

## Rapid, Noninvasive Diagnosis of Visceral Leishmaniasis in India: Comparison of Two Immunochromatographic Strip Tests for Detection of Anti-K39 Antibody

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**Used with blood or serum, a new anti-K39 antibody immunochromatographic strip test (IT-Leish; DiaMed AG) proved sensitive (range, 99 to 100%) and specific (range, 95 to 100%) for the noninvasive serodiagnosis of visceral leishmaniasis in India. Used with serum, the IT-Leish test and the existing Kalazar Detect test (InBios International, Inc.) yielded comparable results for symptomatic infection and identified apparent subclinical infection in 15 to 32% of healthy residents in a region where visceral leishmaniasis is highly endemic.**

One approach to diagnosis of visceral leishmaniasis (VL) (kala-azar) involves an immunochromatographic strip test that detects immunoglobulin G (IgG) antibody to recombinant K39, an antigen expressed by leishmanial species that produce VL (1, 3, 4, 13, 15–19, 21). This rapid, inexpensive, and noninvasive field-suitable test requires one drop of whole blood or serum and in India shows high sensitivity and specificity in the diagnosis of both VL and post-kala-azar dermal leishmaniasis (PKDL) (7, 12, 15–17). At present, only one K39-based strip test (Kalazar Detect; InBios International, Inc., Seattle, Wash.) is commercially available (17). Development of a new K39-based strip test (IT-Leish; DiaMed AG, Cressier sur Morat, Switzerland) provided the opportunity to reevaluate this form of noninvasive serodiagnosis and compare the two strip tests.

This study, conducted at Muzaffarpur and Varanasi, India, the two study sites of Kala-Azar Medical Research Center, Banaras Hindu University (Varanasi, India), was approved by the center's Ethics Committee. As shown in Table 1, samples tested were blood and/or sera from (i) 206 untreated patients with clinically active VL, proved parasitologically by demonstration of amastigotes in splenic smears; (ii) 25 patients with documented PKDL; and (iii) 365 individuals who did not have VL or PKDL, including 150 patients symptomatic with other diseases which produce fever and/or splenomegaly and 215 healthy residents of either a region of low endemicity ( $n = 113$ ; Varanasi, Uttar Pradesh State, India) or a region of high endemicity ( $n = 102$ ; Muzaffarpur, Bihar State, India).

In a single-use package, the new test format (IT-Leish) includes a test strip mounted on a plastic cassette, a detachable tray containing a conjugate well and a wash well, two ampoules of buffer and one (each) pipette, lancet, plastic capillary tube,

and alcohol swab. One drop of buffer was added to the conjugate well, and four drops of buffer were added to the wash well. A preset volume (level with a mark on the capillary tube) of either blood obtained by finger prick or serum was added to the conjugate well and mixed with the buffer for 1 min. The strip was placed upright for 10 min in the conjugate well until the buffer-blood solution had been absorbed and then for 10 min in the wash well. Appearance of a purple upper control line indicated the presence of IgG and correct strip test functioning; a second lower purple line indicated the presence of anti-K39 IgG. The test strip membrane is coated with a band of recombinant K39 antigen and above the band with immobilized anti-protein A antibody to detect IgG; protein A-gold conjugate is used as the detection reagent. Anti-K39 IgG reacts with the protein A-gold conjugate and the mixture moves up the strip by capillary action to react with the K39 antigen, giving rise to a colored band in the test area. The Kalazar Detect test consists of individually packaged strips and a separate bottle containing buffer. According to the manufacturer, this test is not intended for use with whole blood; therefore, only serum was tested by the Kalazar Detect test. One drop of serum and five drops of buffer were mixed in a washerman's tube, and the lower end of the strip was allowed to soak in the solution; the mixture moved up the strip by capillary action. After 10 min, two pink bands indicated the presence of anti-K39 IgG and a positive result (17).

In samples from patients with VL, the IT-Leish test detected anti-K39 antibody in essentially all specimens of blood and serum (sensitivity range, 99 to 100%) (Table 1). The Kalazar Detect test showed a similarly high level of sensitivity (range, 98 to 100%), as it has in other regions of endemicity (4, 6). Other anti-K39 strip tests also have been or are now being evaluated (13, 16, 19, 20), and most (but not all) (20) reports have shown high sensitivity (range, ~90 to 100%). Both test formats also detected anti-K39 in serum from most patients with PKDL.

For symptomatic patients with other diseases, the relevant control group used to test specificity, the IT-Leish test also

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TABLE 1. Anti-K39 strip-test results for patients with VL or PKDL and for control subjects

Group and specimen type (disease) <sup>a</sup>	No. of samples tested	No. of samples positive by <sup>b</sup> :	
		IT-Leish test	Kalazar Detect test
Patients with VL or PKDL			
Blood (VL)	100	99 (99, 94–100)	Not tested
Serum (VL)	100	100 (100, 95–100)	100 (100, 95–100)
Banked serum (VL)	106	106 (100, 96–100)	104 (98, 93–100)
Banked serum (PKDL)	25	24 (96, 78–100)	23 (92, 72–100)
Patients with other Diseases			
Blood	54	0 (0, 0–8)	Not tested
Serum	150	7 (5, 2–10) <sup>c</sup>	4 (3, 1–7) <sup>d</sup>
Healthy individuals from a region of low endemicity			
Blood	113	0 (0, 0–4)	Not tested
Serum	113	0 (0, 0–4)	3 (3, 1–8)
Healthy individuals from a region of high endemicity			
Blood	102	15 (15, 9–23)	Not tested
Serum	102	32 (32, 23–41)	24 (24, 16–33)

<sup>a</sup> Samples listed as blood or serum were freshly obtained from the same individuals. Banked serum denotes frozen samples from previous patients. For patients with other diseases, 54 blood and serum samples were freshly obtained from the same individuals and 96 serum samples had been previously banked. The numbers of patients with other diseases were as follows: tuberculosis, 70; malaria, 44; leprosy, 6; typhoid fever, 10; and chronic myelogenous leukemia, 20.

<sup>b</sup> Where two values are shown in parentheses, the first is a percentage, and the second is the 95% confidence interval.

<sup>c</sup> The seven patients had the following other diseases: tuberculosis (four patients), malaria (two patients), chronic myelogenous leukemia (one patient).

<sup>d</sup> The four patients had the following other diseases: tuberculosis (two patients), malaria (two patients).

performed well, showing 95 to 100% specificity (Table 1). While none of 54 blood specimens was reactive, sera from 7 of 150 patients (5%) with fever and/or splenomegaly due to other causes showed a positive IT-Leish result (3% reactivity [4/150] by the Kalazar Detect test). Thus, clinical caution should be exercised in interpreting a positive result that, for a small number of symptomatic patients, could lead to an incorrect diagnosis of VL and unwarranted treatment. When the result of either strip test is positive, then, clinicians may need to consider other common diseases that may simulate VL (e.g., malaria, tuberculosis, typhoid fever, or human immunodeficiency virus infection) (17). Nevertheless, in India, anti-K39 strip test results for symptomatic patients with suspected VL can be used as the sole diagnostic test and safely direct antileishmanial therapy (17). For symptomatic patients in other regions where leishmaniasis is endemic, the specificity of the Kalazar Detect test as well as other formats has been generally (range, 92 to 100%) (3, 4, 6, 8, 9, 13, 18) but not uniformly (range, 59 to 81%) high (2, 5, 14, 19).

No sample from 113 healthy residents of an area of low endemicity showed a positive reaction in the IT-Leish test; however, results were positive for both blood (15%) and serum (32%) samples from 102 healthy residents of a region of high endemicity (Table 1). Why serum was more often reactive is unclear, but the higher reactivity may simply reflect that concentration of anti-K39 IgG is higher in serum than in whole blood.

We believe the reactions for these healthy individuals are not false positives and likely reflect unrecognized subclinical infection. The Kalazar Detect test also yielded similar results with serum from this patient group (24/102 [24%] reactive), consistent with prior observations from researchers using this test format in the same region (15). There is really no clinical reason to test an asymptomatic individual. Nevertheless, misdiagnosis of VL in a person with previously acquired subclin-

ical infection and a positive anti-K39 strip test result who subsequently develops an illness which mimics VL can be envisioned (15, 17). Thus, though the test avoids an invasive diagnostic procedure, some caution must still be exercised when diagnosing VL on the basis of a positive anti-K39 strip test. Anti-K39 IgG can also persist in the serum for months after successful treatment in kala-azar; therefore, strip tests cannot be used to diagnose suspected relapse of VL (1, 10, 11, 20).

The new IT-Leish test is straightforward to use, and the assay is suitable for field sites since it requires a minimal volume of blood (or serum) and no laboratory sophistication or expertise in interpretation. Individual self-contained packs provide an additional measure of convenience (by contrast, in the Kalazar Detect test, the strip is supplied in a separate pouch, but the buffer is packed separately for use with multiple strips.) Cost and shelf-life for the Kalazar Detect and IT-Leish tests are comparable, with costs being US\$1.00 and 1.20, respectively, and shelf-lives being 1.5 and 1.17 years, respectively.

Overall, results derived from the IT-Leish test appeared similar to those generated with the Kalazar Detect test, an immunochromatographic strip test intended for testing serum and already extensively evaluated in India and elsewhere (2, 5, 7, 17). Anti-K39 strip testing using blood obtained by finger prick is certainly simple and more convenient than that using serum, providing the flexibility of performing the IT-Leish test essentially under any field conditions. While blood may be acceptable for diagnosis of symptomatic cases, the sensitivity of the test may be higher with serum, and when available, serum should preferably be used for strip testing.

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