

# Evaluation of rK-39 strip test using urine for diagnosis of Kala-azar in Bangladesh

## Key Messages:

- Diagnosis of Kala-azar or visceral leishmaniasis (VL) by demonstration of parasites in tissue smears obtained from bone marrow, spleen or lymph nodes is risky, painful, and difficult.
- The rK-39 strip test is widely used for the diagnosis of VL using blood/serum samples in endemic countries.
- The study aim was to evaluate the rK-39 strip test using urine sample as a non-invasive means for the diagnosis of VL. The rK-39 strip test was performed using urine compared with blood/serum samples from 100 suspected VL cases along with 25 disease control (malarial febrile cases) and 50 healthy control (from endemic and non-endemic areas).
- All the VL suspected cases were positive with the rK-39 strip test using serum. The sensitivity and specificity of the rK-39 strip test using urine samples was 95% and 93.3%, respectively, compared to serum based rK-39 test.
- The findings suggest the urine based rK-39 test could be a practical and efficient tool for diagnosing VL patients in rural areas, particularly where resources are limited.

## Introduction:

Kala-azar or Visceral leishmaniasis (VL) is a serious public health problem in Bangladesh where 20 million people (18% of the total population) are at risk with a trend of rising incidence [1]. Diagnosis of VL still relies on clinical manifestations and microscopic confirmation of parasites from aspirates of lymph nodes, bone marrow, and the spleen. These invasive and painful techniques require skilled personnel and are difficult to implement in resource-limited settings. Several less invasive serological tests like IFAT, ELISA and DAT have been evaluated for the diagnosis of VL [2,3,4]. However, a rapid immunochromatographic test (ICT) based on a recombinant 39-amino acid repeat antigen, conserved in the kinesin region of *Leishmania chagasi* and *Leishmania donovani* (rK-39 strip test), gained popularity for the field screening of kala-azar [5] because it was simple, cheap and reliable. Recently, a low molecular weight, heat-stable, and carbohydrate based leishmanial antigen has also been detected in urine of VL patients [6]. It is hypothesized that antibody detection tests, specifically the rK-39 strip test, will also detect anti-leishmanial antibody in urine.

The serum-based rK-39 will be used to validate the value of the urine-based rapid test for the primary diagnosis of VL.

## Study area and population:

In total, 100 suspected VL patients, who were positive with the serum based rK-39 strip test and had fever for at least two weeks, along with other clinical signs, were enrolled in this study from Trishal Upazila (sub-district) Health Complex (UHC) in Mymensingh district, one of the most endemic VL regions in Bangladesh. All VL subjects were treated free of charge in the UHC as per the National Guideline and WHO recommendations. To investigate cross-reaction with other diseases, 25 subjects with malaria were enrolled from a malaria-endemic area. To investigate subclinical infection, 25 healthy controls were enrolled who lived in the endemic area (Trishal) but did not have a past history of VL. Twenty-five (25) healthy controls from non-endemic area were also enrolled for assessing the specificity of the urine rK-39 strip test. The serum rK-39 test was performed again in the field setting, a small laboratory in Trishal, whereas the urine rK-39 test was performed in the Parasitology Laboratory, icddr,b in Dhaka.

## Sample collection and methods:

Finger-prick blood was taken in a capillary tube and transferred to a micro-tube (200 µm). Urine samples were

also collected in a tube containing preservative (Na-azide) and stored at 4°C until transporting to icddr,b. The blood sample was then centrifuged for separation of serum at the field laboratory (Trishal) where the rK-39 strip test (Kalaazar Detect™, InBios Inc., USA) was also performed as per the protocol of the manufacturer. Briefly, 1 drop of serum samples was applied to the base of strips. After being air-dried, 3 drops of the test buffer were added. The appearance of a lower red band (control) indicated the proper functioning of the test while the appearance of an upper red band indicated the presence of anti-rK-39 IgG, signifying a positive test. For the urine assay, 3 drops of urine sample were applied directly to the strip without adding any test buffer. In both the cases the strip was observed after 10 minutes for the test band.



## Results and Discussion:

According to the national guideline for the treatment of VL in Bangladesh, suspected kala-azar cases must be confirmed by a positive rK-39 for serum antibodies or demonstration of parasite in the tissue (bone marrow/splenic puncture) or by PCR. The ICT based rK-39 antibody test has been used widely in Bangladesh for the diagnosis of VL because of its high sensitivity and specificity [5]. According to the instruction of the manufacturer, this test is performed using serum or plasma for which collection of venous blood or finger-prick is necessary. But our study showed excellent sensitivity and specificity levels for the rK-39 dipstick test using a non-invasive procedure, i.e. urine samples. The serum rK-39 strip test was positive in 100 enrolled VL subjects whereas all the healthy controls from the endemic area and non-endemic area and all the diseased controls (confirmed malaria subjects) tested negative. The urine rK-39 strip test was positive in 95 out of

## References

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the 100 VL subjects and in five of the 25 confirmed malaria patients (who tested negative by the serum rK-39 strip test). However, the urine rK-39 tested negative in all the healthy controls from the VL endemic and non-endemic areas. Thus, the sensitivity and specificity of urine rK-39 was found to be 95% (95% CI: 88.2-98.1) and 93.3% (95% CI: 84.5-97.5), respectively, considering the serum rK-39 test result as the gold standard (Table 1). Kappa coefficient (k) for the urine rK-39 strip test was found 0.88.

**Table 1: Comparison of urine and Serum based rK-39 strip test in the diagnosis of clinically suspected VL**

Patient type	Serum rK- 39 test		Urine rK-39 test	
	+ve	-ve	+ve	-ve
No. of VL case	100	0	95	5
No. of Malaria	0	25	5	20
No. of Non endemic healthy control	0	25	0	25
No. of VL Endemic healthy control	0	25	0	25
Total	100	75	100	75

kappa (k): 0.88

The urine-based rK-39 test has great advantages over the serum-based test because of ease of sample collection without causing any discomfort or pain to the subject. The non-invasive urine collection procedure minimizes the risk of blood-borne infections and facilitates the collection of samples from anemic patients, infants and children.

## Conclusion:

The urine rK-39 strip test is a promising non-invasive point-of-care tool for the rapid screening of VL and could be used in remote rural areas where there is a high prevalence of VL.

## Recommendation:

A large scale field evaluation of the urine rK-39 strip test is required before using it as a diagnostic tool for VL patients in different endemic areas.