*Several studies included more than one type of control.

criteria such as cost, feasibility, and sustainability. rK39 dipsticks are cheaper and easier to use than the direct agglutination test. The brand of dipstick did not significantly influence pooled sensitivity and specificity estimates in our analysis. Nevertheless, dipsticks from different brands or from different generations of the same brand are likely to show variable diagnostic performance. Moreover, we did not include the results of recent studies carried out in East Africa and Asia with a newly manufactured rK39 dipstick that may perform better than other dipsticks in East Africa.<sup>11-13</sup>

The development of the direct agglutination test and the rK39 dipstick has brought a major improvement in the diagnosis of visceral leishmaniasis in the field. However, the limited sensitivity of the rK39 dipstick in Sudan and the suboptimal specificity of these serological tests when used for patients with clinically suspected disease necessitate the research and development of better diagnostic tools.<sup>14</sup>

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### What is already known on this topic

The direct agglutination test and rK39 dipsticks have been proposed as the most appropriate serological tests for diagnosis of visceral leishmaniasis in the field

A global evaluation of these tests, including an assessment of the heterogeneity of their performance, is lacking

### What this study adds

The direct agglutination test and the rK39 dipstick showed similar diagnostic performance for visceral leishmaniasis, ranging from good to excellent

The performance of dipstick tests for diagnosis in Sudan needs to be improved

endemic locality using fast agglutination screening test (FAST). *Acta Trop* 2002;83:93-101.

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## Use of single and combined antithrombotic therapy and risk of serious upper gastrointestinal bleeding: population based case-control study

Jesper Hallas, Michael Dall, Alin Andries, Birthe Søggaard Andersen, Claus Aalykke, Jane Møller Hansen, Morten Andersen, Annmarie Touborg Lassen

Editorial by Sung

Department of Clinical Pharmacology, IST, Syddansk Universitet, 5000 Odense, Denmark

Jesper Hallas  
professor

Research Unit of General Practice, Syddansk Universitet

Morten Andersen  
senior researcher

Department of Medical Gastroenterology, Odense University Hospital, Odense

Michael Dall  
registrar

Claus Aalykke  
senior registrar

Jane Møller Hansen  
senior physician

Department of Cardiology, Odense University Hospital

Alin Andries  
registrar

Birthe Søggaard Andersen  
senior registrar

Department of Infectious Medicine, Odense University Hospital

Annmarie Touborg Lassen  
senior registrar

Correspondence to: J Hallas  
jhallas@health.sdu.dk

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### Abstract

**Objectives** To assess the risk of serious upper gastrointestinal bleeding associated with the newer antithrombotic agents used alone or in combination with other antithrombotic drugs; to describe the trends in use of antithrombotic drugs in the background population.

**Design** Population based case-control study.

**Setting** Funen County, Denmark (population 470 000).

**Subjects** 1443 cases of serious upper gastrointestinal bleeding identified during 2000-4; 57 720 age and sex matched controls.

**Main outcome measure** Exposure to low dose aspirin, clopidogrel, dipyridamole, vitamin K antagonists, and combined antithrombotic treatment.

**Results** Adjusted odds ratios associating drug use with upper gastrointestinal bleeding were 1.8 (95% confidence interval 1.5 to 2.1) for low dose aspirin, 1.1 (0.6 to 2.1) for clopidogrel, 1.9 (1.3 to 2.8) for dipyridamole, and 1.8 (1.3 to 2.4) for vitamin K antagonists. Corresponding figures for combined use were 7.4 (3.5 to 15) for clopidogrel and aspirin, 5.3 (2.9 to 9.5) for vitamin K antagonists and aspirin, and 2.3 (1.7 to 3.3) for dipyridamole and aspirin. Other combinations were used too infrequently to allow estimation. The number of treatment years needed to produce one excess case varied from 124 for the clopidogrel-aspirin combination to 8800 for clopidogrel alone. During the study period, exposure to combined antithrombotic regimens increased by 425% in the background population.

**Conclusion** Antithrombotic treatment is becoming increasingly aggressive. Combined antithrombotic treatment confers particular risk and is associated with high incidence of gastrointestinal bleeding.

### Introduction

Aspirin is the mainstay of prophylactic antiplatelet treatment in patients with atherosclerotic disease.<sup>1 2</sup> Clinical trials have established the superiority of combined antiplatelet treatment over aspirin alone in preventing thrombotic outcomes.<sup>3-9</sup> Antiplatelet treatment is increasingly used in combination with vitamin K antagonists in patients who have dual indications for treatment. Unfortunately, safety data on the use of combined regimens are relatively scarce.

We did this population based case-control study to assess the risk of serious upper gastrointestinal bleeding associated with the newer antithrombotic agents used alone or in combination with other antithrombotic drugs. We also aimed to describe the trends in use of antithrombotic drugs in the background population.

### Methods

#### Setting

The data for this study came from three different sources—Odense University pharmacoepidemiological database, the Funen County patient administrative system, and the Danish central person register. Information on reimbursed drug dispensing in Funen County (population 470 000) has been recorded in the pharmacoepidemiological database since 1990. Data on patient contacts came from the Funen County patient administrative system. All Funen County residents have had their secondary care contacts registered since 1977. We used the central person register to extract the controls.



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