राष्ट्रीय वैक्टर जनित
रोग नियंत्रण कार्यक्रम
NVBDCP

GUIDELINES

ON

USE OF rK39

Government of India

Dte. of National Vector Borne Disease Control Progamme
22-Sham Nath Marg, Delhi – 110 054
Ph. No. 011-23967745/23967780, Fax No. 011-23968329/23972884
Website: www.namp.gov.in

GUIDELINES ON USE OF rK39 FOR DIAGNOSIS OF KALA-AZAR IN THE ENDEMIC STATES ON A PILOT BASIS

GUIDELINES ON THE USE OF rK39

1. Preamble:

Visceral leishmaniasis or Kala-azar is a intracellular protozoal infection caused by Leishmania donovani and transmitted by phlebotomine sandflies. Kala-azar is a major public health problem in the areas of its prevalence, principally India and its neighbors Bangladesh and Nepal, and Brazil and Sudan. In India the disease is found in Bihar, Jharkhand, West Bengal and pockets of eastern Uttar Pradesh. A national health programme to eliminate the disease by 2010 is in operation in India. The programme relies on case management, vector control, community involvement in control activities and capacity building as the principal components of the elimination strategy. The Kala-azar elimination programme is a centrally sponsored programme, under which the cost of all materials, i.e. insecticides, diagnostic kits, drugs and cost of operations is borne by the Central Government.

Diagnosis and treatment of Kala-azar are problematic because of a variety of reasons. While treatment is lengthy and relatively costly, definitive diagnosis of Kala-azar requires tissue specimens, which are conventionally obtained by organ needle aspiration for microscopic demonstration of amastigote forms in stained smears. Bone marrow and the spleen and, in some regions, lymphnode are the tissues most often sampled in patients with suspected infection. The diagnostic sensitivity of splenic aspiration is high (95% - 98%), but the procedure carries a risk of bleeding; the sensitivity of examination of bone marrow specimens is considered to be lower (53% - 95%). Organ aspiration and accurate examination of smears also require technical skills that are not uniformly available in rural areas. Culture or PCR testing of aspirate material improves parasitologic yield, but these methods are seldom undertaken outside of research laboratories [(1).]

In the Kala-azar endemic areas of India, Napier's aldehyde test has been used for a long time. The test relies on the jellification caused by the binding of the serum globulins to the formaldehyde. The serum globulins increase in a variety of infections and thus this test is rather non-specific. A positive reaction may also be seen in diseases like, Tuberculosis, Cirrhosis of liver, Malaria, etc. Further, in kala-azar, the test becomes positive only when infection is atleast three months old and may remain positive even after six months of cure.

Therefore a rapid, accurate, field suitable and non-invasive method of diagnosis of kala-azar has long been sought to circumvent the need for obtaining tissue specimens and preceding limitations. A range of assays have been developed to detect antileishmanial antibody. The complement fixation test detects specific antibodies present in the serum. The test though more sensitive as compared to aldehyde test, shows cross reaction in cases of pulmonary tuberculosis, leptomonas leprosy and Mycobacterium infections.

Immuno-flourescent antibody test (IFAT) in which parasite antigen labeled with fluorescent dye is conjugated with serum antibodies and seen under fluorescent microscope has also been widely used.

Indirect hemagglutination test (IHA) is also based on the principle of antigen-antibody reaction. The serum antibodies are conjugated with parasite antigens to observe agglutination.

Enzyme Linked Immunosorbant Assay (ELISA) utilizes soluble antigen or sonicated extract of promastigotes to capture antibodies specific to Leishmania. Though sensitive and specific, it may give cross reactions with infections like, malaria, tuberculosis, leprosy, etc. at very low litres.

Direct Agglutination Test (DAT) another test widely used for serodiagnosis of kala-azar is based on antigen-antibody reaction. Trypsin treated, stained and formalin preserved promastigotes are used as antigen which show agglutination with specific antibodies present in patients serum. The test is performed at room temperature though the antigens are stored under controlled temperature in freezer.

The usefulness of the above mentioned serological tests is limited by, their variable sensitivity or specificity, requirement of electricity, refrigeration, or a well-equipped laboratory and high cost.

Recently developed rapid dip-stick test – rK39 is the option available now to diagnose kala-azar cases at the grass roots in conjunction with the clinical diagnosis.

rK39:

Now a rapid dipstick test based on the recombinant K39 protein is available for rapid diagnosis of kala-azar. K39 is an epitope apparently conserved on amastigotes of *Leishmania* species that cause visceral infection; by use of laboratory ELISA testing, circulating anti-K39, IgG is detectable in 95%-100% of patients who have kala-azar, irrespective of geographic region. Using K39 antigen-impregnated nitrocellulose strips developed for field conditions, fingerstick-obtained blood and serum samples tested from Indian subjects demonstrated a positive anti-K39 immunochromatographic reaction in 362 patients with aspirate-proven kala-azar; with an estimated sensitivity of 100% and a specificity of 97%. The strip testing proved simple to perform and yielded results within five minutes [(1)].

The rapid diagnostic test when evaluated in comparison to the diagnostic performances of DAT, based both on freeze-dried and liquid antigens, on parasitologically confirmed Kala-azar and Post-Kala-azar Dermal Leishmaniasis (PKDL), the sensitivity of the tests was almost 100%, rK39 was found, to be more sensitive. **It was concluded that these tests are comparable to parasitology in terms of their sensitivity and can replace parasitology as the basis for a decision to treat visceral leishmaniasis at peripheral health centers in endemic areas.**

Considering the field evaluation results, the expert committee under the Chairmanship of Dr. S.P. Agarwal, DGHS, considered that the rapid dipstick test based on rK39 can be introduced into the Kala-azar elimination programme.

2. The antigen: The recombinant antigen is a 39-amino acid (rK39) cloned in **Escherichia Coli,** from the C terminus of the kinesin protein of Leishmania major in India. The rK39 rapid diagnostic test has undergone extensive evaluation and has been found to be highly sensitive and specific in the diagnosis of both VL and PKDL.

3. Kala-azar Case Definition for enrolling a subject for dipstick testing is as follows,

> *"a case presenting to a clinician with a fever of more than two weeks duration, with splenomegaly and not responding to the full course of anti-malarials, should be subjected to rK39 dipstick test."*

The rK39 diagnostic test and miltefosine, would be used on a pilot basis in a few kala-azar endemic districts, to facilitate rapid diagnosis of the disease and complete treatment with an easy to administer oral drug, at the community level. These are decisive interventions in kala-azar control, as they are expected to reduce the parasitic load in the community and thereby diminish transmission of the disease, apart from alleviating suffering of communities and generating greater confidence in the control programme. Thus experience gained in the piloting rK39 for rapid diagnosis and miltefosine for treatment of kala-azar, would facilitate their deployment on a foolproof basis in the rest of the endemic areas. The concurrent evaluation, for a feedback to the programme, is thus very important. The expert group on the introduction of rapid diagnosis and miltefosine into the programme had **recommended at least five percent of the suspected kala-azar cases**, conforming to the case definition of kala-azar and reporting to the PHC for rapid diagnosis should be subjected to splenic aspirate examination. The cases, to be referred for splenic aspiration, will be only those reporting to the Additional PHC's within very short distances of the higher centers of diagnosis and treatment. The Chief Medical Officer, of the district will designate such centers, and referrals will only be made to such centers, after ensuring that splenic aspiration is to be conducted by experienced and skilled personnel, in properly equipped laboratories.

In case a suspected patient of kala-azar referred for splenic aspiration is found to be negative for parasites, he/she may be referred to a higher center, according to the discretion of the medical officer.
The data will be recorded in the accompanying format.

4. Exclusion Criteria: The rK39 is not to be used in the following cases:

- Kala-azar relapses
- In cases of kala-azar re-infection
- kala-azar and HIV co-infection

5 Area Selection: Following areas are being piloted for rK39 dipstick diagnosis.

	State	**Districts**
1.	Bihar	Vaishali, Saran, Patna & Muzaffarpur.
2.	West Bengal	South 24-Pargana, Malda, Murshidabad
3.	Uttar Pradesh	Varanasi
4.	Jharkhand	Sahebganj

All Additional PHC's will use the dipstick tests and proper records will be maintained of the use of all materials.

6. Supply of Kits: Each of the districts will be supplied kits numbering 8 times the reported Kala-azar cases of 2004. For example, Vaishali district can be supplied 952 X 8 = 7616 nos. of dipsticks based on the reported incidence of 2004. More kits can be considered to be provided to the districts if there is more demand.

7. Kit Contents : Kala-azar dipstick test strip's is a membrane, pre-coated with a recombinant VL antigen on the test line region and chicken anti-protein A on the control line region. The Kit contains the following:

1. Twenty-five (25) individually pouched Test Strips or twenty-five (25) test strips in a vial with desiccant in the cap.
2. One vial of Chase Buffer solution.

8. Principle: The kala-azar dipstick rapid test is a immunochromatographic assay for **qualitative** detection of antibodies to **L. donovani** in human serum. The assay is for aid in the presumptive diagnosis of VL.

During testing the serum sample reacts with the dye conjugate (Protein A-Colloidal Conjugate, which has been pre-coated in the test device). The mixture then migrates upwards on the membrane chromatographically by capillary action to react with rK39 antigen on the membrane and generate a red line. Presence of this red line indicates a positive result while its absence indicates a negative result.

Regardless of the presence of antibody to VL antigen, as the mixture continues to migrate across the membrane to the immobilized chicken anti-protein A region, **a red line at the control line will always appear.** The presence of this red line serves as a verification for sufficient sample volume and proper flow and is a control for the reagents.

9. Test Procedure:

1. 1 or 2 drops of finger prick blood may be assayed for anti K39 IgG.

2. Remove the Kala-azar dipstick strip from the pouch or vial

3. Place one drop of blood on the absorbent pad on the strip bottom.

4. Place the test strip into a test tube so that the end of the strip is facing downward.

5. Allow the mixture to migrate upto the strip by capillary action.

6. Add 2-3 drops of the Buffer solution provided with the test kit to the pad.

7. Read the results in 10 minutes. It is significant that the background is clear before reading the test, especially when samples have low titer of anti-Leishmanial antibody, and only a weak band appears in the test region (T). Results interpreted after 10 minutes can be misleading.

10. Interpretation:

A positive result: The test is positive when a control line and test line appear in the test area. A positive result indicates that the Kala-azar dipstick detected antibodies to *L.donovani*. **A faint line is a positive result.** The red color in the test region will vary depending on the concentration of anti-Leishmanial antibodies present. The test line for "weakly positive" sera samples may show results between a weak positive red line to a faintly red, almost white background. ("Weakly positive" samples are those with low affinity or low titer antibodies against the recombinant test antigen).

A Negative Result: The test is negative when only the control line appears. A negative result indicates that the Kala-azar dipstick did not detect antibodies to L. donovani.

An Invalid Result: If no lines appear at either the control or test line areas the test is invalid. The test is also invalid if no control line appears, even though a test line is seen. It is recommended to retest using a fresh dipstick and fresh blood sample should be used in such a case.

11. Storage: The sealed pouch or vial containing the test strip along with the buffer vial is designed to be stored at room temperature (20^0C - 28^0C) for the duration of its shelf life. Exposure to temperatures over 30^0C can impact the performance of the test and should be minimized. **The strips should not be frozen.** The test should be **used within one hour after removal from the pouch or vial to prevent exposure to humidity.**

The shelf life of dip-stick is quiet long so the dip-stick can be easily stored in Primary Health Centre (PHC), in the endemic states.

12. Training: Expert Group recommended that training is a crucial input for success of these new diagnostic and treatment strategies. The trainings on rapid diagnosis will be part of the overall capacity building exercise to be undertaken by NVBDCP with the assistance of IMS, Benaras and RMRIMS, Patna and other identified institutes by states. The training of technicians may be undertaken by the Institute of Medical Sciences, Benaras & RMRIMS, Patna, to be carried out in batches of 30 to 40 technicians and may be completed in about 10 batches. Funds for carrying out these trainings can be provided by the NVBDCP. The separate course for the Medical Officers of the Additional PHC's of endemic states can be organized by RMRIMS, Patna.

13. IEC: There is always a tendency to resist a novel strategy. The IEC campaigns to promote compliance of diagnostics and treatment need to be emphasized that the dip-sticks are an easy to perform, simple procedure, and they do not entail any cost to the individual. It may also need to be emphasized that dip-stick diagnosis will be followed by free treatment of the kala-azar disease with miltefosine.

14. Supervision: Supervision is a critical input in the effective implementation of any activity. It is a positive contribution from the supervisor and not just fault finding.

At the district officers level following should be ensured.

- That serological diagnosis of the kala-azar suspected cases with rK39 is carried out after obtaining precise clinical information, and is not applied to random fever cases. The rK39 confirmed cases are to be enrolled for miltefosine treatment, and these strips are not wasted on randomly selected fever cases.
- That test are performed and interpreted strictly according to the national guidelines.
- To ensure that there is no pilferage of the test dipsticks to private channels.
- That the storage of the dipsticks is in the desiccants, kept in cool, safe places, away from excessive humidity and temperature.
- That the reports and records match with the actual beneficiaries of treatment.
- That the community leaders are informed about the new diagnostic procedure, and suspected cases are encouraged to avail of the facility.

NATIONAL VECTOR BORNE DISEASES CONTROL PROGRAMME

MONTHLY PROGRESS REPORT OF THE UTILIZATION OF rK39 DIP-STICKS (KALA-AZAR ELIMINATION PROGRAMME)

PHC ……

District …… **Month ……..**

MONTHLY PROGRESS								PROGRESSIVE TOTAL						
Balance of rK39 test available as on Ist day of the month	No. of rK39 tests received from district during the month	Total stock of rK39 tests during the month	No. of suspected cases detected during the month		No. of rK39 tests (sample) conducted	**No. of cases confirmed with rK39**		Balance of rk39 tests available at the end of the month	Total No. of rK39 tests available upto reporting month	Total No. of rK39 tests used upto reporting month	No. of suspected cases detected upto reporting month		**No. of cases confirmed with rK39 tests upto reporting month**	
			Kala-azar	PKDL		**Kala-azar**	**PKDL**				Kala-azar	PKDL	**Kala-azar**	**PKDL**

Medical Officer I/c :
PHC :

NATIONAL VECTOR BORNE DISEASES CONTROL PROGRAMME

MONTHLY PROGRESS REPORT OF THE UTILIZATION OF rK39 DIP-STICKS (KALA-AZAR ELIMINATION PROGRAMME)

State ……

District …… **Month ……..**

MONTHLY PROGRESS										**PROGRESSIVE TOTAL**					
Name of PHC	Balance of rK39 test available as on Ist day of the month	No. of rK39 tests received from state during the month	Total stock of rK39 tests during the month	No. of suspected cases detected during the month		No. of rK39 tests (sample) conducted	**No. of cases confirmed with rK39**		Balance of rk39 tests available at the end of the month	Total No. of rK39 tests available upto reporting month	Total No. of rK39 tests used upto reporting month	No. of suspected cases detected upto reporting month		**No. of cases confirmed with rK39 tests upto reporting month**	
				Kala-azar	PKDL		**Kala-azar**	**PKDL**				Kala-azar	PKDL	**Kala-azar**	**PKDL**

Chief Medical Officer
District _____

NATIONAL VECTOR BORNE DISEASES CONTROL PROGRAMME

MONTHLY PROGRESS REPORT OF THE UTILIZATION OF rK39 DIP-STICKS (KALA-AZAR ELIMINATION PROGRAMME)

State …… **Month ……..**

MONTHLY PROGRESS										**PROGRESSIVE TOTAL**					
Name of District	Balance of rK39 test available as on Ist day of the month	No. of rK39 tests distributed during the month	Total stock of rK39 tests during the month	No. of suspected cases detected during the month		No. of rK39 tests (sample) conducted	**No. of cases confirmed with rK39**		Balance of rk39 tests available at the end of the month	Total No. of rK39 tests available upto reporting month	Total No. of rK39 tests used upto reporting month	No. of suspected cases detected upto reporting month		**No. of cases confirmed with rK39 tests upto reporting month**	
				Kala-azar	PKDL		**Kala-azar**	**PKDL**				Kala-azar	PKDL	**Kala-azar**	**PKDL**

State Programme Officer
State _____

Referral of 5% of suspected cases for splenic puncture

Additional PHC:- **District:-** **Month: -**

Sl. No	Name of suspected case	Head of Family	Village	Symptoms				Referred to	Result	Diagnosis (If negative)