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Evaluation of Different Approaches in Leishmania Diagnosis

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ABSTRACT

Leishmaniasis is a disease caused by a malaria-like parasite called Leishmania in human and some species of animals. Detection of leishmaniasis has always been crucial for control and treatment of the disease. Different strategies have been approached for detection of leishmania. In this review methods used for detection of leishmania infection have been discussed and compared.

Key word: Leishmania, Diagnosis, ELISA, PCR

INTRODUCTION

Leishmania is a flagellate parasite causing an infectious disease that threaten human beings, both for the high death rates and the economic loss mostly in the tropical and subtropical areas (Das et al. 2008). Today, Leishmaniasis ranks the second only to malaria with ever increasing cases worldwide and its control remains as a serious third world problem becoming a major focus of concern particularly in poor sections of societies (WHO. 2002). In vertebrates the parasite is called amastigote living intracellularly within parasitophorous vacuoles of macrophages and in sand fly vector or media culture, it is living extracellularly named as promastigote (Rogers et al. 2004; Bates 2007). The amastigote is 3-5 µm in length and the promastigote is 15-20µm, which is flagellated and highly motile (Burchmore and Barrett 2001; Handman and Bullen 2002; Weigle et al. 2002; Konecny and Stark 2007; Dostalova and Volf 2012). The first classification of *Leishmania* was based on ecobiological behavior of the parasite including antigenic properties, the geographical distribution, clinical manifestations, tropism and the vector (Marsden and Lumsden 1971; Bray 1974; Pratt and David 1981; Ryan et al. 1990). More than 30 species of the parasite such as *L. panamensis*, *L. donovoni*, *L. chagasi*, *L. infantum*, *L. archibaldi*, *L. garnhami*, *L. pifanoi*, *L. venezuelensis*, and *L. forattinii* are known from which 20 species are pathogenic for humans (Ashford 2000; Mauricio et al. 2000; Cupolillo et al. 2003; Sharma et al. 2005). However, new studies using molecular techniques have altered the status of some of these species; for example, *L. chagasi* is now accounted as a synonym for *L. infantum* (Mauricio et al. 2000) and *L.*

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peruviana is known as an independent species (Banuls et al. 2000). Using the molecular techniques, the World Health Organization (WHO) has published a new taxonomic scheme for *Leishmania* (WHO. 1990).

Leishmaniasis is now included in the list of neglected tropical diseases by WHO (Alvar et al. 2006), which has a strong link to poverty (Feasey et al. 2010). According to WHO, the disease is now prevalent in 88 countries (22 in the new world and 66 in the old world) (Desjeux 2004) out of which 16 are developed, 72 are developing, and 13 of them are among the least developed countries (TDR/WHO. 2012).

An estimated 20 million cases of Leishmaniasis exist worldwide and 367 million are at risk of acquiring the disease. About 1-1/5 million new cases of cutanous Leishmaniasis and 0/5 million cases of visceral Leishmaniasis occurs throughout the world annually resulting in 75,000 deaths (Herwaldt 1999; Desjeux 2004; WHO. 2007; Alvar et al. 2012). However, due to underreporting and misdiagnosis, the number of actual cases is expected to be higher (Collin et al. 2006; Singh et al. 2006; Bhargava and Singh 2012). For example, the mortality rate for VL in Brazil in 2006 was 7.2%. In 2008, the case-fatality rate in Bangladesh was estimated 1.5%, in Nepal 6.2%, in India and South Sudan more than 10% with an increase up to 20% in villages (Zijlstra et al. 1994; Seaman et al. 1996; Kumar et al. 1999; Ahluwalia et al. 2003; Barnett et al. 2005). In East Africa, it causes around 50,000 annual cases, in the form of epidemic outbreaks distributed in scattered displaced populations with a high death rate. Post-kala-azar dermal leishmaniasis (PKDL), which is developed in 5–50% of AVL patients depending on geographical areas, requires lengthy and costly treatment with a low efficacy (Zijlstra et al. 2003; Desjeux 2011).

The highest estimate for CL incidence is for ten countries including Afghanistan, Algeria, Colombia, Brazil, Iran, Syria, Ethiopia, North Sudan, Costa Rica and Peru (Alvar et al. 2012). On the other hand, 67% of the global VL is estimated to occurs in India, Nepal, and Bangladesh (Hotez et al. 2004) and more than 90% of this form of the disease occurs in just six countries including India, Nepal, Bangladesh, Brazil, Ethiopia and Sudan where it killed an estimated 100,000 people out of a population of 280,000 between 1984 and 1998 in southern Sudan (Desjeux 2004; Jacquet et al. 2006; Chappuis et al. 2007). It has also been shown that 90% of CL cases occur in Afghanistan, Algeria, Ethiopia, Sudan, Iran, Iraq, Saudi Arabia, Syria, Brazil, and Peru (Desjeux 2004).Visceral leishmaniasis has shown to be fatal if left untreated where its mortality rate is almost 100%, and even with treatment the case fatality rate ranges from 4% to 10% and even more (Berman 1997; Collin et al. 2004; Bern et al. 2005; Rey et al. 2005; Herrero et al. 2009). The number of Leishmaniasis cases has been increasing in several areas e.g. Brazil, Afghanistan and Aleppo, and in some other countries such as Sudan, the disease spreads from endemic to non-endemic areas (WHO ; Yamey and Torreele 2002; Dujardin 2006; Alvar et al. 2007). The epidemiology of Leishmania has now been influenced by the expansion of human immunodeficiency virus (HIV). For example, in Ethiopia, 30-40% of VL patients are HIV positive (Alvar et al. 2008; Burki 2009). In addition, *Leishmania*-HIV co-infection cases have been reported from 35 countries (Alvar et al. 1997). In European countries such as Spain, Italy, France, and Portugal, up to 9% of the AIDS patients suffer from fatal visceral leishmaniasis (Berhe et al. 1999).

Visceral leishmaniasis (VL), cutaneous leishmaniasis (CL), and rarer manifestations such as mucosal leishmaniasis and post-kala-azar dermal leishmaniasis (PKDL) are the major forms of leishmaniasis in the human in which leishmania parasites are transmitted by female sand flies via anthroponotic or zoonotic cycles (Reithinger et al. 2007). Due to the lack of effective vaccine against *Leishmania* infections, control strategies for leishmaniasis now solely relies on control of the vector, protecting the host from the bite of the sanfly vector, early diagnosis and chemotherapy with pentavalent antimonials as the first-line, or amphotericin B and pentamidine as second-line drugs (Herwaldt 1999; Murray et al. 2000). Miltefosine is also known as the first oral treatment for leishmaniasis, but drug resistance may emerge during treatment (Seifert et al. 2007).

Diagnosis of Leishmaniasis

Diagnosis of Leishmaniasis is based on a combination of clinical symptoms, parasitological detection, immunological tests and molecular techniques (Bhargava and Singh 2012).

Clinical symptom:

In endemic areas, clinical symptoms do not appear in all infected individuals, and a fraction of the infected population remain asymptomatic (Topno et al. 2010) where its prevalence is usually higher than symptomatic infections (Chappuis et al. 2007).

A series of clinical manifestations including long-term unexplained fever, cachexia and hepatosplenomegaly, enlargement of lymphnodes, low blood cell count, hypergammaglobulinemia and pancytopenia can be seen in visceral Leishmaniasis. In the absence of treatment, the patients may also show cough and leukopenia resulting in fatal consequences, (Werneck et al. 2003; Machado de Assis et al. 2012; Pastor-Santiago et al. 2012). In underdeveloped countries, leishmaniasis is associated to poor hygienic conditions and lack of efficient prophylactic measures (Pink et al. 2005). In addition, predictive models using clinical symptoms and serological diagnostic methods for VL have been developed to predict the probability of VL and help its differential diagnosis in patients. The models showed to be useful tools and assist healthcare systems and control programs in their strategical choices (Machado de Assis et al. 2012). In cutaneous Leishmaniasis changes on the skin appearance are the most important symptom, which can lead to the diagnosis of the disease. The symptoms include ulcerative skin lesions developing mainly at the site of the sand fly bite (localized cutaneous leishmaniasis), multiple nonulcerative nodules (diffuse cutaneous leishmaniasis) and destructive mucosal inflammation (mucosal leishmaniasis (ML). However, all forms of the disease need to be confirmed by laboratory tests (Murray et al. 2005). Due to similarities between clinical spectrum of different form of leishmaniasis and other disease with similar clinical spectrum (for example leprosy, skin cancers, and tuberculosis for CL and malaria and schistosomiasis for VL) and presence of such disease in Leishmania endemic areas, differential diagnosis in leishmaniasis is critical and usually completed by other diagnostic tests (Alvar et al. 2012; Bhargava and Singh 2012; van den Bogaart et al. 2012; van den Bogaart et al. 2013).

Parasitological diagnosis (microscopic examination and parasite culture)

The most suitable diagnostic method for leishmaniasis is detection of the amastigote form of the parasite by microscopic examination of tissue aspirates. In preparations after staining with Giemsa or Leishman stain, amastigotes are oval with nucleus and kintoplast. The cytoplasm appears to be pale blue, with a relatively large nucleus that stains with red and the kinetoplast is deep red or violet rod-like body.

In cutaneous Leishmaniasis, the detection of amastigotes by microscopic methods is based on obtaining the smear from the skin lesion biopsy. In cutaneous and mucocutaneous leishmaniasis, the sensitivity of the microscopic examination is low, with a range of approximately 15–70% (Vega-Lopez 2003; Al-Hucheimi et al. 2009).

In visceral Leishmaniasis, the amastigote form can be easily detected intracellularly in monocytes or macrophages by the microscopic examination of Gimsa stained smears of aspirates derived from lymph nodes, bone marrow, liver or spleen (Markle and Makhoul 2004; Bhattacharya et al. 2006). In visceral leishmaniasis, the specificity of this technique is high and the sensitivity varies depending on the tissue used, being higher for liver or spleen (93–99%) than for bone marrow (53–86%) or lymph node (53–65%) aspirates (Siddig et al. 1988). However, there is always a risk of hemorrhage and complication for splenic and liver aspiration, which is also painful and unpleasant for the patients. So, the results are totally dependent on technical expertise and quality of prepared slides/reagents (Osman et al. 1997; Srividya et al. 2012).

The aspirate can also be cultured for recovering the parasite (Markle and Makhoul 2004). The culture method (e.g. Novy-Mcneal- Nicolle medium: an axenic culture medium comprised of a blood_agar slope with a saline overlay incubated at 25˚) is simple, cheap and relatively sensitive, and facilitates the diagnosis but suffers from its vulnerability to contamination. In addition, the culture method of the parasite is usually time-consuming, which makes it not an ideal method for field use (Konecny and Stark 2007).

In occult and sub-clinical infections, both direct microscopy and culture-based methods have a low sensitivity and cannot distinguish between the amastigotes of different species, so that, no species identification can be applied by these methods (Osman et al. 1997; Singh and Sivakumar 2003). In visceral Leishmaniasis the sensitivity of the methods for the splenic aspirates are quite high (98%) but it is lower for other organs indicating a very high level of infection in splenic macrophages. The sensitivity of blood smears as shown in table 1 is lowest because, parasitemia in VL patients is low (Singh and Sivakumar 2003; Allahverdiyev et al. 2005). .

In cutaneous Leishmaniasis, the sensitivity of both culture-based method and direct microscopic examination depends on the species of the parasite, clinical figure of the disease and the technical expertise applied for the tests. In microscopic examination, the sensitivity varies from 42 to 74% for direct stained smear and 33 to 76% for histological sections (Andresen et al. 1996; Aviles et al. 1999). When the microscopic diagnosis and parasite culture are applied together, the sensitivity increases even up to 83% (Bensoussan et al. 2006). The specifity of the methods are reported as high as 100% (Bensoussan et al. 2006). In mucocutaneous Leishmaniasis in particular, the sensitivity of microscopic and culture-based method is quite low due to the organisms are often scant (Rosbotham et al. 1996; Calvopina et al. 2004; Disch et al. 2005). It has also been reported

that in PKDL, the sensitivity of tests for skin lesions was low (17%) but it was higher (30 %) for lymph node aspirates (Osman et al. 1998).

Table 1: Sensitivity and specificity of various laboratory tests used for visceral leishmaniasis adapted from (Singh and Sivakumar 2003).

Investigation	Sensitivity	Specifity
Splenic aspirate smear	80 –98%	100%
Splenic aspirate culture	70-98%	100%
Bone marrow smear	60-85%	100%
Bone marrow culture*	40-50%	100%
Liver aspirate smear	50-75%	98%
Lymphnode smear	40-50%	95%
Buffy coate culture	0-30%	100%
Complement fixation test	70-80%	60-73%
Immunodiffusion test	60-75%	90-95%
CCIEP test	80-90%	50-70%
IHA test	73-75%	80-95%
IFA test	55-96%	70-98%
DAT	90-100%	80-95%
ELISAs **	36-100%	85-100%
* Hampered by high contamination rate of the cultures.		
** Depending on the antigen used		

Immunological tests

Immunological tests are based upon the detection of anti-leishmanial antibodies and leishmanial antigens, which are useful in both individual diagnosis and epidemiological surveys. Serodiagnosis of the disease is sometimes accompanied by shortcomings due to the antibody prevalence in endemic areas specially in post-infected cases, absence of antibody during the incubation period, or cross-reactivity with other pathogenes such as malaria, trypanosoma, schistosoma or leprosy (Kar 1995). A number of methods have been described for immunological test of leishmaniasis (table 2).

Table 2: Results of serologic tests in VL patients

Patient group	FAST %	DAT %	IFA %	ELISA %
Parasitology positive(n=24)	23(95.8)	24(100)	24(100)	23(95.8)
Parasitology positive(n=35)	5(14.3)	4(11.4)	5(14.3)	6(17.1)

Leishmanin Skin Test (LST)

Leishmania Skin Test, also known as the Montenegro reaction, is an important delayed hypersensitivity reaction in cutaneous forms of leishmaniasis. Results of the test become positive after subclinical infection and within weeks-months after successful therapy against VL, indicating a healing or protective response (Zijlstra et al. 1994; Khalil et al. 2005). Also, the usefulness of the test to detect asymptomatic infection is shown in different disease-endemic areas (Alvar et al. 2007; Riera et al. 2008; Gidwani et al. 2009). In VL-endemic areas, the sensitivity of LST in asymptomatic *Leishmania* infections is similar or even higher than that of serologic analyses (Evans et al. 1992; Costa et al. 2002; Riera et al. 2004b; Riera et al. 2008; Hailu et al. 2009; Gadisa et al. 2012). This makes the LST a valuable tool in detecting exposure to *Leishmania* parasites and distinguishes asymptomatic cases in epidemiologic surveys (Alvar et al. 2007; Riera et al. 2008; Gidwani et al. 2009).

The test is applied by the injection of leishmania antigens intradermally and measuring the immunological reactions. The leishmanin antigen is not commercially available. It is a suspension of whole killed parasites (0.5-1 10^7 /ml) or disrupted promastigotes in pyrogen-free phenol saline (250 µg protein/ml). There is no cross-reactions occurring with Chagas' disease, but some cross-reactions are found with cases of glandular tuberculosis and lepromatous leprosy (Singh and Sivakumar 2003). The LST is usually used as an indicator of the prevalence of cutaneous and mucocutaneous leishmaniasis in human and animal populations and successful cure of visceral leishmaniasis, as it remains negative during active visceral leishmaniasis and converts to positive after treatment (Agwale et al. 1998; Zijlstra and el-Hassan 2001; Singh and Sivakumar 2003; Salotra and Singh 2006). In PKDL patients, the test is not useful, as the result does not correlate with the presence of the infection (Zijlstra et al. 2000). In these patients, within weeks-months after successful therapy against VL, the LST results still become positive (Zijlstra et al. 1994; Khalil et al. 2005).

Indirect Flourcent Antibody Test (IFAT):

The Indirect Flourcent Antibody Test (IFAT) is one of the sensitive tests available for diagnosis of leishmaniasis in humans and animals. The sensitivity of the test is accounted for 96% and the specificity 98% (Hommel et al. 1997; Rosati et al. 2003; Boelaert et al. 2004; Boarino et al. 2005; Pastor-Santiago et al. 2012). The test is based on the detection of anti-leishmania antibodies, which appear in the early stages of the disease lasting for 6 to 9 months after the cure. Titers above 1:120 are significant and 1:128 is diagnostic (table 3). The persistent low doses of antibodies indicate a probable relapse of the disease. There would be a cross-reaction with trypanosomal sera, however, it will overcome by using amastigotes as the antigen instead of promastigotes (Singh and Sivakumar 2003).

Table 3: Anti-leishmania antibody determined by IFAT

	IFA Titers						
Patient group	1:64	1:128	1:256	1:512	1:1024	1:2048	Total(n)
Confirmed VL	-	3	4	9	5	3	24
Suspected VL	3	1	1	(-)	-	-	5

Enzyme Linked Immunosorbent Assay (ELISA)

In serodiagnosis of leishmania parasites, ELISA is accounted as a valuable test. The test can be easily applied for either the laboratory analysis or the field diagnosis. Although the sensitivity of the test is high, it is entirely influenced by the antigen used in the test. A number of leishmania antigens used in ELISA is shown in table 4. In visceral leishmaniasis, 39-amino-acid kinase-like protein (rK39) is shown to be a good antigen to be used in ELISA, however crude SLA still seems to be a potent alternative (Kumar et al. 2001; Carvalho et al. 2003; Maalej et al. 2003). In contrast, K39 does not show detectable antibodies in cutaneous or mucocutaneous leishmaniasis (Braz et al. 2002). The titre of antibody to rK39 has a good correlation to the efficacy of chemotherapy in visceral leishmaniasis as during the period in which the disease is active, the antibody level is very low (Kumar and Tarleton 2001; Braz et al. 2002; Singh et al. 2002). In addition rK39-ELISA has a high predictive value for detecting VL in immunocompromised patients, like those with AIDS (Houghton et al. 1998). Some other antigens such as gene B protein (GBP) and recombinant major surface glycoprotein (gp63) from *L. major*, have been tested for detection of cutaneous leishmaniasis (Mosleh et al. 1995; Jensen et al. 1996; Singh and Sivakumar 2003). It has recently been shown that in detection of mucocutaneous leishmaniasis, ELISA using crude SLA or the patient's serum is a valuable test with as high sensitivity as 94.7-100%. The specificity of the test was lower due to the cross-reactivity to chagas disease and/or malaria (Junqueira Pedras et al. 2003). In addition, ELISA using rK39 detects asymptomatic infection earlier than the DAT (Zijlstra et al. 1998). However, Due to the requirement of skilled personnel, sophisticated equipment, and electricity, using ELISA for diagnosing VL is not usual in the endemic areas (Srivastava et al. 2011a).

Table 4: Sensitivity and specifity of enzymelinked immunosorbent assay using different leishmania antigens adopted from (Singh and Sivakumar 2003).

Antigens	**Crude SLA ***	**rK39**	**rH2A**	**rH2B**	**rGBP**	**rLACK**
Sensitivity	100	100	100	100	97	97
Specificity	94	97	91	92	92	84
	rgp63	rP20	rPSA,-2-GST	rPSA-2-TRI-GST	rPSA-2-MBP	Purified LPG **
Sensitivity	86	68	47	36	57	92
Specificity	90	95	96	85	97	92

* : using crude soluble leishmania antigenes (SLA)

** : purified leishmania lipophosphoglycan

In PKDL a set of antigens have been tested in ELISA for detection of the disease (Salotra et al. 2003). The sensitivity and specificity of some of them in *L. donovoni* are shown in table 5.

Table 5: Antigens used in ELISA for detection of anti-leishmanial antibody in PKDL patients sera adopted from (Salotra and Singh 2006).

Antigen	**Sensitivity (%)**	**Specificity (%)**
CLA	86-100	90-100
SLA	83	90-100
MP	100	96.7
rK39	94.5-100	93.7-100
GPB	93-100	83
GRP78	78	90
C-ELISA (D2)	100	100

CLA, Crude l*eishmania* antigen; SLA, soluble l*eishmania* antigen; MP, membrane protein; rK39, recombinant K39; GBP, gene binding protein; GRP78, glucose related protein 78; C-ELISA(D2), competitive ELISA based on D2 (*L. donovani* specific monoclonal antibody)

Direct agglutination test (DAT)

Direct detection of antigen is an excellent method for diagnosing an infection and is more specific than antibody-based immunodiagnostic tests (Figure 1). The direct agglutination test (DAT) is a highly sensitive test for detection of leishmania, which has effectively been used in the field and laboratory (Abdallah et al. 2004).

To detect the antigen, DAT has extensively been evaluated in clinical trials in well-defined cases and controls from endemic and nonendemic regions (Table 6). In different studies, the specificity of 79.1–100% and sensitivity of 60.4–100% have been reported for this test (Sreenivas et al. 2002; Jacquet et al. 2006; Boelaert et al. 2008).

Some recent studies indicated similarity between the results of DAT and rK39-ICT tests for VL diagnosis however, to assess the asymptomatic infection in asymptomatic population higher positivity rates were reported for DAT versus rK39-ICT (Topno et al. 2010; Canavate et al. 2011; Gadisa et al. 2012). Better results for detection of asymptomatic infections were also obtained when either a rK39-based ELISA test or combination of DAT and LST were applied in VL-endemic areas (Zijlstra et al. 1998; Gadisa et al. 2012).

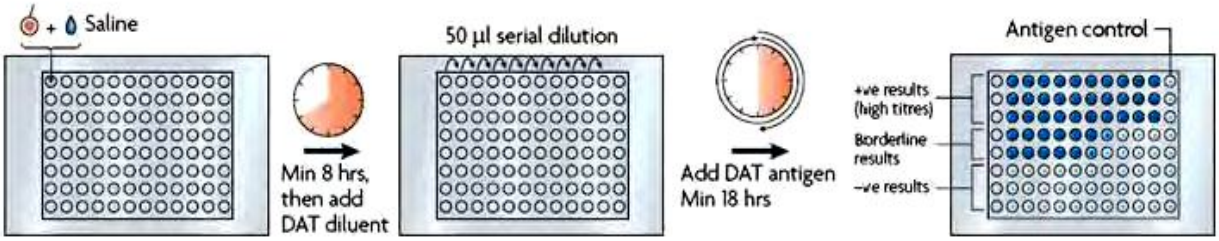
Although the nature of the antibody involved in the test is not yet known, the test is cheap and easy to perform (Hommel et al. 1997; Silva et al. 2005). Stained promastigotes either as suspension or freeze-dried are used as antigens and due to the heat-stability of the freeze-dried promastigotes, they are easier to be used in the field. Major disadvantages of DAT are the long incubation (18 h), the need for a serial dilution of serum and the high cost of antigen (Sundar et al. 2006). In addition, the test is unable to differentiate between clinically active and asymptomatic infections showing positive results long after cure. So that the test cannot be used for diagnosis of cure or relapses (Sundar et al. 2006).

A new method called fast agglutination-screening test (FAST) has recently been developed with 3h incubation for rapid detection (Silva et al. 2005; Hailu et al. 2006). Another method of DAT has also been investigated using patient's urine in endemic and nonendemic areas, showing a comparable sensitivity and specificity to that performed with serum. (Islam et al.. 2004).

Table 6: DAT results for anti – leishmania antibodies in suspect and confirmed VL patient

	Serial dilution series (reciprocal)									
Patient group	800	1600	3200	6400	12800	25600	51200	102400	> 102400	Total
Confirmed VL	-	-	-	-	-	5	4	2	13	24
Suspected VL	1	-	1	1	-	1	1	-	-	4

Figure 1: Direct Agglutination Test (DAT)



Immunoblotting

A variety of immunoblotting method have been described for detection of leishmania antigens, however, these methods are only used in research laboratories. However, due to the low level of antibody in cataneous leishmaniasis, this method is mostly being used in the diagnosis of visceral leishmania (Kumar et al. 2002; Ravindran et al. 2004).

Antigen Detection

Antigen detection in the serum or urine can be an alternative for antibody detection methods in leishmania diagnosis particularly in the immunocompromised patients, where the antibody response is very poor. However, detection of antigen in the patient's serum is complicated by the presence of high level of antibodies, circulating immune complexes, serum amyloid, rheumatoid factor, and autoantibodies. Recently a new latex agglutination test called KATEX has been developed for primary diagnosis of visceral leishmaniasis. The test is simple and easy to perform. Primary studies have shown 68-100% sensitivity and a 100% specificity for the test with patient's urine (Attar et al. 2001). Besides, the test shows similar results in HIV positive patients and because of the simplicity of the test, it has a good potency in monitoring of the patients who have received a medical treatment (Riera et al. 2004a; Sundar et al. 2005). But using antigen detection methods in the field for detection of leishmaniasis still needs further investigation.

Molecular techniques

Some molecular techniques have recently been developed for a more sensitive detection of leishmania parasites (Smyth et al. 1992; Bastrenta et al. 2002; Mary et al. 2004). The main approaches of these techniques is the polymerase chain reaction (PCR) for the detection of Leishmania DNA, which allows a sensitive, specific and fast detection of minute amounts of the pathogen's DNA (Deborggraeve et al. 2008). PCR is based on the amplification of a known and specific sequence of DNA using oligonucleotide primers (typically 20-mers), which specifically bind to the DNA flanking the region of interest. In PCR-based techniques, primers target ribosomal RNA genes (Srivastava et al. 2011b); kinetoplast DNA (Maurya et al. 2005); miniexon-derived RNA genes, genomic repeats (Kuhls et al. 2007), the β-tubulin gene region (Dey and Singh 2007), glycoprotein 63 (gp63) gene locus (Quispe Tintaya et al. 2004), and internal transcribed spacer (ITS) regions (Mauricio et al. 2004). Recent studies have shown that kinetoplast minicircle is an ideal target DNA in leishmania parasite as there are 10,000 copies of the DNA per cell and its

sequence is known for most of species (Aransay et al. 2000). In visceral leishmaniasis, PCR has opened a new window for diagnosis of leishmaniasis using blood samples with high sensitivity, which is very simple to obtain compared to spleen and bone-marrow aspirates. The sensitivity of the test using blood samples is reported as 70-96% (Osman et al. 1997; Salotra et al. 2001). In PKLD, PCR with either lymph node or skin aspirates is more sensitive than microscopic examination for the diagnosis (Osman et al. 1998). The sensitivity of PCR in PKLD patients is also between 93.8-96%. The specifity of the test is 100%, which is even higher than ELISA (Faber et al. 2003; Salotra et al. 2003). In cutaneous and mucocutaneous leishmaniasis the test has also shown better sensitivity compared to other tests; up to 100% for cutaneous and 86.4% for mucocutaneous leishmaniasis (Faber et al. 2003; Disch et al. 2005). However, compared to other diagnostic techniques available, the molecular tools like PCR and real-time PCR are expensive, cumbersome to perform and need to be made more user-friendly and cost-effective in leishmaniasis endemic areas (Deborggraeve et al. 2008; Bhargava and Singh 2012).

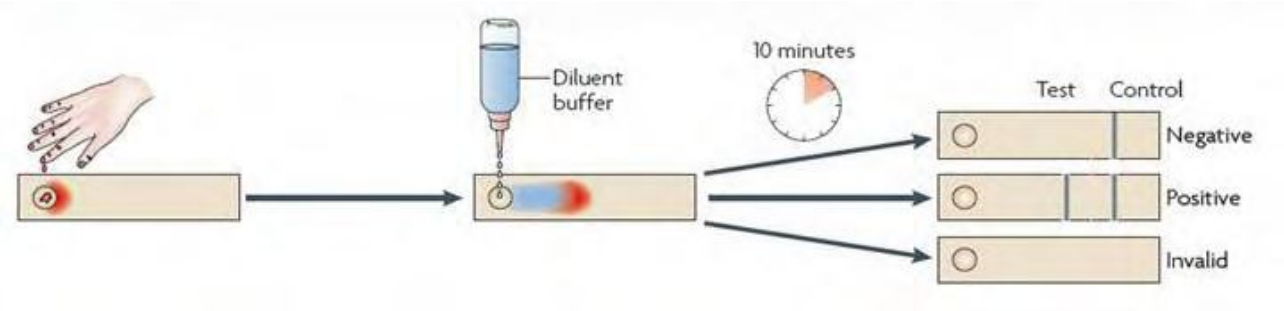
Combination of PCR and ELISA methods has provided promising results for diagnosing visceral leishmaniasis (VL) in blood samples. PCR-ELISA is more sensitive than conventional PCR and demonstrated 100% and 87.2% specificity for healthy controls who had never travelled to a VL-endemic area and controls from a VL-endemic area as references, respectively (De Doncker et al. 2005).

rK39-immunochromatographic test (Dipstick test):

In this test we use 2 protein, A-colloidal gold conjugate and rk39 leishmania antigen. Combination of these two proteins detect anti- leishmania antibody in serum or plasma. Dipsticks are placed in to 50 µl of serum. After 5-8 min, a red control line appears on the test field. If the test is positive, a second line also appears on the test field (figure 2) (Reithinger et al. 2002; Mohebali et al. 2004).

Immunochromatographic test using K39 antigen is a promising method implicated in detection of leishmania. Rrecombinant K39 antigen, which is encoded in the highly conserved kinesin region of *L. chagasi* contains 39 amino acids. In this method, rK39 is fixed on a nitrocellulose paper in an immunochromatographic-based strip test and colloidal gold-protein A is used for detection. The sensitivity of 100% and specificity of 98% in the initial clinical evaluation has been reported. However, the test similar to DAT, in VL cases remain positive for long periods after cure in endemic areas. (Bhargava and Singh 2012). In asymptomatic infection, the sensitivity of the test is less than DAT and LST (Gadisa et al. 2012).

Figure 2: rK39 RDT



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The HIV-VL co-infection is characterized by a high fatality rate and frequent relapses, and cases play an important infectious reservoir (Alvar et al. 2008).

L. tropica was considered to be a strict anthroponosis, but several cases of canine infection have been described (Dereure et al. 1991a; Dereure et al. 1991b; Yahia et al. 2004).