DIAGNOSTIC GAPS AND RECOMMENDATIONS FOR VISCERAL LEISHMANIASIS

Assessment of user needs, use cases, and the diagnostic landscape

PATH

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Acronyms

Ab	antibody
Ag	antigen
CMI	cell-mediated immune
ELISA	enzyme-linked immunosorbent assay
HIV	human immunodeficiency virus
IM	intramuscular
IV	intravenous
KA	kala-azar
LAMP	loop-mediated isothermal amplification
NASBA	nucleic acid sequence-based amplification
PCR	polymerase chain reaction
PKDL	post-kala-azar dermal leishmaniasis
qPRC	quantitative polymerase chain reaction
RDT	rapid diagnostic test
VL	visceral leishmaniasis
WHO	World Health Organization

Executive summary

Visceral leishmaniasis (VL) is a deadly disease caused by infection with the *Leishmania* parasite. The majority of cases are found in South Asia, east Africa, and Brazil. As many as 310 million people are at risk of infection, and it is estimated that between 20,000 and 50,000 deaths result from VL annually. VL is spread through the bite of the sandfly vector, and it can be harbored by human and canine reservoirs. The parasite causes nonspecific symptoms such as fever and splenomegaly; if left untreated, VL typically leads to death.

The World Health Organization (WHO) 2020 goal is to eliminate VL from the Indian subcontinent (i.e., achieve prevalence of less than 1 case per 100,000). The WHO set a number of goals for the Neglected Tropical Diseases (NTD) to be achieved by 2020, and the London Declaration on NTDs backed these goals with commitments from public and private institutions. The 3rd progress report of the London Declaration indicated that "priorities for progress" towards reaching VL goals include early detection of cases, improved access to diagnosis, and scale-up of diagnostic services.

In support of the London Declaration goals, PATH aims to catalyze engagement of the diagnostics industry and product development efforts. As part of this work, PATH assessed needs and landscaped potential solutions to improve diagnostic tools used to support VL elimination efforts. We conducted literature reviews, a product development landscape, and interviews with key stakeholders to identify gaps in current human VL diagnostics as well as emerging solutions. These findings were used to identify use cases for VL diagnostics, determine which tools address specific use cases, analyze progress toward robust diagnostics in the development pipeline, and ultimately to propose recommendations on how to improve availability, access, and adoption of VL diagnostics.

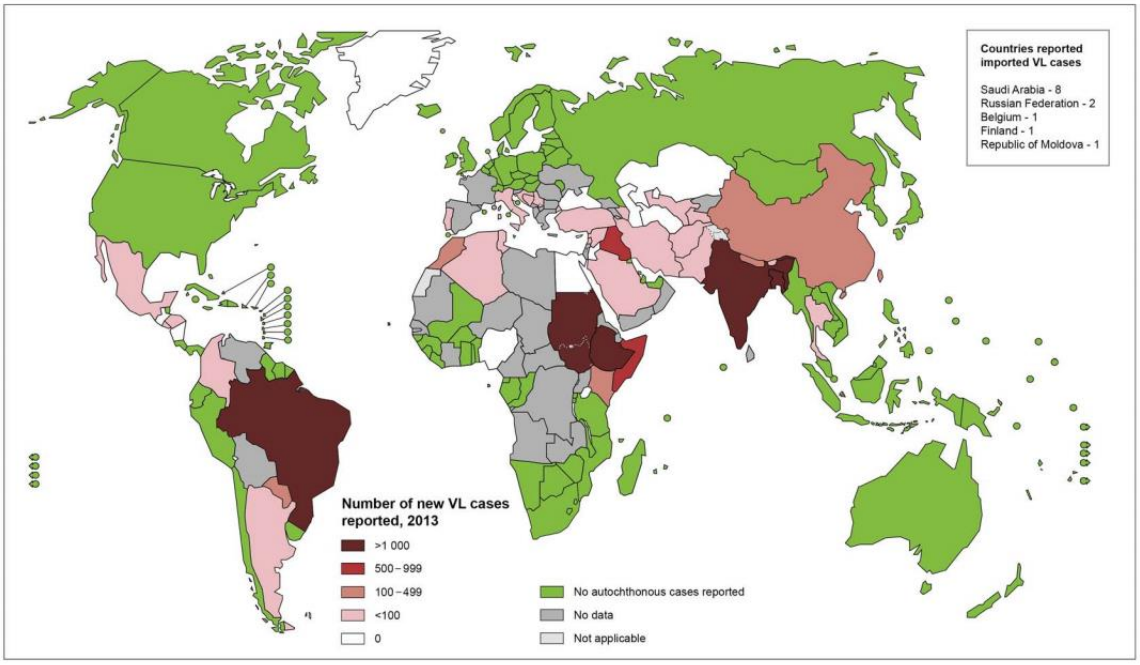
PATH identified four use cases for human VL diagnostics: diagnosing acute infection, diagnosing VL-HIV coinfection, diagnosing post-kala-azar dermal leishmaniasis (PKDL), and treatment monitoring. We found that current tools and methods are likely sufficient for the early case detection needed to support elimination goals. However, current rapid diagnostic tests (RDTs) have limitations and new tools would benefit patients with HIV coinfection and PKDL, as well as improve treatment monitoring. We have developed the following recommendations:

1. **Support ongoing efforts to ensure full access to, and adoption of, current antibody detection RDTs.** Health systems and market research may be needed to support optimal uptake of currently available antibody tests.
2. **Develop an antigen detection test to better diagnose VL-HIV coinfection and monitor treatment.** There is a need for a noninvasive, field-friendly test that can identify active VL infections among HIV coinfected patients, as well as monitor for treatment failure and relapse.
3. **Support research and development needed for next generation antibody detection RDTs.** A noninvasive, sensitive rapid test is needed to enable treatment monitoring and detection of PKDL.

Introduction

Visceral leishmaniasis (VL) is a neglected tropical disease associated with poverty and lack of access to health services and other resources. The disease attacks the immune system, affects bone marrow and internal organs, and causes fever, weight loss, and anemia. Left untreated, VL can have a fatality rate as high as 100 percent within 2 years. Although VL is found around the world, over 90 percent of the cases are concentrated in Bangladesh, Brazil, Ethiopia, India, Sudan, and south Sudan (see Figure 1 for disease distribution). An estimated 300,000 new cases occur each year, resulting in between 20,000 and 50,000 deaths.[1] Most of the VL burden is in India, where 40 percent of all new cases are found.[2] Disproportionately affecting patients in poor and remote areas, as much as 20 percent of patients with VL infection may die before their disease is recognized.[3] It is estimated that 310 million people are at risk of infection in the six most heavily affected countries.[2] However, inadequate surveillance and misdiagnosis mean that the true incidence, mortality, and morbidity of VL are unknown.

Figure 1. Burden and distribution of new visceral leishmaniasis cases. Reported by World Health Organization, Control of Neglected Tropical Diseases as of 2013.[4]



The 2012 World Health Organization (WHO) Neglected Tropical Disease (NTD) Roadmap aims to eliminate VL from the Indian subcontinent, where elimination is defined as achieving prevalence less than 1 case per 10,000 people.[5] Current WHO control strategies include early diagnosis and complete treatment of cases, integrated vector management, effective surveillance through both passive and active case detection, social mobilization, and clinical and operational research as needed.[6] Several factors indicate that elimination of VL as a public health problem in the subcontinent is possible. Transmission is anthroponotic with humans as the sole reservoir of the disease, and there is a single vector, the sandfly,

that is being reduced as a side effect of malaria elimination programs. The disease is geographically focused in border districts in Bangladesh, India, and Nepal. Furthermore, there is strong political will in these three countries to eliminate VL, expressed through the signing of a memorandum of understanding and support from the WHO.[7]

Shortly after the release of the NTD Roadmap, 20 public and private institutions that support global health and international development—including pharmaceutical companies, donors, governments from endemic countries, nonprofit organizations, and others—joined the efforts to reach the 2020 goals for 10 of the 17 diseases, in a document known as the London Declaration on Neglected Tropical Diseases.[8] The London Declaration represents a commitment from these institutions to sustain, expand, and extend programs and interventions to achieve the Roadmap goals for NTDs, including the control of VL.

The London Declaration 3rd Report identified the following strategic priorities for VL: improved surveillance in Bangladesh, India, and Nepal and a scale-up of diagnostic and treatment services in East Africa and Latin America.[1] In response to these priorities, PATH conducted a diagnostic landscape analysis to identify gaps, and evaluated current and emerging VL diagnostics for humans that may provide solutions. This analysis was informed by a review of literature and interviews with organizations and experts in the VL community. The literature review included peer-reviewed publications, policies and guidelines, documents from expert meetings, country case studies, and a review of the technology landscape. The peer-reviewed literature was searched for studies that evaluated human diagnostic tools and algorithms in clinical management. Key grey literature documents include WHO guidelines and control strategies.

Key organizations in the VL community were identified through their roles in global and country-level programs, academic research, and participation in consultative meetings, and through referral from other stakeholders. Stakeholders were interviewed using a semi-structured interview guide focusing on several themes identified through the literature review. These themes included disease progression and treatment, access to care, diagnostic use cases and user needs, and existing technologies and technology gaps. Information from the literature review, product development landscape, and stakeholder interviews was compiled to:

- Identify use cases and understand current VL diagnostic practices and tools associated with each use case.
- Analyze progress toward robust diagnostics for VL across different biomarkers.
- Develop recommendations for steps to improve the availability, access, and adoption of VL diagnostic tools.

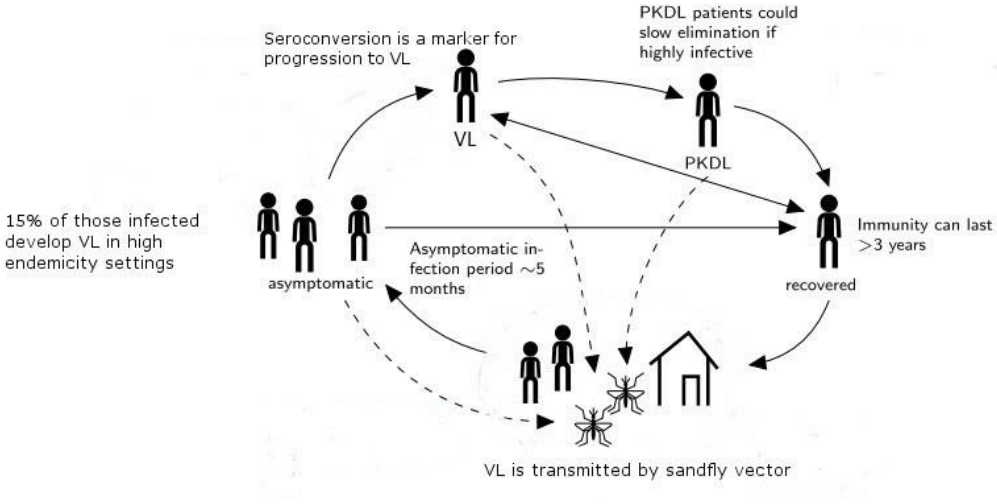
Diagnostic landscape

Disease course and transmission

Visceral leishmaniasis, also known as kala-azar (KA), is caused by a protozoan parasite transmitted to humans by sandflies.[6] VL is one of four clinical syndromes caused by *Leishmania* alongside cutaneous leishmaniasis, mucocutaneous leishmaniasis, and post-kala-azar dermal leishmaniasis (PKDL), a complication of VL characterized by infectious lesions on the skin.[9] This analysis focuses on VL and PKDL due to their inclusion in the goals of the 2020 London Declaration.

The causative agent of disease varies across geographies: *Leishmania (L.) donovani* is responsible for VL cases in East Africa and the Indian subcontinent, whereas *L. infantum* is responsible for transmission in Europe, North Africa, and Latin America.[10] Zoonotic transmission of the disease occurs from animals, usually a dog, to the sandfly vector and then to humans, and is most prevalent in areas of *L. infantum* transmission. Anthroponotic VL is transmitted from humans to the vector, and back to humans, and is prevalent in areas with *L. donovani*.[6] Figure 2 provides an overview of the disease course and progression.

Figure 2. Disease course and progression of visceral leishmaniasis. Adapted from Hollingsworth, et al. [11]



Following an incubation period, VL infected patients generally experience nonspecific symptoms including fever, fatigue, weakness, loss of appetite, and weight loss—symptoms that are shared by a number of other diseases that affect at-risk populations.[13] The parasite invades the host bone marrow, spleen, and lymph nodes, resulting in an enlarged spleen and liver, anemia due to inflammation, and a weakened immune system. If left untreated, VL is usually fatal. As a result of suppressed immunity, death from other common diseases such as tuberculosis, pneumonia, and diarrhea is also more likely in VL patients.[14]

Not all infections progress to disease. Asymptomatic infections may also play an important role as a reservoir for the parasite, and studies suggest asymptomatic infections may be prevalent.[12] The host cell-

mediated immune (CMI) response is a critical factor in determining the course of the infection.[6] Risk factors for infection and progression to symptomatic illness include young age, genetic susceptibility, malnutrition, repeated exposure to sandfly bites, and immunosuppressive conditions such as HIV coinfection.[6]

VL-HIV coinfection is another challenge, as the parasite infection can induce chronic immune activation, resulting in worsening HIV progression, while immune dysfunction caused by HIV can lead to uncontrolled and therefore higher parasite loads.[15] *Leishmania*-HIV coinfection rates range from a reported 2 to 9 percent of VL cases but may be as high as 30 percent in some target areas.[15,16] HIV and *Leishmania* infection have a synergistic damaging effect on host CMI responses, resulting in an increased HIV load and uncontrolled multiplication of the parasite.[15] Coinfection complicates diagnosis and treatment and increases the likelihood of relapse after treatment.[15,17]

VL can be followed by PKDL, a non-fatal skin condition characterized by parasitemia in skin lesions. In India and Bangladesh, PKDL is reported in 5 to 10 percent of patients within months of treatment, whereas in Sudan, it develops in over half of patients within a few weeks.[18] Because the disease presentation differs by region, treatment for PKDL also differs between countries. PKDL in Sudan is often self-limiting so treatment is varied by severity of disease. In India, treatment is required to cure the disease, and a similar treatment regimen is administered for all patients.[19] PKDL patients act as a reservoir for the parasite, particularly between epidemics, and may be a critical factor in the transmission of the disease.[12] Several expert meetings have highlighted a need for a greater focus on PKDL, particularly in South Asia given elimination goals for the region.[20]

Diagnosis

The clinical symptoms of VL are largely nonspecific, including fever, loss of appetite, weight loss, and enlarged lymph nodes, spleen and liver. Due to the nonspecific nature of VL symptoms and varying rates of coinfections, clinical signs and symptoms are often unreliable and inconclusive indicators of VL infection.[21] As a result, confirmatory tests are necessary to diagnose VL. The reference standard for VL diagnosis is demonstration of parasites from tissue aspirates, which is complex and invasive, requiring samples from splenic, bone marrow, or lymph node aspirates.[21] The amastigote forms of the parasite can be seen intracellularly in monocytes or macrophages by microscopic examination of Giemsa-stained specimens. The identification of amastigotes requires expertise and training, causing accuracy to be dependent on the microscopist. Sample collection by needle aspirates also requires medical expertise and infrastructure, precluding diagnosis at the community or primary care level. Methods using samples from the spleen have a greater specificity than methods using samples from either bone marrow or lymph nodes, but they are also more dangerous to the patient and risk fatal internal bleeding.[22]

Field-friendly tests that facilitate more efficient confirmation of VL are currently available. A rapid diagnostic test (RDT) to detect antibodies to the rK39 antigen has demonstrated accuracy and is supported by the WHO to diagnose primary infection along with clinical signs.[23] In a recent Cochrane review, the test demonstrated 92 percent sensitivity and specificity in India compared to clinical confirmation of disease, however, it performed less well in East Africa.[24] In response to the diminished performance in

East Africa, an RDT to detect antibodies to the rK28 antigen was developed and has demonstrated similar sensitivity and specificity to the rK39 antibody detection tests.[25,26] A shortcoming of antibody detection confirmatory tests is the inability to differentiate between acute disease that is fatal if left untreated, asymptomatic infection that is not treated because of the toxicity of current treatment regimens, and past or cured infection that does not require further treatment. Additionally, confirmation of PKDL among individuals who are otherwise healthy is not feasible with the rK39 antibody detection tests.[27]

Timely diagnosis of VL is also critical to the elimination strategy.[7,28] Early case detection and treatment is key to reducing mortality and morbidity as well as to interrupting transmission of VL, as variance in the time it takes to diagnose VL may explain varying rates of incidence around the globe.[29] Case detection can be both passive and active but there are still significant delays in care-seeking, diagnosis, and initiation of treatment.[30,31] Delays can be attributed to low awareness of the disease resulting in misdiagnosis, and care-seeking from providers unqualified to diagnosis and treat VL appropriately.[7] While these point-of-care diagnostics have been introduced in many countries, including India, to expand case identification and improve surveillance efforts, stakeholders suggest that further access and adoption of the available RDTs may be needed.[32] Additionally, adherence to testing algorithms by clinicians may be a challenge with rK39 antibody detection tests.[33] Full uptake and proper use of these tools is essential at all levels of health care in order to achieve regional elimination goals.

Treatment

The treatment of VL requires anti-leishmanial drugs and the management of other bacterial coinfections as well as management of associated anemia or malnutrition. Treatment availability and guidelines vary widely, as illustrated in Table 1. Sodium stibogluconate was the preferred first-line treatment, but its toxicity and development of resistance in India prompted a switch to amphotericin B or miltefosine.[6,21,27] With the exception of miltefosine, drugs must be administered via intravenous (IV) infusion or intramuscular (IM) injection, requiring inpatient treatment for the duration of the regimen, up to 30 days. Miltefosine cannot be used to treat pregnant women.[6] Treatment failure occurs in 3 percent to 30 percent of cases in endemic areas and much more often among patients coinfected with HIV.[34] Ongoing concerns regarding increased parasite resistance to miltefosine, the only oral treatment option, and high rates of treatment failure are indicative of inappropriate and insufficient treatment options.[35]

There is an urgent need for better drugs and shorter treatment regimens.[36] Current drugs deviate significantly from the desired VL treatment, which should be affordable, safe, short-course, and orally administered in an outpatient setting. Additionally, parasite resistance to the drugs should not be evident, and the drugs should be effective for all cases including HIV coinfected patients, who are more likely to experience lower cure rates and adverse reactions due to drug toxicity.[27] Several clinical trials and renewed research and development efforts have been undertaken by various partners and WHO to develop better drugs for VL treatment.[27] Combination therapies are recommended as a way to increase treatment efficacy with current drugs, prevent the development of drug resistance, and reduce treatment duration.[6]

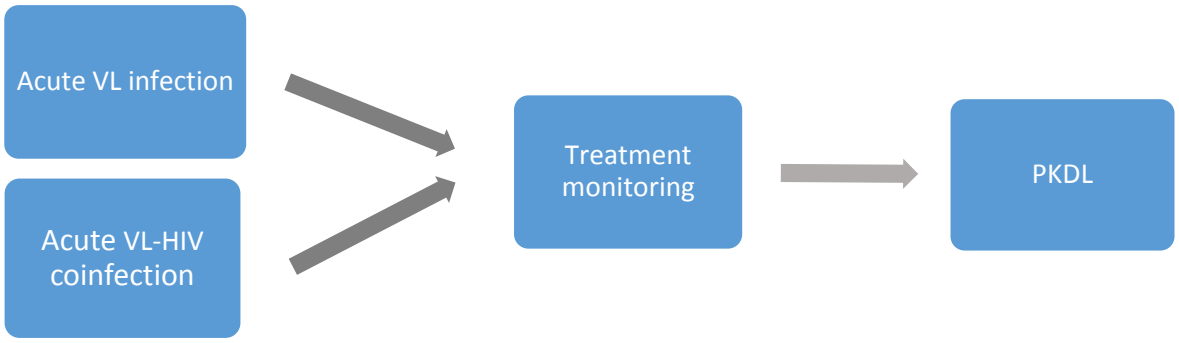
Table 1. Currently available drugs for the treatment of visceral leishmaniasis. Adapted from Mondal, et al.[27]

	Pentavalent antimonials	Amphotericin B	Liposomal amphotericin B	Miltefosine	Paramomycin
Registration	Bangladesh, Brazil, Ethiopia, Kenya, Sudan, India, Nepal, and Uganda	Brazil, Ethiopia, Kenya, Sudan, India, Nepal, and Uganda	Brazil (allowed for use on compassionate basis in other endemic areas)	India	India
Regimen	20 mg/kg daily for 20–30 days (depending on the geographical region)	0.75–1 mg/kg for 15–20 infusions either daily or on alternate days (15–20 mg/kg total dose)	5–20 mg/kg total dose in 1–10 doses over 1–20 days	50–100 mg (for bodyweight of <25 or ≥25 kg) for 28 days, and 2.5 mg/kg for children (India only)	15 mg/kg for 21 days (India only
Administration	Intramuscular (IM) and intravenous (IV)	IV	IV	Oral	IM
Clinical efficacy	36%–95% (depending on the geographical region)	>95% in all regions	>98%; single dose (5–15 mg/kg) 91–100% cure established only in India; treatment failure in Sudan with 20–30 mg/kg in a few cases	94%–97% (India)	94% (India)
Advantages	• Relatively inexpensive • Less toxic than other drugs	• High efficacy • No reported resistance	• Short treatment duration • Less toxic	• Oral administration • Few side effects • Safe in VL-HIV coinfection	• Inexpensive • Few side effects
Disadvantages	• Long duration of treatment • Development of resistance in India	• Long duration of treatment • High toxicity requires hospitalization	• Expensive • Requires slow infusion	• Relatively expensive • Low compliance • Cannot be used during pregnancy • Potential for resistance	• Low efficacy in Sudan • Long duration of treatment • Potential for resistance

Use cases

This analysis identified four use cases for human VL diagnostics: diagnosis of acute infection, diagnosis of VL-HIV coinfection, treatment monitoring, and diagnosis of PKDL (see Figure 3). Case diagnosis and treatment monitoring represent critical use cases for both acute VL infection and VL-HIV coinfection while only a subset of VL infected cases develop PKDL.

Figure 3. Use cases for visceral leishmaniasis diagnostics.



Acute infection

Rapid, field-friendly diagnosis of acute VL infection offers the opportunity to identify cases and immediately initiate treatment. As described above, timely diagnoses of febrile illness can limit disease transmission. Accurate diagnosis further reduces unnecessary and inappropriate treatment for malaria, TB, and typhoid. Due to the prevalence of asymptomatic infection and the toxicity of current treatments, only patients experiencing clinical symptoms undergo treatment. A fever lasting for two weeks, other clinical signs such as splenomegaly, and a positive antibody test together are conditions of diagnosis. Diagnostic support for this use case is largely satisfied by current RDTs.

VL-HIV coinfection

In patients with VL-HIV coinfection, typical clinical signs of VL may not be present and other opportunistic infections may complicate diagnosis.[15] Given the risk of treatment failure and relapse in coinfected patients, noninvasive diagnostic methods are needed to avoid repeated bone marrow aspirations. Also, because of immune suppression, parasitemia may be high while antibody levels may be low causing serologic tests to be of limited value and requiring at least two different tests to be used for confirmation.[34] One option for the diagnosis of VL-HIV coinfection is the screen-and-treat strategy used with other HIV-associated infections. This strategy would require the development of prognostic tools and risk cut-offs to inform treatment decisions as well as safer and better-tolerated treatment options.[17]

Treatment monitoring

A test to monitor treatment is needed to inform clinical decision-making during the complicated treatment regimen and to support ongoing investments in better drugs. To target patients for treatment, there is a need to differentiate between acute and recent infections, which currently available antibody tests cannot do.[37] There is also a need to identify and prevent the progression of parasite resistance. These requirements point to a currently unmet need for a diagnostic tool to identify treatment failure and relapse.[34]

PKDL

Similar to regimens for VL, treatments for PKDL are long and toxic, necessitating greater certainty in the diagnosis in otherwise healthy individuals.[20] Diagnosis of PKDL may include clinical signs, detection of rK39 antibodies to demonstrate history of VL, polymerase chain reaction (PCR), or demonstration of the presence of parasites on a punch biopsy. Because PKDL is a complication of VL, current antibody tests have limited efficacy. Antigen tests developed for urine may not be useful for PKDL because PKDL is characterized by low numbers of parasites localized in the skin rather than systemic infection. Antigen tests using other specimens like nodular aspirate have the potential to be validated and used.[38] Similarly, the rK39 antibody detection tests may be able to detect PKDL infection if used with skin-slit smear samples rather than finger prick blood.[18]

Regional differences in the policy and clinical management of PKDL also impact diagnostic needs. Treatment in Sudan may only be recommended for severe cases, while in India it is recommended for all cases. Therefore, a diagnostic tool to detect PKDL may be more important in the Indian subcontinent than Africa. Treating all PKDL cases in the Indian subcontinent is further complicated because PKDL-infected individuals may not be seeking health care if they feel healthy, aside from the skin lesions.[19]

Current diagnostic tools

Field friendly, point-of-care tests are most desirable due to a lack of infrastructure in regions where accurate diagnosis is needed and where use of more laboratory-based assays may be impractical. See Table 2 for an overview of the diagnostic landscape.

Clinical signs

According to WHO, diagnosis of VL is made by combining clinical signs with parasitological or serological tests,[39] with the clinical case definition as persistent fever and splenomegaly in a person residing in a VL-endemic area.[40] These clinical definitions lack specificity due to overlap with diseases that can be prevalent in VL-endemic areas, such as malaria, hyper-reactive malarial splenomegaly, enteric fever, tuberculosis, brucellosis and hematological malignancies. Given the high cost and toxicity of the available treatment options for VL, starting a course of anti-leishmanial treatment solely on the basis of clinical suspicion is not recommended.

Parasitology

Parasitological confirmation remains the reference method for diagnosis, which entails microscopic identification of the parasite in lymph nodes, liver biopsy, or spleen aspirates, or the buffy coat of peripheral blood or culture, after staining with Giemsa or Leishman stain. The most sensitive method is microscopic analysis with splenic aspiration. However, due to invasiveness and potentially fatal complications, spleen puncture can only be used under highly controlled conditions and is not suitable for peripheral health settings. Bone marrow and lymph node aspirates are other options for parasite confirmation but are less sensitive and these methods are still invasive. Also, parasite confirmation is

challenging in immunocompetent individuals with VL due to low levels of parasitemia. The identification of parasites from aspirates of VL and PKDL patients requires expertise and training, and the accuracy is dependent on the microscopist.[41,42]

Antibodies to VL-specific recombinant proteins and peptides

Current antibody-detection tests, though numerous, have limitations in some target populations, such as patients with diminished immune responses (HIV coinfection) and those with preexisting antibody responses to prior VL infection. The first serological test developed for VL is the direct agglutination test (DAT), which now uses freeze dried VL specimens.[43] Although it is highly sensitive and specific, and its performance does not vary by region, the DAT requires substantial training and expertise and can only be read after a minimum of 8 hours incubation.[23] In 2012, WHO evaluated the diagnostic accuracy of five commercially available VL RDTs with rK39 and rKE16 VL specific antigens and demonstrated variation of sensitivity and specificity between the major endemic regions.[44]

Antibodies to VL rK39

Antibody responses to the recombinant antigen, rK39, have been demonstrated to be a highly sensitive and specific marker of acute VL disease caused by members of the *L. donovani* complex.[45,46] The rK39 antibody detection RDT is a field-friendly, easy to use format that has been extensively tested in many countries.[24] In a WHO supported multicenter trial, the FDA-approved rK39 RDT (Kalazar Detect- Inbios, Seattle) demonstrated excellent sensitivity (>95 percent) and specificity (>90 percent) in the Indian subcontinent (India and Nepal), but only moderate sensitivity (75–85 percent) and specificity (70–92 percent) in East Africa (Sudan, Kenya and Ethiopia).[47] Sensitivity and specificity of the rK39 antibody RDT were determined against a panel of 500 clinical specimens obtained from confirmed symptomatic individuals with positive microscopy on splenic, lymph node or bone marrow aspiration. The rK39 antibody detection test was integrated into the elimination program in the Indian subcontinent in 2005.[7] Since then, VL tests lacking stringent quality controls have appeared in the market, which presents a challenge to current treatment as poor quality diagnostic tools can be as detrimental as poor quality drugs.[6,48]

Antibodies to VL rK28

To overcome the geographical limitations of the rK39 antibody detection test, a recombinant fusion antigen, rK28, was identified and developed into a next generation RDT that showed an improvement over rK39 in Africa without changes to sensitivity in the Indian subcontinent.[25, 26, 49-51] Based on these publications, a number of manufacturers have developed prototypes and products utilizing the rK28 antigen.

Antibodies to VL rK18 and rK26

Though antibody responses against the rK39 and rK28 antigens remain elevated after treatment, antibody responses against two other VL antigens, rK26 and rK18, have been shown to decline after treatment in Ethiopia and Bangladesh, potentially enabling their use as indirect measures of parasite clearance.[37] Also in Bangladesh, patients that had treatment complications such as treatment failure, relapse, or PKDL development did not have the same declining anti-rK26 and anti-rK18 responses as those who were cured.

Further study is needed to determine whether these markers identify patients who would benefit from closer observation due to lack of cure.[37]

VL antigens

The latex agglutination test (KAtex) detects a heat-stable carbohydrate VL antigen in urine. Evaluated across 5 sites in East Africa and the Indian subcontinent, it has shown 64-98 percent specificity and 36-84 percent sensitivity compared to latent class analysis,[47] though other estimates have been determined.[52,53] A rapid decline of antigen levels in urine after treatment suggests KAtex may be a potential test of cure, however, lower sensitivity in clinically suspected patients in Nepal has been observed.[54] KAtex has also shown good to fair performance in HIV-positive patients.[55,56] Although the technical principle of the KAtex test is promising, its current format, requires the boiling of urine and is not easy to use in clinical practice.[42]

Recently researchers developed and assessed two new ELISA-based standardized tests to detect *Leishmania* specific antigens in the urine of patients, the *Leishmania* Antigen Detect™ ELISA (Inbios) and the *Leishmania* antigen ELISA (Kalon).[57] The *Leishmania* Antigen Detect™ ELISA contains antibodies raised against a panel of *L. donovani* antigens while the Kalon products, *Leishmania* antigen ELISA and KAtex, contain polyclonal antibodies to whole promastigotes. Both ELISAs displayed higher sensitivity on samples from Ethiopia, Sudan, Brazil, and Bangladesh compared to KAtex, and comparable declining antigen detection following treatment. These assays could be further adapted to a more point of care lateral flow format, which may have promise for treatment monitoring in endemic regions.[57]

Nucleic acid tests

Nucleic acid amplification methods are both sensitive and specific and can be used on skin, blood, and bone marrow samples of VL, HIV-VL and PKDL patients.[20,58] These methods can be used as a confirmatory test after initial screening by serological tests but are not as field-friendly as RDTs. Also, PCR cannot confirm acute VL disease in patients in endemic areas because of asymptomatic carriers of the infection that would be PCR positive but do not have the disease.[20]

Table 2. Overview of visceral leishmaniasis diagnostic landscape.

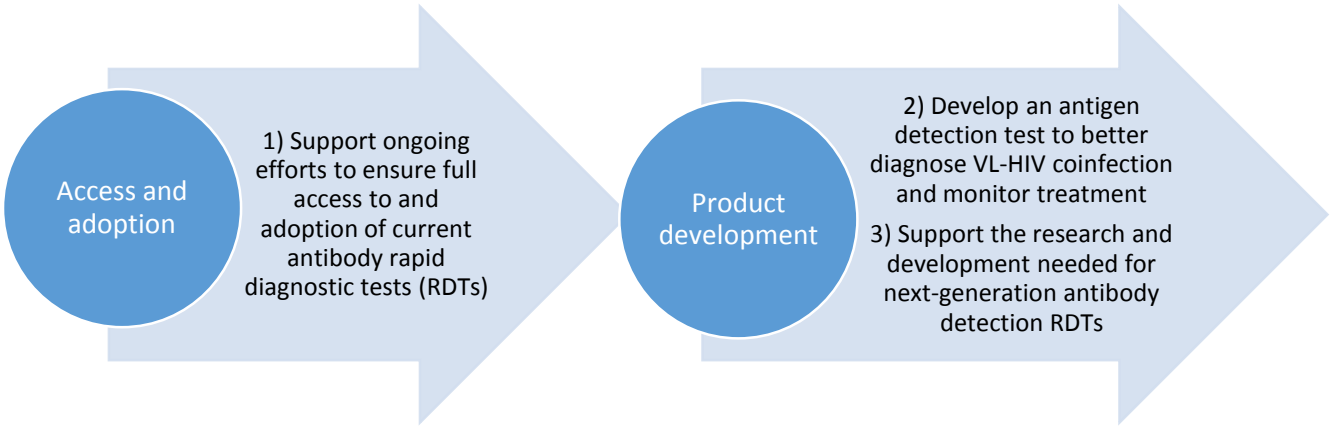
Biomarker	Candidates	Sample type	Format	Stage of product development	Use case	Pros	Cons
Clinical signs and symptoms	Fever with splenomegaly	Exam	Clinical exam	• NA	• Suggestive of VL infection, requires confirmation (microscopy/antibody) • Monitor post-treatment recovery • Suggestive of PKDL	• World Health Organization (WHO) recommends combining clinical signs with parasitological or serological tests	• Diagnosis often delayed by 2-4 months, after other treatments fail (antibiotics, antimalarials)
Demonstration of parasite	*Leishmania donovani* complex	Bone marrow, lymph node, and spleen aspirates	Microscopy, culture	• Lab-based test (gold standard)	• Confirm diagnosis of VL	• Gold standard	• Invasive, can be fatal (spleen puncture) • Expert physician and infrastructure essential • Lower sensitivity (bone marrow and lymph node aspirates)
Antibodies to VL-specific recombinant proteins and peptides	rK39, rK28, rK26- rK18, rKE16, serum for direct agglutination	Whole blood, serum, split aspirate for PKDL	• Lateral flow/RDT • Direct agglutination test (DAT)	• rK39 RDTs available (Inbios, Biorad, CTK biotech) • rkE16 RDTs available (Span) • rk39/rk28 RDT available (CTK biotech) • rk28 RDT in development (Chembio, EMT, InBios) • rk26/rk18 in development (IDRI) • DAT reagents available (KIT)	• Diagnose VL without HIV coinfection (rK39, rK28, rKE16) • Diagnose PKDL (rK26/18 in blood) • Suggest PKDL (rK39 in lesion aspirate, rK39 in blood indicates history of VL, which is necessary for PKDL diagnosis) • Post-treatment monitoring or test for cure (rK26/18)	• rK39 RDT in market • rK28 may have greater sensitivity in Africa • rK26-rK18 antibodies decline post treatment (continued detection if PKDL or relapse) • DAT is sensitive and not dependent on geographic regions	• False negatives for HIV coinfected (low/no antibody response) • rK39: remains positive after treatment; diminished sensitivity in Africa • Cannot differentiate past/ current infection • DAT not as field friendly as RDT • Detects asymptomatic VL in endemic areas

Biomarker	Candidates	Sample type	Format	Stage of product development	Use case	Pros	Cons
VL antigens	Low molecular weight, heat-stable, carbohydrate antigen	Urine	• ELISA • Slide agglutination • RDT	• ELISA in development (FIND-Kalon, InBios) • KAtex in market (Kalon) • RDT in development (FIND-SD)	• Diagnose symptomatic cases with/without HIV coinfection • Post-treatment monitoring (test for cure, detect relapse)	• Amenable to RDT	• High sensitivity required to detect Ag (HIV coinfection and PKDL) • KAtex requires boiling urine, low sensitivity, not as field friendly as RDT • ELISA not as field friendly as RDT
Nucleic acid	qPCR, nested PCR, NASBA, LAMP, *Leishmania* OligoC	Various tissues/ specimens	PCR followed by lateral flow	• qPCR, nested PCR, NASBA in development • LAMP in development (FIND/Eiken) • *Leishmania* OligoC available (CORIS BioConcept)	• Detect acute VL infection • Detect PKDL •	• Sensitive for PKDL with skin aspirates • Sensitive for VL with HIV • Detects low level parasites in blood	• Remains positive after treatment • Detects asymptomatic VL in endemic areas • Different samples for different use cases • Not field friendly

Conclusions

Current tools and methods for VL are likely to enable the acute infection detection needed to achieve regional elimination in the Indian subcontinent. While current antibody detection RDTs may have sufficient performance, ongoing support may be needed to ensure full access and adoption. However, these RDTs are not sufficient in cases of VL-HIV coinfection, to monitor treatment, or to diagnose PKDL, a condition becoming increasingly important with regard to its role in disease transmission. To support regional and global VL goals, PATH offers the following recommendations to the VL research community. Table 3 provides a summary of recommended product attributes of needed diagnostic tools**.**

Figure 4. Proposed diagnostic recommendations to the visceral leishmaniasis research community.



1) **Support ongoing efforts to ensure full access and adoption of current antibody RDTs.** Clinical symptoms are likely to remain important in the initial screening of infection but lack of specificity due to symptom overlap with other common diseases like malaria, typhoid, and tuberculosis mean that the current RDTs play a critical role in identifying acute infection. Further research may be needed to understand the current market and health system factors influencing access and adoption of these tests.

2) **Develop an antigen detection test, which would enable improved diagnosis of VL-HIV coinfection and improved treatment monitoring.**
A test that is both field friendly and does not rely on antibodies could be used to improve diagnosis of VL-HIV coinfection and monitor treatment outcomes for relapse and the development of parasite resistance. The format of current antigen detection tests has operational disadvantages regarding its use in remote areas where VL is prevalent and tests in an RDT format are desirable. Efforts to develop or adapt this product are ongoing and should be supported and monitored.

3) **Support the research and development needed for next-generation antibody detection RDTs.** Current antibody detection tests have limitations in that antibody levels remain high after

treatment, preventing differentiation between current and past infections, which is necessary for treatment monitoring and PKDL detection. Promising research has identified antibodies that decrease after treatment (rk26/18) and furthering these efforts should be supported.

Table 3. Summary of product development recommendations: product attributes of needed diagnostic tools for visceral leishmaniasis.

	Antigen detection RDT	Improved antibody detection RDT (rK26/rK18)
Use cases	• VL-HIV coinfection • Treatment monitoring	• Treatment monitoring • PKDL
Specimen	Urine	Blood
Health system level of use	Tier 2–3	Tier 2–3
Value proposition	A noninvasive, field-friendly test to detect VL-HIV coinfections for immediate treatment initiation and to monitor treatment progress for optimized therapeutic regimens.	A more-sensitive, noninvasive test to determine if active infection is still present after acute VL infection has occurred, such as with PKDL and VL relapse, which necessitate further treatment regimens.

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