Field evaluation of latex agglutination test for detecting urinary antigens in visceral leishmaniasis in Sudan

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تقييم ميداني لاختبار تراص اللاتكس لتحري المستضدات البولية في داء الليشمانيات الحشوي في السودان

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الخلاصة: دُرس اختبار تراص اللاتكس لتحري المستضدات البولية في داء الليشمانيات الحشوي في شرق السودان. وقد أوضحت دراسة 204 مريضاً يشتبه إصابتهم بداء الليشمانيات الحشوي أن حساسية اختبار كاتكس وصلت إلى 95.2٪ وكانت متوافقة توافقاً جيداً مع الدراسة المجهرية للّطاخات، ولو أن توافُقها مع أربعة اختبارات سيرولوجية (مصلية) أخرى لم يكن جيداً. وقد كان الاختبار إيجابياً أيضاً في حالتَيْن مثبتتَيْن على أنهما عدوى بالليشمانيات الحشوية مع عدوى مرافقة بفيروس الإيدز. وقد تمت متابعة جميع الحالات المثبتة الإيجابية لاختبار كاتكس بعد المعالجة، ولوحظ أن الاختبار يصبح سلبياً بعد شهر واحد من استكمال المعالجة، وبالرغم من تمتع اختبار كاتكس بنوعيَّة مقدارُها مئة بالمئة في الشواهد في كلا المناطق الموطونة وغير الموطونة، فإن اختبار التراص المباشر كان إيجابياً في 14٪ من الشواهد الأصحّاء السلبيِّي الكاتكس في المناطق الموطونة. فاختبار الكاتكس يمثِّل إضافة بسيطة للوسائل التشخيصية لداء الليشمانيات الحشوي ولاسيَّما على الصعيد الميداني، كما يمثِّل اختباراً تكميلياً لتشخيص داء الليشمانيات الحشوي في الحالات السلبية اللطاخة مع نتائج إيجابية لاختبار التراص المباشر.

ABSTRACT A latex agglutination test to detect urinary antigens for visceral leishmaniasis (VL) was studied. In 204 patients with suspected VL, *KA*tex had a sensitivity of 95.2% with good agreement with microscopy smears but poor agreement with 4 different serology tests. It was also positive in 2 confirmed VL cases co-infected with HIV. In all *KA*tex-positive confirmed cases actively followed up after treatment, the test became negative 1 month after completion of treatment. While *KA*tex had a specificity of 100% in healthy endemic and non-endemic controls, the direct agglutination test (DAT) was positive in 14% of the *KA*tex-negative healthy endemic controls. *KA*tex is a simple addition to the diagnostics of VL particularly at field level and as a complementary test for the diagnosis of VL in smear-negative cases with positive DAT results.

Evaluation sur le terrain du test d'agglutination au latex pour la détection des antigènes urinaires dans la leishmaniose viscérale au Soudan

RESUME Un test d'agglutination au latex pour la recherche des antigènes urinaires de la leishmaniose viscérale a fait l'objet d'une étude. Chez 204 patients avec suspicion de leishmaniose viscérale, le KAtex avait une sensibilité de 95,2 % concordant bien avec les frottis mais peu avec 4 tests sérologiques différents. Il était également positif dans 2 cas de leishmaniose viscérale confirmée co-infectés par le VIH. Dans tous les cas confirmés positifs au KAtex, suivis de près après traitement, le test est devenu négatif 1 mois après la fin du traitement. Si le KAtex avait une spécificité de 100 % chez les témoins sains de zones endémiques et non endémiques, le test d'agglutination directe était positif chez 14 % des témoins sains de zones endémiques, négatifs au KAtex. KAtex est un simple supplément pour le diagnostic de la leishmaniose viscérale, en particulier sur le terrain, et un test complémentaire pour le diagnostic de la leishmaniose viscérale dans les cas à frottis négatif ayant des résultats positifs au test d'agglutination directe.

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Introduction

Sudan is recognized as one of the most endemic areas for visceral leishmaniasis (VL) in the world. The disease poses a major health problem in many parts of the country, particularly in the eastern and southern states [*1–5*]. Accurate diagnosis and treatment remains the mainstay of intervention control measures. Early diagnosis of VL is of crucial importance, as a successful outcome depends on the initiation of prompt treatment.

While parasitology methods, which use direct demonstration or cultivation of the causative organism, remain the definitive laboratory diagnostic method for VL, serodiagnostic methods are needed to overcome the diagnostic difficulties experienced in some cases. In the past, many patients in Sudan died untreated in hospital, with the diagnosis of VL being made later at autopsy [*6*]. A variety of serological tests for the detection of anti-leishmania antibodies have been described, including indirect immunofluorescence (IFAT) [*7*], enzyme-linked immunosorbent assay (ELISA) [*8,9*], lateral flow using recombinant antigen (e.g. rK39 antigen dipstick) [*10*] and immunoblotting (western blotting) methods [*11*]. These assays are associated with a number of problems including the possible cross-reactivity with other pathogens and the high cost and/or need for sophisticated equipment. The direct agglutination test (DAT) [*12,13*] is a sensitive, specific and simple test but its main disadvantage is that, as with other serological tests, it cannot readily distinguish between active disease, sub-clinical infections or past infections [*14*]. This is a significant drawback to the use of the test in areas where infections are common, since seroconversion does not necessarily signify VL; in the majority of cases the infection remains asymptomatic and only an estimated 5%–20% of infections ever become clinically patent [*15*]. Thus, the initiation of treatment cannot be based on serological grounds alone and parasitological confirmation is not always possible in endemic areas. The use of an antigen detection test would fill this gap, if the test was highly specific and simple to use.

Tests for the detection of antigen would, in principle, provide a better means of diagnosis since the presence of antigen necessarily indicates an active infection and broadly correlates with parasite load. Although a number of papers have reported the existence of circulating antigens and immune complexes in VL [*16–19*], none of them has led to a useful antigen detection assay for VL. Since VL is a chronic infection, the detection of antigens in patient serum is complicated by the presence of high levels of antibodies, circulating immune complexes, serum amyloid, rheumatoid factors and autoantibodies. These problems could, theoretically, be avoided by detecting antigen in the urine [*20–22*]. Recently, a latex agglutination test, *KA*tex (Kalon Biological), has been described for the detection of urinary antigens in VL [*23*]. The test is simple, rapid, cheap and suitable for use in remote rural areas. It has recorded 100% specificity and 81.4% sensitivity in human urine samples, a figure comparable to parasitology results on bone-marrow aspirates [*24*]. In experimentally infected animals, urine antigens were detected as early as 1 week post-infection, and more importantly, the antigen levels started to decline very quickly after treatment.

In the light of these encouraging results, this study was undertaken to evaluate the performance of the *KA*tex test under field conditions in Sudan and to compare these results with those obtained by parasitology and serology techniques. In addition, the

ability of the test to detect asymptomatic pre-patent cases of VL and its prognostic potential were investigated.

Methods

Study area and subjects

The study was conducted in Gedaref state, eastern Sudan, in 3 VL diagnostic and treatment centres: Kassab and Um El-Khair centres in the Rahad river area, and Tabarakallah centre in the Atbara river area. The field study was implemented over 3 visits during the period April 2001 to February 2002.

The following study subjects presenting to the 3 centres were included in the study: all 204 new patients suspected with VL (i.e. with fever for 2 weeks or more plus splenomegaly and/or lymphadenopathy); 7 post-kala-azar dermal leishmaniasis (PKDL) cases identified on a clinical basis [*25*]; 10 patients who had completed their treatment and investigated for test-of-cure (i.e. by parasitology testing at the end of treatment); and 24 cured VL cases (i.e. with clinical cure and a negative test-of-cure) who reported for routine follow-up (within 3–6 months).

For the parasitology testing, lymph node aspiration was used to obtain samples for microscopy to confirm active disease in VL suspects and as a test-of-cure.

All confirmed VL cases (i.e. with positive lymph node smear and/or positive DAT result) were treated with generic sodium stibogluconate (Albert David Ltd, Calcutta, India); the regimen was an intramuscular dose of 20 mg/kg/day for 30 days. If the test-of-cure was negative at the end of treatment and if the patient recovered clinically then the patient was considered cured.

Confirmed VL cases with positive *KA*tex were subjected to active follow-up for a period of 1 to 3 months after completion of treatment in order to evaluate the prognostic potential of *KA*tex.

To investigate sub-clinical infection, blood samples were obtained from 58 healthy endemic controls (i.e. those living in the endemic area but with no past history of VL). They were recruited from the Rahad river area of Gedaref state by interview and clinical screening of household members for VL. In addition, 100 non-endemic healthy controls (i.e. subjects with negative past history of VL or history of travelling to VL endemic areas) were recruited from the blood bank at Khartoum Teaching Hospital and were studied together with the endemic controls to assess the specificity of the test.

To investigate cross-reaction with other diseases, a cohort of 16 subjects with confirmed active pulmonary tuberculosis (TB) (positive smear for acid-fast bacilli) and 20 subjects with malaria (positive blood film) were recruited to the study. The TB cases included 13 cases from a non-endemic area (Khartoum) plus 3 cases from the endemic area (Gedaref). They were recruited by clinical screening of the patients who presented with TB to El-Shab Hospital, Khartoum and Um-el Khair Centre in Gedaref. The malaria cases included 5 cases from Khartoum (non-endemic area) and 15 cases from Gedaref (endemic area). They were recruited by clinical screening of the patients who presented to the above-mentioned facilities for malaria.

To study co-infection, 95 individuals of the sample investigated for VL who agreed to it were tested for human immunodeficiency virus (HIV). Informed written consent was obtained from all patients agreeing to HIV testing.

Laboratory testing

Venous blood (5 cm^3 from adults and 2 cm^3 from children) was collected from VL sus-

pects. Serum samples were separated by centrifugation and stored at –20 °C. In addition, finger prick blood spots were collected on filter papers (Whatman chromatography paper no. 3) from the healthy individuals in Um El-Khair village and stored at 4 °C. A urine sample (5–10 mL) was collected from all study subjects in 2 sterile containers: one was immediately used for field testing and the other one kept at –20 °C for subsequent testing.

The *KA*tex kit (Kalon Biological, Aldershot, UK) containing latex beads coated with IgG anti-leishmania antibodies was used. The test was performed according to Attar et al. [*23*]. Each urine sample was boiled for 5 minutes before testing and cooled at room temperature. Fifty µL of the prepared latex reagent was mixed with 50 µl of neat urine sample on a black glass slide (provided with the kit) and rotated and rocked consistently for 2 minutes in both clockwise and anticlockwise directions to ensure complete mixing. The degree of agglutination was recorded as:

++++ Most of the latex agglutinated and moved to the edges.

+++ Reaction resembled chalk dust scattered onto surface.

++ Clear agglutinated particles against a background of granular latex.

+ Agglutination could just be noted compared with the negative control.

– No agglutination observed.

Only the first 3 grades of agglutination were regarded as positive.

For comparison with *KA*tex, 4 different serological tests were used: DAT, IFAT, ELISA and immunoblotting.

DAT was performed on the sera of all patients who reported to the 3 centres according to Harith et al. [*26*], as well as on the eluates from filter paper serum samples from healthy endemic controls in Um El-Khair village.

The antigens for IFAT, ELISA and immonoblotting were prepared at the Laboratory of Immunology and Genetics of Parasitic Diseases, University of Marseille, France. Antigens were produced from a *Leishmania* strain isolated in Barbar village, Gedaref state, and characterized as *L. donovani donovani* by zymodeme analysis [*27*].

IFAT was performed according to the procedure of Edrissian et al. [*28*]. Results were considered positive if fluorescence was observed at a 1/50 dilution.

ELISA was carried out according to Mary et al. [*29*], using a leishmania lysate as coating antigen. The cut-off point for ELISA was determined by the mean + 2SD) of the absorbance for the control sera.

Immunoblotting was also carried out as described previously by Mary et al. [*29*]. The test was considered positive if antigen-14 and/or antigen-16 were recognized.

Screening for HIV was performed on stored and coded sera for the detection of specific antibodies to human immunodeficiency virus HIV-1 and HIV-2 using an ELISA kit (Novum, Dietzenbach, Germany). Confirmation of reactive samples was performed using 2 tests: a rapid test (HIV-SPOT Kit, Diagnostic Biotechnology, Bangkok, Thailand) and another ELISA (Vironostika HIV Uni-Form 11 plus 0, Organon Tecnica, Durham, USA). Only samples positive in the 3 tests were considered positive for HIV.

Data analysis

The data were entered into *Epi-Info*, version 6. The sensitivity and specificity of *KA*tex were estimated by comparing it with parasitology results as the reference standard in the 2 × 2 contingency table. A pa-

tient with a positive lymph node aspirate was considered as a VL case. Agreement of the *KA*tex result with that of other serological tests was assessed by Cohen's kappa coefficient, κ. Interpretation of κ was made according to Landis and Koch [*30*]: 1.00–0.81 excellent, 0.80–0.61 good, 0.60–0.41 moderate, 0.40–0.21 weak, 0.20–0.00 negligible agreement.

Results

A total of 204 new VL suspects at Om El-Khair, Kassab and Tabarakallah treatment centres were studied. They included 137 (67.2%) males and 67 (32.8%) females, with ages ranging from 1 to 55 years (mean 19.4 ± SD 12.7).

The diagnosis was confirmed with a positive lymph node smear in 62 of the 180 patients (34.4%) on whom parasitological testing was performed (Table 1). *KA*tex was positive in 43.6% (89/204), ELISA in 85.5%, IFAT in 90.4%, DAT in 96.1% and immunoblotting in 95.2% of the enrolled patients. *KA*tex was positive in 59 of the 62 cases with positive smears (the gold standard), giving a sensitivity of 95.2%. The test was negative in all 58 endemic and 100 non-endemic controls indicating 100% specificity. The κ coefficient showed there was a good agreement between the results of *KA*tex and those of parasitology testing ($P < 0.001$). However, no significant agreement was observed with the various serological tests (Table 1).

Table 1 **Comparison of number of patients testing positive with *KA*tex, lymph node smear microscopy and serology tests**

Diagnostic test	*KA*tex		κ-value	SE	*P*-value	Concordance
	Negative No.	Positive No.				
LN smear						
Negative	90	28				
Positive	3	59	0.65	0.07	< 0.001	Good
DAT						
Negative	7	1				
Positive	108	88	0.04	0.02	0.04	Negligible
IFAT						
Negative	14	4				
Positive	95	74	0.07	0.04	0.04	Negligible
ELISA						
Negative	16	8				
Positive	70	72	0.08	0.05	0.06	Negligible
Immonoblotting						
Negative	6	2				
Positive	86	74	0.04	0.03	0.12	Negligible

LN = lymph node.
DAT = direct agglutination test.
IFAT = indirect immunofluorescence.
ELISA = enzyme-linked immunosorbent assay.

A cohort of 37 confirmed cases with a positive *KA*tex were actively followed-up for 1 month (19) and 3 months (18) after completion of their treatment. In all cases, *KA*tex became negative after 1 month and patients were considered clinically and parasitologically cured.

The results for the former VL cases are shown on Tables 2–4. In the 24 patients investigated after completion of their treatment (Table 2), *KA*tex was strongly positive (+++ or higher) in all the 5 cases who remained smear-positive when investigated for a test-of-cure. Most of the smear-negative patients (17/19) had a negative *KA*tex; only 2 were positive (with ++ and +++). Of the 10 ex-VL patients who reported for follow-up (Table 3), the test was positive (++) in only 2. All the 7 PKDL cases had negative *KA*tex except 1 patient with a strongly positive test (++++) in whom the LN smear was also positive (Table 4).

DAT was positive in 8 (13.8%) of the 58 *KA*tex negative healthy endemic controls in the Rahad river area. Seven samples had a titre of > 3200 and 1 sample had a titre of 1:3200. In addition, 2 samples had a borderline titre (1:1600).

In the Um El-Khair treatment centre (endemic area), *KA*tex was positive in 7 of 15 confirmed malaria cases; only 2 of these were parasitology-proven VL cases. On the other hand, *KA*tex was negative in all confirmed malaria cases (5) studied in Khartoum state (non-endemic area). The test was also negative in all confirmed TB cases (13 in the non-endemic and 3 in the endemic areas).

HIV infection was confirmed in 8 of the 95 VL suspects who agreed to be tested. The *KA*tex test was strongly positive in 3 of these 8 individuals; 2 of these were parasitology-confirmed VL cases.

Discussion

Clinicians are often faced with a diagnostic dilemma as the clinical features of VL include fever, lymphadenopathy, hepatosplenomegaly, weight loss and hypergammaglobulinaemia, features which are common to other co-endemic diseases. Therefore, the availability of a sensitive, non-invasive diagnostic method that can specifically identify VL early in the disease process is essential.

The sensitivity of the *KA*tex test in the present study (95.2%) was higher than that recorded by Attar et al. (80%) [*23*]. This may be explained by the fact that, in our study, the test was performed on fresh urine samples, while in the previous study samples had been stored for variable periods of time. The sensitivity was considerably higher than in a recent study performed by Rijal et al. in Nepal [*31*] and this may be due to the method of analysis, since they compared *KA*tex to microscopy on splenic aspirates which are more sensitive than lymph node biopsies. The poor agreement with other serological results in our study could in fact be explained if a considerable number of the smear-negative patients were true VL cases, and thus showed positive in the serology. In that case, our sensitivity figure for *KA*tex would be overestimated. On the other hand, the specificity of *KA*tex (100%) was in agreement with previous reports [*23*,*31*].

Cross-reactivity with urine samples from confirmed malaria and TB patients in Khartoum state was not observed. On the other hand, although the test was negative in Um El-Khair centre (in an endemic area) in the 3 confirmed TB cases with negative smears, it was positive in 5 of the 15 con-

Table 2 **Demographic, smear microscopy and serology findings in visceral leishmaniasis (VL) cases investigated for test-of-cure (*n* = 24)**

Sex	Age	VL test results						HIV test
	(years)	LN smear	DAT	*KA*tex[a]	ELISA	IFAT	Immuno-blotting	
M	16	+	ND	++++	+	++	+	–
M	17	+	+	++++	+	++	+	–
M[b]	32	+	+	++++	+	+	++	–
F[b]	45	+	ND	+++	ND	ND	ND	–
F[c]	8	+	+	+++	ND	ND	ND	–
F	40	–	ND	+++	ND	ND	ND	–
M	20	–	ND	++	ND	ND	ND	–
M	4	–	ND	+	+	+++	++	–
M	23	–	+	+	+	+	+	–
M	28	–	ND	+	+	++	+	–
M	8	–	–	+	ND	ND	ND	ND
F	22	–	+	+	+	+	–	–
M	7	–	+	+	–	–	–	–
M	10	–	+	+	–	++	++	–
M	10	–	+	+	–	–	–	–
M	10	–	+	+	+	+	++	–
M	40	–	–	+	ND	ND	ND	–
M	2	–	ND	+	ND	ND	ND	–
M	23	–	ND	+	ND	ND	ND	–
M	21	–	ND	+	ND	ND	ND	–
F	50	–	ND	–	ND	ND	ND	–
F	35	–	ND	–	ND	ND	ND	–
M	21	–	ND	–	ND	ND	ND	–
M	13	–	ND	–	ND	ND	ND	–

[a]*Both + and – indicate a negative test.*
[b]*This patient had post-kala-azar dermal leishmaniasis (Table 4).*
[c]*Isolate was obtained and typed by isoenzymes analysis.*
LN = lymph node.
DAT = direct agglutination test.
ELISA = enzyme-linked immunosorbent assay.
IFAT = indirect immunofluorescence.
ND = not determined.

firmed malaria cases in whom the parasite (*L. donovani*) could not be identified by microscopy using lymph gland aspirates. Due to the low sensitivity of the lymph node smear compared with other parasitological methods [*24*], this finding would need to be further validated with more sensitive parasitological tests in order to rule

Table 3 **Demographic, smear microscopy and serology findings of ex-visceral leishmaniasis (VL) cases who reported for follow-up (*n* = 10)**

Sex	Age (years)	VL test results						HIV test
		LN smear	DAT	*KA*tex[a]	ELISA	IFAT	Immuno-blotting	
M	19	–	+	++	–	+	++	–
M	13	–	ND	++	ND	ND	ND	ND
M	15	–	+	+	–	–	+	–
M	9	–	+	+	–	–	–	–
M	20	–	+	+	–	–	++	–
M	20	–	+	+	+	–	++	–
F	10	–	+	+	–	+	++	–
M	17	–	+	+	–	+	+	–
M	27	–	ND	+	+	+	–	–
M	4	–	+	+	ND	ND	ND	–

[a]*Both + and – indicate a negative test.*
LN = lymph node.
DAT = direct agglutination test.
ELISA = enzyme-linked immunosorbent assay.
IFAT = indirect immunofluorescence.
ND = not determined.

Table 4 **Demographic, smear microscopy and serology findings of post-kala-azar dermal leishmaniasis cases (*n* = 7)**

Sex	Age (years)	VL test results						HIV test
		LN smear	DAT	*KA*tex[a]	ELISA	IFAT	Immuno-blotting	
M[b]	32	+	+	++++	+	+	++	–
F	27	ND	ND	+	+	+	++	–
F	24	ND	+	–	+	++	++	–
F	29	ND	+	–	+	++	++	–
M	5	ND	+	–	+	++	++	–
M	5	ND	+	–	+	+	+	–
M	3	ND	+	–	+	++	+	–

[a]*Both + and – indicate a negative test.*
[b]*This patient was investigated for test of cure (Table 2).*
LN = lymph node.
DAT = direct agglutination test.
ELISA = enzyme-linked immunosorbent assay.
IFAT = indirect immunofluorescence.
ND = not determined.

out or confirm the possibility of co-infection.

Our current results indicate that *KA*tex was negative in all healthy endemic controls who reacted positively in the DAT in spite of the fact that they did not report any past history of VL. In view of these findings, it may be assumed that the test cannot detect pre-patent or sub-clinical infection, which contradicts earlier reports based on animal experimental studies [*23*]. Although this fact, if validated by further studies, limits its usefulness as an epidemiological tool, it emphasizes its diagnostic potential in VL-endemic areas where the inhabitants, including VL cases, develop humoral immune responses to *L. donovani* infection, and the infection remains asymptomatic in the majority of cases [*32*]. Unfortunately, a firm conclusion could not be drawn from the present study, as the studied sample size was too small.

The longitudinal active follow-up for 1 and 3 months of treated confirmed VL cases showed that the test became negative in all the cases who had a positive test prior to treatment. In addition, the test was also negative in 8 of the 10 smear-negative ex-VL cases who reported to the centres for follow-up. Furthermore, of the 24 cases that have been studied for a test-of-cure, the test was strongly positive in all the 5 cases who were found to be smear-positive, and was negative in 17 of the 19 smear-negative cases, which suggests that *KA*tex may be useful in the early detection of treatment failure. These findings are in accordance with previous reports by Attar et al. [*23*], who observed a rapid decline of antigen levels in the urine of experimentally infected animals at week 12, with the test becoming negative before the end of the course of treatment. Other workers recommended the use of a competitive ELISA [*33*] and rK39 recombinant antigen [*34*] for the prognostic evaluation of VL and the success of drug treatment. The question of how rapidly the *KA*tex turns negative during the course of treatment of VL cases needs to be clarified.

An anti-leishmania serological response may not develop in immunocompromised patients with VL, including those with HIV infection. VL has emerged as an AIDS-associated opportunistic infection [*35,36*]. The rK39 dipstick test has shown promise both for the diagnosis and monitoring of patients with HIV infection and VL [*37*]. In this respect, *KA*tex, being an antigen-based detection assay, would be expected to be a useful tool, but this needs to be clarified. Our data indicate that the test was positive in the 2 parasitology-confirmed cases co-infected with HIV infection. These preliminary results may predict the diagnostic potential of this test in this important emerging problem in the Sudan [*38,39*].

It is interesting to note that, with the exception of 1 smear-positive patient, the test was negative in all the 7 PKDL cases who had not been subjected to parasitological study in the treatment centres due to the absence of clinical features suggestive of VL. Furthermore, the test was also negative in all PKDL cases who had been detected in the epidemiological study. These findings may indicate that a positive result for *KA*tex is not only associated with VL disease status, but is also related either to the visceralization of the parasite or to the parasite load.

The strong agreement between *KA*tex and lymph node aspirate smear results indicates that, unlike DAT [*14*], the test may discriminate between active disease and infection (including sub-clinical and past infections). Thus, being a rapid test in addition to its specificity, *KA*tex can be extremely useful for complementing DAT for the detection of the disease in the consider-

able number of cases for whom parasitological confirmation cannot be achieved, particularly when this depends on lymph gland aspiration, the least invasive but also the least sensitive procedure [*40*].

We conclude that *KA*tex is a point-of-care test, which represents a useful addition to the diagnostic and therapeutic algorithms for VL, and is particularly well suited for use in field conditions. Further studies are recommended to evaluate the performance of the test in the diagnosis of HIV co-infected cases.

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References

1. De Beer P et al. Outbreak of kala-azar in the Sudan. *Lancet*, 1990, 335:224.
2. De Beer P et al. Killing disease epidemic among displaced Sudanese population identified as visceral leishmaniasis. *American journal of tropical medicine and hygiene*, 1991, 44:283–9.
3. El-Safi S et al. *The current situation of kala-azar in Gadarif area, eastern Sudan.* Tunis, Archives de L'Institut Pasteur de Tunis, 1994.
4. Seaman J, Mercer AJ, Sondorp E. The epidemic of visceral leishmaniasis in western Upper Nile, southern Sudan: course and impact from 1984 to 1994. *International journal of epidemiology*, 1996, 25:862–71.
5. El-Safi S et al. Recent observations on the epidemiology of visceral leishmaniasis in Atbara River Area, Eastern Sudan: the outbreak of Barbar El-Fugara village. *Microbes and infection*, 2002, 4:1439–47.
6. Veress J et al. Morphological observations on visceral leishmaniasis in the Sudan. *Tropical geographical medicine*, 1974, 26:198–203.
7. Badaro R, Reed SG, Carvalho EM. Immunofluorescent antibody test in American visceral leishmaniasis: sensitivity and specificity of different morphological forms of two *Leishmania* species. *American journal of tropical medicine and hygiene*, 1983, 32:480–4.
8. Hommel M et al. Micro-ELISA techniques in the serodiagnosis of visceral leishmaniasis. *Annals of tropical medicine and parasitology*, 1978, 72:213–8.
9. El-Safi SA, Evans DA. A comparison of the direct agglutination test and enzyme-

linked immunosorbent assay in the serodiagnosis of leishmaniasis in the Sudan. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 1989, 83:334–7.

10. Badaro R et al. rK39: a cloned antigen of *Leishmania chagasi* that predicts active visceral leishmaniasis. *Journal of infectious diseases*, 1996, 173:758–61.
11. Al-Assad et al. The significance of blood levels of IgM, IgA, IgG and IgG sub classes in Sudanese visceral leishmaniasis. *Clinical and experimental immunology*, 1994, 95:294–9.
12. El-Harith A et al. A simple and economical direct agglutination test for serodiagnosis and sero-epidemiological studies of visceral leishmaniasis. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 1986, 80:583–6.
13. El-Harith A et al. Improvement of a direct agglutination test for field studies of visceral leishmaniasis. *Journal of clinical microbiology*, 1988, 26:1321–5.
14. Zijlstra E et al. Direct agglutination test for diagnosis and sero-epidemiological survey of kala-azar in the Sudan. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 1991, 85:474–6.
15. Hommel M. Visceral leishmaniasis: biology of the parasite. *Journal of infection*, 1999, 39:101–11.
16. Sehgal S, Aikat BK, Pathania AGS. Immune complexes in Indian kala-azar. *Bulletin of the World Health Organization*, 1982, 60:945–50.
17. Galvao-Castro B et al. Polyclonal B cell activation, circulating immune complexes and autoimmunity in human American visceral leishmaniasis. *Clinical experimental immunology*, 1984, 56:58–66.
18. Azazy AA, Devaney E, Chance ML. A PEG-ELISA for the detection of *Leishmania donovani* antigen in circulating immune complexes. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 1994, 88:62–6.
19. Azazy AA, Chance ML, Devaney E. A time-course study of circulating antigen and parasite-specific antibody in cotton rats infected with *Leishmania donovani*. *Annals of tropical medicine and parasitology*, 1997, 91:153–62.
20. Kohanteb J, Ardehali SM, Rezai HR. Detection of *Leishmania donovani* soluble antigen and antibody in the urine of visceral leishmaniasis patients. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 1987, 81:578–80.
21. Senaldi G et al. Serological diagnosis of visceral leishmaniasis by a dot-enzyme immunoassay for the detection of a *Leishmania donovani*-related circulating antigen. *Journal of immunological methods*, 1996, 193:9–15.
22. Urnovitz HB et al. Urine-based diagnostic technologies. *Trends in biotechnology*, 1996, 14:361–4.
23. Attar ZJ et al. Latex agglutination test for the detection of urinary antigens in visceral leishmaniasis. *Acta tropica*, 2001, 78:11–6.
24. Siddig M et al. Visceral leishmaniasis in the Sudan: comparative parasitological methods of diagnosis. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 1988, 82:66–8.
25. Zijlstra EE, El-Hassan AM, Ismael A. Endemic kala-azar in Eastern Sudan: post kala-azar dermal leishmaniasis. *American Journal of Tropical Medicine and Hygiene*, 1995, 52 299–305.
26. El-Harith A et al. Evaluation of cleaving agents other than trypsin in DAT for further improving diagnosis of visceral

leishmaniasis. *Journal of clinical microbiology*, 1995, 33:1984–8.

27. Pratlong F et al. Sudan: the possible original focus of visceral leishmaniasis. *Parasitology*, 2001, 122:599–605.

28. Edrissian GH et al. Application of the indirect fluorescent-antibody test in the serodiagnosis of cutaneous and visceral leishmaniasis in Iran. *Annals of tropical medicine and parasitology*, 1981, 75: 19–24.

29. Mary C et al. Western blot analysis of antibodies to *Leishmania infantum* antigens: potential of the 14-kD and 16-kD antigens for diagnosis and epidemiologic purposes. *American journal of tropical medicine and hygiene*, 1992, 47(6):764–71.

30. Landis J, Koch JJ. The measurement of observer agreement for categorical data. *Biometrics*, 1977, 33:159–74.

31. Rijal S et al. Evaluation of a urinary antigen based latex agglutination test in the diagnosis of kala-azar in Eastern Nepal. *Tropical medicine and international health*, 2004, 9(6):724–9.

32. Badaro R et al. New perspectives on a subclinical form of visceral leishmaniasis. *Journal of infectious diseases*, 1986, 154:1003–11.

33. Chatterjee M et al. Diagnostic and prognostic potential of a competitive enzyme-linked immunosorbent assay for leishmaniasis in India. *Clinical diagnostic laboratory immunology*, 1999, 6:550–4.

34. Singh S et al. Diagnostic and prognostic value of rK39 recombinant antigen in Indian leishmaniasis. *Journal of parasitology*, 1995, 81:1000–3.

35. Peters BS et al. Visceral leishmaniasis in HIV infection: clinical features and response to therapy. *Quarterly journal of medicine*, 1990, 77:1101–1.

36. Rosenthal E et al. Visceral leishmaniasis and HIV co-infection in Southern France. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 1995, 89:159–2.

37. Houghton RL et al. A cloned antigen (recombinant rK39) of *Leishmania chagasi* diagnostic for visceral leishmaniasis in human immunodeficiency virus type 1 patients and a prognostic indicator for monitoring patients undergoing drug therapy. *Journal of infectious diseases,* 1998, 177:1339–44.

38. El-Safi S, El-Khidir I. *HIV/leishmania co-infection in the Sudan (preliminary results)*. Paper presented at the at the Consultative Meeting on Leishmania/ HIV co-infection, Minorca, Spain 26–29 September 1998.

39. El-Safi S, El-Khidir I. *HIV/leishmania co-infection in the Sudan.* Paper presented at the Pan African Workshop on Asthma, Allergy and Immunology, Zimbabwe, 24–27 September 1999.

40. Zijlstra E et al. Kala-azar: a comparative study of parasitological methods and the direct agglutination test. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 1992, 86:505–7.