# Diagnosing the biggest parasitic killers:

## A review of available diagnostic tools for malaria and visceral leishmaniasis

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**The international effort to control malaria has received decades of global funding and resources, and for this reason rapid diagnostic testing has been rolled out to many communities with great success. Historically, malaria diagnosis has relied heavily on microscopic analysis of blood smear slides - but in the last decade there has been a huge rise in the implementation of rapid diagnostic tests (RDTs). Due to their excellent convenience and affordability, RDTs have enhanced access to accurate diagnosis. On the other hand, visceral leishmaniasis is classified as a neglected tropical disease and affects the poorest people in remote areas, which makes diagnosing the disease even more challenging. Whilst microscopy is incredibly accurate, acquiring the tissues required for biopsy is far more invasive than taking the blood sample required for the diagnosis of malaria. Serological testing, whilst not appropriate for malaria diagnosis, maintains high sensitivity and specificity and can be easily deployed to rural health outposts. Discussed herein are new diagnostics which present an incredible opportunity for the advancement of the control of these diseases and represent a major step towards elimination goals. Indeed, successful control of both diseases will require an integrated approach that considers multiple molecular and serological or antigenic techniques.**

### Introduction

Diagnosis is the first step in controlling the spread of disease. Diseases such as malaria and visceral leishmaniasis affect people across the world and cause immense suffering and disability if not properly diagnosed (as seen in Box 1 and 2). Combined, these diseases account for almost 415,000 deaths recorded in 2019, which re-enforces the need for new technology to diagnose and treat patients quickly and accurately (WHO, 2020, DNDi, 2021).

To this end, the World Health Organisation has identified seven criteria to aid the development of new tools to diagnose disease: The acronym ASSURED stands for Affordable, Sensitive, Specific, User-friendly, Rapid and robust, Equipment-free and Deliverable to end-users. For new tools to be effective in real-word settings, they must adhere to these criteria. There are many complex biological and sociological differences between malaria and visceral leishmaniasis infections, which means the technology used to diagnose the diseases must be different. Rapid diagnostic tests (RDTs) are very effective in diagnosing malaria cases, and are also affordable and accessible to poor, rural communities. However, to diagnose visceral leishmaniasis it will be more practical to use specialised technology, such as serological testing. For both diseases, microscopy is a very accurate and useful tool, but it will be difficult to find the resources to implement it on a large scale. A brief schematic of this review can be seen in Figure 1.

Specific and sensitive testing limits unnecessary administration of frontline treatments, which subsequently prevents the expansion of drug resistance. Prompt diagnosis of disease also contributes to the rapid administration of treatment to patients, thus reducing clinical manifestation and further transmission. Diagnosis of malaria has historically relied on parasite identification

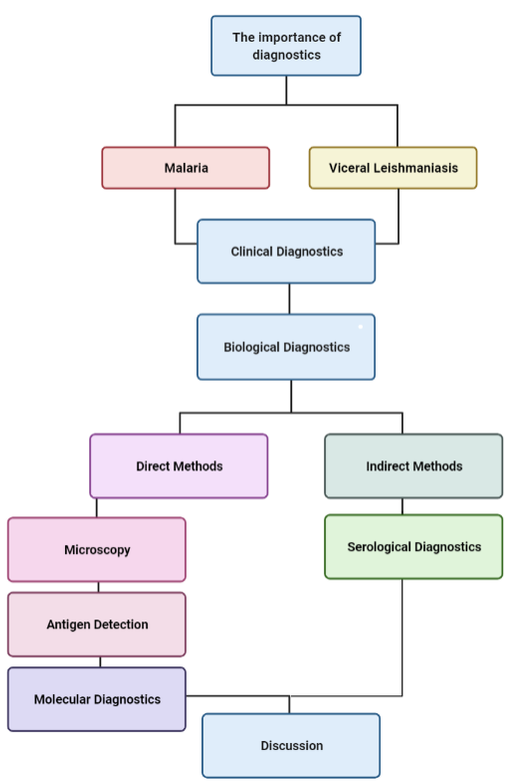


**Figure 1.** A brief schematic of the this review.

by microscopy, but research into new diagnostic tools for malaria have expanded the field into molecular diagnostics as well as antigen detection.

Visceral leishmaniasis, a neglected tropical disease (NTD), is identified as requiring innovative and intensified disease management (IDM) i.e., diagnosis, treatment and follow up is difficult and costly to manage, and the people affected are in rural areas where diagnosis is difficult to

access (WHO, 2020). To date, diagnostic and monitoring tests for NTDs such as visceral leishmaniasis are limited compared to diseases such as malaria, which receive greater treatment and research funding (Kosack *et al.,* 2017).

Understanding the biological and sociological causes of these discrepancies will be invaluable in improving malaria and visceral leishmaniasis diagnosis and have been reviewed here.

**Box 1. Malaria**

*Plasmodium* parasites are transmitted by the bite of female *Anopheles* mosquitoes, and cause the disease known as malaria. Mild symptoms include fever and chills, though without prompt treatment, the disease may progress to a more serious form which often leads to death. Severe symptoms include anaemia, respiratory distress, and cerebral malaria. WHO estimates 229 million cases in 2019 and 409,000 deaths, of which 94% were reported in the WHO Africa region. Children under 5 are at most risk of malaria infection, due to their lack of acquired immunity, and accounted for 67% of worldwide malaria deaths (WHO, 2020).

**Box 2. Visceral leishmaniasis (VL or kala-azar)**

VL is transmitted through the bite of female hematophagous sandflies, specifically phlebotomine species. Of the multiple diseases caused by *Leishmania* parasites, VL is considered the deadliest, with mortality rates as high as 100% within 2 years if treatment is not administered (WHO, 2020). Symptoms include fever, anaemia, and hepatosplenomegaly. The disease is responsible for the second largest number of deaths related to parasitic infection, after malaria (DebRoy *et al.,* 2017). VL is endemic in many regions across the world which have high population density settings, and where poverty is widespread. 90% of the global burden is concentrated to East Africa (Ethiopia, Kenya, South Sudan, and Sudan), Somalia, India, and Brazil (WHO, 2020). The cost of treatment and inability to work drives people in impoverished regions further into economic deprivation.

## Clinical diagnostics

Clinical diagnosis is the determination of a disease via observation of the clinical symptoms presented by a patient. It is likely that clinical diagnostics are limited to resource-poor settings with inadequate health infrastructure, as this method is inexpensive and requires no laboratory equipment. It is worth noting that clinical diagnostics have largely been superseded by biological diagnostics, which are superior in both specificity and sensitivity. In fact, diagnosis by clinical symptoms alone is complicated by polyparasitism and co-endemicity of multiple diseases, as well as the non-specific nature of malaria symptoms.

Similarly, clinical diagnostics are not likely to be used unaided by other diagnostic techniques for the detection of visceral leishmaniasis, due to the costly, invasive, and toxic treatment that is required. Diagnosis is made through a combination of parasitological and serological tests, with clinical signs, such as hepatosplenomegaly, aiding identification (WHO, 2020).

## Biological diagnostics - direct methods

### Microscopy and culture

Malarial infection may be diagnosed microscopically by visualising *Plasmodium* parasites in thick and thin blood smears. Light microscopy remains the 'gold standard' diagnostic tool to which other diagnostics are compared, although important advances in diagnostic methods mean that there are tests which greatly exceed the sensitivity and specificity of blood smears. As an established method, there exists a huge amount of experience and knowledge in the field of microscopy and the results are highly specific (Rodulfo *et al.,* 2007).

Furthermore, microscopy allows for the quantification of parasite density, so that the efficacy of treatment may be monitored. In areas of polyparasitism, microscopy is an effective tool for diagnosing multiple coinfections at the same time, and so its importance as part of an integrated disease management plan should not be underestimated.

However, the quality of microscopy-based diagnosis is unpredictable, as it relies on the variable skill of technicians and the ability to identify species at low levels of parasitaemia or in mixed species infections (Tangpukdee *et al.,* 2009). Importantly, microscopy is often not 'deliverable to end users' in rural healthcare settings where there are no electricity or laboratory resources, and due to its laborious nature is not suitable for high throughput screening (Wangai *et al.,* 2011).

It is also possible to diagnose visceral leishmaniasis via light microscopy. This involves biopsy of relevant tissues, usually splenic aspirates, or bone marrow, followed by identification of the amastigotes of Leishmania species under microscopic examination. Though sensitivity is higher in diagnosis from splenic aspirates (95%) compared to bone marrow (60-85%), the aspiration procedure can occasionally lead to fatal haemorrhage (Sundar & Rai, 2002). Bone marrow extraction is much safer, but the procedure is resented by patients and requires adequate medical facilities. Culture of Leishmania is possible but is rarely necessary in routine diagnostics.

### Antigen detection

An alternative approach to light microscopy centres on the biochemical detection of proteinaceous epitopes presented by a pathogen. Malaria rapid diagnostic tests (RDTs) detect specific antigens in the blood produced by *Plasmodium* parasites. RDTs currently target the histidine-rich protein-2 (HRP-2) antigen as well as two plasmodium enzyme-based detection assays: plasmodium lactate dehydrogenase (pLDH) and plasmodium aldolase.

The use of RDTs has expanded rapidly in recent years ahead of microscopy; from 40% of malaria tests in sub-Saharan Africa in 2010 to 75% in 2017, due to its simplicity, rapid receipt of results and inexpensiveness (Rapid Diagnostic Tests, 2019). As RDTs require little training or resources, they can be implemented on a community level, extending into rural areas and peripheral health posts. For this reason, they are extremely useful, ASSURED tools, as seen in Figure 2. However, RDTs are

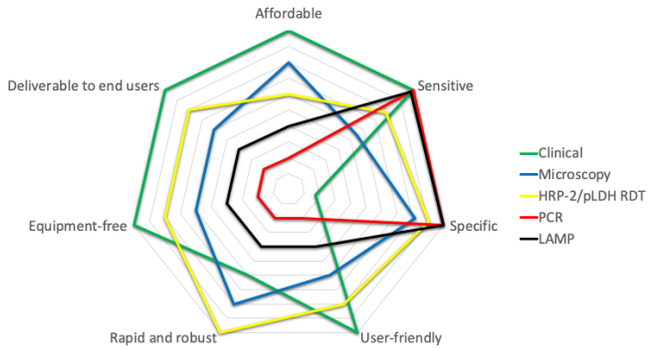


**Figure 2.** ASSURED criteria for each of the common malaria diagnostic method is represented on a radar chart. Quantitative data for specificity and sensitivity was extracted from Mfuh *et al.,* (2019), Berzosa *et al.,* (2018) and Sirichaisinthop *et al.,* (2011).

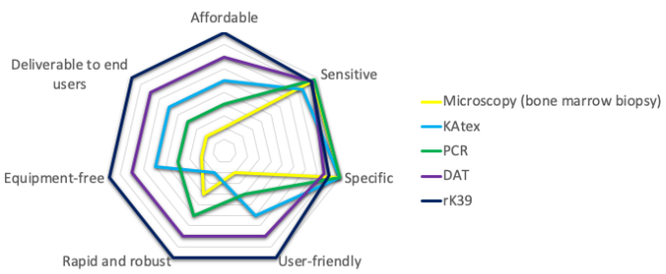


**Figure 3.** ASSURED criteria for each of the common visceral leishmaniasis diagnostic methods is represented on a radar chart. Quantitative data for specificity and sensitivity was extracted from Ghatei *et al.,* (2009) and Khatun *et al.,* (2017).

susceptible to stockout shortages, whereas the reagents required for microscopy last longer (Hasselback *et al.,* 2014).

There is also concern over persistent HRP-2 antigenemia after parasite clearance, the resultant false positives, and the inability of the test to monitor response to therapy (Iqbal *et al.,* 2004). Additionally, consideration must be given to the effectiveness and sensitivity of HRP-2 RDTs in areas with a high prevalence of pfhrp2/3 gene deletions. pLDH tests have reported higher specificity due to the short half-life of lactate dehydrogenase compared to HRP-2, but a purported lower sensitivity, which makes HRP-2 the preferred test in *P. falciparum* dominant areas (Coldiron *et al.,* 2019).

In the case of visceral leishmaniasis, there is urgent need for a rapid diagnostic test that maintains high specificity and sensitivity, with improved deliverability to end users. KAtex, a rapid latex agglutination test that detects urinary antigens in boiled urine, is effective for diagnosis in immunocompromised patients, unlike serological tests. However, sensitivity is too variable to be the ideal diagnostic test, and boiling urine is impractical and time-consuming (Sundar & Singh, 2018).

### Molecular diagnostics

Despite its superior sensitivity and specificity, most resource-poor settings preclude the use of PCR, for reasons including limited financial resources and inadequate laboratory infrastructure (Hänscheid & Grobusch, 2002). It has however been argued that the expense of implementing PCR diagnostics, even in rural and peripheral healthcare settings, is less that the financial burden of misdiagnosis and the incorrect administration of antimalarials (Wangai *et al.,* 2011). An important strength of molecular diagnostic techniques is that they remain the only method of detecting drug resistant mutations, an advantage that may become more important as resistance threatens the efficacy of artemisinin-based combination therapy.

Molecular-level tools for visceral leishmaniasis diagnosis may be useful in areas that are approaching elimination goals, as more of the population becomes susceptible to infection. It will become increasingly important to identify all cases that maintain transmission with a highly sensitive and specific molecular diagnostic test (Sundar & Singh, 2018).

## Biological diagnostics - indirect methods

### Serological diagnostics

To date, a serological antibody test that detects the presence of *Plasmodium*, and observes ASSURED principles, has not yet been developed for point-of-care diagnostics. Serological testing is not an appropriate diagnostic tool due to the time it takes for antibodies to develop, as well as the persistence of antibodies after parasite clearance (Sulzer & Wilson, 1971). This makes antibody tests impractical for routine diagnosis of acute malaria, especially in endemic areas where a large proportion of the population is seropositive.

There are multiple well-developed serological tests for the detection of antileishmanial antibodies, notably enzyme-linked immunosorbent assay (ELISA) and direct agglutination test (DAT). DAT is not only expensive, but it requires trained technicians and has a subjective endpoint. To confirm diagnosis in patients with low DAT titres, splenic aspirates may need to be performed (Cañavate *et al.,* 2011). Consequently, several immunochromatographic tests which require minimal equipment and are more suited to decentralised diagnostics have been developed using the rK39 Leishmania antigen. Both tests have high sensitivity (DAT 94.8% and rK39 93.9%), but specificity is higher in rK39 (90.6% compared to 85.9%) (Cañavate *et al.,* 2011). For this reason, rk39-based diagnostics are excellent tools for accurate diagnoses in resource-poor settings. However, it should be noted that the test shows decreased sensitivity in East Africa (Bangert *et al.,* 2018).

## Discussion

It will be effective to increase the use of RDTs in malaria diagnostics, especially in areas of high transmission and to reduce the burden in rural settings which lack the equipment and infrastructure for laboratory-based diagnostics. RDTs, due to their user-friendly nature, encourage active involvement from the community and provide an opportunity to move diagnostics away from the

laboratory and nearer to the patient. As a diagnostic tool, they are the most promising, as demonstrated by Figure 2.

However, there is a need for better investment into microscopy, which, when performed to a high standard, provides critical information for speciation, co-infection, and quantification of parasite burden. Though further investment should not detract from the implementation of RDTs in decentralised health posts, better training and facilities for existing healthcare services will provide greater opportunity for species-specific and polyparasitic treatment plans.

The current situation for visceral leishmaniasis diagnostics bears some similarities to malaria, such as the need for rapid, affordable, and deliverable tests to both urban and rural resource-poor settings. Serological tests, rather than antigen detection tests, such as rK39 are the most promising diagnostic that should be scaled up for high-throughput use, as shown in Figure 3.

Further research into the use of alternative molecular diagnostics should be conducted as multiple countries approach and set elimination goals. It is necessary to understand the contribution of sub-microscopic infections in malaria transmission, and which nucleic acid amplification-based diagnostic will be most cost effective to accelerate elimination.

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