CHAPTER – TWO
Kala-azar has pillaged parts of India for over one hundred years, ever since its first report as Burdman fever (1862 – 1872) in the Garo hills of Assam, followed by an intensified full scale epidemic situation in Bihar between 1935- 1937. The disease has undergone through repeated epidemic periodicity since then at the interval of every 10-12 years, and currently, its importance as an issue in public health is well known (Peters & Prasad 1983). By and large, the epidemic has remain localized in northern parts of Bihar.

Kala-azar was almost eradicated from Bihar in 1960 due to effective control strategies of National Malaria Eradication Programme (status report on kala-azar-directorate of Health Services, Bihar, 1988) and people began to forget the unpleasant epidemics of kala-dukha (a name given to kala-azar when it was identified first in Bihar in 1892 at Purnia). The state, however could not remain much longer in absence of the disease, which resurged again in 1971 due to its simmering transmission and increased number of vector (Sanyal, 1979). An analysis of Kala-azar status in Bihar
from 1981, reflects an increase in magnitude and direction of its migration. Magnitude of kala-azar has been found rarely constant due to rapid increase in mortality status amongst kala-azar sufferers. The disease which was previously confined to northern parts of Bihar has started migrating from north to south of Bihar now known as Jharkhand on one hand and from west of Bihar to east of west Bengal and subsequently from eastern Bihar to Bangladesh and Bhutan. Bihar is the worst affected state with exacerbations every 15-20 years since the 1930’s. Due to the absence of an effective surveillance system in Bihar, only patchy data are available for outbreak before 1977-78. However, Sanyal et al (1979) collected data between 1933-37 and 1956-60 from hospital and dispensary returns and found that 16 districts of old Bihar had reported kala-azar during 1955-60.

The different control programs initiated in Bihar included

1. Opening of kala-azar treatment centers.
2. Mass spraying of DDT under the National Malaria Control Program (NMCP) and the National Malaria Eradication Program (NMEP).

3. Kala-azar control program with UNDP assistance.


The three previous control programs had only a temporary effect on the occurrence of the disease in the state. The kala-azar epidemic in the state affected four districts (Vaishali, Muzaffarpur, Samastipur and Sitamarhi) and the north central and north east districts in 1992. In the inter epidemic year of 1981, minimum cases were reported due to the initiation of a control program in 1977-78. The number of cases reported per 10,000 populations was 6.9 during the 1991-92 epidemic and 3.68 in 1996. Ten districts of the state, mainly in north Gangatic plane had reported more than 75 % of the total cases for several years. In 1996, these 10 districts reported 80 % of the total cases, with 33 districts accounting for all the cases. Due to administrative and socio-political
reasons, several new districts were created in 1996 and this had led to difficulties in analyzing the subsequent district wise data. However, most of these districts were located in north and south of river Ganges. Due to migratory nature the disease has crossed international boarder and now established in Nepal, Bangladesh and Bhutan.

The disease was feared long before its real knowledge was made. Cunningham (1885) misquoted the amastigotes as member of mycetozoa (from Peters & Kendrick 1987). It was in 1903 when two Indian Scientists Leishman and Donovan discovered amastigotes in viscera of Kala-azar cases separately and recognized them as morphologically related to Trypanosomes. This marked the beginning of recognition of Visceral Leishmaniasis (VL) in India. However, it was Ross (1903) who gave the name of the parasite associated with Indian kala-azar as Leishmania donovani. Soon after, Leishmanial nature of splenic anaemia in Mediterranean region (Lathorie 1909) confirmed the presence of Visceral Leishmaniasis (from Peters and Kendrick, 1987).
The causative organism, Leishmania is a diagenetic parasitic protozoa having mammals and phlebotomine sandflies (Diptera: psychodidae: phlebotominae) as the two hosts. Leishmania has evolved to get sheltered in mammals, after inoculation of the organism during evolution of the blood sucking habit of primitive sandflies (Peters and Kendrick, 1987). Mammals act as a supply of blood for the sandfly and also a source of leishmanial infection from the amastigotes in their blood or skin (Peters and Kendrick, 1987). In the vertebrate host, the promastigotes, gain entrance into cells of reticulo-endothelial system in which it transforms into amastigotes (1.5 x 2.5μm - 3.0 x 6.5μm in size). Spread of infection occurs with the rapture of the heavily parasitized cells due to multiplication of amastigotes by binary fission, and the ingestion of the liberated amastigotes by another macrophages (Mauel & Bahin 1982). The duration of existence of promastigotes, inoculated by sandflies in the extra-macrophage environment at the site of the bite was found dependent on the rate of recruitment of macrophages at the site. Later it was made known that only a few promastigotes were capable of infection after the bite.
The host specific cell-mediated immune (CMI) response has an important role in controlling the infection. In VL patients, the inability to control *L. donovani* infection is associated with a profound T-cell unresponsiveness to *L. donovani* antigens (Sacks et.al, 1987) and the production of interleukin 10 (Ghalib et. al, 1993). IL 10-producing CD25-Foxp3<sup>-</sup> T cells were recently implicated in the pathogenesis of human VL in India (Nylen et. al, 2007). The crucial role of the CMI response is illustrated by the increased risk of developing clinical illness in cases of malnutrition or concomitant immunosuppressive diseases (Murray, 1997). Other risk factors for developing clinical illness have been identified and include young age (Devies and Gavgani, 1999), decreased production of interferon gamma and polymorphisms in the promoter of the tumor necrosis factor gene. The gene encoding solute carrier family 11 A1 (SLC 11 A1 formerly NRAMP 1), which regulate macrophage activation, and polymorphisms in the gene encoding IL1 were also associated with underlying susceptibility to VL in Sudan, highlighting the key role of innate immunity in driving the adaptive host immune response (Blackwell et. al, 2004, Davies et. al, 2003).
Some earlier works (Young et. Al, 1924; Stauber, 1966 Ghose and Ghose, 1987) have established animal model and enabled the scientists to conduct in vivo experiments. Later on, it also becomes possible to conduct experiments under laboratory conditions with establishment of in vitro methods. However, these advancements came too early in time to be understood and no work of significance was done until very recently. This was mainly due to lack of knowledge of Leishmania biology in the early years which limited the scope of control measures and also resulted in the occurrence of several epidemics in eastern India, particularly in north Bihar. The other reasons for failure of control measures at the beginning were non-availability of adequate knowledge on environmental, vector, clinical and immunobiology of kala-azar. Diagnosis of the disease depended only on non-specific techniques, which rarely detected disease at an earlier stage.

The recent control strategy for visceral leishmaniasis includes vector control, the use of insecticide – impregnated materials, active case detection and treatment and reduction of parasitic reservoir. In indian
subcontinents, the asymptomatic infection and PKDL patients are considered as reservoir. The documentation of a significant prevalence of asymptomatic infection in the South-East Asian subcontinent is fascinating and is obviously pertinent from the epidemiological as well as from the clinical point of view. The reported ratio of asymptomatic infections to VL clinical cases are widely varied as 4:1 in Kenya (Schaefer et. al, 1995), 5 to 6:1 in Ethiopia (Ali and Ashford, 1994), 1.2-11:1 in Sudan (Khalil et. al, 2002) 13:1 in Iran (Devis et.al, 1999), 18.1 in Brazil (Evan et.al,1992) and 50:1 in Spain (Moral et.al,2002). Population based epidemiological studies have also been recently initiated in the Indian subcontinents covering mainly India, Nepal and Bangladesh. A recent study reveals asymptomatic infection as 9:1 in the highly endemic foci in India, where 1 in 50 asymptomatic infections developed into active disease over a period of 18 months (Ostyn et. al;2011 ). Another study in Bihar has reported prevalence of asymptomatic cases as 110 per 1,000 persons and the rate of progression to symptomatic cases as 1:3 (Topno et.al,2010). Another monthly follow-up of the asymptomatic cohort study in Vaishali district of Bihar has revealed a disease conversion rate of 23.1 per 100 persons.
within a year (Das et.al, 2011). These discrepancies could reflect differences in parasite virulence and host population characteristics. The asymptomatic VL persons in part, however, are the most important determinant of infection prevalence (Bern et.al, 2010). The other group of reservoir is constituted by PKDL patients.

The methods used for the decrease of vector population in endemic area are an important control measure strategy. Sandflies are susceptible to the same insecticides as Anopheles mosquitoes, the malarial vector. Residual insecticides spraying of houses and animal shelters were shown to be efficacious in India (Kaul et.al, 1994), where the vector Ph.argentipes is restricted to areas in and around the house. But resistance to Ph.argentipes to DDT will create problem in near future (Singh et.al, 2001). The next strategy for the control of VL is use of insecticide impregnated materials. The use of such material could concomitantly prevent VL. There is limited evidence that bed nets provide protection against VL. Case control studies conducted in Bangladesh and Nepal showed that sleeping under a non-impregnated bed net during the warm
months was a protective factor against VL (odds ratio = 0.20, p = 0.001, odds ratio = 0.69, p = 0.01 respectively) (Bern et al., 2000; Bern et al., 2005). Despite low usage, the mass distribution of ITN’s in Sudan was accompanied by a 27% reduction in the incidence of VL in an observational study (Ritmeijer et al., 2007). A large prospective randomized controlled trial testing the efficacy of long lasting ITNs to prevent *L. donovani* infection and VL is underway in Nepal and India under the Kalanet-project. Depending on the sleeping traditions of the population and the biting habits of the local vector, other insecticides impregnated materials such as curtains and blankets should be evaluated for use in VL prevention, as some have been shown to provide efficient protection against cutaneous leishmaniasis (Reyburn et al., 2000, Kroeger et al., 2002). The next and most important strategy for control of VL is early diagnosis and treatment. It is essential for both individual patients and for the community. The treatment outcome is worse in VL patients having bad general health. Adult patients in Sudan with severe anaemia, malnutrition and long duration of illness were shown to be at an increased risk of death (Collin et al., 2004). Untreated VL patients act as a reservoir
for parasites and therefore contribute to disease transmission in anthroponotic VL areas. Early case finding and treatment is therefore considered an essential component of VL control (Desjeux, 2004; Boelaert, 2000; Guerin et al, 2002).

In the absence of early detection of disease at population level, it is necessary to direct the control measure for the prevention of contact between man-vector to control the longitudinal spreading of diseases. The method required a complete understanding of behavior of vectors with respect to environment and socio-economic pattern of population. Much of the impact of climate on vector born diseases can be explained by the fact that sandfly is exothermic and therefore, subject to the effects of fluctuating temperatures of their reproduction, development, behavior and population dynamics. Temperature can also affect pathogen development within vectors and interact with humidity to influence survival rate of vector and, hence, vectorial capacity. Leishmaniasis is transmitted by a number of different vectors and the responses of these vectors to climatic changes will also vary. Although leishmaniasis is often
associated with drier condition, sandfly vectors need high humidity as well as moderate temperatures and specific soil types in order to thrive. Their limited flight range also requires that they must be in close proximity to humans for transmission of the disease (Cross et.al, 1996).

In Bahia state of Brazil, the number of Leishmaniasis patients increased after a 2-years lag following droughts associated with El Nino (Franks et.al, 2002). The spatial analysis of Thompson et.al(2002)hypothesized that increases in leishmaniasis cases in Ceara, Brazil were associated with a drought that stimulated human emigration to urban areas and clustering of people around water supplies a fact that increased risk for human as the limited water sources and high humidity associated with them also resulted in concentration of vector population (*Lutzonia longipalpis*) around these same sites. The computer aided analysis of transmission pattern in South Wes Asia (Cross et.al,1996) and study of climate environment variable in Sudan (Thomson et.al,1999) suggested that *Ph.orientalis* occupies a “climate space” with 400-1200 mm annual rainfall and an annual mean maximum temperature between
34ºC – 38ºC. Ecological niche modeling in Brazil is providing insights into the ecological basis for difference in the distributors of these sand fly species (Peterson & Shaw, 2003). Studies in Colombia suggest that under climate change scenarios, the increased frequency of droughts is likely to increase the incidence of leishmaniasis (Cardenas et.al, 2006). A temperature based model predicts that global warming could greatly increase both the geographical and seasonal distribution of sand fly vectors in South West Asia (Cross & Hyams, 1999). It has been reported that higher temperature not only accelerates the development of sandfly vectors but also shortens the development time of Leishmania parasites in the vectors (Rioux et.al, 1985, Kasap & Alten, 2006, Rioux et.al, 1985) showed that raising the temperature significantly increased the overall proportion of infected sandflies, speeded up the multiplication of promastigotes in the midgut, controlled the movement of parasites forward into the thoracic midgut (from 15ºC) and encouraged the attachment of the flagellates to the wall of the stomodaeal valve (from 20ºC). The similar reporting has also been reported from Iran and the workers suggested that the larvae of the second generation hibernate
through winter and the first adult population appears in the mid to late June of the next year. The highest population density of sandflies was observed in early August, followed by a rapid decrease in September. These workers further suggest that specific knowledge of vector phenology is the centre piece of the effective vector control campaigns (Oshaghi et.al,2009).

The extrinsic incubation period for *L. donovani* is 6-9 days but intrinsic incubation period is variable due to variation in eco—climatic factors and immunity of host. The establishment of a strict seasonal correlation is difficult due to the highly variable incubation period. The intrinsic incubation period of kala—azar in man is a debatable issue since its emergence as a major public health problem in India. Napier (1926) vaguely sumrised that the intrinsic incubation period of kala-azar in man may be about two months based on the annual kala-azar incidence curve in Assam before knowledge of the role of sandfly in the transmission of disease. Swaminathan et.al,(1942)were first time established the role of *Ph. argentipes* in disease transmission. Manson Bhar (1982) was of the
opinion that the incubation period may vary from 10 days to 3 months. The incubation period of kala-azar was also reported about nine months or more (Verghese and Rahman, 1993), and 8—10 months (Rahman et.al, 1988) or it may be of more than 2 years.

Emphasis to analyse Leishmania immunobiology was made at a time when many eastern states of India, particularly north Bihar had undergone many episodes of epidemics taking millions of lives residing in the endemic areas. The ineffectiveness of use of anti Leishmanial therapies particularly the antimonials to control the level of infection was the most unpleasant situation. An increase in number of unresponsive cases to drug and in some cases, resurgence of the old infection (relapses) on part of chemotherapeutical failures were taken as serious threat to the available control measures. Unfortunately this situation is no better still today.

The drugs available for treatment remain sodium stibogluconate and meglumine antimoniate as the first choice therapy of kala-azar with polyene antibiotic-amphoteronon-B and the synthetic diamidine
pentamidine as second line drugs. Sen and Guru, (1983) suggested that variations in the absorptions, distribution, metabolism, and excretion of the drug may lead the parasite being exposed to lower concentration of the drug. Concurrently, Bryceson (1983) defined 5 categories of antimonial resistance i.e. primary unresponsiveness to initial SSG treatment; slow response during treatment; relapse after adequate initial response to treatment; secondary unresponsiveness in relapse and acquired resistance to drug by the parasite. Sen and Guru, (1983) mentioned that 30 % of cases during an epidemic of kala-azar in Bihar (1977-1980) were unresponsive and had taken incomplete treatment as well. Earlier, Peters (1981) observed that 45.7 % of cases were unresponsive to SSG from Bihar state itself. These reports reflected that ‘drug unresponsiveness’ is the major problem associated with treatment and control of the kala-azar disease. Extension of treatment durations was taken initially as the major criteria to resolve this issue (Rocha et.al, 1980). These problems later led a few group of scientists to emphasize on issues such as, why host is unable to take the benefit of immunity and whether the parasite is insensitive because of failure of immunity to process the
drug to be effective against the parasite (Sen and Guru, 1983). Characterization of Leishmania-parasite by Western Blot and other technique are being attempted in many studies. A few attempts have also been made to examine gene amplification in many gene locus of Leishmania. Of all these attempts, immunological studies have little been worked out and needs in depth analysis since there is the natural system of host defense and any defect in this system may have an important bearing on the development of unresponsive trend in kala-azar. Most of the evaluations on the immune responses were made when patients could be diagnosed only after appearance of symptoms in them. Now it is known that at the time of clinical manifestations disease becomes already matured, which enables the *Leishmania* to induce profound impairments. Therefore, the recent studies have begun to develop non-sophisticated, sensitive and a specific test for identification of early cases of kala-azar so as to characterize the immune response in an appropriate way, classical presentation of fever, splenomegaly and anaemia along with family histories are the basis of diagnosis of Leishmaniasis. Confirmation of disease is done by parasitological techniques by demonstration of
*Leishmania donovani* in bone-marrow or spleen aspirates of patients. Later it has been shown that the classical hematological findings i.e. R.B.C. and W.B.C., high lymphocyte and monocytes, anaemia etc. and low albumin and high globulin ratio (A/G ratio in patient 0.55 : 1, in healthy 1) with greatly raised IgG are also significant clinical changes associated with the kala-azar infection (Haldar et.al,1983). Manzoor et.al,2008 analysed hematological findings in relation to clinical findings of VL in hazara division in Pakistan. They reported that the VL patients were characterized by anaemia (100%) followed by neutropenia, lymphocytosis and thrombocytopenia.

Manan and Nadeem (2010) studied hematological parameter in patients of visceral leishmaniasis and reported that they have characteristically decreased hemoglobin, total leucocytic count, platelets count, very high ESR, relative lymphocytosis with micro cystic, hypochromic red cells.

Preeti et.al(2013) studied the haematological parameters in patients of VL in Ludhiana and suggested that mean haemoglobin of
patients was 6±1.3gm/dL (range 5-7.1 gm/dL). Leucopenia was observed. Total leucocyte count (TLC) was 3.6 ± 2.4 × 10^9/L (range 2.3 – 3.6 × 10^9/L). Thrombocytopenia was observed with mean platelet count as 88.7 ± 62.2 × 10^9/L (range 43 – 152 × 10^9/L). Pancytopenia was observed in 87 % of patients.

However, the parasitological examination on the large number of individuals have been found rarely practical and hence need for development of a suitable “on the spot procedure” was felt to identify the disease. Serological techniques are among priorities in this regard. Most of the earlier tests were non-specific and incapable of showing high accurate degree of seropositivity on one hand and in distinguishing current over infection from the past on the other hand.

Various in vitro antigen-antibody reaction were developed simultaneously which represented aggregation of antigen and antibody in broadly two ways viz., precipitation or agglutination. Agglutination reactions were established using particulate antigens. Development of indirect haemagglutination test (IHA) by Stavitsky (1954) was supposed
once as landmark in the field of serodiagnosis. Though application of Fluorescent antibody technique in the beginning of it’s establishment by Coons et.al(1941), failed to draw global attention. However the test come back later on with applicability, when it was felt that straight localization of specific sites of antigen and antibody reaction by labeling antibodies with fluorescent dye could permit their direct detection.

Later, introduction of Enzyme Linked Immunosorbant Assay (ELISA) enabled serodiagnosis to take an new shape because the test was high specific (voller et.al,1976). The theory of ELISA is based on coupling serum antibodies to an enzyme peroxidase followed by addition of colorless substrate that is converted by the enzyme in to colored product. The amount of colored product is proportional to the amount of antigen-antibody complex (Engvall, 1980). ELISA, which competed successfully with radioimmuno assay, was used for the first time in the study of Leishmaniasis by Hommel(1976). Since then it has been used in study of both old world Leishmaniasis (Hommel et.al,1978, Edrission and Darabian, 1979; Roffi el.el,1980, Ho et.al,1983 etc.) and new world Leishmaniasis
(Luzzio et al, 1979; Anthony et al, 1980; Guinareus et al, 1983) in Visceral Leishmaniasis. Maria et al (1990) obtained percent sensitivity and specificity of 76 and 100 respectively in sera infected from *L. mexicana*. Chaudhary et al (1990) however, obtained a slightly raised ELISA response in subjects with other diseases. Cross reactions of *Leishmania* parasites with the sera of patients infected with a variety of other parasites has also been reported in indirect Fluorescent assay (Mantossian et al, 1975; Harith et al, 1987 and indirect haemagglutination assay (Bray and Lainson, 1967). Choudhary et al, (1990) recommended use of ELISA despite some cross reactions by using only 25 mg soluble antigen per well of Microtitre ELISA plate, instead of use of 2-8 µg antigen per well (Mohammed et al, 1986). On the basis of work on ELISA, it was equally true that use of purified specific antigen can enable ELISA in reducing cross reacting epitopes for other protozoal infection (Chaudhary et al, 1990).

Serodiagnosis immediately came up with unique break through when Harith et al (1986) observed the presence of high level of agglutinating antibodies as a feature of distinction in kala-azar. Getting
enough enthusiasm he evaluated Direct agglutination test (DAT) as a method of diagnosis of Visceral Leishmaniasis (VL) in Kenya with good results. It seems justified to mention here that Dr. Abdella Harith had suggested the researcher to select DAT as test of choice for field studies in 1989. Suitability and stability of DAT with regards to sensitivity and specificity was later proved all over the world. On analyzing the serological reactivities and parasitological findings in cases of suspected Leishmaniasis, Mengistu et.al(1992) observed DAT and Immunoblot with greater sensitivity percentage of 80 as compared to 60 % sensitivity observed in ELISA. This sensitivity was comparable to those observed in previous studies (EL safi & Evans, 1989); Mengistu et.al,1990). Recently, Lal Suman et.al,(1992)reported 100 % sensitivity and specificity of DAT using Harith antigen during a sero-survey study of an endemic village of kala-azar in Bihar, India. These studies on the applicability of DAT revealed the usefulness of the test in predicting onset of kala-azar infection, if history of Leishmaniasis were present. Some reports however, was exceptionally not encouraging as DAT gave a negative liter in four patients confirmed parasitologially.
The diagnostic test of VL based on the principle of antigen antibody reaction. The antibody detection tests are principally based on the detection of specific anti-leishmanial antibodies but all have two major limitations. First, though serum antibody levels decrease after successful treatment, they remain detectable up to several years after cure (De Almeida Silva et al., 2006; Hailu, 1990, Bern et al., 2005). Therefore, VL relapse cannot be diagnosed by serology. Second, a significant proportion of healthy individuals living in endemic areas with no history of VL are positive for anti-leishmanial antibodies owing to asymptomatic infections. The sero-prevalence in healthy populations varies from <10% in low to moderate endemic areas (Schaefer et al., 1995; Koirala et al., 2004, Schenkel et al., 2006) to >30% in high transmission foci on in household contacts antibody based tests must therefore always be used in combination with standardized clinical case definition for VL diagnosis.

Serological tests based on indirect fluorescence antibody (IFA), Enzyme-linked immunosorbent (ELISA) or western blot have shown high diagnostic accuracy in most studies but are poorly used for field study (Ho
et.al,1983, Sinha & Sehgal, 1994). Two serological tests have been sufficiently validated- the direct agglutination test (DAT) and the rk-39, immunochromatographi test (ICT). The DAT is a semi quantitative test that uses microtitre plate in which increasing dilutions of patient serum or blood are mixed with stained killed *L. donovani* promastigotes (Harith et.al,1986; Harith et.al,1987). If specific antibodies are present, agglutination is visible after 18 hrs. with the necked eye. This test has been extensively validated in most endemic areas. The studies were included in a recent meta analysis which gave sensitivity and specificity estimates of 94.8% (95% CI, 93.9-98.7) (Chappuisel,et.al,2006). The performance of DAT was influenced by neither region nor by the leishmania species. Freez dried antigen is more robust than liquid antigen (Sunder et.al,2006, Jacquet et.al,2006). The DAT is simpler than many other test but it requires equipment such as microtiter plates and micropipette, well trained laboratory technicians and regular quality control. The storage of the antigen at 2-8ºC, once it has been dissolved and the prolonged incubation time are draw backs. The fast agglutination screening test (FAST) is a simplified (single serum dilution at a cut-off of
1:800 or 1:1600) and more rapid (2-3 hrs) version of the DAT, and its diagnostic accuracy seems comparable (Schoone, 2001; Silva et. al, 2005), but further validation is needed.

rk 39 is a 39-amino acid repeats. It is a part of kinesine protein in *Leishmania chagasi* and which is conserved within the *L. donovani* complex. An rk 39 based ELISA showed very high sensitivity (93-100%) and specificity (97-98%) in many VL endemic countries (Braz et. al, 2002; Singh et. al; 1995; Kurkjian et. al,2005). The test was thus developed into an ICI or dipstick format that was more suitable for field use. A meta analysis that included 30 validation studies of rk 39 ICT showed sensitivity and specificity estimates of 93.9% (95% CI, 87.7-97.1) and 95.3% (95% CI, 88.8-98.1), respectively (Chappius et. al,2006). Recently the excellent diagnostic performance of rk 39 ICT was confirmed in India and Nepal (Sundar et.al,2006, Sinha et. al,1994, Sunder et. al, 2007). However this test has been shown to be less accurate in East Africa (Koirala et. al.2004, Veeken et. al, 2003), for reasons that remain unclear Sudanese patients seem to develop lower liters of antibodies against rk 39.
than do Indian patients, although the format of the test might be a factor, as other brands of ICT performed in this region (Chappuis et.al, 2005).

The rk 39 ICT’s are easy to perform, rapid (10-20 min), cheap (around us & 1 per test) and give reproducible results. They are currently the best available diagnostic tool for VL for use in remote areas and their wide distribution and use within an appropriate VL diagnostic algorithm should be promoted. The case management strategy of the VL elimination program planned for the Indian subcontinents; which is based on the treatment of suspected clinically affected individuals who have positive rk39 ICT results, is supported by solid scientific evidences. Given that several counterfeit VL ICTs have already been found in the Indian subcontinents, the need for rigorous quality standard and regulations of diagnostics should be addressed at the same time. Recently the use of rk39 rapid ICT was used for the detection of VL from saliva of a person by a group of workers (Manisha et.al, 2012). They evaluated the presence of anti rk39 antibody in human saliva being noninvasive to replace the invasive procedure of diagnosis. They performed ELISA and ICT assays in
300 subjects and reported sensitivity in saliva as 83.3 % by ELISA and 82.5 % by ICT, compared with 100 % for both ICT and ELISA for serum. The reported specificity for saliva was 100 %, 95.5 % and 88.6 % with ELISA and 91.48 %, 91.57 % and 84.06 % with ICT in non-endemic, endemic and different disease respectively. In serum specificity was reported 97 %, 88.5 % and 89 % by ELISA and 100 %, 94.7 % and 95.5 % by ICT in non—endemic, endemic and different diseases. They recommended non-suitability of detection from saliva due to low sensitivity.

Theoretically the diagnosis based on antigen is more accurate than the diagnosis based on detection of antibody concentration in serum as they avoid cross-reactivity of past infection and principally depend on the present infection. A latex agglutination test detecting a heat stable, low molecules weight carbohydrate antigen in the urine of VL patients has shown promising initial results (Sarkari et.al,2002). Several studies conducted in east Africa and the Indian subcontinents showed good specificity but only low to moderate (47—87 %) sensitivity (Kurkjian et.al,2005; Sunder et.al,2007, Sunder et.al, 2005). The latex agglutination
test correlated well with cure in a high proportion (97-100%) of patients during anti leishmanial treatment (Sunder et.al, 2005; El Safi et.al, 2003). Apart from its low sensitivity, there are two practical limitations, the urine must be boiled to avoid false +ve reactions and it is difficult to distinguish weakly positive from –ve results, which affects the reproducibility of the test (Chappuis et.al, 2005; Chappuis et.al, 2006). Work to improve the format of this test is going on.