MALARIA DIAGNOSTIC TECHNOLOGY LANDSCAPE

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N.B. All monetary figures are presented in US dollars ($) unless otherwise stated.

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Executive Summary

The *Malaria Diagnostic Technology Landscape* is published annually and is prepared as part of a broad and on-going effort to understand the technology landscape for malaria. This document will be published annually and will be followed by a semi-annual update once a year.

This document describes the role of malaria diagnostic tests, unmet needs in malaria diagnosis, and factors considered in diagnostic test selection, followed by a review of existing malaria diagnostic tests and new technologies in the development pipeline. The technologies described include those for patient management, as well as those that may be more suitable for surveillance, especially in the context of elimination.

In general, the material in this landscape was gathered from publicly available information, published and unpublished reports, papers, and prospectuses, and interviews with technology developers.

Background

Malaria is a preventable and very treatable parasitic disease, however, an estimated 225 million cases occur each year across 106 countries. The malaria burden is highest in sub-Saharan Africa, where one in five childhood deaths is caused by malaria.[1] With increased investment in malaria control efforts, malaria has decreased dramatically in the past decade. One recent review of fevers in Africa found a 50% reduction (from 44% to 22%) in the proportion of fevers caused by malaria.[2]

Because the symptoms of malaria (fever, headache, fatigue) mimic those of other common illnesses, effective and reliable diagnostic tests are needed to guide treatment. In resource-poor settings, where malaria diagnostic tests are often lacking, it has been customary to assume that most fevers are malaria and to treat them presumptively (also known as clinical diagnosis). When malaria incidence is high and malaria diagnostic tests were not widely available, this approach to fever was appropriate. However, given the current decline in incidence of malaria and increased availability of point-of-care (POC) tests for malaria, presumptive treatment no longer makes sense. It results in massive overuse and misuse of antimalarial medicines for non-malaria illnesses.

In light of the decreasing incidence of malaria and concern about overuse of antimalarial drugs, policy makers, donors, and national malaria programmes have recently launched several initiatives to scale-up malaria diagnosis. In 2010, at the global level, the World

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[1] *World Malaria Report 2010.* Geneva, World Health Organization, 2010. Available at: http://www.who.int/malaria/world_malaria_report_2010/en/index.html. Accessed on 5 December 2011.

[2] D'Acremont V, Lengeler C, Genton B. Reduction in the proportion of fevers associated with *Plasmodium falciparum* parasitemia in Africa: a systematic review. *Malaria Journal.* 22 August 2010.

Health Organization (WHO) changed its guidelines for malaria diagnosis, recommending for the first time that *all* suspected cases of malaria be confirmed with a diagnostic test before treatment. Due to recent progress in scale up of testing in several countries, the Roll Back Malaria Partnership recently set global targets for universal access to diagnosis in the public and private sectors, as well as in the community.

The Role of Diagnostic Tests

The most common use of malaria diagnostic tests (MDTs) is to establish the presence of malaria in sick individuals who present with symptoms of malaria. A positive result in a patient who does not have signs of severe illness can be easily treated on an outpatient basis with an effective antimalarial drug.

Additionally, diagnostic tests play a role in malaria surveillance activities. Malaria tests are used for monitoring the clinical burden of disease through health facility reporting of all malaria cases. Tests are also used to estimate malaria prevalence through surveys, in which a sample of the population is tested to estimate the overall prevalence of malaria.

Due to decreases in transmission that have been achieved in some areas, malaria elimination is back on the agenda. In connection with this, additional surveillance activities are expanding the use of existing diagnostic tests and creating demand for new technologies.

Unmet Needs in Malaria Diagnosis

Access to Testing

In the past few years, malaria diagnostic test use has grown significantly, especially in African countries, where malaria rapid diagnostic tests (RDTs) are increasingly being used not only at health facilities but also in the community.

Despite increasing use, access to diagnostic testing for malaria remains quite low in sub-Saharan Africa, where the burden of disease is highest. In the public sector, the percentage of suspected malaria cases (i.e. suspected based on symptoms) that were actually tested was less than 20% in 21 of 42 countries.[3] Although large proportions of people, in some countries the vast majority, turn to the private sector for fever care, malaria diagnostic testing in the private sector is uncommon.

Increasing access to malaria diagnostic tests has far reaching public health implications. With regards to antimalarial drugs, testing permits improved targeting of medicines to patients who have malaria, thereby reducing wastage and exposure of patients to drugs they do not need. Testing also provides a more accurate picture of the disease burden:

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[3] *World Malaria Report 2010.* Geneva, World Health Organization, 2010. Available at:
http://www.who.int/malaria/world_malaria_report_2010/en/index.html.

Currently, many fevers are reported as malaria cases even though malaria has not been confirmed with a diagnostic test, and it is likely that a large proportion of these fevers are not malaria. Lastly, if malaria tests are increasingly used to rule out malaria, the true cause of potentially severe illnesses can be investigated and managed appropriately.

Diagnostics for Special Population Groups and Situations

In addition to the general need to increase access to malaria diagnostic tests, there are several unmet needs in malaria diagnosis, for which existing technologies (i.e. microscopy and RDTs) are largely inadequate. These include tests that: Screen for detection of malaria in pregnancy; measure low-level transmission and detect asymptomatic malaria infections for use in elimination campaigns; assist with the differential diagnosis of fever and management of non-malaria fever; and assist with diagnosis and treatment of the liver stage of *P. vivax* malaria. The first two may be addressed by some of the technologies in the development pipeline, which are discussed in this report. Technologies addressing the latter were not specifically researched for this report.

Malaria Diagnostic Test Selection

There are several characteristics and factors that are typically considered when decisions are being taken as to the choice of a particular diagnostic test platform. Desirable characteristics for diagnostic tests vary depending on the epidemiology, infrastructure and systems available, and goals of testing. It is unlikely that any one test meets all of a malaria programme's needs.

With respect to patient management, it is important to have a test that is amenable to widespread deployment, even to the most remote and poorly resourced settings. Important characteristics, therefore, include portability, ease of use, and robustness under adverse environmental conditions. Secondly, because malaria can be acute and life-threatening, it is important to have an accurate, high-quality test that will detect all clinical cases of malaria and will produce results within minutes so that treatment can be started promptly. Lastly, because many populations affected by malaria are poor, and because worldwide testing volumes are potentially enormous, affordability is critical for malaria diagnostic tests.

With respect to surveillance, the key characteristics of a malaria diagnostic testing platform depend on prevalence and the goals of the malaria programme. In malaria control settings, the scale up of routine diagnostic testing is expected to improve the quality of surveillance data substantially, and tools for capturing and analysing this data in a timely manner may be important. In elimination settings, surveillance activities may require tests capable of measuring low-level transmission and for active case detection. In both cases, high sensitivity at low parasite densities is critical. In monitoring transmission, a low per-test cost

and high throughput are important characteristics. In active case detection, portability, robustness, and rapid results are critical due to the need to treat infections immediately.

Technology Landscape

A variety of technology platforms and scientific approaches to diagnosing malaria exist or are being developed. Currently, there are two technologies in widespread use for routine patient management: Microscopy and malaria RDTs. In addition, there are some sophisticated laboratory-based tests that are occasionally used to investigate complex cases or for research purposes.

Microscopy, which involves direct visualization of parasites, sets the standard against which other malaria diagnostic tests are evaluated. In expert hands and ideal settings, it is highly sensitive and specific and provides invaluable information. Under typical field conditions, however, the performance of microscopy is compromised due to: Poor quality microscopes, slides, and stains; insufficient training and supervision; interruptions in electricity; insufficient time to examine slides; and an absence of quality assurance systems. While improvements are possible, they need consistent financial and human resource investments, which are often lacking.

Malaria RDTs are lateral flow tests (i.e. strip tests) that employ antibodies to detect antigens produced by the malaria parasite. RDTs are truly POC tests: They require no laboratory, power, or special instruments; results are available rapidly; they are disposable and inexpensive; and they can be performed by health workers with minimal training. Recent WHO product testing have demonstrated there are a number of RDTs that perform as well as operational microscopy and are acceptable for use in routine malaria case management.

There are also a number of technologies for malaria diagnosis in the development pipeline that employ a variety of technology platforms and scientific approaches to diagnosing malaria. Among them are improvements to existing technologies, such as systems to automate and simplify microscopy, efforts to improve the stability, quality, and sensitivity of RDTs and rapid tests that use urine as opposed to blood as a sample, and efforts to simplify nucleic acid detecting technologies such as polymerase chain reaction (PCR) and loop-mediated isothermal amplification (LAMP). There are also several platforms that take advantage of scientific approaches, that have not previously been used widely, to detect malaria. These include systems that detect haemozoin and spectroscopic approaches to detection of malaria. Finally, serology is a technique that is being explored for use in monitoring low-level transmission.

Introduction

The *Malaria Diagnostic Technology Landscape* is published annually and is prepared as part of a broad and on-going effort to understand the technology landscape for malaria. This document will be published annually and will be followed by a semi-annual update once a year.

This document is structured as follows:

- The Background section provides a brief overview of malaria disease management and the role of malaria diagnostic tests in patient and programme management. This section also summarizes recent trends in malaria that affect the need for diagnostic tests as well as some of the major unmet needs in malaria diagnosis.

- The Malaria Diagnostic Technology Landscape includes a description of factors considered in malaria diagnostic test selection, followed by a review of existing malaria diagnostic tests and new technologies in the development pipeline. The technologies described include those for patient management, as well as those that may be more suitable for surveillance, especially in the context of elimination. Point-of-care (POC) tests have received more consideration than laboratory based testing systems largely because of the need for widespread deployment of malaria diagnostics and the need for rapid results. In addition, this report focuses on those technologies that are actively being developed and commercialized, as opposed to technologies in the very early stages of development.

Methodology

The *Malaria Diagnostic Technology Landscape* is compiled by Jennifer Daily with support from UNITAID. In general, the material in this landscape was gathered by the author from publicly available information, published and unpublished reports and prospectuses, and interviews with developers and manufacturers.

With regards to the technology review, significant prior work (reports, literature etc.) has been done to describe existing malaria diagnostic technologies and this is summarized below. For existing technologies, the methodology largely involved review of existing reports supplemented by expert interviews and targeted literature searches. In contrast to existing technologies, very little in-depth work has been done previously on the malaria diagnostic pipeline. Key informant interviews, along with literature and internet searches were used to identify new technologies actively being developed and commercialized. (Due to the nature of this work and the timeframe for the report, a totally exhaustive search was not possible.)

Once products were identified, detailed information on these new technologies was obtained primarily through conversations with technology developers, as well as through publications, where they exist. In some instances, technologies were identified but the developers were not available to provide additional information. Because these products are in the development phase, the ultimate performance and operational characteristics may change by the time the product is launched. Similarly, projections of market launch will shift as time goes by, as will price estimates.

Malaria Background

Globally, half of the world's population across 106 malaria endemic countries is at risk of malaria with the largest burden of illness and death occurring in Africa. In 2009, there were an estimated 225 million cases of malaria,[4] down from 244 million in 2005, and an estimated 784,000 deaths caused by malaria.

Disease and Case Management

Prompt diagnosis and effective treatment are the cornerstones of malaria case management; if effectively diagnosed and treated at an early stage, patients recover rapidly. However, if ineffective treatment is given or treatment is delayed, particularly in *P. falciparum* malaria, individuals may rapidly progress to severe malaria, which requires hospitalization and may be fatal, if left untreated.

The nature and degree of illness from malaria will vary by an individual's background level of immunity, which is determined by the extent of malaria transmission where they live. People living in stable or high transmission areas are infected frequently, however children generally develop some immunity (e.g. they may have parasites circulating in their blood but they will not have symptoms of malaria). Regions of stable and high transmission are largely found in Sub Saharan Africa. In areas of unstable transmission (Asia and Latin America, and increasingly parts of Southern Africa and the Horn of Africa), populations are less likely to develop immunity and people of all ages are at risk of suffering from severe disease, if not promptly treated. Epidemics are also a major risk in these areas. While the correlation between illness, immunity and parasite density (the number of parasites in a drop of blood) is not perfect, in general people with low immunity (young children and people living in areas of unstable transmission) will be sick at low parasite densities. Adults living in higher transmission settings will have developed immunity and, and while they may have many parasites circulating in their blood, they will have no symptoms of malaria.

Although diagnostic tests are recommended to confirm malaria before treatment, the symptoms of malaria (fever, headache, fatigue) are non-specific and mimic those of other illnesses, and malaria diagnostic tests are not readily available in many places where patients seek care. In malaria endemic regions, malaria is often clinically suspected on the basis of fever (e.g. clinical diagnosis or presumptive treatment) and is massively over-treated resulting in overuse and misuse of antimalarial medicines for non-malaria illness.

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[4] The 2010 World Malaria Report includes a wide uncertainty interval, ranging from 169–294 million cases (5–95th percentiles), due to mathematical extrapolations from incomplete reported data of unknown quality. Not only is reporting by NMCPs largely incomplete, but also many of the 'malaria cases' reported by NMCPs are based on clinical diagnosis which leads to overestimation of malaria.

Trends in Malaria Management

The last decade has seen a dramatic reduction in the burden of malaria. This is largely attributable to increased global funding for malaria control, investment in preventative measures such as long-lasting insecticidal nets (LLINs), and the scale up of effective antimalarial treatments, in both the public sector and, increasingly, in the private sector through the Affordable Medicines Facility-malaria (AMFm) subsidy programme.[5] With the recent progress in control, elimination of malaria—defined as the interruption of local transmission—is increasingly possible in areas where transmission has been reduced. Surveillance activities associated with elimination campaigns are expanding the role of malaria diagnostic tests and creating a need for new technologies.

The decreasing prevalence of malaria means that a large proportion of fevers, previously assumed to be caused by malaria, are not actually caused by malaria. At the same time, the availability of malaria rapid diagnostic tests (RDTs), which are portable and simple to use, makes widespread access to malaria testing possible for the first time. This has in turn led to a renewed focus in the malaria community on malaria diagnostic testing, an area that has generally lagged behind prevention efforts.

In 2011, the Roll Back Malaria Partnership set new targets for universal access to malaria diagnosis in the public and private sectors, as well as in the community. The scale up of diagnosis is under way and altering the approach to malaria case management by allowing for better targeting of treatment and improved management of non-malaria fever.

Other recent trends in malaria that affect diagnosis are resistance to artemisinin and the increasing importance of *P. vivax* malaria. In the past five years, resistance to artemisinin, one of the partner drugs in artemisinin combination therapies (ACTs), was noted in South East Asia; aggressive programmes to contain resistance have begun. Although the extent of the spread is unclear, it serves as a reminder of the need to eliminate the use of monotherapies, to confirm malaria with a diagnostic test, and to treat only those who actually are infected with malaria in order to maximize the useful life of these drugs, which are currently the mainstay of treatment programmes.

In the future, it is likely that we will see a shift in global focus from predominantly *P. falciparum* malaria to *P. vivax*. Although *P. falciparum* is generally considered to be more deadly than *P. vivax*, *P. vivax* represents a considerable burden as it is the most widely distributed malaria species: An estimated 2.85 billion people live at risk of *P. vivax* infection, the majority in the tropical belt of central and south east Asia.[6] The symptoms of *P. vivax* are similar to those of other malarias, however, *P. vivax* can relapse months and years after treatment because it remains dormant in the liver for extended periods. Currently, there are no diagnostic tests capable of detecting the dormant liver stage infection. Additionally,

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[5] Affordable Medicines Facility for Malaria (AMFm) is hosted by the Global Fund to Fight AIDS, Tuberculosis and Malaria.
[6] Guerra CA et al. The international limits and population at risk of Plasmodium vivax transmission in 2009. *PLoS Neglected Tropical Diseases,* 3 August 2010, 4(8):e774.

there is limited access to tests capable of screening individuals for a common genetic deficiency that causes adverse reactions to the drug used to treat the liver stage disease.

The Role of Diagnostic Tests in Malaria

The most common use of malaria diagnostic tests is to diagnose malaria in individuals seeking care for fever and other symptoms of malaria. Additionally, diagnostic tests are used for malaria surveillance and increasingly for active case detection[7] in malaria elimination campaigns.

Patient management

By far the most common use of malaria tests is to establish the presence of malaria in patients who are ill and have symptoms that are consistent with malaria. A positive result in patients with no signs of severe disease can be easily treated on an outpatient basis with an effective antimalarial drug (usually ACT). In addition to providing a qualitative result (positive/negative for malaria), diagnostic tests may provide information on which species is present and on parasite density.

Clinically, differentiating between species is relevant in settings where multiple species are present and the available treatment for malaria actually differs by species.[8] The parasite density,[9] if available, generally correlates to severity of infection and may affect treatment decisions (e.g. a clinician may decide to hospitalize, rather than treat on an outpatient basis, a child who does not appear extremely ill, if their parasite density is extremely high and due to the risk that the child's condition may rapidly deteriorate.) If available, information on the parasite lifecycle (i.e. the presence of particular forms of the parasite in the blood) may provide clinically useful information about the stage of infection and the patient's prognosis. Occasionally, diagnostic tests are used to monitor a patient's response to treatment; after taking an effective antimalarial, the parasite density should decrease.

As the burden of malaria declines, relying on clinical diagnosis is no longer appropriate and diagnostic tests become increasingly critical to providing quality health care as many of the fevers are no longer caused by malaria. In response to the changing epidemiology of malaria, in early 2010, the World Health Organization (WHO) updated its policy on malaria

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[7] Active case detection refers to a proactive approach whereby health workers go into the community to detect malaria infection in individuals who may not have symptoms of malaria.

[8] According to the WHO's summary of Malaria Policies, most Latin American, Asian, and western Pacific countries have different treatments for *P. falciparum* and *P. vivax*. In Africa, due to the lower clinical burden of non-*falciparum* malaria in the majority of countries, only Ethiopia and Eritrea officially have different treatments for *P. falciparum* and *P. vivax*. See http://www.who.int/malaria/publications/treatment-policies/en/index.html. Accessed 8 November 2011.

[9] Parasite density refers to the volume of parasites in a given quantity of blood, usually expressed as the number of parasites per microliter of blood (e.g. 5,000 parasites/μl) or as the percentage of red blood cells infected with parasites (e.g. 1% parasitemia). The density depends on a number of factors, including the species of parasite, genetic and immunological factors of the patient, the duration of the malaria infection, and the effectiveness of any treatments already taken. Parasite densities vary tremendously, and densities at all levels may lead to clinical illness, depending on the patient, and may contribute to transmission.

diagnosis, recommending that all cases of suspected malaria be confirmed with a diagnostic test before treatment. In practice, this policy represents a major departure from the long-standing approach of presumptive treatment (also known as clinical diagnosis), where people may be wrongly diagnosed with malaria on the basis of symptoms (fever, chills, etc.). For example, one review of malaria diagnostic practices, including 15 African countries, found that on average, 61% of cases clinically diagnosed as malaria were not actually malaria.[10]

As transmission rates decline to very low levels, malaria programmes aim to maximize the number of cases that are picked up through the health system and be sure that any cases that are identified are truly malaria. Therefore, it becomes increasingly important to have an accurate test that does not miss any cases that present at health-care facilities (e.g. high sensitivity at low parasite levels) and that correctly identifies positive cases of malaria (e.g. few false positives).

Surveillance

Surveillance is the cornerstone of malaria control and elimination programmes as it enables programme managers to monitor the effectiveness of programmes and can help identify populations requiring additional interventions. The clinical features of malaria are non-specific, therefore a confirmatory test is necessary and makes it possible to form an accurate picture of the disease burden. The role of diagnostics and focus of malaria surveillance activities depends on the local epidemiology, as well as available systems and technologies. In general, as the prevalence of malaria declines and programmes shift from control to elimination strategies, surveillance activities increase. Although, in theory, a diagnostic test should be used for all surveillance, in practice, as the prevalence of malaria decreases, diagnostics play an increasingly central role in surveillance activities.

When the burden of malaria is high, surveillance focuses on the burden of disease, in particular the clinical burden (i.e. how many people are *sick* from malaria rather than how many people are *infected* with malaria), so indicators of morbidity and mortality are emphasized. In the case of malaria, this is accomplished primarily by health facilities reporting malaria cases and deaths, supplemented by periodic surveys. In addition, overall childhood mortality rates and other measures of morbidity (e.g. anaemia rates) may be monitored as proxies for malaria. Health facility reporting has several limitations. First, the definition of what constitutes a *case* is not always clear. Often, it has been based on *suspected cases* (e.g. cases not confirmed with a diagnostic test) and leading to overestimation of the disease burden. With the increased emphasis on diagnosis and scale up of rapid diagnostic tests (RDTs), data are likely to become more accurate as only *confirmed cases* of malaria are reported.

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[10] Amexo M, et al. Malaria misdiagnosis: effects on the poor and vulnerable. *The Lancet*, November 2004, 364:9448.

Because not all health facilities submit reports, there are limitations in the completeness of reporting. Additionally, individuals who receive treatment through the private sector or who self-treat/self-medicate are not captured by these systems. To supplement health facility reporting and capture a more accurate picture of malaria, national surveys are conducted every 3–5 years. These surveys often include questions about the coverage of specific interventions (e.g. availability of diagnostic tests, LLINs, etc.) and include performing a diagnostic test to determine the prevalence of malaria.

Figure 1: The Goal of Surveillance From Malaria Control to Elimination

PHASE	Control → Elimination
PURPOSE OF SURVEILLANCE	**Monitor impact of control measures; early detection of outbreaks to reduce morbidity and mortality** / **Real time surveillance to avoid outbreaks** / **Detect and respond to all new infections to avoid onward transmission** / **All new cases (imported and local) detected and treated to prevent reestablishment of local transmission**

Source: *Jennifer Daily*

As transmission declines, the purpose of surveillance changes (as depicted in Figure 1 above), and the role of diagnostic tests becomes increasingly important. As areas move towards elimination, the surveillance measures described in Figure 1 may continue but will be supplemented by additional activities aimed at identifying all infections and halting onward transmission of malaria. These may include closer monitoring of case reporting to prevent outbreaks, identification of foci of transmission (i.e. areas with higher transmission in need of targeted interventions), and screening populations for asymptomatic infections (i.e. detecting infections, regardless of whether the individual has symptoms) because *any infection* can cause onward transmission. As areas move towards elimination, more proactive approaches to identifying malaria infections are often implemented. For example, health workers may perform follow-up visits in the community for cases that present to clinics. During these community visits individuals who reside or work in proximity to the confirmed case are tested to see if they have been infected with malaria. Health workers may also routinely screen certain high-risk populations for malaria infections. In the end, diagnostic tests are required to establish that malaria transmission has been interrupted.

Also falling under the category of surveillance is the need to monitor the development of drug resistance to medicines for malaria. Diagnostic tests for resistance are essential for

drug resistance surveillance, however these are beyond the scope of this document and not discussed.

Unmet Needs in Malaria Diagnosis

Unmet Needs: Access to Malaria Diagnostic Testing

Although malaria diagnostic testing is increasing, substantial scale up is required if universal access to testing is to be achieved. Indeed, most individuals living in areas affected by malaria who have a fever do not have proper malaria diagnosis before treatment: this gap in access to malaria diagnosis is particularly marked in the African public sector and in the private sector, where worldwide, many people receive malaria treatment based on having a fever.

Globally, the proportion of malaria cases that are confirmed by a diagnostic test has increased in the past decade, although there is tremendous variation by region. While diagnostic testing is lowest in Africa, the region of the world with the highest burden of disease, the proportion of reported cases in Africa confirmed with a diagnostic test has risen substantially from less than 5% at the beginning of the decade to approximately 35% in 2009. Despite the efforts, low rates persist in the majority of African countries.

Although many individuals seek care in the private sector, use of diagnostics in this sector is extremely limited due to a number of factors including unaffordable prices, limited awareness, little incentive for the private sector to offer testing, local regulatory and policy issues, and a need for extremely user-friendly test formats and packaging appropriate for the private sector.

The scale up of diagnostic tests has many benefits, chief among them improvements in the quality of medical care for malaria and for febrile illness more broadly; the targeting of ACTs, which reduces drug expenditures and helps maximize the useful life of these important drugs; and improvements in the quality of malaria surveillance data.

Unmet Needs: Diagnostics for Special Population Groups and Situations

In addition to the general need to increase access to malaria diagnostic tests, there are a few overarching unmet needs in malaria diagnosis, for which existing technologies are largely inadequate. These include: screening tests for detection of malaria in pregnancy; tests that measure low-level transmission and tests that detect asymptomatic malaria infections for use in elimination campaigns; tests that assist with the differential diagnosis of fever and management of non-malaria fever; and tests related to the diagnosis and treatment of the liver stage of *P. vivax* malaria. The first two may be addressed by some of the technologies in the development pipeline that are discussed in this report. Technologies addressing the latter were not specifically researched for this report.

Placental Malaria

Among the groups most vulnerable to malaria are pregnant women. Malaria in pregnancy can cause maternal anaemia, miscarriage, stillbirth, and low birth weight. In low transmission areas, there is an increased risk of severe malaria and death. In high transmission areas, malaria infection has adverse effects on fetal growth; however, it is often asymptomatic in pregnancy or has mild, non-specific symptoms. Each year, up to 125 million pregnancies occur in malaria endemic countries and malaria in pregnancy is responsible for as many as 100,000 infant deaths every year.[11]

The biology of a *Plasmodium falciparum* malaria infection in a pregnant woman differs from that of a non-pregnant individual in ways that are dangerous to the mother and fetus and that make diagnosis of malaria during pregnancy challenging. In all malaria infections, the *Plasmodium* parasites infect the body's red blood cells. During pregnancy, the *P. falciparum*-infected cells sequester in the placenta,[12] (i.e. the infected cells become attached to the placenta rather than circulating in the peripheral blood). Dangers to the mother and fetus, such as maternal anaemia and low birth weight, occur when malaria parasites infect the placenta. The sequestration also has the effect of reducing the number of infected cells circulating in the peripheral blood that can be detected by traditional malaria diagnostic methods. One recent study showed 5.6% of woman had malaria in the peripheral blood, while 60.5% had infection in the placenta.[13]

Further complicating the detection of malaria in pregnancy is the effect that the infection has on a pregnant woman: Many pregnant women who are infected with malaria may have no classical symptoms of malaria. The effect that malaria infection has on pregnant women is governed by a number of factors, not all of which are completely understood. In general, a women's immunity may be compromised during pregnancy, thereby increasing her risk of developing severe complications from malaria. However, a pregnant women's acquired immunity to malaria also depends on transmission intensity (as in the case of any adult), as well as the number of times she has been exposed to malaria during previous pregnancies. Typically, in endemic settings, pregnant women are more susceptible to symptoms of malaria in their first pregnancy and less susceptible to malaria symptoms in future pregnancies. Pregnancy associated immunity does not appear to eliminate the infection, but does seem to maintain it at a low parasite level; however, the level of parasitemia that is actually harmful to the mother and fetus is not clear.

To reduce the risk of malaria in pregnant women and foetuses, WHO recommends intermittent preventive treatment of malaria in pregnancy (IPTp) in areas of stable malaria transmission. This involves administration of two or three doses of an antimalarial drug

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[11] Malaria in Pregnancy Consortium website, http://www.mip-consortium.org/. Accessed September 26, 2011.
[12] Others species of malaria do not appear to sequester to any significant degree in the placenta or other tissue.
[13] Anchang-Kimbi JK, et al. Diagnostic comparison of malaria infection in peripheral blood, placental blood and placental biopsies in Cameroonian parturient women. *Malaria Journal*, 8 June 2009, 8:126.

(usually sulfadoxine-pyrimethamine, or SP) to all women attending routine antenatal clinics, regardless of whether they have any symptoms of malaria. However, the current IPTp programmes, which are policy in 37 countries, are being reassessed.[14] As the burden of malaria decreases, many women who receive the drugs will not have malaria parasites and the risk of adverse effects from the antimalarial drug becomes a concern. In addition, the efficacy of SP is declining in many countries due to increasing drug resistance. As a result of these changes, researchers and policy makers are exploring new strategies for reducing the effects of malaria on pregnant women. In addition to new effective drugs to replace SP, strategies that involve screening all pregnant women for malaria on a regular basis and treating only those who have parasites (known as intermittent screening and treatment, or IST) are being evaluated. The question then becomes, what is the best diagnostic test for screening? Although research in this area is somewhat limited, today's technologies routinely used for case management (microscopy and RDTs) are probably not sensitive enough to detect all cases of placental malaria.

Malaria Elimination: Measuring Low-level Transmission and Detection of Asymptomatic Infections

While existing tests will have a role in elimination campaigns, there is an emerging consensus that there is a need for improved technologies to support malaria elimination efforts. Specifically, the following activities require improved diagnostic tests:[15]

- Monitoring transmission in low prevalence areas. A low-cost, high-throughput screening test is needed to conduct large population surveys that are used to monitor progress over time and to identify hot-spots (i.e. foci) of continued transmission. Desirable characteristics for a diagnostic supporting survey include: high sensitivity; a low limit of detection (important for ensuring that *all infections,* any of which may be sources of onward transmission even if they are not symptomatic, are picked up); and high specificity, (because the absence of false positives results is critical in understanding the presence or absence of transmission).

- Active case detection and screening of high-risk populations. These involve proactively searching for malaria infections in the population and immediately treating any cases found. There are a variety of ways in which this kind of proactive case detection occurs. For example, follow up of anyone in close contact with someone who has confirmed malaria or mass screening of high-risk population groups, such as migrant workers, who move from an area of high transmission to low transmission. In these situations, it is likely that many of the infected people will

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[14] Chico M et al. Intermittent preventive treatment of malaria in pregnancy: at the crossroads of public health policy. *Tropical Medicine and International Health*, 2011, Volume 16 No 7, pp 774-785.

[15] This section draws from: malERA Consultative Group on Diagnoses and Diagnostics. A research agenda for malaria eradication: diagnoses and diagnostics. *PLoS Medicine*, 2011 Jan 25; 8(1):e1000396.

have very low parasitemias and no symptoms. The ideal test for these situations would have a low limit of detection and be highly sensitive, rapid, and portable.

Differential Diagnosis of Fever

An expanding, yet thus far unmet, role for diagnostic tests in malaria case management is to provide guidance on the differential diagnosis of fever. The decreasing burden of malaria in many areas means that the vast majority of patients who are tested for malaria will not have malaria. For health workers, this means that an alternative diagnosis should be sought, which can be challenging given the limited skills and diagnostic tools available in resource-constrained settings, as well as patient expectations. It is likely that the difficulty in managing non-malaria fever actually limits use and acceptance of the existing malaria diagnostic tests (e.g. a negative malaria test result may be ignored and the antimalarial drug still given).

The common causes of non-malaria fever vary tremendously. In general, however, the majority of fevers are likely to be self-limiting and patients will recover without any specialized treatment, while some proportion of non-malaria fevers may need specialized treatment (e.g. antibiotics). There are two main schools of thought about what the ideal test for fever should be:

- Some experts recommend a multiplex point-of-care (POC) test that detects several common causes of fever at one time (e.g. malaria, dengue, and influenza). Among the challenges of this approach are: first, deciding what to test for, which involves identifying the most common causes of fever in different geographical settings; and second, identification or discovery of the most relevant biomarkers for these causes of fever (pneumonia being one of the most important alternative causes of illness without any specific biomarker).

- Another approach involves development of a POC fever test that is more of a triaging tool providing information on management of the patient rather than pinpointing the exact cause of fever. Proponents of this approach suggest that this type of test would be clinically useful and have a greater public health impact, because it would allow the front line health worker to decide whether to hospitalize/refer the patient or treat them on an outpatient basis. This type of technology would perhaps include a malaria test and biomarkers for severity of disease. It may also include information that helps differentiate broadly between bacterial versus viral infections. This type of diagnostic would be especially useful for management of febrile children whose condition can deteriorate rapidly.

In higher transmission settings, differential diagnosis of fever is further complicated by the protective immunity from malaria that is common among adults and older children (i.e. individuals with immunity may be infected and have malaria parasites circulating in their blood, however, they will not have any symptoms of malaria, therefore they do not have

clinical malaria). In these circumstances, a positive malaria test would indicate the presence of parasites and would generally warrant treatment with an antimalarial; however, the parasites may not be the cause of illness and the clinician should examine the patient for other causes of fever. A test that detects a biomarker of clinical malaria, rather than malaria infection, may be useful in these situations.

Although not a primary focus of this report, the research done for this report did not reveal any diagnostic platforms that diagnose malaria and assist with the diagnosis of non-malaria fever that are on the market or in the later stages of the development pipeline.

Diagnosis and Treatment of Liver Stage Malaria

As mentioned previously, as control measures are successful against *P. falciparum*, other malarias, *P. vivax* in particular, are expected to become increasingly important, especially in countries that are pursuing malaria elimination. There are two challenges associated with diagnosis and treatment of *P. vivax* malaria, both relating to the liver stage of the disease. Even after treating a primary infection, *P. vivax* (and *P. ovale*) malaria can remain latent in the liver for significant periods of time and cause relapse unless the individual is treated successfully with drugs that specifically target this liver stage of the disease. Treatment with primaquine, the drug used today to treat the liver stage, is not extensive due to poor compliance to a 14-day course of treatment and the potential for drug-induced adverse reactions in certain individuals. This results in a potentially large reservoir of asymptomatic but infected individuals, who may experience relapses and cause onward transmission of malaria. Currently, there is no way to diagnose the latent stage of the disease, as there is no biomarker for *P. vivax* hypnozoites, the stage of malaria parasite lifecycle that is responsible for the liver stage of the disease. Although elimination of *P. vivax* malaria is likely to be impossible without a biomarker and diagnostic tests for this latent stage of disease, there is little research being done in this area.

A second issue related to the liver stage of *P. vivax* has to do with adverse reactions to treatment. Primaquine, the drug used to treat the liver stage of malaria, thereby preventing relapse in *P. vivax* and *P. ovale* malaria, is dangerous for individuals who have Glucose-6-phosphate dehydrogenase (G6PD) deficiency, a hereditary condition that causes haemolytic anaemia (i.e. premature destruction of red blood cells when the body is exposed to certain drugs or stress caused by infection).

G6PD deficiency is one of the most prevalent disease causing mutations worldwide, affecting hundreds of millions of people, with certain ethnic groups being more affected than others. There are a large number of types of G6PD deficiency, each with different degrees of impairment, ranging from mild to severe. The degree of deficiency is associated with the severity of any adverse events caused by primaquine.

Due to the dangers associated with primaquine, it is generally not administered without knowledge of a person's G6PD status. Currently, most G6PD testing is conducted in a laboratory and there are very few POC options for rapid screening prior to treatment.

Therefore, very few patients are aware of their G6PD status. In practice, the lack of availability of G6PD tests also contributes to the limited use of drugs for the liver stage of *P. vivax* and results in a potentially large reservoir of infected individuals who may experience relapses.

Although important for management of *P. vivax* and *P. ovale* malaria, diagnostic tests for G6PD were not a primary focus of this report and were not researched for this report.

Malaria Diagnostic Test Selection

Before discussing tests in depth, this section considers the characteristics of malaria diagnostic tests (MDTs) that are typically considered when decisions are being made as to the choice of test. Desirable characteristics for diagnostic tests vary depending on the epidemiology and the goals of testing (e.g. patient management, active case detection, and so on). It is unlikely that any one test meets the need of every programme.

Performance Characteristics

In malaria diagnostic testing, the performance of the test is of utmost importance. In general, malaria tests are designed to distinguish infected from uninfected individuals. The key performance characteristics are sensitivity, specificity, and the limit of detection (LOD).

Sensitivity refers to the probability (percentage) that patients with an infection will have a positive result using the test under evaluation, as compared to the result of the reference or 'gold standard' test.[16] As the sensitivity of a test increases, the number of false negatives decreases. In malaria, a high sensitivity has always been important as a missed diagnosis may have serious consequences.

Specificity is the probability (percentage) that patients without the infection will have a negative result using the test under evaluation, as compared to the result of the reference or 'gold standard' test. As the specificity of a test increases, the number of false positives decreases. Due to the concerns about overtreatment and a desire to improve the quality of care, the specificity of a diagnostic test is now becoming a priority for many malaria programmes.

Another parameter often used to describe the performance of MDTs is the LOD, which refers to the lowest quantity of parasites that can be detected in a sample.

In terms of performance characteristics for malaria patient management, the World Health Organization's (WHO) *Guidelines for the Treatment of Malaria* recommend that malaria diagnostics have 95% sensitivity at 100 parasites (p)/µl.[17] For screening and surveillance in elimination settings, more sensitive tests are desired. One recent expert group suggested a minimum detection threshold of 20 parasite/µl and a sensitivity of ≥ 95%[18] for these settings.

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[16] The reference, or 'gold standard', is the best available approximation of a true result and is used as the reference method for assessing the performance of other test methods. In malaria diagnosis, thick and thin film microscopy performed by accredited expert microscopists has been considered the gold standard and is commonly used as the reference method when evaluating other malaria diagnostic tests. However, PCR is usually more sensitive for detection and species identification. As such, PCR is often included in evaluations as an additional reference method.

[17] *Guidelines for the Treatment of Malaria, Second Edition*. Geneva, World Health Organization, 2010. Available at: http://www.who.int/malaria/publications/atoz/9789241547925/en/index.html.

[18] malERA Consultative Group on Diagnoses and Diagnostics. A research agenda for malaria eradication: diagnoses and diagnostics. *PLoS Medicine*, 2011 Jan 25; 8(1):e1000396.

Of note, WHO's product testing of malaria rapid diagnostic tests (RDTs)[19] employs several alternative measures of MDT performance that have become widely used in the malaria community when describing test performance.[20] These measures include a panel detection score (also referred to as a detection rate) and a false positive rate. The panel detection score is a number between 0 and 100, calculated as the proportion of times a malaria test gives a positive result against samples positive for malaria in a panel[21] at a specific parasite density (e.g. four tests at 200 parasite/µl).[22] A false positive rate is the percentage of all tests of a particular product that gave a positive result when it should not have.

Operational Characteristics

In addition to performance, the operational characteristics of a MDT have a significant impact on test adoption and use. Table 1 presents several of the key operational characteristics for MDTs.

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[19] Reports from the WHO product testing of malaria RDTs:
- Malaria Rapid Diagnostic Test Performance: Results of WHO product testing of malaria RDTs: Round 1 (2008). Geneva, World Health Organization, 2009. Available at: http://apps.who.int/tdr/svc/publications/tdr-research-publications/rdt-performance.
- Malaria Rapid Diagnostic Test Performance: Results of WHO product testing of malaria RDTs: Round 2 (2009). Geneva, World Health Organization, 2010. Available at: http://apps.who.int/tdr/svc/publications/tdr-research-publications/rdt_round2.
- Malaria Rapid Diagnostic Test Performance: Results of WHO product testing of malaria RDTs: Round 3 (2010-11). Geneva, World Health Organization, 2011. Available at: http://apps.who.int/tdr/svc/publications/tdr-research-publications/rdt_round3.

[20] Sensitivity and specificity are only established during field trials of a diagnostic test. The metrics used in the WHO product testing of malaria RDTs are for laboratory-based evaluations.

[21] In order to evaluate the ability of a particular test to detect *Plasmodium* antigen, several panels of specimens were assembled for the WHO product testing of malaria RDTs. These panels include wild-type panels comprising *P. falciparum and P. vivax* samples derived from infected patients and culture panels comprising *P. falciparum* specimens that were grown in the laboratory.

[22] The panel detection score/detection rate is a combined measure of a) the ability of a particular test to detect *Plasmodium* antigen in a specimen, and b) the consistency of this result across two or more tests (RDTs from the same lot or from different lots). Note that the panel detection score/detection rate is not the sensitivity or the positivity rate of the test.

Table 1: Operational Characteristics of MDTs

Characteristic	Explanation
Type of technology and format	As described later in this report, a variety of technologies and scientific approaches, (ranging from magnification and direct visualization of the parasite, measurement of the light patterns produced by bi-products of the parasite, detection of parasite nucleic acid, and so on), are possible for malaria diagnosis, each method has advantages and disadvantages, in terms of performance and operational characteristics. With regards to testing format, MDTs include disposable tests, as well as portable, table top, and large laboratory instruments. For patient management, disposable and portable formats allow tests to be widely deployed and to reach those who need them, particularly those in remote areas without health facilities. For prevalence surveys, where samples may not be processed immediately but collected and processed at a central laboratory, larger instruments may be acceptable. With regards to instruments, some instruments are designed only to diagnose malaria while others are platforms that can be used to investigate other diseases and conditions. A platform that has multiple applications may be advantageous, depending on the relevance of the other applications to the local setting.
Output	In addition to a qualitative result (positive/negative for malaria), malaria diagnostics may provide other information, including species of the parasite, stage of parasite development, and quantification of parasite density. The device may also measure additional parameters, such as haemoglobin.
Turnaround time and capacity	The turnaround time (or time to result) and the number of tests that may be processed at a time and in one day varies greatly. Many of the portable and disposable malaria devices process one sample at a time in a matter of minutes. Larger instruments tend to have the ability to process multiple samples, but may take longer. For patient care, results are ideally available within minutes, allowing for treatment of the patient during their visit. Unless patient volumes are high, devices that process one sample at a time are acceptable and likely to be more efficient for malaria case management. For some surveillance activities, samples are collected in the field and processed later. The ability, therefore, to process a number of samples at once is beneficial and a fast turnaround time is less important. For active case detection, it is usually desirable to have an immediate result so that treatment can be administered immediately.
Sample requirements and stability	Common samples used for malaria diagnosis include capillary and venous blood. In addition, the use of alternate sample types (urine, saliva) and non-invasive techniques are being explored. The most common sample collection method for malaria testing is fingerprick blood, collected by pricking the finger (or the heel in infants) with a lancet and capturing blood drops on a slide, filter paper, or with a small capillary tube or similar device. For malaria patient management, the sample is collected and processed

	immediately because results are needed rapidly. As a result, long-term sample stability is not a critical operational characteristic. However, for surveillance, stability of the sample is an important criteria when samples are being collected in the community and then transported to a central laboratory for processing.
Environmental requirements for device and reagents	Malaria is common in tropical and subtropical environments, therefore the stability of the test kit and the ability of the device to operate in extreme heat and humidity is critical. Long-shelf life at extreme temperatures is also important due to the nature of supply chains, which, especially in the case of remote areas affected by malaria, can be quite long and poorly controlled.
Protocol complexity	Protocol complexity refers to the number of steps required to collect the sample, prepare it for testing, transfer it to the testing platform, initiate and monitor the testing process, and interpret the results. In general, the health and laboratory systems of many areas affected by malaria are overburdened and suffer from shortages in trained staff capable of preparing samples, performing complex tests, and interpreting results. Therefore, testing processes that involve simple sample collection, limited sample preparation, require minimal supervision during the testing process, and are easily interpreted are advantageous.
Cost per Test	Tests must be affordable for those at risk of infection. From an individual patient's perspective, malaria affects the poor disproportionately and their ability to pay for a malaria test is limited. From a public health systems perspective, MDT budgets are growing: because so many people live in areas affected by malaria and suffer from fever, many millions of tests are needed on an annual basis. Even as malaria prevalence comes down, the overall fever rate is likely to remain stable and testing will still be required for the vast majority of fevers.
Cost per instrument	Similar to the per test cost above, a low cost per instrument is important especially considering the need for widespread deployment of MDTs.
Power requirements	In many situations where MDTs are needed, there may not be a constant source of centrally distributed electricity. Even in large cities power cuts are frequent. Therefore, to avoid the use of expensive generators and devices to stabilize the power supply, tests that do not require power are required. For devices needing power, low-power utilization and the ability to use battery or solar power are advantageous.
Training and technical sophistication	Tests vary in their degree of sophistication and recommended level of training required to collect and prepare the sample, perform the test, and interpret the result. A variety of test operators representing a range of skill sets are possible—from highly skilled laboratory technicians to lay persons. However, laboratory human resources shortages are common in many areas of the world affected by malaria, and there is increasing interest in the deployment of MDTs within the private sector or in the patients' home; therefore techniques that can be performed by lay people are needed. The amount of and differing lengths of time required to train an operator are also important criteria for test utilization and the

	quality of test results. Related to the technical sophistication of a test and required training is support from a vendor. Often, vendors that offer technical support and training and that have a local presence are preferred.
Durability and maintenance	For testing platforms that include a portable device, robust construction with durable components and few moving parts is important. Furthermore, the vendor's plan to address non-functioning devices (i.e. will devices be serviced on site or will non-functioning devices be exchanged by the vendor) is often considered.
Infrastructure requirements	People seek care for malaria both within the health system and outside of it. The infrastructure and personnel available within different settings has an important impact on which diagnostic tests are available and most appropriate. Within the health system, there are generally four or five levels of laboratory services.[23] Level I: Primary health post and health centres that predominantly serve outpatients. These facilities may not have formal laboratories, per se, and clean water, refrigeration and electricity may or may not be available. Often these facilities do not have a dedicated laboratory technician, and a limited menu of diagnostic tests are available (rapid tests, simple microscopic examinations, POC glucose/haemoglobin measurements) with diagnostic testing performed by a nurse or an assistant. Level II: These include district/primary hospital laboratories that serve in-patients as well as outpatients. Usually these facilities will have a laboratory staffed by one or more trained laboratory technicians. In addition to tests performed at Level I labs, more sophisticated instruments are often available for full blood counts, chemistry panels, HIV monitoring. Level III: This level includes the laboratories at regional and provincial hospitals. These facilities have dedicated lab space, automated analysers and a separate microbiology space, and uninterrupted power supply systems. Formally trained technicians and technologists staff these labs. Level IV: These included national and multi-country reference laboratories that possess the infrastructure, equipment, information systems, and logistical systems of sophisticated reference laboratories. They play a central role in management of the national laboratory system, as well as in surveillance, clinical trials, and evaluation of new technologies. Although it varies by country, there are two other important settings where malaria diagnostic tests may be performed, in the community and in the private sector. In some areas, village or community health workers perform malaria diagnosis. These health workers are often lay-persons who have one or more weeks of training and receive periodic supervision and resupply from a health facility or nongovernmental organization

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[23]Drawn from: Consultation on Technical and Operational Recommendations for Clinical Laboratory Testing Harmonization and Standardization, Maputo, Mozambique, 22–24 January 2008. Geneva, World Health Organization, 2008. Available at: http://www.who.int/hiv/amds/amds_cons_tech_oper_lab_test.pdf.

	(NGO). Outside the health system, in the private sector, individuals seek care for malaria within a wide variety of settings and infrastructures, and from a wide range of personnel, some highly skilled, others with no formal training.
Results display and storage	The results display on MDTs ranges tremendously. At one end of the spectrum is microscopy, which requires a visual scan for parasites across hundreds of microscopic fields. At the other end of the spectrum is a "positive/negative" readout on a device screen.
	In general, a simple, unambiguous output is preferred in resource-constrained settings. When the readout is visual or requires interpretation by a human reader/evaluator, an element of subjectivity is introduced to the test and, depending on the complexity of the interpretation, may require additional time and operator training. Automation of results interpretation and display reduces the labour requirement of a test, as well as the potential variation between operators.
	In addition to results display, a variety of functions can be incorporated into testing platforms, including results storage, wireless transmission of results, and printing capacity.
Quality assurance and Quality control	Quality, including regulatory approvals, product evaluations, availability of controls and external quality assurance programmes, is an important factor in adoption of diagnostic tests.
	With respect regulatory approvals, the regulatory framework in many resource-constrained countries is often ambiguous and poorly enforced. Often, policy makers in resource-constrained countries will look to approvals from stringent regulatory authorities (e.g. the United States Food and Drug Administration [US FDA], the European Union's [EU] conformity mark, or CE Mark). In malaria, however, the cost of obtaining an FDA approval is often prohibitive, particularly if the testing platform is used exclusively for malaria and lacks other disease applications for which there may be a more profitable developed world market. Even when a technology platform receives an FDA approval, this approval is usually for an application other than malaria—the malaria assay itself is unlikely to undergo FDA evaluations. Due to the risk classification system used by the EU system, the CE Mark requirements for malaria diagnostic tests are not very stringent and do not include a full quality evaluation.
	WHO plays an important role in providing guidance on new technologies. For example, the WHO Prequalification (PQ) programme for diagnostics reviews products and identifies those whose quality is deemed sufficient for UN procurement tenders. Many national programmes will look to the WHO PQ programme in the absence of FDA or similar approvals.
	With regards to performance evaluations, there is one major programme, the WHO product testing for malaria RDTs, for evaluating malaria diagnostics. However, this programme has been designed for antigen detecting tests and would not apply to the majority of tests in the development pipeline.
	The compatibility of a test with any existing, external quality assurance

	(QA) programmes and the availability of quality controls (QC) from commercial sources or public health laboratories are also important considerations.
	In general, the availability of a standardized test kit from a commercial manufacturer (as opposed to a protocol developed by a laboratory "in house" for performing a test) reduces the QA/QC burden on poorly staffed laboratories in resource-constrained countries, especially when deploying a test across numerous sites or in settings where testing volumes are high. A test kit from a reputable manufacturer is more likely to have been through a stringent regulatory review and includes a well-validated testing protocol, quality controls, and technical support.

Malaria Diagnostic Technology Landscape

A variety of technology platforms and scientific approaches to diagnosing malaria exist or are being developed, as shown in Figure 2. These include: direct visualization of the parasite using microscopy; detection of antigens produced by the parasite; testing for parasite nucleic acid; detection of haemozoin, a byproduct of the parasites digestive pathway; spectroscopic approaches that involve analysis of how molecules in malaria samples interact with specific wavelengths of light; and serology, involving the detection of antibodies to malaria. Each has its advantages and disadvantages, and it is unlikely that any one test is suitable for all situations.

For most approaches there are existing technologies (some are more widely used than others) and technologies in development. Of the existing technologies, microscopy and RDTs are by far the most widely deployed tests. In general, the methods that are less widely used are laboratory-based and are methods that do not, in their current form, lend themselves to near patient testing (e.g. they are not portable or disposable, lack rapid turnaround time, are not simple to use, etc.) Many of the laboratory-based methods are currently reserved for specialized use or for surveillance purposes; however, if field-ready formats are developed, they may have potential for widespread use.

Figure 2: Technology platforms for malaria diagnosis

Technology Platforms for Malaria Diagnosis

★ Multiple developers/suppliers
* Predominantly in house methods
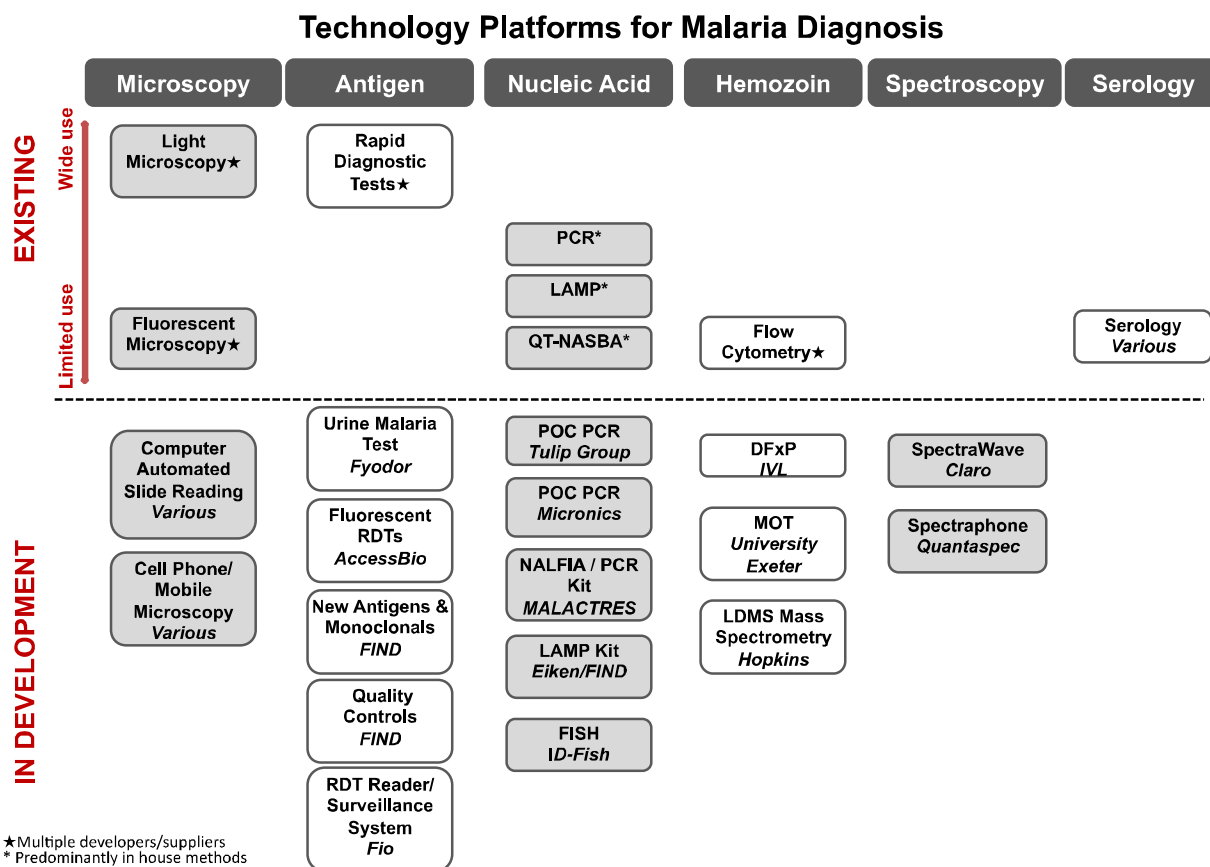
This section of the document is structured around the approaches outlined in Figure 2 above. For each approach (e.g. microscopy, antigen detection, etc.), technologies currently in use/existing are described first, followed by technologies in the pipeline/in development. For most technologies in development, additional information on key operating characteristics is available in an appendix.[24] In addition to the methods described below, there are a number of technologies in the very early stages of development (for example, there are at least 17 recipients of Gates Foundation Grand Challenge Grants with malaria diagnosis technologies) whose progress will be important to follow.

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[24] These descriptions are based primarily on the available information from developers. In some cases, the technology developers could not be reached or did not respond to requests for information, so descriptions are limited.

Microscopy

Microscopy Technology Currently in Use

Traditional Light Microscopy

Microscopic examination of slides for presence of malaria parasites has been the standard for malaria diagnosis since it was first introduced nearly 100 years ago. In settings where the most basic laboratory is available, examination of dye-stained blood smears for malaria parasites using a light microscope is common.

The process is relatively simple. It involves collecting a drop of blood from a fingerprick on a glass slide, staining the slide, allowing it to dry, and examining the slide using a standard laboratory microscope. Both a thin smear and a thick smear may be prepared. In the thin smear a very small quantity of blood is spread on a slide such that the cells do not overlap; the slide is fixed and stained so the cells are intact. In the thick smear, a larger drop of blood is spread onto a slide such that cells are layered on top of each other concentrating the cells in a relatively small area, and then stained. Because the thick film contains more cells, it is examined first to search for malaria parasites. The thin film is used to get a closer look at the parasites themselves; that is, to look at their shape to determine the species of malaria present. A skilled microscopist can identify the species of malaria, ascertain the developmental stage of the parasites (certain forms of the parasite suggest early stage infection or severe infection), and count the number of parasites in a given quantity of blood (higher numbers of parasites are associated with severity. After treatment with an effective antimalarial drug, the parasite density should rapidly decrease).

Microscopic diagnosis, in ideal settings, is highly sensitive and specific. In expert hands, microscopy is considered the 'gold standard' against which other malaria diagnostics are evaluated. In ideal conditions, an expert microscopist can detect parasites at densities fewer than 10 µl of blood. However, under typical field conditions, the performance of microscopy is compromised due to poor quality microscopes, stains, and slides; insufficient training and supervision; interruptions in electricity; insufficient time to stain and examine slides; and an absence of quality assurance systems. Staining and interpretation are labour intensive (30 minutes per slide) and require considerable expertise, particularly for species identification and in cases of low parasite density.

From a cost perspective, when caseloads are high, microscopy is inexpensive. Microscopy involves an upfront purchase of microscopes (good quality microscopes from leading suppliers cost $ 1,000–$ 1,500), on-going training (a refresher training course every three years for all microscopists), supervision and quality assurance, and purchase of relatively inexpensive consumables and reagents.[25] From a laboratory systems perspective, microscopy is advantageous because it is has applications for other diseases and it is widely

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[25] Supplies needed include: Lancets, alcohol swabs, cotton gauze, glass slides, Giemsa stain and other common laboratory chemicals, staining vessels and glassware for measuring liquids, immersion oil, lens cleaners, tally counters, and timers.

available; nearly every lab has a microscope and all laboratory technicians receive training in microscopy. Although microscopy quality assurance and quality control suffer from lack of investment (QA/QC for microscopy is human-resource intensive and many public laboratory systems are understaffed), there are well-established methods for monitoring the quality of testing.

Although a number of companies manufacturer microscopes, a few global optics companies—Olympus, Zeiss, and Nikon, particularly—are known for the quality of their objective lenses and production systems and dominate the microscope market. For example, a survey of 90 microscopes in one African country found 16 different manufacturers, but two-thirds are from three leading manufacturers.[26] A number of significantly less expensive (e.g. < $ 500) microscopes are available; however, their quality and durability has not been proven on a widespread basis. In general, quality and durability are important criteria for microscope procurement; a prerequisite for reliable malaria diagnosis is a good quality microscope, stains, and slides. Other factors affecting product selection are price and the installed base (i.e. the types of microscopes and consumables that are currently working well in a country and are familiar to its technicians).

Recently, in order to address the need for an artificial light source for high quality microscopy, light-emitting diode (LED) light sources have been incorporated into microscopes or portable LEDs are used in conjunction with traditional microscopes. LEDs are an inexpensive (< $ 100) and reliable light source with a long lifespan (~50,000 hours). In addition, LED power consumption is low allowing for battery operation or solar power.

Despite the simplicity of the technology, a general lack of sustained investment in microscopy services means that the quality of results varies from operator to operator. Indeed, in settings where microscopy is available, it is not uncommon for clinicians to treat without a test and/or to ignore microscopy results, suggesting a lack of confidence in microscopy. While improvements are possible (for example, through participation in External Quality Assurance Programs, regular training using the recently updated WHO Microscopy Curriculum, implementation of the WHO Quality Assurance guidelines, or participation in recognized accreditation programmes for microscopists), these require consistent financial and human resource investments that are often lacking in health systems. With regards to scalability, reliable microscopy is generally limited to health facilities and, in most health systems, it would be impractical to scale and provide appropriate quality assurance, supervision, and training beyond those facilities.

Fluorescence Microscopy

Over the years, technological efforts to improve the performance and operational challenges associated with traditional light microscopy have been developed. Among the

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[26] Mundy C, et al. Evaluation of microscope condition in Malawi. *Transactions of the Royal Society of Tropical Medicine and Hygiene, Sept-Oct 2000,* 94(5):583-4.

methods most widely explored is the use of fluorescence. Fluorescence microscopy is based on the use of certain fluorescent dyes, usually acridine orange (AO), that become incorporated into the parasite nucleic acid during the staining process thereby staining parasitized red blood cells. When viewed with a light of a specific wavelength using a fluorescent microscope, the nucleus fluoresces.

In general methods, using fluorescence can reduce the time required to scan microscopic fields for malaria parasites. However, uptake of these methods for malaria has been limited for a number of reasons. Considerable training and experience is needed for microscopists to distinguish between parasitized cells and other cell or debris-containing nucleic acid, because the fluorescent stain being used does not discriminate. It is also difficult for microscopists to differentiate between species with these methods. Microscopy QC, which often relies on storing slides for re-reading by a supervisor, is also a challenge with the fluorescent methods, as stained slides must be read immediately or the dye will fade or precipitate. Operationally, traditional fluorescent microscopes are more expensive than light microscopes and they require more upkeep. Fluorescent microscopes use a mercury vapour light bulb, which has a short life span and is susceptible to damage from fluctuating power supplies that are common in resource-constrained settings. In the past, several papers have been written on use of acridine orange stains, and one commercial kit (Quantitative Buffy Coat, marketed by QBC Diagnostics) exists that involves collection of blood into AO-coated capillary tubes that are centrifuged and examined with a fluorescent microscope.

More recently, to address the higher cost and operational challenges of traditional fluorescent microscopes, several companies have developed LED-based fluorescent microscopy systems, available as stand-alone microscopes (available for < $ 2,000 for resource-constrained settings) or as add-on adaptors to conventional microscopes. LED-based fluorescent microscopes produce a spectrum of light that excites fluorescent stains. They also have a long lifespan (~50,000 hours compared to < 500 hours for a conventional mercury vapour bulb) and their power consumption is low, allowing for battery operation or solar power.

Several affordable LED fluorescent microscope systems have been developed recently and marketed primarily to the tuberculosis (TB) diagnosis market. Among the many LED technologies marketed for malaria diagnosis and having undergone published evaluations is the CyScope[27] (approximately $ 1,400 for high burden countries, $ 0.65 per test) a portable (< 3 kg) battery operated microscope marketed by Partec that employs an LED light for both fluorescent and white-light illumination. The CyScope is used with specially prepared slides coated with reagents that include the fluorescent dye. Other commercial products using LED

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[27] Sousa-Figueiredo JC, et al. Investigating portable fluorescent microscopy (CyScope) as an alternative rapid diagnostic test for malaria in children and women of childbearing age. *Malaria Journal,* 27 August 2010, 9:245; and Nkrumah B, et al. Comparison of the novel Partec rapid malaria test to the conventional Giemsa stain and the gold standard real-time PCR. *Journal of Clinical Microbiology, August 2010,* 48(8):2925-8.

lights also exist. To date, though, none of these products has been adopted widely for malaria diagnosis in developing countries.

Microscopy Technology in the Pipeline

Globally, there are multiple efforts under way to improve microscopy, not only for malaria but also for other health applications. Current efforts to improve malaria microscopy focus on reducing the size and cost of microscopes and on automating the process to improve efficiency and objectiveness of reading slides. Most of these approaches require preparation of a blood smear, the image capture and interpretation processes are automated by the instruments to varying extents. Although a number of groups are working in this area, research for this report did not uncover any products on the market or in the later stages of development. In addition the examples provided below are illustrative and by no means exhaustive.

Broadly speaking, areas that are being explored can be grouped into two categories: Computer automated slide reading and cell phone-/mobile-based microscopy.

Computer Automated Slide Reading

The goal of using computers to automate the reading of malaria smears is to provide an objective and reliable result and to improve efficiency. Reducing or eliminating human involvement in interpretation of slides reduces variability and subjectivity. In addition, limiting the amount of human intervention required to process slides improves efficiency and reduces the labour requirements of microscopy.

Various groups are working to develop these systems and several have been reported in the literature. Typically, thin smears are made and stained as they would be for traditional microscopy. The slides are then put under a microscope, illuminated and focused, and a digital image is taken. Image processing software and algorithms are used to interpret the images, including detecting the presence of parasites, determining the species present, lifecycle stage, and quantifying the parasite density. Work to refine programmes that fully automate and reliably interpret microscopy images is under way, although no technologies in the late stages of development or on the market were identified in preparing this report.

Cell phone-/mobile-based microscopy

Closely related to the automated interpretation of microscopy images is the miniaturization and incorporation of microscopes into cell phones. Some of these technologies are based on miniature lenses, while others take advantage of lens-free approaches. For example, a group at the University of California–Berkeley has developed the CellScope, a microscope attachment for a cell phone, and has published a journal article on its ability to capture

images of malaria-infected red blood cells from smears.[28] A group at the University of California–Los Angeles is developing a lens-free, light-weight microscope based on digital holography that has a large field of view, which enables faster scanning of smears for parasites. While some of these technologies rely on transfer of images to a remote location for interpretation by a trained technician, others use computer software in the cell phone to automate the interpretation of images.

Antigen Detection (Rapid Diagnostic Tests)

Antigen detecting tests employ antibodies to detect antigens produced by the pathogen through lateral flow immunochromatography, a simple technique with many diagnostic test applications (e.g. human immunodeficiency virus [HIV] RDTs, influenza tests, etc.). Broadly speaking, the technique combines principles of chemistry and immunology and is based on the use of antibodies to detect antigens (or vice versa for some diagnostic tests). The test format involves the capillary diffusion of a sample through a membrane to which reagents are attached. As the sample migrates up the membrane, it interacts with reagents producing reactions that can be visualized with the naked eye. Most immunochromatographic tests provide a qualitative result on the basis of the presence or absence of a particular analyte, although progress has been made in semi-quantitative and quantitative assays with the use of a handheld device that reads the RDTs.

Malaria Rapid Diagnostic Test Technology Currently in Use

Overview and Operational Characteristics

Malaria rapid diagnostic tests (RDTs) are immunochromotagraphic tests that detect antigens produced by the malaria parasite. With over 200 malaria RDTs on the market, a large number of companies currently supply malaria RDTs. These companies are diverse, vary in size and years in operation, provide a range of diagnostics business lines, have varying degrees of vertical integration, and are broadly, geographically located. Among the leading suppliers to the public sector market are AccessBio (USA), Premier Medical Corporation (USA), Standard Diagnostics (Korea), ICT (South Africa), and Orchid Biomedical (India).

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[28] Breslauer DN, et al. Mobile phone based clinical microscopy for global health applications. *PLOS One* July 2009;4(7):e6320.

Figure 3: Examples of malaria RDT cassettes
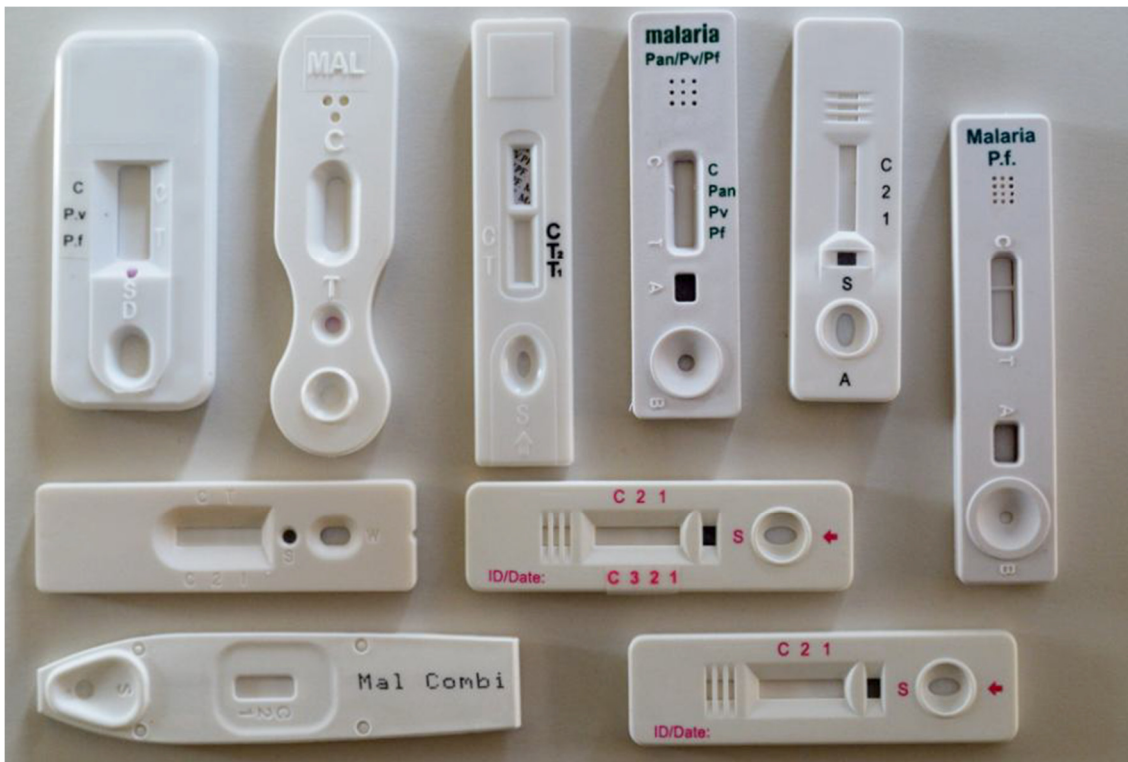
Source: *Gillet P. Malaria Rapid Diagnostic Tests: Laboratory aspects in the diagnostic setting, 2011 (Doctoral dissertation, Maastricht University).*

In the case of malaria RDTs, antibodies are used to detect antigens produced by malaria parasites. There are currently three antigens detected by malaria RDTs:

- HRP-II (histidine rich protein): A water soluble protein produced by the asexual stages and young gametocytes of *P. falciparum*.

- pLDH (parasite lactate dehydrogenase): An enzyme in the glycolytic pathway produced by the sexual and asexual stages of all parasites.

- Aldolase: An enzyme of the glycolytic pathway produced by all parasites.

RDTs use specific antibodies, called monoclonal antibodies (MAbs), that have been manufactured to specifically bind to one of these three antigens. Table 2 describes these antibodies.

Table 2: Monoclonal Antibodies in Rapid Diagnostic Tests

Monoclonal Antibody	What it detects
HRP-II (histidine rich protein)	*P. falciparum* malaria only
pLDH (parasite lactate dehydrogenase)	Many pLDH antibodies exist, the most commonly used ones are: 1) *P. falciparum*-only detecting pLDH 2) *P. vivax*-only detecting pLDH 3) *Plasmodium genus* detecting pLDH (also called pan-malaria; detects any species and does not differentiate between species)
Aldolase	*Plasmodium genus* (also called pan-malaria; detects any species and does not differentiate between species)

Malaria RDTs use one or more antibodies to detect: only one species of malaria, (*P. falciparum*- or *P. vivax*-only detecting test); all species of malaria (pan malaria tests); or to differentiate between species of malaria (i.e. combination tests). Today, there are several types of tests based on different, commercially available combinations of antibodies.

RDTs are available in dipstick, card, and cassette formats, although the cassette is easiest to use and is by far the most common. In the cassette format, a test strip is encased in a plastic housing.

RDTs are simple to perform, and although each RDT has specific instructions with regards to the volume of blood and buffer and time to results, the process is generally similar. The first step is lancing the finger and transferring a drop of blood to the test using a disposable blood transfer device that is included in the RDT kit. Buffer solution, also provided in the kit, is then added to the RDT. After 15-25 minutes results appear as a visible line. Depending on the number of species detected, RDTs will have two or more lines in the result window. Figure 4 depicts a typical RDT cassette format: on the right are separate wells for adding the blood sample and buffer. The results window contains two lines: the control line (far left) indicates that liquids have migrated down the test strip; the test line would only appear in the case of a positive result.

Malaria RDTs are relatively inexpensive; in public sector bulk procurement, the most basic *P. falciparum* tests cost $ 0.40–$ 0.60. Tests that detect more than one species cost up to $ 2.00 per test.[29] The operational characteristics of RDTs make them highly valued, as they are truly POC tests: they require no laboratory infrastructure, power supply, or instruments; they are disposable; and they can be used by health workers with limited training and skills.

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[29] These are public sector bulk procurement prices.

Figure 4: Schematic Representation of a Malaria RDT Cassette
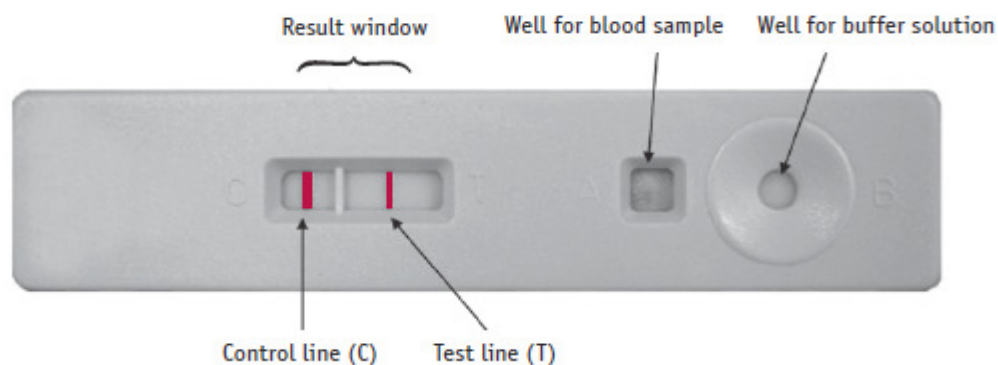
Source: *Good Practices for selecting and procuring rapid diagnostic tests for malaria, World Health Organization, Geneva, 2011.*

Malaria Rapid Diagnostic Test Components and Reactions

Figure 5 depicts the different components of a malaria RDT lateral flow test. As the diagram depicts, the test strip comprises several pads and a nitrocellulose membrane that are applied to a plastic backing. Nitrocellulose is a porous membrane commonly used in lateral flow tests. The size of the membrane's pores has an important effect on the capillary flow rate of the sample and reagents along the test strip, and hence on the timing of the reaction. During RDT manufacturing, reagents, including monoclonal antibodies, are bound to the nitrocellulose membrane and the pads. As the sample migrates along the test strip, the reaction occurs as follows:

1. *Application of Sample and Buffer to the Strip*: The sample is applied to the sample pad, followed by application of the buffer, most often to an area just adjacent to the sample pad. During these first steps, red blood cells are lysed (ruptured) to release their contents, including malaria antigens. The buffer and/or pads may contain blocking agents to prevent false positive reactions. The liquid sample and buffer migrate down the nitrocellulose test strip by capillary action.

2. *Conjugate Pad/Signal Antibody*: The sample passes through the conjugate pad. The pad contains the detection antibody (HRP-II, pLDH, or aldolase monoclonal antibody) that has been conjugated (attached) to a signal, most commonly colloidal gold. This antibody-gold conjugate is often called the signal antibody. If any malaria antigen is present in the sample, it will bind with the antibody-gold conjugate.

3. *Test Line/Capture Antibody*: The antigen-antibody-gold complex then migrates further down the nitrocellulose strip to a test line, to which an antibody specific to the antigen (e.g. HRP-II, pLDH, or aldolase) has been bound. This antibody is often called the capture antibody. If antigen is present in the sample, the capture antibody binds to another part of the antigen-antibody-gold complex. Because the complex contains gold, a reddish signal will appear along the test line as it accumulates at the

line. The intensity of this line depends primarily on the amount of antigen present in the sample and the amount of antibody that has been placed on the strip.

4. *Control Line*: Excess antibody-gold complex that was not bound to the antigen or to the capture antibody moves further down the test strip where it crosses a control line, where another antibody has been placed. This antibody will bind with the antibody-gold complex, irrespective of the presence of any malaria antigen being attached to the antibody-gold complex. As antibody-gold accumulates, a reddish signal will appear along the control line.

5. *The absorption pad* at the end of the test strip absorbs excess liquid off the nitrocellulose membrane to allow the capillary flow to continue in the right direction and at the right rate for reactions to occur. These pads reduce any possible backflow of liquids, which makes reading results difficult.

Figure 5: Schematic Drawing of the malaria RDT lateral-flow strip.
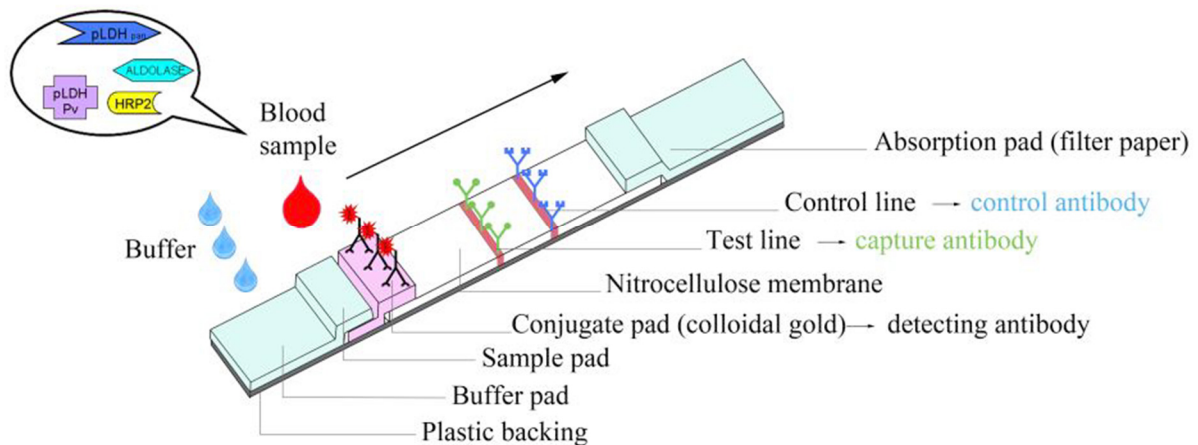
Source: *Gillet P. Malaria Rapid Diagnostic Tests: Laboratory aspects in the diagnostic setting, 2011 (Doctoral dissertation, Maastricht University).*

Rapid Diagnostic Test Performance

Evaluating the performance of a malaria RDT is a technically challenging, complex, and costly process. Before 2009, it was extremely difficult to establish the performance of malaria RDTs. Although hundreds of studies (manufacturer and independent) had been conducted, poor study design and inadequate results reporting made it difficult to interpret results.

In 2009, WHO completed the first round of product testing for malaria RDTs.[30] This evaluation, a landmark for malaria RDTs, directly compared the performance of dozens of

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[30] The product testing programme is co-sponsored by the Foundation for Innovative New Diagnostics (FIND), the Special Programme for Research and Training in Tropical Diseases (TDR) and the WHO Global Malaria Programme (GMP). Testing is

malaria RDTs and concluded that there were many, commercially available RDTs that performed as well, if not better than, operational microscopy.

To date, three rounds of product testing evaluating 120 tests from more than 25 different suppliers have been completed. Of the tests reviewed, 35 meet WHO's most stringent recommendations for procurement.[31] The results of a third round of product testing, released in October 2011, demonstrated that many RDT manufacturers continue to improve their products. This round had 23 resubmissions of tests from previous rounds and these tests either maintained excellent performance or improved substantially. After the first two rounds of testing, the number of *P. vivax* detecting tests that met WHO procurement recommendations was limited; however after this third round of product testing, there are many RDTs that meet current WHO recommendations, including several options for *P. vivax* detection. A fourth round of product testing began in June 2011 and is currently under way. Future rounds of the product testing programme are dependent on funding availability.

In July 2011, a Cochrane Review[32] was conducted to assess the accuracy of RDTs for detecting clinical *P. falciparum* malaria in people living in endemic countries who presented to health facilities with symptoms of uncomplicated malaria. The review analysed results from 74 trials conducted in countries in Africa, Asia, and South America. It concluded there are several commercially available RDTs that demonstrated acceptable performance (> 90% sensitivity and 90% specificity) across a variety of transmission settings. The review found HRP-II-based RDTs to be slightly more sensitive than pLDH-based tests, although the differences were not statistically significant. With regards to specificity, pLDH tests were more specific than HRP-II-based tests; the difference was slight, but statistically significant. The authors of the study suggest that the lower specificity of the HRP-II tests may be due to the persistence of HRP-II antigens in an individual after successful treatment for *P. falciparum* malaria. The review did not identify any differences between commercial brands of RDTs.

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performed at the US Centers for Disease Control and Prevention (CDC). Reports from the WHO product testing of malaria RDTs include:

- Malaria Rapid Diagnostic Test Performance: Results of WHO product testing of malaria RDTs: Round 1 (2008). Geneva, World Health Organization, 2009. Available at: http://apps.who.int/tdr/svc/publications/tdr-research-publications/rdt-performance.
- Malaria Rapid Diagnostic Test Performance: Results of WHO product testing of malaria RDTs: Round 2 (2009). Geneva, World Health Organization, 2010. Available at: http://apps.who.int/tdr/svc/publications/tdr-research-publications/rdt_round2.
- Malaria Rapid Diagnostic Test Performance: Results of WHO product testing of malaria RDTs: Round 3 (2010-11). Geneva, World Health Organization, 2011. Available at: http://apps.who.int/tdr/svc/publications/tdr-research-publications/rdt_round3.

[31] The WHO Global Malaria Program (GMP) has issued guidance for malaria RDT procurement based largely on the WHO product testing for malaria RDTs, and nearly all public sector procurement is in line with these recommendations.

[32] Abba K, et al. Rapid diagnostic tests for diagnosing uncomplicated P. falciparum malaria in endemic countries. *Cochrane Database Systematic Reviews*, 6 July 2011;(7):CD008122. The Cochrane Collaboration is a non-profit organization that prepares systematic reviews of evidence about health-care interventions to inform policy and practice.

Limitations of Malaria Rapid Diagnostic Tests

Although their adoption and use is increasing, malaria RDTs have several limitations as compared to other technologies for diagnosing malaria. Some of these deficiencies are considered to be more significant (e.g. lack of quality controls) than others (e.g. persistent antigenemia, speciation). These are discussed in more detail below.

Quality Control For most diagnostic tests there are technologies and well-established methods for checking the quality of the tests at the central level (i.e. evaluation of tests prior to purchase), when they are delivered to the country, at intermediate points in the distribution chain, and at the point of service. These technologies and methods have been developed by international bodies, public health laboratories, and/or are commercially available. With respect to malaria RDTs, practical methods and technologies to enable quality control testing are inadequate. While programmes exist at the international level to evaluate tests prior to purchase (i.e. the WHO product testing programme for malaria RDTs) and to evaluate RDTs when they are delivered to the country (i.e. WHO lot testing programme[33]) these programmes face an uncertain future as their funding is time-limited. Moreover, there are no practical means of confirming the performance of an RDT in the field.

Rapid Diagnostic Test Heat Stability. Little data exists on RDT heat stability and actual conditions of use (i.e. temperature and humidity). In general, RDTs are at risk of deterioration and reduced sensitivity when they are exposed to heat and humidity for prolonged periods. Malaria RDTs are generally labelled to be stable at 4° to 30°–37° C for 18–24 months. Conditions in some malaria endemic settings will at times exceed these manufacturer recommendations; however, the effect on RDT performance has not been closely analysed. Despite the lack of formal evaluations, the WHO product testing and WHO lot testing programmes, which both include limited heat stability studies, are building confidence in the ability of RDTs to withstand heat and humidity.

Limit of Detection / Sensitivity While the sensitivity of high performing RDTs is thought to be acceptable for diagnosis of malaria in people with symptoms (i.e. clinical cases of malaria), it may not be adequate for detecting low-density infections. As discussed above, the ability to detect low-density, asymptomatic cases is needed in low-prevalence areas that are screening populations for infections.

Differentiating between Current and Previous Infections (i.e. Persistent Antigenaemia) Although they are the most widely used type of RDT, HRP-II based RDTs may not distinguish between an active and previous malaria infection because the HRP-II antigens can persist in the bloodstream for several weeks after successful treatment, resulting in a positive RDT

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[33] The WHO and partners operate a lot testing programme for malaria RDTs that is designed to detect major flaws in RDT performance. Lot testing involves taking a sampling of RDTs from each lot (or batch) of RDTs and sending them to one of two international reference laboratories for QC checking. The testing is designed to detect major flaws in RDT performance and to supplement batch release testing at the manufacturing level and in-country quality control testing. As with the WHO product testing programme, funding for this programme after 2011 is not certain.

even though the individual does not have an active infection.[34] In practice, this complicates the differential diagnosis of fever: clinicians must take into account the patient's recent history of malaria treatment and consider alternative causes of fever. In addition, this means that HRP-II RDTs are not effective for monitoring response to treatment. Unlike HRP-II, it appears that pLDH and aldolase antigens are more closely correlated with active infection (cleared within 5-6 days after elimination of viable malaria parasites from the blood[35]), and as such may be more useful in differentiating between active and previous infections and in monitoring response to treatment.

Geographic Variability in Antigens Expressed by Malaria Parasites Although HRP-II based RDTs are the most widely used type of malaria RDTs, geographic variation in expression of the HRP-II antigen is well documented and in some areas, South America in particular, malaria parasites do not express the HRP-II antigen. As a result, an HRP-II based RDT may miss a case of malaria. To date, this problem has only been documented in certain geographic areas and pLDH-based tests are preferred in these areas.

Speciation Although RDTs have proven to be fairly adept at detecting *P. falciparum* malaria and differentiating it from other forms of malaria, there is significantly less data on their ability to distinguish between the other species (i.e. *P. vivax, P. ovale, and P. malariae*) of malaria.

Malaria Rapid Diagnostic Test Technology in the Pipeline

Several efforts are under way to improve upon existing malaria RDT technologies. These involve the use of urine as a sample rather than blood; efforts to enhance the signal and sensitivity of RDTs by use of fluorescent dyes and a handheld reader; the development of new monoclonal antibodies, and the development of quality controls for RDTs. What follows is a description of three efforts to develop and commercialize new technologies. Although they were not specifically identified during the research for this report, other researchers and companies are reportedly pursuing similar efforts to improve RDTs.

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[34] In clinical practice, for example, a person who was sick with confirmed malaria successfully completes treatment then becomes ill again (with malaria or another illness) and returns to the clinic. This patient might test positive for malaria (HRP-II antigen) for some period of time, and the clinician would not know symptoms were attributable to failed previous treatment, a new infection of malaria, or another disease all together. In general, the issue of HRP-II persistence is more relevant in high transmission areas than in low transmission areas. In high transmission settings, frequent infections are more likely; with positive RDTs clinicians would need to distinguish between potential treatment failure, new infection, or persistent antigen from an effectively treated infection and alternative cause of illness. In lower transmission zones, it is less likely that an individual would be infected repeatedly, and a new febrile illness is likely to have a non-malaria etiology or represent a treatment failure.

[35] *Good Practices for selecting and procuring rapid diagnostic tests for malaria*, World Health Organization, Geneva, 2011. Available at http://www.who.int/malaria/publications/atoz/9789241501125/en/index.html. Accessed on 13 December 2011.

Urine Malaria Test (Fyodor)

Fyodor Biotechnologies, based in Maryland, is developing a urine-based test for the diagnosis of malaria in individuals with fever. In response to the large proportion of malaria that is managed at home, Fyodor's intention is to develop an easy-to-use test that allows POC providers or individuals with symptoms of malaria (e.g. fever) to test for malaria at peripheral health centres or in point of care settings in order to determine the need for and to guide targeted treatment.

Fyodor's urine test is a simple one-step dipstick assay that uses immunochromatographic technology to detect malaria proteins that are shed in the urine of persons with fever. The testing process is simple: it involves collection of about 5 drops (100-200 µl) of urine, dipping the test into the urine sample, and reading the results after 10 minutes. The result is read visually: one line indicates no malaria and two lines indicate malaria. The first generation product will detect *P. falciparum* only; a second generation product that will detect both *P. falciparum* and *P. vivax* malaria is in development. Fyodor expects the first generation device to cost $ 1.50.

Originally developed at Johns Hopkins University, Fyodor licensed the technology in 2008, and expects to launch its first generation product in 2013. Through collaborations with the University of Maryland Baltimore, The Johns Hopkins University, and others, Fyodor is currently implementing a series of clinical validation studies in Africa. A final large-scale clinical trial is planned for 2012. Currently, the company expects to submit its malaria RDT to the WHO Prequalification of Diagnostics programme in 2012.

For more information on this technology, see Appendix 1.

Fluorescent Rapid Diagnostic Tests (AccessBio)

AccessBio, founded in 2001 and based in New Jersey, specializes in lateral flow tests. Access has extensive experience in the malaria diagnostics market as one of the leading global malaria RDT suppliers, under the CareStart Malaria brand.

In response to the need for improved sensitivity and LOD in malaria RDTs, AccessBio is developing a lateral flow test that generates a fluorescent signal. The use of fluorescence improves the RDT's detection system by making even minute quantities of parasite antigen (bound to the monoclonal antibody and captured on the signal line) detectable. The technology is similar to traditional RDTs, except that monoclonal antibodies (HRP-II or pLDH) are coated onto tiny particles that contain europium instead of being attached to colloidal gold. Europium is a metal that fluoresces when viewed with an ultraviolet light. In order to read the results, the RDT must be inserted into an RDT reader that converts the fluorescent signal into a digital read out.

The testing process is similar to a standard RDT: a fingerprick sample of blood is collected and transferred to the disposable RDT. Buffer solution is added and after 15 minutes the RDT is read using the reader. To date, the assay has been evaluated in the laboratory using cultured and clinical specimens, results suggest that fluorescent RDTs will be at least 100 times more sensitive than traditional RDTs.

AccessBio expects the fluorescent RDTs to be available in 2013. Prices have not yet been established, however AccessBio is forecasting that the RDT will cost slightly more than traditional RDTs and that the reader will cost $ 500–$ 1,000 depending on the level of customization (e.g. printer attachments, WiFi enabled etc.)

New Antigens and Monoclonal Antibodies for Rapid Diagnostic Tests (FIND and others)

The Foundation for Innovative New Diagnostics (FIND), in collaboration with partners, is working to develop new antigens and monoclonal antibodies (MAbs) to address some of the shortcomings of RDTs. Specifically, they aim to improve the heat stability of tests by improving the binding agents, identify antigens that are highly conserved and consistently expressed, and identify antigens that can be used to monitor response to treatment (e.g. antigens that are highly expressed and rapidly cleared from the body immediately after an infection is cleared). Currently, FIND and the Royal Tropical Institute (KIT) in Amsterdam have three new target antigens in the pipeline which are still in the early stages of research and discovery.

A limited number of other research organizations and manufacturers are also reportedly working to improve existing or develop new target antigens and antibodies for use in malaria RDTs.

Quality Controls for Rapid Diagnostic Tests (FIND and partners)

FIND, in collaboration with ReaMetrix, a private Indian diagnostics research and development (R&D) company, is developing positive control wells (PCWs) for malaria RDTs that address the need to check the performance of RDTs in the field. PCWs are small plastic wells coated with a small amount of recombinant parasite antigen (i.e. a genetically engineered parasite antigen). When reconstituted with water and applied to an RDT, the recombinant antigen solution produces a positive reaction on the RDT. PCWs are intended to be used by heath workers at the point of service to check that tests perform at a minimally acceptable level. A PCW prototype exists and is in the final stages of laboratory testing. Following laboratory testing, large-scale field trials are planned. ReaMetrix expects to manufacturer the PCWs, and specifications will be available for manufacturer elsewhere. Marketing plans, pricing, and the product launch date are dependent on funding for demonstration trials and have not yet been finalized.

FIND is also spearheading work on recombinant antigen panels to address the need for RDT quality controls at the international, national, and possibly manufacturing levels. Additional

development work is needed and further stakeholder consultations on specifications and mode of use, before these products will be available. It is expected that the panels would simplify and reduce the cost of the WHO product testing and the WHO lot testing programmes for malaria RDTs, enable national-level lot-testing, and that additional formats might be developed to allow for quality control testing on a much broader scale (e.g. at national reference laboratories or by manufacturers). Manufacturing, distribution, pricing, and product launch dates are dependent on funding and have not been finalized.

Malaria Surveillance Using a Rapid Diagnostic Test Reader and Cloud Information Services (Fio Corporation)

In response to the need for improved infectious disease data and improved RDT interpretation, Fio Corporation, a private Canadian company, has designed a system that integrates a smartphone-based RDT reader with information services, utilizing widely available technologies: malaria RDTs and mobile phone networks at the point of care, and web browsers at programme management offices.

The reader, to be used by health workers, manages the RDT processing steps and automates the readout of RDTs. It captures clinical and diagnostic data (e.g. RDT results and patient information), which are automatically geo-localized and transmitted to a secure central database. A web portal, available to programme managers and public health stakeholders, supports various types of cloud information services, including real-time surveillance, analysis, monitoring and evaluation. The system's two-way communication also allows programme managers to distribute to readers information and directives, such as outbreak alerts, standardized clinical protocols, and treatment updates.

The Fio system has other disease applications and is being initially marketed for select malaria RDTs. It has had successful field tests and is expected to launch in early 2012. Other disease targets, including HIV, dengue, and hepatitis, will follow.

Nucleic Acid Detection: Polymerase Chain Reaction (PCR)

Nucleic acid detection refers to the detection of parasite genes (DNA/RNA) in a sample. These relatively new laboratory techniques, developed in the past 25 years, are highly sensitive, capable of detecting nucleic acid in minute quantities (i.e. a single molecule in a specimen), and as a result they have revolutionized diagnostic medicine in many fields. With respect to malaria, several highly sensitive techniques for detecting the nucleic acid of the malaria parasite have been developed or are in the pipeline. Among these nucleic acid detection systems, PCR is the most commonly used and mature.

Polymerase Chain Reaction Technology Currently in Use

Polymerase Chain Reaction-based tests

Polymerase chain reaction (PCR) tests detect malaria parasites in a blood sample by multiplying the nucleic acid present in a sample. This process, called amplification, is accomplished through the use of special reagents that catalyse gene replication and through precise control of the environment in order to create favourable conditions for the reactions. In one cycle of PCR, it is theoretically possible to double the amount of target gene present; the cycle is typically repeated several times to produce large quantities (i.e. millions of copies) of the target gene. The product of this amplification process is then analysed for the presence of malaria using a variety of detection methods. PCR is able to detect extremely low parasite densities, surpassing microscopic and antigen detection methods in sensitivity and specificity. With regards to limit of detection, PCR can detect as few as 1 to 5 parasites/μl of blood as compared to 50-100 parasites/μl for microscopy or RDTs.

Currently, PCR requires a very well-equipped laboratory and technicians trained in molecular biology. As a result, malaria PCR is used for research, surveillance, and as a reference standard against which other methods are evaluated. Even in facilities with PCR capacity, it is generally not used to diagnose patients, as the results are not immediately available to the clinician and it is expensive. However, PCR is sometimes used to investigate complex cases; for example, to establish species after diagnosis has been made with microscopy or RDT or to assess a patient's response to a particular drug treatment.

Generally speaking, malaria PCR involves the following steps:

1. <u>Sample preparation</u>: Extraction and concentration of nucleic acid from a blood sample is usually needed to ensure that the nucleic acid in the sample is accessible for interaction with the reagents and so that amplification proceeds without interference or inhibition. The extent to which a sample must be purified varies based on the particular assay. A variety of methods are possible for extracting and concentrating nucleic acid, including commercial kits or automated machinery for high volume settings.

2. <u>Amplification</u>: This step requires making multiple copies of parasite nucleic acid and is done using specialized reagents and a thermal cycling machine that varies the temperature required for reactions to take place. A number of different amplification instruments are currently available and are largely interchangeable. Selection of the appropriate equipment depends on needs; for example, for patient care, a low throughput but rapid time-to-results instrument is desirable. In a surveillance study, with multiple samples, however, a high throughput instrument would be preferable.

3. <u>Detection</u>: The products of the PCR amplification must then be detected. A large number of approaches to detection are possible; these vary by laboratory and by PCR protocol. In some cases, the product of the amplification is processed using another laboratory method (for example, enzyme-linked immunoabsorbent assay, or ELISA). In other instances, the PCR instrument automates the detection process.

A number of PCR assays have been developed for the detection of malaria genes in whole blood. There are three main types of malaria PCR: conventional PCR; nested PCR; and real-time or quantitative PCR.

- **Conventional PCR** is the original method of malaria PCR that was developed 25 years ago and first used for malaria in the early 1990s. Parasite-specific genes are amplified and detected, and the output is a qualitative result for malaria.

- **Nested PCR** uses two rounds of PCR to do species identification. One commonly used approach to nested PCR amplifies the 18S rRNA gene, which is present in all four species of malaria. In the first round of PCR, reagents replicate this gene. A second round PCR is then performed using species-specific reagents, allowing for identification at the species level. PCR products are subsequently analysed by various the detection methods.

- **Real-time PCR (RT-PCR)** involves amplification of the parasite gene and detection in the same closed tube, and real-time monitoring as the reaction progresses. RT-PCR provides a semi-quantitative result, differentiates between species, and can be done in 1 hour (not including 30 minutes for sample preparation), as compared to > 6 hours for conventional and nested PCR methods. RT-PCR also has a lower risk of contamination because amplification vessels are closed, with amplification and detection occurring simultaneously in the same vessel. RT-PCR methods lend themselves to quantitative analysis because the concentration of circulating nucleic acid correlates to the density of parasites in the blood, although precise correlations with parasitemia, as determined by microscopy, are difficult to make.

Real-time PCR is probably the most commonly used PCR method today as it requires less handling, is less prone to contamination, and, although it is generally more costly on a per test basis, has advantages due to its automation and reduced labour requirements.

Despite its many advantages, PCR is reserved for specialized uses for a variety of reasons, including:

- Lack of standardization: While at least one commercial kit for malaria diagnosis is available, commercial kits do not appear to be widely used. Most laboratories use methods largely based on published protocols, however, they frequently make small changes to the method of sample collection and storage, of sample preparation, in the selection of reagents and the amplification conditions, and in the analysis of amplified product. As a result, there is very little standardization in malaria PCR methods.

- Cost: Although the cost varies greatly based on the approach, PCR generally requires setting up a laboratory with several rooms for processing, as well as purchase of several instruments and laboratory equipment for sample preparation, thermal cycling, and detection. In general, it may cost several tens of thousands of dollars to equip a full laboratory from scratch (including instrumentation for PCR, general laboratory instruments and equipment, equipment to ensure stable power, laboratory set up, and so on). The costs per test range as well—one paper estimated a range of US$ 0.35 – US$ 5.00 per test.[36]

- Contamination: The high sensitivity of PCR creates a challenge for laboratories because even a small amount of genetic material can contaminate a specimen and be amplified several fold. Careful sample handling and design of the laboratory (e.g. separate rooms for sample handling and processing) are critical to reducing contamination. With conventional and nested PCR, there is considerable pre- and post-reaction processing sample handling and a high risk of sample contamination during post-reaction processing. RT-PCR uses a closed vessel system that reduces the risk of cross contamination.

- Power: Thermal cyclers require a stable source of power.

- Trained technicians: The testing process requires highly trained laboratory technicians, and, depending on the level of automation and the type of PCR (e.g. nested PCR being more cumbersome than conventional PCR), involves several processing and monitoring steps.

- Cold chain: Although the samples for PCR are generally quite stable (for example, blood spots collected on filter paper can be stored for extended periods of time), most of the reagents necessary to perform PCR require cold chain storage.

- Time to results: Conventional and nested PCR methods are time consuming (> 6 hours to result).

Polymerase Chain Reaction Technology in the Pipeline

Several initiatives to address some of the limitations related to PCR are under way, including the development of POC PCR instruments, methods of simplifying purification of DNA away from other sample components, development of instrument free amplification, and development of detection systems that are appropriate for resource constrained settings.

Although there are several generic POC PCR platforms currently on the market and in the pipeline, most do not have malaria-specific assays. The research for this report found two POC PCR platforms with malaria-specific assays as well as one malaria PCR kit with simplified sample preparation and detection systems. These three technologies are described below.

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[36] Erdman, LK, et al. Molecular Approaches for Diagnosis of Malaria and Characterization of Genetic Markers of Drug Resistance. In: *Molecular Microbiology: Diagnostic Principles and Practice,* 2nd Ed. Washington, DC, ASM Press, 2011.

MicroPCR (Tulip Group and Bigtec Labs)

The Tulip Group, based in Goa, India, comprises several diagnostics companies specializing in lateral flow tests, reagent kits, and instruments for a variety of diseases and clinical laboratory analyses. Tulip has extensive experience in the malaria diagnostics market as one of the leading global malaria RDT suppliers for over 12 years, primarily under the Paracheck and Parascreen brands.

In response to the need for more sensitive tests for specialized groups (i.e. pregnant women, elimination settings) the Tulip Group, in collaboration with Bigtec Labs and under the banner of Molbio Diagnostics, is developing a portable quantitative RT-PCR platform and a quantitative duplex assay for *P. falciparum* and *P. vivax* malaria. Currently, sample preparation is done on a semi-automatic device (an automated disposable cassette version is under validation). The purified sample is then transferred to a microchip that is inserted into the microPCR device for real-time amplification and detection. The commercial launch for the malaria assay is expected in early 2012 and pricing for the device and individual assay are currently being finalized.

The malaria assay is currently being validated in India. A pilot study has also been conducted with good results in Kenya and large-scale field testing and validation in other countries where malaria is highly prevalent are expected to begin in late 2011. Collaborations have been established with national reference labs (like the National Institute of Malaria Research [NIMR] in Bangalore, India) and major hospitals for clinical evaluation and validation studies. In addition to malaria, microchips for other diseases will be available (e.g. assays for dengue/chikungunya duplex, typhoid, Hepatitis B, and H1N1 are expected to be launched in early 2012, assays for TB and Hepatitis C are under validation and an HIV assay is in advanced stage of development.)

For the microPCR device, the procedure for regulatory approval in the EU and its CE Mark is under way and, at a later stage, 510(k) pre-market clearance by the US FDA will be sought in the US. For the chips, country level approvals or registrations will be sought where required, starting with India, the EU, and other countries. Endorsement from WHO will also be sought.

For more information on this technology, see Appendix 1.

PanNAT™ Malaria Assay (Micronics)

Micronics, founded in 1996, is developing POC molecular diagnostic tests for infectious diseases that take advantage of novel microfluidics technologies. Micronics is currently developing the PanNAT™ Assay system, a platform that provides sample input to nucleic acid detection results and can be used for different pathogens (when nucleic acid is the method used for detection of the pathogen) using microfluidics cartridges. The first assay being developed for this system is an *E. coli* test that will initially be marketed in the

developed world. Among the other assays in development for the PanNAT™ system is the PanNAT™ Malaria Assay that combines high sensitivity and specificity into a POC format.

The PanNAT™ system is a portable, battery or mains-powered, fully automated PCR system with primers, molecular beacon fluorescent probes and all other reagents contained within a microfluidics cartridge. Processing involves collection of a fingerprick blood sample onto an assay-specific disposable cartridge that is then inserted into the PanNAT™ device. The nucleic acid purification, amplification, and detection processes all take place after the cartridge has been inserted into the instrument. In the Micronics design, as the sample moves through the cartridge containing reagents, the reactions occur. Thermo-cycling occurs through use of reagents in the cartridge that, when activated, rapidly heat and cool certain sections of the cartridge. The closed-system design reduces the chance of contamination and allows the test operator to walk away from the device while the sample is being processed. The price of the instrument and assay are not yet known, however Micronics is forecasting $ 1,000–$ 4,000 for the instrument and $ 3.00–$ 4.00 per test in volume production.

As a result of the recent purchase of Micronics by Sony, the expected launch date for the malaria PanNAT™ system is being reviewed. Currently, the PanNAT™ platform and *E. coli* assay are expected to begin multi-country evaluations in the coming year, and FDA submission is planned in 2012. The malaria assay has been developed in the laboratory, but has yet to undergo field trials. Timing of trials and commercial launch of the malaria assay is therefore likely to be 2013-2014.

For more information on this technology, see Appendix 1.

Nucleic Acid Lateral Flow Immunoassays (MALACTRES Consortium)

MALACTRES[37] is a consortium dedicated to research for improved diagnostics and treatment of malaria with a special focus on artemisinin resistance. In connection with this, it is developing a molecular test, for detection of malaria and possibly drug resistance markers, that is more readily adapted to resource-constrained settings than traditional PCR methods.

Although the MALACTRES assay requires use of a traditional PCR amplification instrument, several simplifications have been made for resource-constrained settings, including: i) it is a direct PCR, meaning it uses whole blood and does not require any sample preparation / extraction of DNA; ii) after performing traditional PCR amplification, detection of DNA is done using a disposable lateral flow test device, called a nucleic acid lateral flow immunoassay, or NALFIA; and iii) a commercial kit will be available that contains primers, reagents, and the lateral flow device required to run the test.

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[37] MALACTRES is an acronym for multi-drug resistance in malaria under artemisinin combination therapy: Assessment of specific markers and development of innovative, rapid and simple diagnostics. See: http://www.ist-world.org/ProjectDetails.aspx?ProjectId=0030c4ebae3842e2b37fbb3cfcf60ff2&SourceDatabaseId=018774364ea94468b3f4dec24aa1ee53. Also: http://www.malactres.eu/. Accessed on 13 December 2011.

The consortium developing the product comprises several European and African Institutes,[38] led by the Royal Tropical Institute in Amsterdam, the Netherlands, as well as the UK-based company Foresite Diagnostics, which will manufacture the test. Foresite Diagnostics was spun out of a UK government research laboratory in 2007 as a contract developer and manufacturer of lateral flow devices. It has extensive experience in developing highly precise lateral flow tests for a health care, agricultural and veterinary applications. Foresite will manufacture the NALFIA device and package it along with all primers (sourced from a company to be determined) and reagents necessary to perform the malaria assay in a kit. The kit is expected to cost around $ 2.00 per test in volume production. Marketing and distribution plans are not yet final.

The test is designed to address the need for a molecular test that is more field adaptable than traditional PCR approaches for use in malaria detection, especially submicroscopic levels, as well as possibly markers of drug resistance. Therefore, the test is at present not targeted for use in routine case management but would be primarily used to investigate complex clinical cases, and for surveillance, research, and clinical trials. The test requires a well-equipped laboratory that has the infrastructure to support PCR, a thermocycler for performing PCR, and trained laboratory technicians. However, the consortium will be looking at the use of solar powered miniaturized PCR equipment that should enable the implementation of the test in less sophisticated settings.

The platform is undergoing trials in Africa and with an Asian trial scheduled to begin shortly with results available in mid-2012, at which point the test kit would likely be commercially available. Results to date suggest that the assay's performance is comparable to traditional PCR methods that require purification and instrument based detection systems. The consortium has been funded through an EU contract and is seeking funding for additional demonstration projects. No regulatory plan has been established as of yet.

For more information on this technology, see Appendix 1.

Nucleic Acid Detection: Isothermal Methods

Isothermal Technology Currently in Use

Isothermal nucleic acid methods amplify DNA/RNA at a stable temperature, obviating the need for PCR thermal cyclers, which are relatively expensive. Isothermal methods that have been used for malaria include loop-mediated isothermal amplification, or LAMP and, to a lesser extent, quantitative nucleic acid sequence-based amplification, or QT-NASBA.

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[38] Other consortium partners include: London School of Hygiene and Tropical Medicine; Wageningen University in the Netherlands; Institute for Tropical Medicine in Belgium; Centre Muraz in Burkina Faso; Kilimanjaro Christian Medical Center in Tanzania; and University of Benin City in Nigeria.

Loop-mediated isothermal amplification (LAMP)

Loop-mediated isothermal amplification (LAMP) is a diagnostic test platform developed 20 years ago by Eiken Chemical, a Japanese company that retains control of the intellectual property rights for LAMP. Commercial LAMP test kits have been developed for numerous viral, bacterial, and protozoan pathogens, and several academic papers have recently reported on LAMP assays for malaria.

The LAMP procedure beings with a sample preparation step to extract DNA, followed amplification and detection of DNA though reactions at a constant temperature of 60°–65° C using a heating block or water bath. During the LAMP process, large quantities of DNA are amplified, enabling simpler end-point detection as compared to PCR methods. In addition, the DNA sequences are amplified in such a way that the products fold into looped structures causing the reaction mixture to appear turbid.

Following amplification, detection may be accomplished through various methods: the LAMP amplification procedure can produce a large amount of amplified product, therefore the reaction's turbidity may be visually observed; to enhance detection, a dye may be added that will fluoresce when sufficient bi-products of the LAMP reaction have been formed and can be read either with or without a UV light; or to eliminate the subjectivity involved in visual detection, an instrument that measures turbidity (turbidimeter) or fluorescence may be used.

To date, LAMP methods have not been widely validated or used routinely in the field. However, initial studies suggest that LAMP achieves sensitivity and specificity comparable to PCR and well above microscopy or RDTs and has many operational advantages over PCR, including:

- Although sample preparation for LAMP can be done using similar methods as used in PCR, simpler methods are also possible with LAMP because the polymerase used to catalyse the LAMP reactions is less susceptible to inhibition than the polymerases used in PCR.

- LAMP operates at a stable temperature and does not require thermal cycling instruments.

- The time-to-results for LAMP (<1 hour) is considerably shorter than many PCR methods that take several hours to complete.

- LAMP is less prone to contamination than PCR as it can be carried out in a closed system.

- LAMP may be less expensive than PCR on a per test basis.

- LAMP is technically less complex than PCR, therefore training and infrastructure requirements are lower than PCR.

Although it has many operational advantages over PCR, in its current format LAMP is likely to be a laboratory based test as it employs several small laboratory instruments, requires stable power (although it may be possible to use rechargeable power sources), requires several reagents/consumables for sample collection, preparation, and testing (some of which may need cold chain storage), and, relative to methods like RDTs, it is a moderately complex test comprising several steps that should be executed by dedicated technicians who have been trained in the method. In addition, LAMP does not have the ability to sequence DNA.

To date, published LAMP methods are considered 'in-house methods', whereby researchers used a combination of commercially available reagents from multiple suppliers and/or in-house procedures and reagents. However, the first commercially available reaction kit for LAMP is currently being developed (see below for more detail). The availability of a commercial kit has several advantages for resource-constrained settings, including: provision of a quality assured kit that includes reagents, some consumables, quality controls, and validated instructions in one box (which obviates the need to source many components from different vendors and the need for laboratories to develop their own quality controls); the availability of a well-validated test procedure; and customer support and training from a large company. Collectively, these advantages mean that LAMP can be performed reliably by technicians with a lower level of training in molecular diagnostics than in the case of 'in-house methods', which require that technicians have troubleshooting skills and the ability to develop quality assurance methods.

Quantitative Nucleic acid sequence-based amplification) (QT-NASBA)

Quantitative nucleic acid sequence-based amplification (QT-NASBA) uses RNA rather than DNA for amplification. It employs a series of enzymatic reactions to produce RNA amplification without the need for thermal temperature cycling. It achieves high sensitivity and specificity, with a low limit of detection comparable to PCR methods. Although QT-NASBA platforms are used quite frequently for HIV testing, it is only occasionally used in malaria. No commercial kits are available for QT-NASBA. One advantage of QT-NASBA, when compared with malaria PCR and LAMP, is its ability to discriminate between gametocytes and asexual forms of malaria.

Isothermal Technology in the Pipeline

LAMP Malaria Diagnostic Kit (Eiken Chemicals, FIND, and HTD)

Eiken Chemicals, FIND, and the Hospital for Tropical Diseases in London (HTD) are developing a malaria LAMP diagnostic kit that is expected to be commercially released in 2012. The test is based on development of new primers for detection of mitochondrial DNA of *P. falciparum* and for *Plasmodium* genus (i.e. pan-malaria). An initial laboratory study

suggests that the method will have sensitivity similar to PCR and higher than previously published malaria LAMP assays, with a reaction time of 40 minutes or less. The assay is currently undergoing clinical trials in Uganda and London, and will be sold as a reagent kit by Eiken. Eiken also markets a sample preparation kit called the PURE Method that can be used in combination with the malaria assay kit.

The Eiken malaria LAMP test kit is designed to address the need for a test that achieves far higher sensitivity and specificity than microscopy or RDTs, but is more field adaptable than PCR approaches. The testing process involves a sample preparation step to extract DNA from a blood sample, transfer of the sample to a reaction tube, insertion of the reaction tube and controls into a heating block for amplification, and detection of the result visually by fluorescence or using a real-time turbidimeter. The first generation platform will be low throughput; a high throughput test is in the early stages of development.

Potential applications of the kit include: as a reference method in developing countries; replacement for microscopy, especially in settings where volumes are low and retaining microscopy skills is a challenge; and, once a high throughput platform is developed, as a tool for surveillance and screening when a highly sensitive assay is needed (e.g. detection of asymptomatic infections in screening programmes or detection of malaria in pregnant women).

For more information on this technology, see Appendix 1.

Nucleic Acid Detection: Fluorescent In-situ Hybridization (FISH)

Fluorescent in-situ hybridization (FISH) is a technology developed in the mid-1980s, it involves the use of fluorescent probes to detect the presence or absence of specific DNA/RNA sequences. In short, a probe is prepared (a short sequence of DNA/RNA that matches a portion of the DNA/RNA that one would like to detect in a sample) and labelled by attaching a fluorescent dye. The probe is added to a slide containing the sample and under favourable conditions it will bond with its naturally occurring DNA/RNA counterpart in the sample (if that DNA/RNA is present). Because the probe is labelled with fluorescent dye, a fluorescent microscope is used to view the sample after the reaction to see if the probes have bound to DNA/RNA in the sample.

Fluorescent in-situ Hybridization Technology in the Pipeline

Malaria FISH Assay (ID-FISH Technology)

ID-FISH Technology Inc. expects to launch its malaria fluorescent in-situ hybridization (FISH) assay in early 2012. ID-FISH Technology is based in California and was founded in 2001 to develop nucleic-acid based diagnostic test kits for infectious diseases. The malaria assay is its first product and has been developed primarily with funding from the US National

Institutes of Health (NIH). The technology employed is Fluorescent in Situ Hybridization (FISH), which takes advantage of fluorescent probes that bind with parasite RNA causing malaria infected cells to fluoresce when viewed under a fluorescent microscope.

The company will market three test kits which contain probes and other reagents required to detect all species of *Plasmodium* (P-genus FISH kit), *P. falciparum* (PF-FISH Kit), and *P. vivax* (PV-FISH kit). In addition to differentiating between species, the assay can provide quantitative results (i.e. parasite density, similar counting technique as with traditional light microscopy) and information on morphology (i.e. stage of parasite lifecycle).

The test requires a trained technician to perform multiple steps using bench-top laboratory equipment, and as such it is a laboratory-based method most suitable for reasonably well-equipped laboratories. In brief, the assay procedure consists of several steps, including: fixation of blood smears that have been prepared from whole blood treated with a proprietary reagent; hybridization which involves the addition of probes that will "hybridize" (i.e. bind) with the parasite nucleic acid if present; a washing step to remove unbound probes; counterstaining to ease reading of the smear; and viewing of the processed smear under a fluorescent microscope. The testing process takes about 1.5 hours from sample collection to completion. The test requires an incubator and fluorescent microscope equipped with special filters (together < 5,000). Each test kit containing enough reagent to perform 50 tests, is expected to cost $ 100, or $ 2.00 per test.

Initial laboratory studies to evaluate the sensitivity and specificity of the FISH assay have been performed. Compared to expert microscopy and PCR, the FISH assays sensitivity is better than expert microscopy and its specificity is comparable to that of PCR. The limit of detection is estimated to be similar to that of PCR, 1–2 parasites per µl blood.

ID-FISH expects to launch the test kit in early 2012.

For more information on this technology, see Appendix 1.

Haemozoin Detection

Several new platforms based on detection of haemozoin are in development for malaria diagnosis. Haemozoin was discovered and linked to malaria in the 1800s, however it has not been used as a primary means of diagnosing malaria. While it is possible to see haemozoin in certain stages of parasite's lifecycle using microscopy (in this case, it is commonly referred to as malaria pigment) it is not always detectable by traditional microscopy. The technologies described below take advantage of unique properties of haemozoin, including its optical properties (haemozoin crystals scatter and depolarize light in a unique way and differently than a red blood cell) and magnetic properties (it is slightly magnetic due to its derivation from iron-containing haemoglobin). One possible advantage of haemozion-based technologies is the potential to develop a non-invasive test, whereby haemozoin

measurements would be taken directly through the skin obviating the need for a blood sample.

Haemozoin Technology Currently in Use

Haematology Analysers

Haematology analysers are primarily used to conduct full blood counts, one of the most widely requested clinical laboratory tests. Automated haematology analysers are large laboratory based instruments that are widely available in laboratories in the developed world and are increasingly available, especially in reference and regional laboratories, in the developing world. Although they are not specifically designed to detect malaria, several groups have explored the use of automated haematology analysers for malaria detection. Because various technology platforms are available, several different approaches have been attempted. Thus far, the Abbott Cell-Dyn, Coulter GEN-S and LH750, and the Sysmex XE-2100 analysers are among the instruments that have been studied by researchers for malaria diagnosis.[39]

In general, abnormalities in the blood counts of patients with malaria have been observed leading to further exploration of the use of haematology instruments to reliably diagnose malaria. The most commonly reported approach is based on the Cell-Dyn analyser's recognition of cells containing haemozoin that produce a particular scatter of light when cells pass through a flow cytometer's channel. Other abnormalities associated with malaria have been reported for the Coulter and Sysmex analysers.

The performance of these techniques has varied greatly, however none of them seems to have high enough sensitivity to be the sole diagnostic test for malaria. For example, for haemozoin detecting methods, papers have reported 49–98% sensitivity and 82–97% specificity.[40] In addition, results are generally analysed manually; i.e. by visual inspection of a plot on the instrument's display monitor with certain patterns considered to represent haemozoin containing cells.

One possible use suggested by researchers is to develop 'alarm systems' for abnormal results that may be associated with malaria. Because complete blood counts are frequently requested in febrile patients, this type of alarm system may be useful in detecting malaria when it is not suspected by the clinician. An alarm system would flag suspicious samples, making the system more automated and user-friendly. This, however, requires additional work to develop algorithms and software adjustments. If an abnormal result was received that was indicative of malaria, it is still likely that a confirmatory test would be performed.

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[39] Campuzano-Zuluaga G, et al. Automated hematology analysis to diagnose malaria. *Malaria Journal*, Nov 2010; 30;9:346.
[40] Erdman, LK, et al. Molecular Approaches for Diagnosis of Malaria and Characterization of Genetic Markers of Drug Resistance. In: *Molecular Microbiology: Diagnostic Principles and Practice,* 2nd Ed. Washington, DC, ASM Press, 2011.

Haematology analysers are in widespread use and the cost per test is comparable to other common laboratory investigations. Despite these advantages, validation and uptake of malaria diagnosis using haematology analysers has been limited. This is due to number of factors, including: variable performance; the cost of automated analysers (several tens of thousand dollars); the size and complexity of operating these instruments; and the need to develop algorithms to identify and classify abnormalities indicative of malaria, allowing for automated interpretation of results.

Haemozoin Technology in the Pipeline

DFxP (Intellectual Ventures)

Intellectual Ventures Lab (IV) is currently developing a MDT that combines the convenience of a POC format with the performance of expert microscopy and molecular methods at a low cost per test. IV intends for the device to be used for patient management, as well as for mass screening and detection of asymptomatic cases in an elimination setting.

IV is a privately-held company created in 2000 by former senior executives at Microsoft. IV's business focuses on inventions, including: development of ideas conceived in-house and investment in existing externally conceived inventions. IV's work spans an enormous variety of applications, from cancer treatments to new battery technologies. An external investor-funded "Global Good Fund" supports IV's work to develop technologies and products for developing world settings, including several malaria technologies.

The device, called Dark-Field Cross Polarization (DFxP), detects haemozoin. In developing DFxP, IV has combined, optimized, and automated two microscopy methods: dark field and cross polarization microscopy. Both methods have existed for years, however neither has been widely deployed for malaria diagnosis. DFxP has several advantages over either method on its own: in terms of performance, the signal to noise ratio of the combined system is 50. On their own, both dark field and cross polarization microscopy require a trained user who can identify haemozoin crystals. The DFxP system uses image processing software and produces automated images of haemozoin that are significantly easier to interpret. Additionally, unlike microscopic methods that require preparation of a blood smear on a glass slide and fixation, DFxP requires no sample preparation.

IV's early prototypes of the DFxP system have performed well in the laboratory and in preliminary field studies in Africa. Additional engineering design work and field studies are planned. IV expects the product to be ready for commercialization within 1-2 years.

For more information on this technology, see Appendix 1.

Magneto-optical Technology (MOT) (University of Exeter)

The magneto-optical technology (MOT) development is led by the University of Exeter in the United Kingdom (UK), and originally in collaboration with several partners, including the Universities of Coventry (UK) and Uppsala (Sweden), the Royal Tropical Institute (Netherlands), and the companies Philips Research Eindhoven, Metis Instruments, and Euroad. The project began in 2005 and was funded first by the European Commission and subsequently by the Bill and Melinda Gates Foundation.

The MOT test is designed to be a portable rugged POC device, simple enough to be used by a low-skilled health worker and inexpensive enough to compete with microscopy and RDTs. The first-generation device uses fingerprick blood samples to detect haemozoin. The technology involves applying a magnetic field to a sample, causing alignment of any haemozoin crystals present (in the absence of a magnetic field the haemozoin crystals orient randomly). The MOT device then employs polarized lasers to compare the transmittance of light before and after application of the magnetic field to the sample. A photo-detector in the device measures the change in transmittance of light and a microprocessor interprets the change in light and provides the result to the test operator.

A prototype of the portable device has undergone laboratory studies and a small-scale manufacturing run is under way at Exeter to support preliminary field studies, which are expected to begin in early 2012. Currently, the intellectual property for MOT is owned by Exeter. Commercialization will occur through licensing of the technology to a commercial partner or by spin-out of a company from the university. The targeted price per instrument is < $ 500 and per test is < $ 0.05 (when mass produced). The expected launch date is 2013–2014.

The university group has also developed a second-generation technology that is non-invasive, taking measurements through the fingernail and removing the need for blood samples. An early prototype of this instrument has been evaluated in Kenya; additional engineering design work is under way to miniaturize the device (although in its final form it may be the size of a large shoebox) and to speed up the patient interface and processing time, as the existing device requires the patient to remain still for > 1.5 minutes.

For more information on this technology, see Appendix 1.

LDMS Mass Spectrometry (Johns Hopkins University)[41]

Researchers at Johns Hopkins University have also developed an approach to detecting haemozoin that employs laser desorption mass spectrometry (LDMS). This method involves directing a laser pulse of ultraviolet (UV) radiation at the sample. If present, haemozoin crystals in the sample will absorb the radiation causing release of certain ions and molecules

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[41] Draws from: Feldman, A. et al. Rapid Detection of Malaria Parasites. *The Johns Hopkins APL Technical Digest,* 2010, Volume 29, Number 3.

from the blood sample. The ions are analysed by their mass/charge ratio; they produce a characteristic mass spectral signature that is used to classify the sample as positive or negative for malaria.

The testing process involves collection of a small amount of whole blood from a fingerprick, dilution of the blood in water, and spotting of the sample onto a metal plate that is air dried and scanned by the instrument. No other consumables, other than a lance and blood collection device, are needed. The metal plates are inexpensive, washable and reusable. Field and laboratory studies using LDMS suggest that it may achieve higher sensitivity than microscopy and therefore be useful for large scale population screening as required in elimination or in pregnant women; however, in its current form it requires sophisticated laboratory based instruments that are not amenable to resource-constrained settings. At the moment, it does not appear that a portable field-ready mass spectrometry instrument for malaria diagnosis is being actively developed/commercialized.

Spectroscopy

Spectroscopy involves the absorption of particular wavelengths of electromagnetic radiation (EMR) (e.g. light) by molecules in a sample. The way different molecules interact with particular wavelengths of EMR is unique and provides information about characteristic features of the molecules. This information is used to classify and characterize the sample.

There are many spectroscopic techniques, differing in the regions of the electromagnetic spectrum (e.g. UV light, visible light, infrared radiation, microwave, etc.) analysed. In general, spectroscopic instruments contain a stable source of radiant energy, which passes through a wavelength selector and filter in order to isolate the desired portion of the spectrum and to focus it on the sample. A photodetector then measures the light that has passed through the sample, this data is subsequently compared to that of a reference spectrum in order to classify the sample and provide a result.

The two devices described below involve spectroscopic techniques for diagnosing malaria.

Spectroscopy Technology in the Pipeline

SpectraWave and SpectraNet (Claro Scientific)

Claro Scientific, based in Florida, began operations in 2006 to commercialize a reagentless POC diagnostics system based on optical profiling technology. The system has broad applications, however, in response to the need for improved malaria and anaemia diagnosis and management, Claro is developing a malaria diagnosis and complete blood count system which is expected to be available in 2014–2015.

Claro's diagnostics system combines two technologies: i) the SpectraWave instrument for sample preparation, multidimensional spectral (MDS) analysis, and transmission of the

sample data file; and ii) SpectraNet a computer software and database system that analyses, interprets and stores the sample data and delivers the results of the analysis. The technology is differentiated from existing spectroscopic approaches because it takes advantages of several optical analysis methods to collect up to 1 million quantitative data points about the sample. The software then analyses the sample profile using an integrated interpretation model. Within the medical field Claro is developing the malaria/anaemia system as well as a bacteria identification system that will initially be marketed in the developed world. Other non-medical applications include industrial, environmental, and biodefense uses.

With regards to malaria and anaemia, Claro has conducted a series of studies on *P. falciparum* malaria with the University of South Florida. These studies used transmission measurements to optically characterize the physical and chemical changes to red blood cells that occur during the course of malaria infections, including the changes as the parasite develops from one stage to another. This optical characterization work forms the basis of the software programme for malaria diagnosis. At the same time, Claro has partnered with Florida Blood Services to develop technologies capable of providing complete blood count analysis.

The process for conducting a malaria/anaemia test involves collection of a fingerprick blood sample that is directly injected into the device and diluted with saline. The system operates without reagents and within five minutes it provides quantitative malaria results and blood count parameter results. Although its final form is continuing to be refined, the system is designed to be rugged and portable, and an early prototype fits in a case that is the size of a large briefcase. Initial studies have demonstrated a LOD of 200 parasites/ µl, however Claro expects to achieve a significantly lower LOD with the addition of additional spectral data (adding angular scattering and fluorescence data) and optimization of sample preparation. Blood count parameters perform within the acceptable ranges for automated systems currently on the market. The device is expected to cost $ 10,000–$ 15,000 and the cost per test is expected to be < $ 0.50.

For more information on this technology, see Appendix 1.

Spectraphone (Quantaspec)

Founded in 2004 and based in Vermont, QuantaSpec is a research and development (R&D) company that creates infrared spectroscopy technologies for detecting pathogens and chemicals with applications for global health, national defence, homeland security, and food safety. To date, the company has received multiple R&D contracts from the US Departments of Defense and Homeland Security, and the US FDA, as well as the private sector. Its malaria diagnostic technology has been developed through a US Army Medical Research contract.

QuantaSpec's technology is a molecular detection system that, based on an initial laboratory evaluation, is expected to achieve sensitivity and specificity far higher than that of microscopy, with a limit of detection that is comparable to PCR-based assays. The test has been developed on a sophisticated and expensive laboratory based infrared spectroscopy system. The testing method requires preparation of a Giemsa stained thin blood smear from a fingerprick blood sample on a glass slide that has a special reflective infrared coating (similar to microscopy). The slide is inserted into an instrument that measures the infrared spectrum of the sample on the slide.

Analysis of the data generated by the sample is based on the principle that every molecule has a unique infrared signature, meaning certain wavelengths of light are absorbed or reflected. A computer-based algorithm is applied to automatically interpret the infrared spectrum of the sample and provide a result to the test operator. Algorithms have been developed to classify the sample as positive for malaria, differentiate between *P. falciparum* and *P. vivax* species, and differentiate chloroquine-sensitive and chloroquine drug-resistant parasites. Early laboratory results have been promising. Additional studies are under way using a larger panel of clinical samples.

QuantaSpec is currently raising funds for development of the first prototype of the Spectraphone, a handheld device based on the technology described above. QuantaSpec expects to launch the Spectraphone in 2014, it will partner with a large manufacturing company to mass-produce the device. Quantaspec is also seeking funding to validate the technology on unstained and unfixed smears in order to minimize sample preparation and the need for reagents.

In addition to malaria, QuantaSpec is working on applications of this platform for diagnosing breast cancer and detecting hospital-acquired bacterial and fungal infections. As the ability to detect a wide variety of diseases and pathogens using a single sample is developed, QuantaSpec expects the device to be used for fever management more broadly.

For more information on this technology, see Appendix 1.

Serology

Malaria serology[42] refers to the use of antigens to detect antibodies. The human body produces antibodies in response to an infection and these antibodies provide some protection from disease. Each time a person is infected, antibodies are boosted and, over time, the antibodies are lost; the kinetics of this immune response depend primarily on age

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[42] This section draws from two sources: 1) WHO technical consultation: Parasitological confirmation of malaria diagnosis, 6–8 October 2009. Geneva, World Health Organization, 2009. Available at: http://www.who.int/malaria/publications/atoz/9789241599412/en/index.html and 2) Drakeley C. and Cook J. Potential Contribution of Sero-Epidemiological Analysis for Monitoring Malaria Control and Elimination: Historical and Current Perspectives. In: Rollinson D. and Hay S. editors: Advances in Parasitology, Vol. 69, Burlington: Academic Press, 2009, pp. 299-352.

and transmission intensity. As such, antibodies represent a marker of exposure to malaria; a person who has not been infected with malaria will not have malaria antibodies.

Although initially developed as a diagnostic test, serologic tests are not used for diagnosing malaria for two reasons: it is not possible to distinguish between current and past infections, and antibodies to malaria parasites are not present during the acute phase of an infection; they appear several days later. Serologic tests for malaria are, however, used to detect exposure to malaria, because antibodies to malaria parasites remain in the body long after an infection has been cleared.

The detection of exposure to malaria has several applications, including screening blood at blood banks to assess prior malaria infection (primarily done in developed countries using commercially available ELISA kits). With elimination back on the global agenda, and many countries looking for effective means of monitoring transmission as malaria prevalence drops, the use of serology for surveillance purposes is gaining attention. In this case, a population is screened for exposure to malaria, which serves as a proxy for transmission. The relative exposure level to malaria can be compared across different geographies, age groups, or periods of time, and may be used to monitor and evaluate programmatic interventions (e.g. a drop in antibody levels would indicate successful interventions), to identify foci of transmission, or to confirm elimination of malaria from an area.

Serological tests for malaria have many advantages for population screening. They are species specific and detect antibodies at very low concentrations. They are relatively inexpensive and are amenable to a high throughput format, which are advantageous characteristics because population surveys generally involve a large number of samples. Since serological tests were last deployed widely (in the late 1960's-70's malaria eradication campaigns) advances in technology, namely the ability to mass produce specific antigens, have allowed for greater standardization in testing and have replaced more primitive and less precise methods of preparing antigens from infected monkeys or cultures.

There are several techniques used for malaria serology (indirect fluorescent antibody test [IFAT], microarrays, RDTs), the most practical for population based screening in resource-constrained settings is ELISA. ELISA is a commonly used diagnostic platform, familiar to many laboratories in developing countries due to its widespread use in diagnosing HIV. ELISA is a laboratory-based test, requiring a reasonably well-equipped laboratory and technicians trained in the method who perform a number of steps during the sample preparation and reaction processes. Although protocols vary by laboratory, a general summary of the process is as follows:

- **Sample preparation**: The test sample is a few drops of fingerprick blood (< 10 µl). For malaria population surveys, the process involves collection as serum or, more commonly, as dried blood spots collected on filter paper. Samples need to be diluted to certain levels before performing the reaction.

- **Reaction**: Antigens (most often recombinant) are coated onto the bottom of each well of a 96-well plate. The serum samples are then added to the wells and incubated. If antibodies to the antigen are present, they will bind to the antigen in the plate. After incubation, a series of buffer rinses removes any unbound matter from the wells.

- **Detection**: A second antibody with an enzyme attached to it is added to the wells; this antibody will bind to any human antibodies present in the well. It is incubated, and then excess matter is washed with a buffer. Finally, an enzyme substrate is added which causes production of a coloured product in wells containing a malaria antibody/antigen complex (i.e. positive for malaria antibodies). The colour change is detected using an ELISA plate reader that measures the amount of colour produced, which is proportional to the amount of antibody present.

The equipment required to run an ELISA is standard laboratory equipment for many academic and reference laboratories and includes pipettes, 96-well plates, and ELSIA plate readers. Some protocols require use of an incubator. The most basic ELISA plate reader costs > $ 1,000. Reagents require cold storage, and, for longer-term storage, samples would ideally be frozen.

The time-to-results depends on the number of samples to be tested. A small number of samples can be processed in one day. For population surveys, though, several hundred or thousands of samples need to be processed. One well-trained technician might take a month to prepare the samples and to test each for a few antigens, as is commonly done in surveys (see below). Higher throughput is possible with more staff and training.

Although serology is increasingly used for surveillance purposes in malaria-endemic countries, a number of challenges remain.

- **Identifying the optimal set of antigens.** Each individual's immune response to malaria differs as do the parasites and antigens present during an infection. When only one antigen is used, it is possible to mischaracterize an individual as not having had malaria. It is, therefore, common to run multiple ELISAs with more than one antigen. The optimal set of antigens needs additional R&D; a combination ELISA would minimize workload and optimize output.

- **Little standardization in the assay.** Most of the laboratories conducting serological tests for malaria are research laboratories using in-house protocols and favoured antigens. Although commercial ELISA kits are available from several companies (Cellabs, Lab21, Diamed, Standard Diagnostics, Tulip Group, etc.), these are relatively expensive compared to in-house methods (> $ 5.00/sample versus $ 0.20 per sample) and the antigens used by any one kit have not been designed with populations surveys in mind. The lack of a widespread standardized assay and inexpensive commercial kits limit the uptake of the method and make comparison between studies difficult.

- **Lack of availability of mass-produced antigens.** The growing demand for serological surveys requires large quantities of standardized recombinant antigen, as well as appropriate positive and negative assay controls. Currently, research labs produce antigens; however, these labs do not have the capacity to or interest in producing antigens on a large scale.

- **Standardization of results interpretation.** Because the ELISA reader produces a quantitative measure of the presence of antibodies (which depends on the amount of antigen in the well, the amount of sample added, etc.), the interpretation of results needs additional work to define standard cut offs for positive and negative reactivity levels and to generate a simple read out usable in malaria control programmes.

The London School of Hygiene and Tropical Medicine is currently leading the field and is involved in several efforts to use serology to track malaria transmission and is actively working to develop and standardize an ELISA test for population surveys. Other groups also working in this area include the Institute for Tropical Medicine (Belgium) and the University of California-San Francisco.

Conclusion

Although malaria diagnostic testing (MDT) has increased substantially over the past decade, there is still significant ground to cover, if universal access to malaria diagnosis in the public and private sectors as well as at the community level is to be achieved. Given that nearly half of the world's population lives at risk of malaria, diagnostic tests for routine patient management must be affordable and widely deployable; in other words portable, easy to use, and robust enough to withstand extreme heat and humidity. MDTs must also be accurate and rapid due to the acute and life-threatening nature of malaria disease.

In addition to the need to expand access to testing such that it is universally available, there are also several unmet needs in malaria diagnosis that require technical advances, including the need for more sensitive tests for screening pregnant women and for detection of infection in asymptomatic individuals, as in the case of malaria elimination campaigns. Also, elimination campaigns are creating the need for technologies capable of monitoring the prevalence of malaria in low-transmission settings.

Summary of Technologies

Today, the primary means of diagnosing malaria are microscopy and, increasingly, malaria rapid diagnostic tests (RDTs). Microscopy, in expert hands is considered to be the gold standard for malaria diagnosis; however, high quality microscopy is not widely available due to a number of operational challenges. As such, the current expansion in access to malaria diagnosis is primarily being driven through increased use of RDTs. In the case of RDTs, there are hundreds of products on the market, an increasing number of which have demonstrated acceptable performance through the World Health Organization (WHO) product testing programme for malaria RDTs. RDTs are relatively inexpensive, and are truly point of care tests, making them an increasingly common means of diagnosing malaria.

Microscopy and RDTs both have their limitations; foremost among them is maintenance of quality. In the case of microscopy, the continued investment in quality is difficult to sustain, due to financial, human resource, and logistical challenges. With regards to RDTs, quality challenges relate largely to the lack of practical technologies and methods for performing quality control at all levels of the value chain.

Although alternative, higher performing diagnostic technologies are currently available for malaria diagnosis, such as polymerase chain reaction (PCR), at the moment these are largely in-house methods that have relatively long time-to-results, require highly trained staff, and must be performed in sophisticated, well-equipped laboratories. As such, these existing technologies are not well suited for the large-scale, highly decentralized testing associated with routine malaria care, and their use is limited to specialized case investigations and research.

The pipeline for new diagnostic technologies for malaria includes a variety of efforts that employ a wide range of technology platforms and scientific approaches for detection of malaria. Some of these approaches address the unmet needs described above, while others are more likely to be used for routine patient management. The majority of tests in the pipeline focus on ease-of-use, either by simplifying existing molecular methods or by developing devices that minimize operator involvement and produce rapid results. The tests vary in terms of format, cost, and output, with some approaches providing a qualitative result for malaria and others providing more detailed information on species, lifecycle, and parasite density. Although the majority of devices use fingerprick whole blood samples, one company is developing a urine test and other developers are exploring non-invasive approaches.

Future Directions and Implications

In contrast to the past few decades, today's malaria diagnosis landscape is very dynamic. With a decreasing burden of disease, the need for and role of malaria diagnostic testing is expanding, both with regards to patient management and to surveillance. In 2010, WHO changed its policy on diagnosis, recommending diagnosis of *all* suspected cases of malaria, changing the paradigm dramatically for clinicians working in the field who had for many years equated all fever with malaria. In 2011, the Roll Back Malaria Partnership set targets for universal access to diagnosis in the public and private sectors as well as at the community level. Indeed, this vision for universal access implies that MDTs are poised to be among the most decentralized diagnostic tests used worldwide, akin to pregnancy or glucose tests in the developed world.

In the immediate future, there will be a continued move away from clinical diagnosis and increasing reliance on diagnostic tests to confirm malaria. Decentralization of diagnosis through community case management of malaria will follow the public facility based scale up of testing. In addition, there is growing interest in expansion of diagnostics to the private sector, with potential models for deployment being an area of active operational research. For both community and private sector use, a fast, affordable point-of-care (POC) test is critical, as are mechanisms for monitoring the quality of testing.

The expansion in diagnostic testing for malaria will improve the accuracy of surveillance data and likely lead to decreased estimates of malaria prevalence. Despite these decreases, demand for testing will remain high because most fevers in areas where malaria is a potential risk will still need to be tested in order to confidently rule out malaria. The majority of tests will be negative for malaria and finding the correct diagnosis for these non-malaria fevers presents a new challenge for clinicians and health systems in malaria endemic areas. A major implication, therefore, of improved access to malaria diagnosis is the need to improve the ability to diagnose and treat non-malaria fever. This involves revisiting protocols for fever management, studies to investigate the common causes of

fever, reviews of treatment options for non-malaria fever, and possibly demand for new diagnostic technologies that assist with the differential diagnosis of fever.

The decreasing burden of malaria is also creating a need for new technologies for low-transmission settings. Although the appropriate mix of surveillance activities and implementation strategies are still being defined and will vary from place to place, in general there is a growing need for tests that can detect asymptomatic infections and monitor the burden of malaria as cases come down. Sensitivity at low parasite densities, portability, and rapid results are important factors for active case detection. For measuring low-level transmission, high throughput, sensitivity, and affordability are critical considerations.

With regards to pregnant women, the changes in the burden of malaria and the efficacy of antimalarial drugs used to prevent malaria in pregnancy is contributing to a growing need for tests for screening pregnant women. Although the most effective strategy for managing malaria during pregnancy is an area of on-going research, the existing diagnostic tools used for routine case management are not ideal for screening this population group. New technologies must be able to detect clinically relevant infections, have a short time to results, be portable, and be robust enough to withstand challenging environmental conditions.

Lastly, with the anticipated expansion in access to malaria diagnosis, tremendous improvements in the quality and timeliness of malaria surveillance is possible. This is likely to be an area of increasing work, as systems are need for capturing, storing, analysing, and disseminating this data. In order to take advantage of the potential for improvements in public health and programme management that high quality surveillance data affords, strategies for rapidly interpreting this data and translating it into action are needed. Several of the new technologies reviewed in this report intend to incorporate data storage and remote transmission capability; one of the technologies deals exclusively with surveillance data capture and analysis.

What should the landscape look like?

In the future, we can expect increasing use of MDTs, as diagnosis is scaled up first in the public sector and then in the community and private sectors. This should lead to improved management of fever overall; however, in order to realize the benefits of diagnostics, the quality of the testing and appropriate care following diagnosis must be ensured.

Initially, the expansion in diagnosis will come from existing technologies, namely RDTs. The contribution of traditional light microscopy to expanding access to malaria testing will likely be limited, although it is an important technology, with relevance to malaria and other diseases, and continued investment in existing microscopic services is warranted. With respect to malaria RDTs, continued investment in quality is vital to test adoption and

acceptance by patients and providers. Strengthening the management of fever more broadly is also needed to maximize the public health impact of tests.

In addition to existing malaria diagnostic tests, several new technologies for malaria diagnosis are in the development pipeline and are expected to contribute to the expansion of testing in the coming years. In 2012, it is expected that several improvements to detecting parasite nucleic acid will come to market. These may be incorporated into health facilities with laboratories and will be used largely for specialized case investigations, research, and possibly surveillance. In 2013 and beyond, more novel products are expected, including less invasive tests, high throughput screening platforms, and platforms that take advantage of technologies that have not been widely used in the past for diagnosing malaria (i.e. detection of haemozoin, spectroscopy). Some of the POC technologies described in this report may play a significant role in patient management, while other technologies are likely to fill gaps in unmet needs for diagnosis in special populations and the expanding role of malaria diagnostic tests in surveillance activities.

It remains to be seen how the technologies in the development pipeline will perform and how their cost and ease-of-use will compare to tests already on the market. New products coming to market for routine patient management will need to compete with RDTs in terms of striking the right balance between cost and added value. For devices that take advantage of technological approaches not yet used to diagnose malaria, robust studies may be needed to demonstrate performance across a variety of settings and populations. Quality is a driver in adoption of new tests and, for new tests, the regulatory pathway and procedures for independent evaluation are uncertain. This will need to be addressed, if these new technologies are going to play a role in expanding access to testing.

In summary, the future landscape for malaria diagnosis is likely to differ from today's landscape, which is dominated by microscopy and RDTs, with very occasional use of PCR. In the future, it is likely that each national malaria programme will employ a different mix of both existing and new technologies, depending on their local epidemiology, health systems, and goals for malaria.

Appendix 1: Operational Characteristics of Malaria Diagnostic Platforms Currently in Use

Urine Malaria Test (Fyodor)

Platform Characteristics	
Type of technology	Disposable one-step urine dipstick based on immunochromatographic detection of malaria parasite proteins in urine.
Output	First generation product is a two-line test that will differentiate "fever due to *Plasmodium falciparum* malaria" from fever due to some other cause. The results are visible: one line indicates fever not due to not malaria, two lines is positive for malaria. Second generation product (2014) will be a three line test that detects both *P. falciparum* and *P. vivax* malaria.
Performance	The test is designed to detect the presence of malaria proteins present in urine during fever. In preclinical studies the test achieves > 90% sensitivity and 90% specificity for the detection of P. falciparum malaria. In testing so far, the urine test has a comparable LOD (as determined by parasitemia) as current blood-based tests. Definitive studies to establish LOD are expected to be completed in 2012.
Turnaround time/ Capacity	Test results are available in 10 minutes.
Sample needed/ Stability	The device requires about 5 drops (100–200 µl) of urine. The test is intended to be performed immediately after sample collection.
Environmental requirements	Stability studies of the urine test have not been completed, however is being designed with stability in mind and is anticipated to have a 12 month or longer shelf life and recommended storage conditions of 25–30° C.
Testing protocol	The urine malaria test is a one-step test with no requirement for sample preparation. The testing protocol is: i) collect urine sample; ii) open packaging and dip test into sample; iii) allow test to dry for 10 minutes; iv) read results.
Cost/Test	Preliminary estimate: $ 1.50/test
Cost/Instrument	No instrument.
Power requirements	None
Training/Technical sophistication	Test procedure is simple, no sample preparation, blood draws, or buffers are required. It is designed for point of care use by lay people.

Durability/ Maintenance	N/A; disposable test.
Infrastructure requirements	No infrastructure required; test is designed for point of care use at all levels of the health system.
Result display and storage	Results appear as visible lines on the test strip. No sample or results storage required.
QA/QC	Fyodor intends to submit the product to the WHO Pre-Qualification programme, and product manufacturing will be done only at manufacturing sites which qualify and operate under the internationally recognized International Standards Organization (ISO) standards.
Availability	Expected in early 2013.

Malaria Surveillance Using a Rapid Diagnostic Test (RDT) Reader and Cloud Information Services (Fio Corporation)

Platform Characteristics	
Type of technology	The Fio System comprises the Fio Smartreader, Portal, Database, and Cloud Information Services. The Fio Smartreader is a mobile, ruggedized smartphone-based RDT reader. It requires a SIM card and is compatible with any mobile phone network. The Smartreader is designed to read and interpret standard RDTs and to capture a high-resolution image of the RDT at the optimal incubation time. It also uploads geo-localized, time-stamped data (diagnostic, image, demographic, workflow) to Fio's Database. The device's two-way-communications allows for downloading of directives (clinical protocols, data surveys, alerts) from health programme managers to health workers. The Portal is a web interface to Fio's Database and Cloud Information Services, accessible to managers via a web browser. The portal is designed to enable: • Real-time monitoring/management of quality control, Smartreaders, user workflow, diagnostic and other data; • Secure storage, retrieval, and export/import of data to other databases; • Data-mining, mapping, graphing, statistical charting, analysis, surveillance, and reporting; • Secure dissemination of clinical protocols, surveys, alerts, and other two-way communication to Smartreaders; and • Use of quality control tools to identify health workers in need of remedial training through tracking and trending of diagnostic performance and reviewing high-resolution images of RDTs captured at the time of interpretation.
Output	A digital dataset that includes: Qualitative digital interpretation of commercially available RDTs; demographics, survey, GPS coordinates, date, and time; and high resolution digital image of RDT at optimal incubation time.
Performance	Preliminary data (from US NIH Center of Excellence in Malaria Research, Colombia) using commercially available malaria RDTs suggests Smartreader concordance with expert visual read of RDT is 99%. Sensitivity and specificity are functions of the RDT being read by the Smartreader.
Turnaround time/ Capacity	The time it takes to run one test is a function of the incubation time for the RDT being read by the Smartreader. The Smartreader can process and track eight RDTs concurrently. Depending on RDT incubation time, estimated maximum throughput per Smartreader is 30 to 50 RDTs per eight hour day.
Sample needed/ Stability	Sample type, volume, and stability vary with RDT being read.

Environmental requirements	Smartreader is designed for altitude up to 2000 meters, temperature 5° C–40° C; maximum relative humidity 80% for temperatures up to 31° C; decreasing linearly to 50% relative humidity at 40° C. RDT environmental requirements are a function of RDT being read.
Testing protocol	i) Run daily control; ii) prepare RDT; iii) auto-detect RDT model and start Smartreader incubation timer; iv) Smartreader incubates the RDT for specified time; v) Smartreader provides result to operator on screen; vi) dataset automatically transmitted to Fio Cloud over cell phone network.
Cost/Test	Per test cost depends on the RDT being read by the Smartreader.
Cost/Instrument	Smartreader cost included in commitment to Fio cloud services, which scales to number of tests per year.
Power requirements	Internal battery recharged via power outlet or solar panel. 4 days of operation per charge. Mains supply voltage of 100-240Vac, -/-10%, 47–63 Hz.
Training/Technical sophistication	Designed to be performed by low skilled health workers, ½ day of training required for a new test operator and/or self training via on-screen tools.
Durability/ Maintenance	The device is expected to last at least three years; non-functioning devices will be exchanged.
Infrastructure requirements	Appropriate for use in community and at health facilities of all levels.
Result display and storage	Results are displayed on the Smartreader's screen. Local storage on Smartreader of 200 datasets. Datasets are transmitted and deleted from Smartreader memory when in cell phone network range. Fio Cloud has unlimited storage capacity.
QA/QC	Quality control functions include: • Smartreader performs an internal quality check once daily • Smartreader identifies RDT and sets optimal incubation time • Automatic detection of invalid RDTs (i.e. no control line, sample or buffer processing errors) • Compatible with 2D barcodes which could enable automatic detection of expired RDTs External quality control functions include: • Data analysis tools on Fio Portal can be set to identify potential quality anomalies such as excessive invalid rates, unusually high positivity/negativity rates relative to other Smartreaders. • Remote quality control read of RDT images through the Fio Portal to identify technicians in need of remedial training. • Custom reporting tools enable remote monitoring and evaluation of programmes and impact of specific interventions.
Availability	2012

MicroPCR (Tulip Group and Bigtec Labs)

Platform Characteristics	
Type of technology	The microPCR device is a portable (dimension: 210mm × 160mm × 110mm, weight: ~1kg) real-time PCR device that takes micro-PCR chips (microchips). In the first generation product, sample preparation is done independently using semi-automatic device/ automatic disposable cassette. Second generation devices will integrate sample prep into device. The core technology used in the platform is fluorescent probe based real time PCR. Specific genes from *P. vivax* and *P. falciparum* are amplified in a duplex reaction format. The reaction is done in a disposable microchip, with integrated thermal cycling capabilities, to enable faster turnaround time. All reagents are pre-loaded in a stabilized form on the chip, making it user friendly and robust. As the microchips are disposable and self-sealing, the reactants do not come in contact with the device, eliminating contamination. The device has real time fluorescence monitoring capability with a touch screen /PDA phone interface for user input and data output. The device is powered by a rechargeable lithium ion battery pack.
Output	The assay differentiates between *P. falciparum* and *P. vivax* malaria and can provide a quantitative result (parasites/µl etc.). Future generation assays may incorporate additional species of malaria.
Performance	Performance evaluation studies have shown that the microPCR is much superior to current diagnostic methods such as microscopy and RDT. The sensitivity and specificity is estimated to be > 99% with a lower limit of detection as 2 parasites/µl of blood.
Turnaround time/ Capacity	Total turnaround time, including sample preparation, would be 45–60 minutes, with the microPCR run time of 30–45 minutes per sample. As the sample processing is done in parallel to microPCR, about 12 samples could be analysed per 8 hour shift.
Sample needed/ Stability	50 µl of human whole blood. It could be either fingerprick / venous blood. The specimen could ideally be processed immediately after collection. If preservation is required, the specimen can be froze and stored for up to 3 days.
Environmental requirements	The individually packed, disposable microchip could be stored at +2° to +30° C for 1 year. Ideal conditions for operation of the device would be at temperature of 20° to 30° C and relative humidity between 40–80%.
Testing protocol	The first generation of the technology includes a sample prep stage followed by transfer of the purified sample to the microchip for loading into the device. Steps include: i) fingerprick/venous blood collection; ii) transfer of blood to the processing device; iii) transfer of purified sample to the microchip; iv) load microchip into device and run assay; v) read result.

Cost/Test	Pricing TBD
Cost/Instrument	Pricing TBD
Power requirements	Rechargeable lithium ion battery pack 5A, 110/230 V AC Charger
Training/Technical sophistication	A medium skilled operator could perform the test. The training time expected is 1–2 days.
Durability/ Maintenance	The microPCR device is designed with durability in mind; if repairs are needed the plan is to swap out non-functional devices for new ones.
Infrastructure requirements	Due to the sample processing steps, the first generation technology is most appropriate for a laboratory setting, e.g. from a basic lab in a district hospital to higher levels of the system, where a technician is available to perform the necessary steps. However, it would be possible to use it outside of a laboratory at a health clinic or in the community due to its portability and battery power. The second generation platform will integrate the sample prep rendering the device more robust for use at even lower levels of the health system.
Result display and storage	The test result would be displayed on the device screen. The device stores > 1,000 test results internally. Results can also be transmitted to remote locations, pushed to a central server in encrypted form for future analysis and disease surveillance through GSM, WiFi networks, and can be printed through WiFi or an optional blue tooth printer.
QA/QC	Plans for regulatory/pre-market approvals: • License from Indian FDA • ISO 13485 CE mark (regulatory approval in the EU) • 510(k) pre-market clearance by the FDA in USA • Country specific registrations
Availability	Q1 2012

Platform Characteristics	
Type of technology	The PanNAT™ system combines microfluidics and PCR techniques into a portable machine *(12 x 12 inches)*. It uses disposable microfluidics cartridges *(4 x 3 inches)*. All sample prep, amplification and detection occur in the cartridge once inserted into the PanNAT™ device. The process combines silica membrane DNA capture, PCR based amplification, and end point molecular beacon fluorescence detection. The PanNAT™ device provides for the on cartridge fluid movement, heat cycling, optical detection, and result interpretation software.
Output	Qualitative result for *Plasmodium* genus (i.e. pan-malaria) and *P. falciparum* malaria.
Performance	Analytical detection of 5–10 parasites/µl compared to 50–100 parasites/µl for microscopy or RDTs.
Turnaround time/ Capacity	~30 minutes per sample; one sample processed at a time
Sample needed/ Stability	50 µl fingerprick whole blood sample*.*
Environmental requirements	Cartridges will be stable for 2 years at room temperature.
Testing protocol	i) Collect fingerprick blood sample; ii) transfer sample to cartridge using a capillary tube; iii) insert cartridge into PanNAT device to initiate the test; iv) wait 30 minutes to read results. Note: it may be possible to lance the patient's finger and collect blood sample directly onto cartridge.
Cost/Test	Targeting $ 3.00–$ 4.00 per test
Cost/Instrument	Targeting < $1000–$ 4000 per instrument
Power requirements	Instrument designed to run for 8 hours on battery, recharged with mains electricity, a car battery, or inexpensive solar panel.
Training/Technical sophistication	Designed to be performed by low skilled health workers. Approximately < ½ day of training would be required to operate the device.
Durability/ Maintenance	The device has maintenance free design. Non-functioning devices will be exchanged by the company.
Infrastructure requirements	Device is designed to be used anywhere, including rural settings and health facilities.

Result display and storage	Results are displayed on an LCD screen. The PanNAT™ device is Wi-Fi enabled and can store up to 350 test results before prompting the user to download or delete the results.
QA/QC	The PanNAT™ platform will have FDA approval; approvals for the malaria assay are TBD. Each assay contains biplexed endogenous internal positive controls within the cartridge.
Availability	Malaria assay and platform likely to be available in 2014.

Nucleic Acid Lateral Flow Immunoassays (MALACTRES Consortium)

Platform Characteristics	
Type of technology	Commercial PCR kit containing primers, reagents, and the lateral flow device required to run the test. Direct PCR method, no purification of whole blood sample required. Amplification is performed on a traditional PCR thermocycler, followed by detection using disposable NALFIA (nucleic acid lateral flow immunoassays). The primers used in PCR process have ligands attached; antibodies on the NALFIA bind to the ligands.
Output	The output is qualitative. A variety of primers that detect different strains and species of malaria are anticipated. For malaria detection, primers are available that detect *Plasmodium* genus (i.e. pan-malaria) and *P. falciparum* malaria. Several primers for markers of drug resistance are being developed. The NALFIA is a generic strip that can detect up to three different items, depending on the combination of primers used, allowing for customization of the assay and flexibility depending on needs.
Performance	To date, trials have shown sensitivity and specificity that is comparable to traditional PCR based assays.
Turnaround time/ Capacity	1 hour to process a sample from start to finish; possible to process 25 samples/hour, or 200 in one day.
Sample needed/ Stability	1 µl fingerprick whole blood sample. Samples are stable at room temperature for a few days, longer term storage is possible with refrigeration or freezer storage.
Environmental requirements	The NALFIA are expected to be stable for several years at 30° C. Storage requirements for reagents and test kits is 4° C.
Testing protocol	The test involves: i) collection of a fingerprick blood sample; ii) transfer into tubes containing primers for PCR; iii) insertion of the tube into the PCR instrument; iv) after 40-50 minutes transfer of PCR product to lateral flow test strip using pipette; v) wait 5 minutes for reaction and read results.
Cost/Test	Final prices have not been determined; target price for kits is approximately $ 2.00.
Cost/Instrument	Several PCR instruments are available and cost varies depending on the instrument selected.
Power requirements	The detection system requires no power; PCR amplification instruments require a stable source of mains electricity.
Training/Technical sophistication	The test requires several steps, and should be performed by a trained laboratory technician. The primary skills required include pipetting. Approximately 1–2 days of training would be needed to train operators.

Durability/ Maintenance	The NALFIA is a disposable device.
	Several PCR instruments are available; useful life and maintenance requirements vary by model.
Infrastructure requirements	The technology requires a well-equipped laboratory with fridge (4° C) and stable electricity, as well as trained technicians. Therefore it is most suited for regional and reference level laboratories.
Result display and storage	Results are read visually.
	Disposable device, no storage capacity (though used device can be stored for later reference.)
QA/QC	There are no plans for regulatory approvals at this time, although the kits will be manufactured in conformance with ISO 13485 standards.
	The NALFIA has a control line that indicates that the amplification was successful and that the lateral flow strip is functioning properly.
Availability	2012 kits will be available for direct purchase from manufacturer; distribution/marketing plans TBD.

Loop-mediated Isothermal Amplification Malaria Diagnostic Kit (Eiken Chemicals, FIND, and HTD)

Platform Characteristics	
Type of technology	Loop-mediated isothermal amplification (LAMP) is a bench-top platform using isothermal DNA amplification technology, whereby parasite DNA is amplified at a stable temperature and the bi-products of amplification detected using a real time turbidimeter or visually by fluorescence. A simple method for sample preparation is available through the "PURE Method" kit from Eiken. After collection of blood from a fingerprick (or filter paper) the process involves transferring the sample into a lysis tube, boiling and attaching the tube to a purification device, squeezing the device to transfer the processed sample to the LAMP reaction tube using a dropper cap that fits onto the purification device. The procedure takes < 10 minutes.[43] The diagnostic kit includes novel primers targeting mitochondrial DNA that in initial studies exhibited superior sensitivity and specificity as compared to other LAMP methods. Each reaction tube in the kit contains the primers and all other reagents needed to run the assay. Controls are included as part of the reagent kit and are required for each run.
Output	Qualitative result for *P. falciparum* or *Plasmodium* (i.e. pan-malaria). Additional species specific tests are under development
Performance	Preliminary study reported a limit of detection of 5 parasites/µl; > 93% sensitivity as compared to PCR and 100% specificity. Larger scale trials are under way.
Turnaround time/ Capacity	Time-to-result is < 1 hour, including sample preparation: > 10 min and 30–40 minutes to run the assay. Current platform (8-well format) processes six patient samples at a time plus two controls for one set of primers; approximately 24 patients can be tested per day (four runs per day). A high throughput platform is in development based on a 96-well format.
Sample needed/ Stability	30 µl of whole blood collected in a heparin tube. Dried blood spots are also possible, with additional elution step required to prepare sample. Samples are stable at room temperature for a few days and longer-term storage is possible using filter paper or refrigeration/freezing.
Environmental requirements	Reagent stability has not be established yet through studies; however the aim is for reagents to be stable at room temperature for 24 months There are no temperature or humidity requirements for device operations.

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[43] An alternative, lower cost preparation uses a heating step at 95C to denature haemoglobin followed by a centrifugation step. Commercially available DNA extraction kits commonly used for PCR (e.g. Qiagen kits) can also be used.

Testing protocol	1) Transfer sample to the Pure Method Eiken tube; 2) squeeze tube to mix contents; 3) transfer sample to reaction tube using dropper cap; 4) insert tube into heating block or into turbidimeter to 65° C for 30–40 minutes; 5) read result in real time with turbidimeter or at the end of the reaction by fluorescence.
Cost/Test	Preliminary estimates for developing countries > $ 2 per patient for reagent tubes; $ 10 for Eiken Pure Method sample preparation kit
Cost/Instrument	Heating block = ~$ 10,000, lower prices may be possible with increasing volumes. Real-time turbidimeter = ~$ 10,000.
Power requirements	Instruments require electricity, battery operation is possible.
Training/Technical sophistication	< 2 days training for lab technicians, primary skills required include: sample collection, biosafety, and basic microbiology lab skills
Durability/ Maintenance	Several heating blocks and turbidimeters are available, maintenance and useful life vary by model.
Infrastructure requirements	Laboratory based technology appropriate for district hospital level and higher. Potential for field based use in specific circumstances such as surveys, when technician available.
Result display and storage	Results are qualitative and are typically read visually by fluorescence, or read by turbidimeter and archived using specific software.
QA/QC	The test kit will be CE-marked. The reagent kit includes positive and negative controls.
Availability	2012 for low throughput version.

Malaria Fluorescent in-situ Hybridization Assay (ID-FISH Technology)

Platform Characteristics	
Type of technology	The assay employs fluorescent in-situ hybridization (FISH) technology which involves the use of a fluorescently labelled probe to detect specific sequences of nucleic acid in a sample. FISH is a laboratory-based test that is performed on blood smears and requires an incubator and fluorescent microscope equipped with special filters. Three different diagnostic test kits containing all reagents required to process 50 samples will be available. P-genus FISH kit will detect all species of *Plasmodium*, the PF-FISH kit will detect *P. falciparum*, and the PV-FISH kit detects *P. vivax*.
Output	The assay detects all species of malaria or may differentiate between P. falciparum and *P. vivax* malaria. It can provide a quantitative result and morphological information that can be used to assess the parasite's stage of development.
Performance	Laboratory studies demonstrate sensitivity and specificity better than expert microscopy. The lower limit of detection is estimated to be 1–2 parasites/µl blood.
Turnaround time/ Capacity	1.5 hours from collection of sample to completion. Recommended to process 10 samples at a time. Assuming a microscopist can read 10 slides per hour, about 60 samples per day can be processed with one microscope and two technicians performing the assay and reading the results.
Sample needed/ Stability	3 µl whole blood collected in EDTA tube is required for the assay. Once blood collected it should be used immediately or stored at 2°–8° C for up to 24 hours. Once methanol fixed smears are prepared with blood treated with the proprietary ID-FISH SPR reagent, the smears are stable for a year at room temperature when stored in a dry dark place.
Environmental requirements	The reagent kits are stable for two years unopened at 15°–30° C. Refrigeration of kits recommended in hot climates. After opening the kit, the shelf life is six months.
Testing protocol	The assay protocol requires multiple steps, in general they are as follows: (i) preparation of methanol fixed blood smear from EDTA whole blood mixed with slide preparation reagent; (ii) hybridization of the fixed smear with probes at 37° C; (iii) perform three washes; (iv) air dry; (v) add mounting medium; (vi) leave in dark place for 10 minutes; (vii) view under fluorescent microscope.
Cost/Test	$ 100.00/kit capable of performing 50 tests.
Cost/Instrument	Incubator ~$ 800 Fluorescent Microscope with special filters ~$ 4,000

Power requirements	The incubator and fluorescent microscope require a stable power source. Due to the sensitivity of mercury vapour bulbs used in the microscope to power fluctuations, a power stabilizer might be needed.
Training/Technical sophistication	The assay requires a laboratory technician, experience with microscopy is helpful. A test operator familiar with microscopy can be trained to perform the assay in one day; for those unfamiliar with malaria microscopy 5 days training is expected.
Durability/ Maintenance	Fluorescent microscope would last > 10 year if well serviced and maintained. Mercury vapour bulbs used by the microscope last approximately ~70 hours, replacement cost is ~$ 25.
Infrastructure requirements	Laboratory based technology appropriate for district hospital level and higher.
Result display and storage	Results are read visually using a fluorescent microscope.
QA/QC	The kit will be FDA cleared. Positive and negative controls can be prepared in-house at the laboratory or can be purchased from the manufacturer.
Availability	Q1 2012

DFxP (Intellectual Ventures)

Platform Characteristics	
Type of technology	DFxP is an automated microscope-based device that uses a dark-field illuminator and cross-polarizers to capture the scattered and depolarized light from haemozoin. Haemozoin is a byproduct of the malaria parasite's consumption of haemoglobin and its presence in peripheral blood indicates infection with malaria. After loading a fingerprick blood sample in a disposable sample chamber into the device, the DFxP microscope screens many different fields of view using a dark-field illuminator and cross-polarizers; an image processing software identifies and quantifies haemozoin crystals present in the sample. Final form has not been engineered, however, IV expects device to be both compact and portable. The device uses disposable sample collection chambers.
Output	Device provides a quantitative result for haemozoin content, which in preliminary laboratory studies is correlated to parasitemia. The device does not differentiate between species.
Performance	In preliminary laboratory studies the detection limit for infected red blood cells is 1–5 parts per million, equivalent to 5–25 parasites/µl. Trials to establish sensitivity and specificity are planned, with small-scale field studies likely in 2012.
Turnaround time/ Capacity	< 3 minutes Hundreds of scans per day possible.
Sample needed/ Stability	Current prototype uses 20 µl blood from a fingerprick, possible that future versions will require less blood.
Environmental requirements	The device does not use reagents, no need for cold chain. The device components are also expected to be highly stable in hot and humid conditions.
Testing protocol	i) Collect fingerprick blood sample (< 20 µl blood) into disposable sample collection chamber; ii) insert chamber into device and press start; iii) wait 3 minutes to read results.
Cost/Test	Competitive or better than current RDTs.
Cost/Instrument	Unknown at this time. Targeted for field availability.
Power requirements	Planned to be battery powered.
Training/Technical sophistication	Device is intended to be used by a low skilled health worker, < ½ day training required to operate the device.
Durability/ Maintenance	Device is still in the prototype phase and is being designed for rugged field conditions.

Infrastructure requirements	The device is intended for use out in the community as well as in health facilities at all levels.
Result display and storage	Final form has not yet been designed. Results likely to be displayed on an LCD screen.
QA/QC	Regulatory approvals TBD once commercial partner has been selected. Device QA/QC TBD.
Availability	To be determined, potentially 2013–2014 years depending on commercialization plan and regulatory/quality approvals.

Magneto-optical Technology (University of Exeter)

Platform Characteristics	
Type of technology	The magneto-optical technology (MOT) device is portable, (about the size of a credit card machine that can be brought to a table), and uses disposable sample cells. The technology is based on detection of haemozoin. After invasion of malarial parasites into red blood cells the parasites digest haemoglobin. The heme component is toxic to the parasite so it converts it into haemozoin which are rod-shaped crystals. The test takes advantage of two properties of haemozoin: i) the crystals are weakly magnetic because they are derived from haemoglobin and contain iron; and ii) due to their shape, haemozoin crystals have unique optical properties. The MOT test involves applying a magnetic field to a sample, causing alignment of any haemozoin crystals present (this phenomenon is called the Cotton-Mouton effect, in the absence of a magnetic field the haemozoin crystals orient randomly). The MOT device then employs polarized lasers to compare the transmittance of light before and after application of the magnetic field to the sample. A photo-detector in the device measures the change in transmittance of light that would indicates the presence of haemozoin and a microprocessor interprets the change in light and provides the result to the test operator.
Output	Read out will be a qualitative result for *Plasmodium* genus (e.g. pan-malaria), does not differentiate between species. Device also quantifies the haemozoin content, which based on preliminary laboratory studies is correlated to parasitemia levels. Future field trials will look at correlation between haemozoin and parasitemia levels, and the commercialized device may include an optional quantitative read-out.
Performance	Targeting > 90% sensitivity and specificity at 100 parasites/µl. Improvements to the LOD are theoretically possible; however, cost of final product will increase with use of more sophisticated technologies.
Turnaround time/ Capacity	1 minute per sample, one sample processed at a time.
Sample needed/ Stability	50 µl fingerprick blood. The sample is meant to be processed immediately, under laboratory conditions settling of the blood starts to occur after about 15 minutes.
Environmental requirements	The device uses standard electronic components and is designed to operate in tropical conditions. The device case will be hermetically sealed, with the exception of the sample space. The device uses a disposable sample cell and a lysing agent, neither of which requires cold chain storage.

Testing protocol	i) Collect fingerprick blood sample (50 µl); ii) transfer sample to disposable sample cell; iii) add lysing agent (50 µl); iv) insert sample cell into device; v) read result in 1 minute. Currently, fingerprick blood sample and lysing agent are pipetted into the sample cell, additional work and field studies will aim to simplify this process, perhaps by including lysing agent in disposable sample cell and collecting blood directly into sample cell.
Cost/Test	Targeting < $ 0.05 for disposable sample cell and lysing agent when mass-produced.
Cost/Instrument	Targeting < $ 500/device
Power requirements	The device uses a lithium iron cell battery capable of performing > 50 measurements with one charge; likely that commercialized device will perform > 100 measurements and will include a solar charger.
Training/Technical sophistication	Device intended to be used by low skilled health worker, major skill required is sample collection and transfer to device. Approximately ½ day training required to operate the device.
Durability/ Maintenance	Device is designed for rugged field conditions; expected to last > 2 years. A 'dummy' sample cell that will be used to calibrate the instrument periodically. Non-functioning devices will be exchanged.
Infrastructure requirements	Device is intended for use in the community as well as at all health facility levels.
Result display and storage	LED read out for results. GPS and mobile communications technology may be built in, enabling remote diagnosis and software updates.
QA/QC	The approach to quality / regulatory approvals is TBD. Blinded field trials are planned in collaboration with well-respected malaria laboratories. Dummy sample cells will be used to calibrate the instrument. In addition, self-checking routines are likely to be included in the operational software of the microprocessor.
Availability	2013–2014

SpectraWave and SpectraNet (Claro Scientific)

Platform Characteristics	
Type of technology	Claro's integrated diagnostic system combines two technologies: i) the SpectraWave instrument for whole blood sample preparation, multidimensional spectral (MDS) analysis, and transmission of the sample data file; and ii) SpectraNet a computer software and database system that analyses, interprets and stores the sample profile and delivers results. SpectraWave is designed to be portable. Although its final form will be refined based on results from 2011 form factor and market studies conducted in Africa, an early prototype fits in a case (approximately 18 x 10 x 6 inches).
Output	Parameters measured include: • Malaria: Detection of malaria; speciation of malaria parasite; identification of the life-cycle stage of the parasite; and quantification of infection (% of RBC) • Blood Count Parameters: Haemoglobin, hematocrit, mean corpuscular volume, mean corpuscular haemoglobin concentration, red blood cell count, white blood cell count, differentials, platelet count.
Performance	Malaria LOD is currently ~200 parasites per microliter of blood although Claro expects to achieve lower LOD through optimization of sample preparation and inclusion of data collected using additional optical methods. Blood count parameters perform within the acceptable ranges for automated systems currently on the market
Turnaround time/ Capacity	< 5 minutes/sample. Initial platform will analyse one to five samples at a time. Technology will be scaled into instruments capable of multiple sample analysis in future. Minimum capacity is 96 samples/day (assumes an 8 hour day, including sample preparation).
Sample needed/ Stability	100 µl blood sample from a fingerprick or venous puncture Sample is directly injected into SpectraWave and diluted in one ml saline. A sample from a fingerprick needs to be analysed immediately; venous sample from an EDTA tube must be analysed within 24 hours.
Environmental requirements	The platform does not require any reagents. Components are highly stable and designed to withstand wide variation in temperature and humidity. Optical systems have travelled on a Mars mission.
Testing protocol	System start-up will provide on-board diagnostic checks of both the sample preparation and the spectral acquisition hardware and software. Daily start-up takes < 3 minutes to complete. The testing protocol is: i) collection of fingerprick/venous blood sample; ii) load sample through the intake port on the system; iii) press the start button; iv) read results. All sample preparation and analysis will take place without further

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	operator intervention.
Cost/Test	The system has been developed to operate without reagents and consumables to manage the cost per test to below $ 0.50.
Cost/Instrument	$ 10,000–$ 15,000
Power requirements	Direct source 12V or rechargeable battery
Training/Technical sophistication	All testing can be performed by a lay person, with minimal training < ½ day.
Durability/ Maintenance	SpectraWave is designed to provide a long service life due to robust components and little to no moving parts. Based on current prototypes and component selection criteria, the system is expected to last more than 5 years. Claro will have a combination of a cross-shipping policy for units that are non-functional and on-site training for minor system issues and maintenance. SpectraWave will also have on-board internal system diagnostics that run upon start-up each day. This will identify and resolve any system issues that arise. In addition, these diagnostics will allow remote access to SpectraWave that Claro and end-users can use to resolve issues quickly.
Infrastructure requirements	The platform has been designed to be robust, fully-automated and easy to use and is therefore appropriate for use at all levels of the health system.
Result display and storage	Results will be displayed in a format relevant to the test being conducted e.g., positive or negative for infections, numerical counts for RBC, etc. Results will be shown on the SpectraWave screen, have the ability to be printed using an on-board printing system, or transferred electronically to another device (e.g. via USB drive, internet connection or laboratory information system). Optical profiles can be stored in SpectraNet for patient monitoring, epidemiological analysis and rapid development of new tests.
QA/QC	SpectraWave will have its own onboard QA/QC systems that will ensure proper operation and self-calibration. In addition these systems will keep an internal log file that will be used to preempt, identify, and resolve maintenance and system related issues. The following regulatory approvals will be sought: • Malaria/anaemia system (CE mark) • Whole blood analysis system (FDA and CE Mark) • Bacterial identification and resistance system (FDA and CE Mark)
Availability	System is at laboratory prototype stage. Optical bench can be deployed to other laboratories for capturing profiles from prepared samples in 2012. Integrated system is expected to be available for launch within three to four years depending on funding for manufacturing scale-up and regulatory approval.

Spectraphone (Quantaspec)

Platform Characteristics	
Type of technology	Portable device employing infrared spectroscopy to detect malaria and other pathogenic organisms in blood. Sample preparation consists of making a thin smear on a specially coated slide and staining the smear with Giemsa. The device analyses the sample's infrared spectrum and a computer-based algorithm is applied to identify the presence of *Plasmodia*. Although the technology has been developed using Giemsa stained slides, the developers aim to create a reagent-less system and have plans to validate the system on unstained and unfixed smears.
Output	Qualitative result for *Plasmodium,* differentiates between *P. falciparum* and *P. vivax* malaria. Drug resistance for chloroquine has been established, other drug resistance theoretically possible. Moving towards febrile illness diagnosis not just malaria diagnosis, differentiation of 14 bacterial species and 2 fungal species have been demonstrated.
Performance	To be determined for the SpectraPhone handheld device. In pre-clinical studies of the laboratory based system, The sensitivity was 98.8% to 100% (95%CI) for differentiating *Plasmodia* infected blood from Salmonella infected blood and uninfected blood with a specificity of 95.4% to 100% (95% CI). The sensitivity and specificity for detecting *P. falciparum* was 98.4% to 100% and 97.7% to 100% (95% CI). The sensitivity and specificity for *P. vivax* was 95.4% to 100% and 98.8% to 100% (95% CI). The sensitivity and specificity for detecting either chloroquine-susceptible strains and chloroquine-resistant strains was 97% to 100% and 98.7% to 100% (95% CI). The lower limit of detection is a single parasite.
Turnaround time/ Capacity	The goal is 1 minute/sample. One sample is processed at a time. Hundreds of samples can be processed in one day.
Sample needed/ Stability	The sample collected is a fingerprick blood sample (~10 µl) collected on a specially coated slide. The process is identical to preparing a slide for routine microscopy: the slide is stained with Giemsa and fixed in methanol. Although extensive studies have not yet been conducted on sample stability, experience suggests that the samples would be stable for several months at room temperature; furthermore the samples would not be as vulnerable to interference due to the unique spectra of infrared light associated with different organisms (e.g. the spectra of dust differs greatly from that of a parasite)
Environmental requirements	No cold chain requirements for the slides or Giemsa. The device should be used in a non-condensing environment.

Testing protocol	The testing process involves i) collection of a fingerprick blood sample on a specially coated slide; ii) staining and fixation of the slide; iii) insertion of slide into instrument for analysis; iv) readout of result.
Cost/Test	Estimated to be $ 0.12 per diagnosis (when manufactured at scale).
Cost/Instrument	Targeting $ 1,000 to $ 2,000 per device (when manufactured at scale).
Power requirements	The handheld system will be battery powered and rechargeable by solar cell.
Training/Technical sophistication	The major skill required to perform the test is collection of blood and preparation of a smear. The test is designed for use by a minimally skilled health worker with limited training (< ½ day).
Durability/ Maintenance	The device is expected to have a 10-year service life, and the manufacturer has a worldwide service network.
Infrastructure requirements	The device is intended to be used in the community and at all levels of the health system. If Giemsa staining is required, it would most likely be used at health facilities where a technician and small laboratory space is available.
Result display and storage	Results are displayed on a video screen. Test results can be stored or reported wirelessly to the national health ministry with time and date stamp, GPS location, and test result.
QA/QC	Regulatory strategy and market approval strategy still under development. Internal quality controls can be provided by manufacturer. External quality control is similar to that for visual microscopy.
Availability	2014

Appendix 2: Malaria Technologies in the Pipeline

Malaria Technologies in the Pipeline*
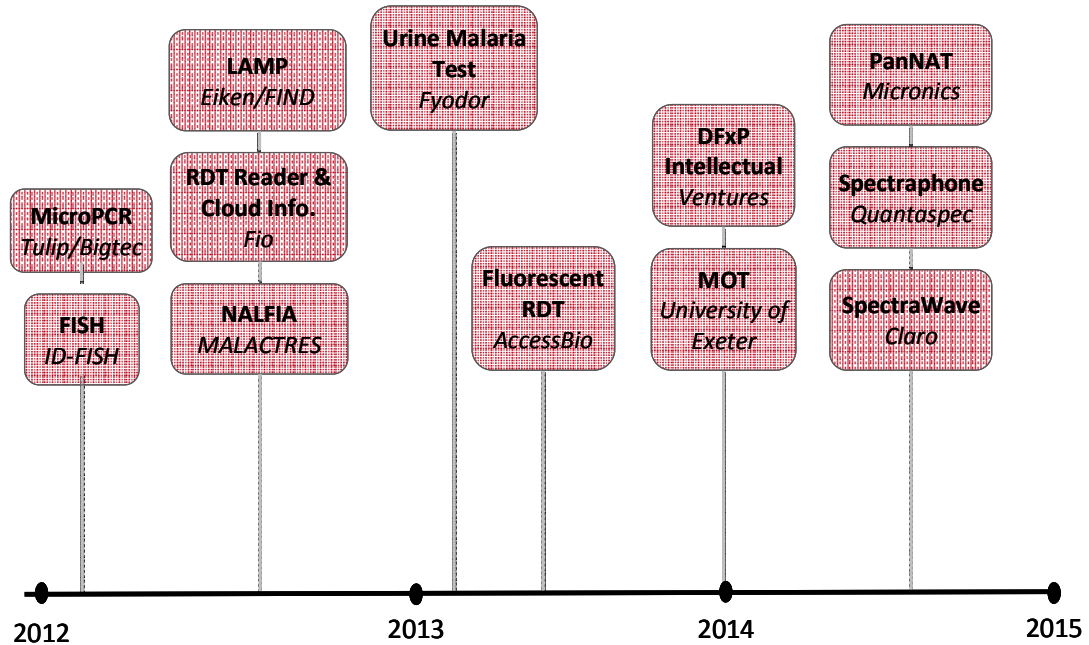
*Estimated. Timeline and sequence may change. Dates are based on estimated availability of product from manufacturers. Availability in individual countries may differ due to local distribution capacity, regulatory policies etc.

Appendix 3: Glossary of Terms and Acronyms

ACT	Artemisinin-based combination therapy
CE / CE marking	A mark placed on products in the European Economic Area that indicates the product conforms with requirements of EU directives. CE stands for Conformité Européenne (European Conformity).
Cross polarization microscopy	A contrast enhancing microscopy technique that employs two polarizers, one on either side of the specimen, to improves the quality of the image obtained with birefringent (or doubly refracting) materials.
Dark field microscopy	A contrast enhancing microscopy technique that brings out fine details in the sample using a special condenser to block out most of the bright light and illuminate the specimen with oblique light. This set up produces a field of bright luminescent objects against a dark background.
ELISA	Enzyme-linked immunoabsorbent assay. An immunoassay that uses specific antibodies to detect antigens or antibodies. The antibody containing complexes are visualized through enzymes that are attached to the antibody. Addition of substrate to the enzyme-antibody-antigen complex results in a coloured product.
FDA	Food and Drug Administration (USA)
FIND	Foundation for Innovative New Diagnostics
FISH	Fluorescent in-situ hybridization
G6PD	Glucose-6-phosphate dehydrogenase. Glucose-6-phosphate dehydrogenase (G6PD) is an enzyme in the human body that is essential for basic cellular functions.
HTD	Hospital for Tropical Diseases (UK)
Haemozoin	A malaria parasite produces haemozoin crystals as a byproduct of its metabolism of haemoglobin: After infecting a person, the parasites enter red blood cells and feed on haemoglobin, an iron-bearing molecule that plays a key role in supply of oxygen throughout the body. The parasite is unable to use the iron-containing part of haemoglobin, and sequesters it in the form of tiny crystals called haemozoin. The presence of haemozoin in a patient is a strong indication of malaria infection.
KIT	Koninklijk Instituut Voor de Tropen/Royal Tropical Institute (the Netherlands)
LAMP	Loop-mediated isothermal amplification
LLIN	Long-lasting insecticide-treated mosquito nets
MDT	Malaria diagnostic test
Microfluidics	Microfluidics involves the reduction of the volume of reagents and samples required to perform a test and the movement of these small amounts of fluids in miniature channels integrated into a device. In diagnostic assays, microfluidics cartridges are designed to move a small volume of the sample being analysed

through a series of channels and chambers. Among the advantages of microfluidics is a substantial reduction in the volume of sample and reagent required to perform an assay, often allowing for reduced time to result and lower overhead costs.

Molecular beacon fluorescent probe	Molecular beacons are hairpin-shaped probes that fluoresce when bound to a specific nucleic acid.
MOT	Magneto-optical technology
MAb	Monoclonal antibody. An antibody produced from a single clone of cells. It has a uniform structure and specificity.
NIH	National Institutes of Health (USA)
POC	Point of Care
PCR	Polymerase chain reaction. A laboratory method developed in the mid-1980's that allows for a particular segment of nucleic acid to be copied limitlessly. This copying (or amplification) makes it easier to detect minute quantities of nucleic acid in a sample.
QA/QC	Quality assurance / Quality control
QT-NASBA	Quantitative nucleic acid sequence-based amplification
RDT	Rapid diagnostic test
Thermal cycler	Laboratory instrument used to achieve the rapid changes of temperature required for PCR. The thermal cycler contains a thermal block with holes for tubes containing the samples. The thermal cycler is programmable to precisely control temperature increase/decrease steps, the length of time that a reaction is held at a particular temperature, and the number of cycles that are completed.
Turbidimeter	A laboratory instrument for measuring the loss in intensity of a light beam through a solution due to particle formation.
WHO	World Health Organization