

Tools for the Diagnosis of Malaria in the Era of Elimination with a Special Emphasis on Molecular Assays and Emerging Technology Platforms

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Abstract: Malaria in humans is mainly caused by infection with five *Plasmodium* species (*Plasmodium falciparum*, *P. vivax*, *P. ovale*, *P. malariae* and *P. knowlsi*). In 2015, an estimated 214 million new malaria cases and 584 000 deaths were recorded globally due to malaria. The ability to accurately detect malaria infection in all individuals and in all populations is fundamental to achieve elimination and eradication goals through effective treatment. *P. falciparum*, which can be fatal, must be identified promptly and differentiated from the other *Plasmodium* species that cause malaria. The gold standard microscopic detection and identification of *Plasmodium* in Giemsa-stained thick and thin blood smears and Rapid diagnostic immuno-chromatographic tests (RDT) remained routine malaria diagnosis tools in disease endemic areas. Although, in emergency situation, prompt and effective diagnostic methods using routine diagnostics are essential for the management and control of malaria, highly sensitive and accurate molecular techniques are needed in the era of elimination. Traditional methods for diagnosing malaria remain problematic; therefore, new technologies have been developed and introduced to overcome the limitations of traditional methods. This review details the currently available diagnostic methods and new technologies for malaria.

Key words: *Plasmodium* • Malaria • Diagnosis • Elimination

INTRODUCTION

Malaria has been a major public health problem, particularly in the tropical and subtropical parts of the world [1-3, 8]. Globally, an estimated 3.3 billion people in 97 countries and territories are at risk of being infected with malaria and developing disease [4]. Moreover, a 1.2 billion are at high risk and according to the latest estimates, 198 million cases of malaria occurred globally in 2013 and the disease led to 584 000 deaths representing a decrease in malaria case incidence and mortality rates of 30% and 47% since 2000, respectively [4]. An estimated 90% of all malaria deaths occur and in children aged less than 5 years, who account for over 75% of all deaths [4-7].

In malaria endemic areas, asymptomatic malaria parasite carriers especially adults are not uncommon and, as potential gametocyte carriers, represent an important

reservoir for malaria transmission [8, 9]. Asymptomatic infection is defined as the presence of malaria parasites in peripheral blood in absence of symptoms [10] and has been described to be prevalent in regions highly endemic for malaria [10, 11].

Generally, severe or complicated malaria has been a focus for epidemiological studies because it is the principal cause of malaria-related deaths. Researchers and clinicians have established diagnostic criteria based on the clinical manifestations upon disease onset, which has aided in forming an integrated approach to improving the management and treatment of severe malaria [12]. And of course in areas of high transmission, this full spectrum of clinical severity is primarily observed in children as severe malaria is negatively correlated with age due to the development of exposure-related immunity in adults [13, 14].

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Diagnosing asymptomatic malaria is not as straightforward due to the obvious lack of clinical manifestations and low parasitemia [15]. Asymptomatic malaria is prevalent in malaria endemic regions and has become a serious cause for concern as efforts are increasing towards eliminating the parasite [16]. Particularly, sub patent malaria is still transmissible and will complicate elimination of malaria in high transmission regions. For example, a study in Senegal suggested that more than 90% of exposed individuals are likely infected with chronic asymptomatic malaria [17], a situation in which the majority of this population can then inadvertently act as a reservoir for malaria transmission. In addition, asymptomatic cases provide a fundamental reservoir of parasites and they might become gametocyte carriers, contributing in the persistence of malaria transmission [18].

Therefore, the presence of asymptomatic cases is a big challenge for the management of the elimination programme in any malaria endemic area. In order to achieve a successful elimination, detection of all parasite carriers by active case detection and then treatment of all reservoirs must be considered to interrupt the malaria transmission in endemic areas. Today, due to implementation of large-scale effective control measures, many countries are experiencing dramatic declines in disease burden. With this success has come a shift in the end goal from control to elimination [19, 20]. When the goal is elimination, accurate detection of persons infected with malaria parasites becomes essential consideration [21]. Standard diagnosis systems depend on diagnosis by microscopy, a method that is technically challenging, labor-intensive and often inaccurate in operational settings. More recently available rapid diagnostic tests (RDTs) provide convenience and ease of use, but they have limitations in specificity, sensitivity, species identification and cost [22]. However, both techniques have limited sensitivity. Molecular detection techniques for malaria have a much higher sensitivity and are increasingly revealing the widespread presence of infections with parasite densities below the detection threshold of either microscopy or RDTs [23-25]. Moreover other diagnostic techniques such as Automated blood cell counters (ACC) and Mass spectrophotometry [26], Flow cytometry [27, 28], Microarray [29, 30], QBC [31-33] and Biosensors [34] has been proposed for the diagnosis of malaria in different settings and conditions. These results fundamentally challenge our current view of malaria epidemiology and burden of infection as well as if

combination of diagnostic technologies is needed to address particularly asymptomatic reservoirs for a success of elimination

Its significant concern for control and elimination programmes could be alleviated by early detection and monitoring of transmission using super sensitive diagnostic technologies. Hence, this work is aimed to review the sensitivity and detection potentials of different laboratory diagnostic technologies toward asymptomatic reservoirs for better monitoring of control strategies and elimination of the diseases at large.

Attributes of Existing Malaria Diagnostics

Microscopy: More than 100 years later from its discovery, microscopic detection and identification of *Plasmodium* species in Giemsa-stained thick blood films (for screening) and thin blood films (for species' confirmation) remains the gold standard for laboratory diagnosis of malaria. Owing to the fact that malaria is over diagnosed when relied on clinical diagnosis alone [35, 36], demonstration of the presence of malaria parasites under microscopy before selection and treatment with anti-malarial drugs is fundamental to reduce malaria morbidity and mortality [37, 38]. Over diagnosis malaria (diagnosis of malaria when it is in fact absent) [35], by clinical approach looks attributable to the high variability and overlap of malaria symptoms with that of a number of other common illnesses such as pneumonia, meningitis and sepsis [39-42]. Diagnosis of malaria by microscopy is labor intensive and technically challenging [43] however its advantages are relative accuracy, low costs and ability to quantify parasites and monitor clearance [44] among others. Microscopy using thick blood film can diagnose most symptomatic malaria it can also detect some asymptomatic cases depending on the level of parasitemia, number of microscopic fields examined and the skills of microscopist [45, 46]. However, the detection potential and sensitivity of microscopy is generally lower than PCR [47-50], importantly in non -endemic areas, low transmission settings, as well as in asymptomatic and sub patent malaria, microscopic sensitivity is even lower.

Rapid Diagnostic Tests (RDTs): Rapid diagnostic tests (RDTs) based on malaria parasite antigen detection are now a key tool in the case management of clinical malaria. Globally, the number of RDTs distributed has increased from fewer than 200 000 in 2005 to more than 160 million in 2013, almost by 99% [4]. RDTs are simpler to use, rapid (offer results within 15-20 minutes) and require no

Table 1: More on attributes of Microscopy in relation to clinical and subclinical/asymptomatic malaria

Attribute	Ref.
Sensitivity is (4-20 parasites/ μ l of blood) in Giemsa stained-Thick film	[50]
Possibility of Misdiagnosis and inappropriate to detect low grade infections. This probability is higher in Low parasitemia, low transmission settings and for asymptomatic malaria cases	[24, [47-49]
Mean number of parasites detectable = 50-100 parasites/ μ l with average microscopist. Underestimating malaria infection rates, especially cases with low parasitemia and asymptomatic malaria is highly probable	
Improve selective treatment of non-malarial fevers and reduce drug resistance. Applies for clinical malaria cases at health care facilities	
Microscopy alone Underestimate the true picture of plasmodia in community. This could affect malaria control and monitoring transmission difficult	
The risk of species mis- mach and under-reporting of mixed infections is high. This greatly affects our understanding of malaria epidemiology and subsequent control and elimination	
Requires skilled microscopist, high quality reagents, labor intensive and time consuming especially if low parasitemia has to be diagnosed	
Today only considered as traditional gold standard due to a questionable reliability at low level of parasitemia	
Indeed, a cost effective allows species identification and quantification and neither complex sample preparation nor advanced technology is required	

electricity, expensive equipments or extensive guidance. Therefore, they are highly suited to the detection of malaria in resource limited remote areas, especially at lower level peripheral health facilities where routine microscopy is absent or of poor quality.

Moreover, the use of RDTs in large-scale surveys is preferable for therapeutic reasons because they provide point-of-contact diagnosis and, if required, immediate treatment. Today they are being utilized for the surveillance of malaria (Both asymptomatic and symptomatic) at school settings as well as in community followed by immediate treatment with Arthemisin based Combination Therapy (ACT). The recent doubling in WHO African region of malaria testing service is mainly due to increases in a number of RDTs [4] and indeed RDTs had contributed a lot to an overall reduction in malaria burden worldwide, particularly in Africa where facility for microscopy and PCR procedures are limited. Despite, this tremendous contribution in achieving testing of all symptomatic individuals for prompt treatment and significant reduction in malaria morbidity and mortality, RDTs has got a number of short fallings when the goal is towards monitoring transmission and elimination. In malaria elimination settings it is critical to detect all infections, including those with low and sub-microscopic carriers as they constitute reservoirs in the community for momentous proportion of mosquito infection and are responsible for an ongoing transmission.

If the question is to address such silent, low grade parasitemia, infections in low transmission areas as well as in asymptomatic individuals, a test with super sensitivity shall come in to play. Atkinson et al in Solomon Islands had highlighted difficulties in detecting people who carried a low density of parasites and were mostly

asymptomatic using available diagnostic tools of microscopy and RDTs, similar report was made in Temotu, Solomon Islands [24].

It has been also reported that the proportion of low density (<200 parasites/ μ l) infections in symptomatic persons is higher in low-transmission than in high transmission areas and also higher in *Plasmodium vivax* than in *P. falciparum* infections suggesting that a significant number of symptomatic cases could be left undetected using RDT or Microscopy in low transmission settings. Such cases called sub-microscopic infections could represent both asymptomatic carriers and symptomatic individuals with low parasite density.

Sub-microscopic infections are more important contributors to transmission in areas with low or very low transmission intensity (under ~0.5%) than to sustain transmission in areas of high transmission intensity. For instance Okell and her colleagues [23] reported that sub-microscopic infections are important in sustaining transmission in areas where slide prevalence is low (<10 – 20%). More importantly, sub-microscopic carriers will become increasingly important as current control programmes continue to successfully reduce transmission intensity . I.e As malaria transmission declines and countries progress towards malaria elimination the need to detect sub-microscopic infections is becoming increasingly important, since low-density infections among symptomatic and asymptomatic persons is likely to increase, which may limit the utility of RDTs.

Many studies had also reported that, the sensitivity of RDTs depends on parasite species, while effective malaria control and eliminations programs calls for careful species identification, however RDTs does not allow species identification except for *P. falciparum* and could

Table 2: Some molecular assays and their plasmodium detection limits

Assay method	Target gene	Sensitivity (%) or Detection limit(P/μL), or infolds	Reference
Conventional PCR	mitochondrial DNA	0.5/ μL	
qPCR	18S rRNA, cox1, cytb	0.02-3	
Nested PCR	18S rRNA	97*	
	18S rRNA, dhfr-ts, 28S rRNA, stevor	0.1-10/ μL	
qReal Time PCR	P.f high-copy telomere associated repetitive element 2 (TARE-2) and the var gene acidic terminal sequence (varATS, 59 copies/genome)	0.03 to 0.15 parasites/μl blood and were 10× more sensitive than standard 18S rRNA qPCR	
	18S rRNA Yes	0.002-0.02	
qNASBA	18S rRNA	0.02 P/ μL*	
		50fold sensitive*	
restriction fragment length polymorphism analysis (RFLP) and denaturing high performance liquid chromatography RFLP-dHPLC	cytochrome b genes	Two-three fold higher than microscopy	

*Compared to microscopy, P/ μL = Number of parasite per microlitre of sample

not allow quantification of parasite density. It is relatively insensitive to *P. vivax* than *P. falciparum*. RDT methods also reveal a big variation in performance up to 20% to 99%. Moreover, to select most reliable RDT for malaria, quality of the product should be assessed prior to testing in real life as quality of RDT could determine its sensitivity and specificity. RDT products are assessed against samples of known malaria parasite species and density, with each product assigned a panel detection score that is based on the sensitivity and reliability of the results.

A quite clear variation in sensitivity among different products of RDTs is also evident. The results from Central African Republic (CAR) support this where it was confirmed that the RDTs perform well at higher parasite concentrations (> 500 parasites/μl) than at low parasitemia suggesting that RDTs may not be suitable for the detection of low parasitemia for elimination purposes.

Molecular Assays: Polymerase chain reaction (PCR) is a DNA-based molecular technique used for the detection of plasmodia. It is more sensitive than microscopy and RDT and has been widely used for diagnosis, confirmation of diagnosis, epidemiology studies and drug efficacy assessment. In theory, PCR is capable of detecting a single parasite in a blood sample and its sensitivity often is only limited by the volume of the blood. PCR provides accurate determination of parasite species, better sensitivity in detecting low density of parasites and better detection of mixed species/strain infections. The introduction of PCR based procedures has highlighted the low sensitivity of microscopy for parasitemia level of <50/ μL. PCR has gained superiority in its detection potential of malaria parasites even in low transmission settings. In a low transmission settings of Temotu

Province, Solomon Islands [24] a significant proportion (40% of *P. f* and 65.6% of *P. v*) infections were identified to have parasite density below 100/μL by Microscopy. And PCR detected considerably more infections (3.3 fold higher) than microscopy suggesting a large number of subjects had sub-microscopic parasitemia. On top of its higher detection potential, PCR methods more readily detect mixed infections.

In Thailand for, instance between 1/3 and half of mixed infections were only detected by PCR than microscopy. According to a study conducted on asymptomatic refugees from endemic areas to Canada, it was shown that all refugees were negative by microscopy and 3.1% among them were PCR positive. This suggests the usefulness of PCR in the diagnosis of asymptomatic malaria to help achieve control and eradication efforts towards the disease. PCR methods are also useful for detection and identification of minority plasmodium species such as *P. malariae* and *P. ovalae*, which are often underestimated by microscopy. More on attributes of PCR procedures with a focus on their role for malaria elimination and monitoring genetic diversity reviewed in. Molecular detection systems have improved the detection potential and species identification as finest species-specific primers or probes became available.

Modifications of the conventional PCR has made the precise quantification of parasites possible and technologies like multiplex PCR allowed simultaneous detection of multiple gene targets using multiple primers at a time in a single PCR experiment. With a highly sensitive assays it is now possible to detect plasmodia in blood sample 1-4 days earlier than by microscopy using peripheral blood smear although not all molecular techniques are equally sensitive or far sensitive than

microscopy. It is also important to note that the parasite density may be imperfectly correlated with severity of illness; it is only in some settings that people with more parasites would have more severe outcomes. At a given level of parasitemia the degree of illness can tremendously vary among individuals because of as such factors including age, genetics and immunity and setting (endemicity) level of the diseases. Hence persons with RDT and or Microscopy positive are more likely to have clinical illness where as many individuals with low level infections or low density parasitemia only detected by molecular approaches may remain asymptomatic. Rather asymptomatic persons (which are only diagnosed by molecular assays) represent a significant population for elimination efforts as they may keep on transmitting the parasites to mosquitoes. Many studies had produced a comparative data evaluating Molecular based assays with but not limited to Microscopy and RDTs. Moreover many researchers has inclined towards the relevance of molecular assays and a need to develop new tools to address asymptomatic malaria and gametocyte carriage owing to the fact that the world is now towards a move to eradication of malaria. As part of monitoring transmission interruption is has also become important to detect stage specific DNA such as gametocytes. The importance of low gametocyte carriage has become well understood following the introduction of molecular tools that are able to detect, quantify and characterize gametocyte genetics. Many Molecular detection tools that amplify gametocyte RNA have been developed. Reverse transcriptase PCR (RT-PCR), quantitative nucleic acid sequence- based amplification (QT-NASBA) and Real time loop-mediated isothermal amplification (RT-LAMP) has been used targeting DNA encoding gametocyte-specific RNA transcripts such as *pfs25* mRNA. Such molecular methods targeting gametocyte genes perhaps have a sensitivity of as low as 0.002/microlitre or 2 gametocytes/ ml.

Molecular approaches such as PCR are very good at detecting mixed infections and *P.falciparum* infections than Microscopy. Parasite prevalence determined by microscopy and estimated by PCR [24] in one comparative study done on pfHRP2 and PCR for the detection of plasmodia in a low prevalence setting in rural Zambia showed RDT's failure to detect low density *P.falciparum* suggesting a need for a more sensitive method to identify parasite reservoirs and achieve malaria elimination.

Loop-Mediated Isothermal Amplification (LAMP): Loop-mediated isothermal amplification (LAMP) of DNA is relatively recent and a novel molecular technology

platform. The LAMP technology amplifies previously determined genes and can be used to detect any pathogen. LAMP may be considered as an alternative to polymerase chain reaction (PCR) for the detection of nucleic-acid sequences (DNA and/or RNA). Both methods amplify and detect DNA, but unlike traditional PCR, LAMP does not require a thermocycler or gel imaging system. Results can be recorded by real-time turbidimetry or through visual detection of fluorescence. Amplification and detection of the target nucleic-acid sequence is essentially completed in a single step, by incubating the mixture of sample, primers, DNA polymerase with strand displacement activity and substrates at a constant temperature. LAMP provides high efficiency, with DNA being amplified 10⁹-10¹⁰ times in 15 - 60 minutes. Therefore LAMP can provide a result faster than traditional PCR and can be performed in basic laboratories without the need for specialized infrastructure [109]. One of the LAMP prototypes is non-instrumented LAMP (NINA-LAMP) where the NINA H.V6 prototype heater is used to produce isothermal conditions suitable for LAMP procedure. An exothermic chemical reaction coupled with a phase change material (PCM) provides temperature control to the NINA device [123].



Fig. 1: Photograph and cross sectional view of the NINA H.V6 prototype heater device. The reusable-housing platform is designed to heat micro PCR tubes using a commercial thermos with manufactured inserts. Five sample wells are surrounded by the phase change material (PCM) chamber and the exothermic reaction takes place below the PCM at the bottom of the stainless-steel insulated thermos. The PCM is used to buffer the exothermic reaction and provide a constant temperature to the sample wells. Disposable magnesium iron alloy (MgFe) and saline cartridges can be simply dropped into the housing to activate the device

The diagnostic performance of LAMP and its relevance to malaria elimination programs has been studied by many researchers in different settings. In Uganda Hopkins and colleagues and Mohon have both shown a 0.1-10 parasite per microliters detection limit using LAMP. In both cases they targeted 18S rRNA, mitochondrial DNA for amplification. In zanzibar comparison was made between PCR, RDTs and Microscopy. Among asymptomatic low density parasite carriers and found a promising result suggesting LAMP for point of care testing (POC) in field setting and its suitability to Mass screening and treatment (MSAT). LAMP was found to detect a significant number of individuals who were diagnosed negative with RDT and Microscopy. Moreover Loop-mediated isothermal amplification (LAMP) offers a field-friendly alternative to PCR and is less time consuming than PCR and can be performed using heat-blocks, with results read by eye under UV light. It has been successfully developed to detect malaria in a field-stable format. A study in Northern Thailand has also demonstrated a 100% sensitivity and specificity of LAMP for *P.falciparum* in comparison to nested PCR (nPCR). The specificity of LAMP for *P.vivax* was 96% in this study. However, microscopy had only 65% sensitivity and 98% specificity in relation to nPCR suggesting LAMP was as reliable as nPCR but far more reliable than microscopy in the detection of plasmodium DNA in Thai samples. Despite a need for further optimization, LAMP makes use of molecular diagnosis of plasmodium in technical resource limited settings. As it constitutes a useful technology combining a high sensitivity and applicability in resource limited areas as well as its robust use of DNA polymerase with low sensitivity to inhibitors and set of three primers that are able to produce a high level of DNA products within short time enables the method more suited to field settings and mass screening for elimination purposes. LAMP has a high negative and positive predictive values comparable to nPCR. It also has similar sensitivity and specificity with nested PCR. More importantly and as an additional quality LAMP is simpler, faster with ability to detect low parasitemia and thus becoming a potential point of care test, which is valuable surveillance tool for guiding elimination efforts. Moreover, Loop mediated isothermal DNA amplification (LAMP) is highly sensitive and specific, faster than PCR, requires minimal processing and instrumentation and allows result detection with the naked eye. Thus LAMP has thus a potential application as tool for population screening in malaria elimination campaigns. Recent finding in Ethiopia shows that, LAMP is highly sensitive tool for the detection of plasmodium at genus and species level as

well as for the differentiation of non-falciparum malaria. It could be a critical diagnostic modality for eradication efforts in low endemic settings compounded to the fact that the results of LAMP are easy to read and interpret compared to microscopy, thermo stability of the lyophilized reagents and minimal or no extensive training required to perform the test.

Biosensors: To date, a different type and quality biosensor devices has been developed for the detection of plasmodium biomarkers, including those of antigen markers such as HRP II and pLDH which were previously detected by RDT-ICT technology. The biosensor technologies employ the principle of electrochemical impedance spectroscopy and antigen-antibody interactions followed by immunosensor based detections. The detection limits and sensitivities dependent on the type of sensor used. Biosensors based on electrochemical impedance spectroscopy (Fig. 2) such as aptasensors developed by Lee and colleagues targeting pLDH had detection limit of 108.5fM for *P. vivax* LDH and 120.1fM for *P. falciparum* LDH.

By making use of antibody raised against HRP II of *P.falciparum*, electrochemical immunosensors such as disposable amperometric HRP II sensors have also been developed. Immunosensors such as constructed by modifying disposable screen printed electrodes with multiwall carbon nanotube (MWCNTs) and Au nano particles has a highest level of immunosensing potential with a detection limit of 8ng/mL. In one study the sensitivity of such a method was reported to be 96% compared to RDT Parachek pf which showed a sensitivity of 79% using Microscopy as a comparator. Immunosensors based on magnetic micro and nano particles are also developed towards HRPII and interestingly magnetic nano particles after being coupled with electrochemical magneto- immunosensors yielded better detection limit up to 0.36 ng/mL that individual technologies. Another class of biosensors are optical biosensors based on immunocapture spectrophotometric assays. A pLDH assay (Fig 3) had shown a detection limit of 50parasites/ μ L.

Piezoelectric Biosensors, another type immunosensors based on measuring HRPII quantitatively in a small volume of sample (fig 4) have also been described. Biochemical biosensors are however more prioritized and a focus of studies owing to the fact that they are portable and require small sample volume as opposed to PCR platforms. Moreover, common advantages of biosensors compared to RDTs lies on their reusability, quantitation and stability for operational matters.

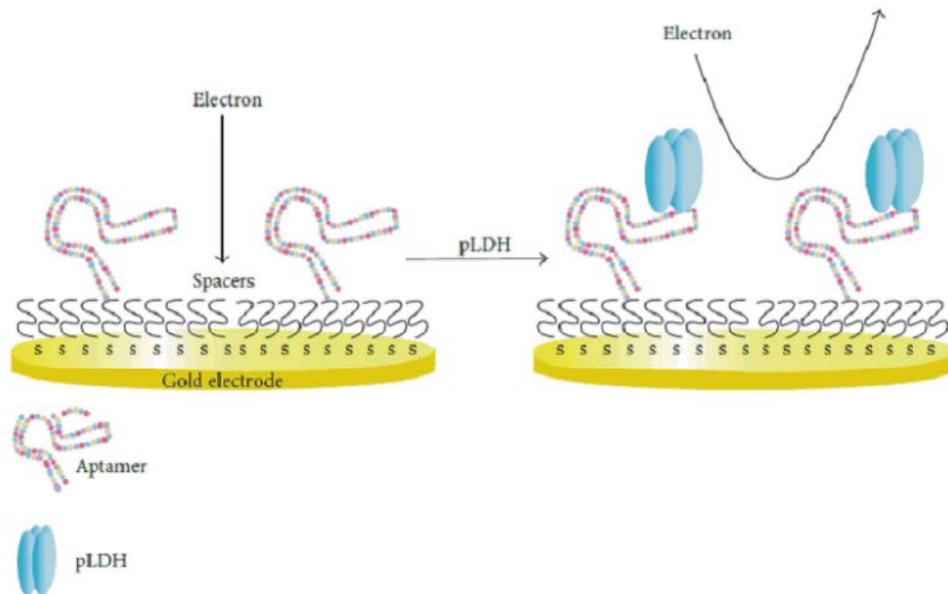


Fig. 2: Electrochemical impedance spectroscopy for the detection of pLDH. The aptamer is shown as a chain of different colored circles, representing the four bases A, T, G and C. Capture of pLDH by aptamer results in a decrease in electron transfer to electrode. The pLDH aptasensor can distinguish between malaria *P. vivax* and *P. falciparum* and has a detection limit of 1 pM [adapted from Ref.124]

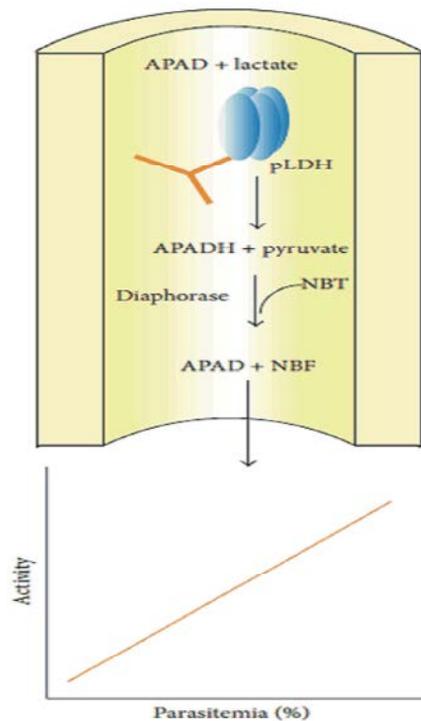


Fig. 3: Plasmodium lactate dehydrogenase (pLDH) immunocapture assay. pLDH is immobilized using a monoclonal antibody. The enzyme activity measured using a coupled enzyme assay that generates APADH. APADH reduces nitro blue tetrazolium, a chromogenic substrate, using an enzyme diaphorase. The activity is quantified spectrophotometrically at 650nm and plotted as a function of percentage parasitemia. It has a detection limit of 50parasites/ μ L

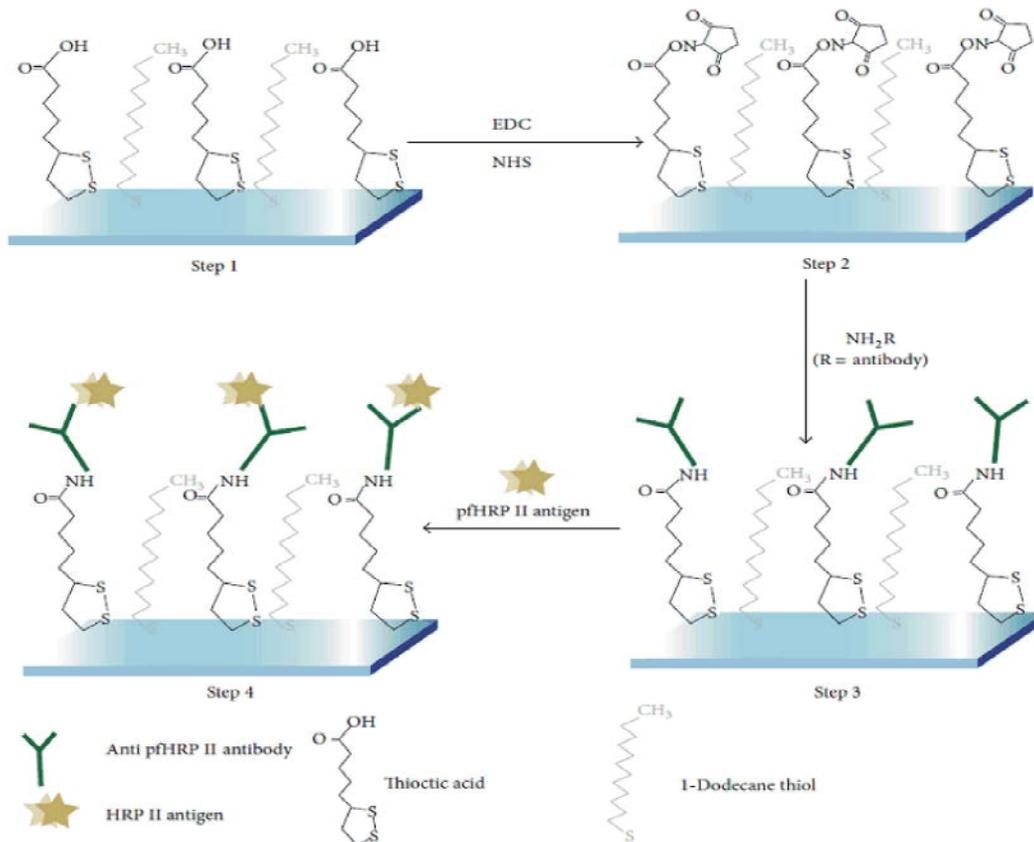


Fig. 4: The diagram shows preparation of piezoelectric immunosensor for HRP II. The mixed self-assembled monolayers (SAMs) of thioctic acid and 1-dodecanethiol were formed on gold surface of quartz crystal. The rabbit anti-PfHRP II antibodies were coupled on mixed SAM modified gold surface of quartz crystal via NHS/EDC activation method. The amount of HRP II molecules bound on the sensitive area of the electrodes is quantitatively measured as a decrease in resonant frequency.

Advanced Microscopes: As traditional microscopy relies mainly on the knowledge, technical skills and judgment of the readers, there occur a considerable discrepant results between inter and intra-reader in parasite counts. Moreover in elimination settings errors with microscopy are likely as it is difficult to maintain proficiency of microscopist due to low level parasitemia. However it is critical to detect all infections including sub clinical infections to achieve elimination in such settings which are characterized by low parasite density in infected persons.

Having identified the pitfalls of traditional microscopy efforts has been made to improve the performance and objectivity of reading by making use of automation to aid read the slides. Automations for slide preparation and staining could thus benefit in standardizing the preparation of blood slides and increase the reliability and quality of blood slides. Other

technology solutions include automated image capture and reading based on using mobile phones to capture microscopy images and transfer them to a central database for assessment and computer-based algorithms to analyze images captured from microscopic examination of stained thick or thin blood smears used to identify and quantify malaria parasites.

Cell phone microscopy such as CellScopes have been developed that enable mobile phone-mounted light microscope to image *P. falciparum* infected RBCs. Fluorescent microscopy such as CyScope based on certain fluorescent dyes' differential affinity for the plasmodium DNA enhance the visual detection of parasites due to a strong fluorescence when excited by UV light at an appropriate wavelength. Compared to conventional light microscopy, CyScope uses only a small volume of blood (5 μ l), is less labor-intensive and faster to use, therefore having a better turn-around time.

Table 3: Comparisons among Conventional and advanced microscopic technology platforms

Method's attributes	Light microscopy	QBC	SyScope	Flow cytometry
Sensitivity and specificity	Highly dependent on microscopist	Higher than traditional microscopy	Wide ranged	Variable
Limit of detection	5-10/μL for experts and >100 for average microscopist	≤ 15/μL	400 parasites/μL	≤ 1/sample
Quantification	Yes	No	Yes	No
POC suitable?	Yes	Yes	Yes	No
Sample volume for a test	10 μL	50-65 μL	5μL	NA
Throughput	1 Slide	Up 20 batch centrifugation, although tubes read individually	1 slide per test	1 per test
Time-to-result	30-60 Minutes per each slide	8-15 Minutes	5 minutes per test	Poor correlation with parasitemia
Species identification	Yes	No or limited	No or limited	No
Targets	Whole parasite	DNA	DNA	Hemozoin
Need electricity	Yes	Yes	Yes but has rechargeable battery	Yes
Cost per test	Low(0.12-0.4 USD)	Higher(2.5USD)	0.5USD	High
Technical demand	High	Minimal training/easy	Minimal	High

POC: Point of Care, QBC: Quantitative Buffy Coat

It also requires less training and expertise. Because it can operate on batteries, it is ideal for fieldwork and areas with no electricity. On the other hand, the Cyscope is not suited for species differentiation. Moreover, CyScope makes use of glass slides already coated with lypholized flouresence dye. Hence does not require special reagent preparations and storage conditions.

A Quantitative buffy coat (QBC) method is another extension of fluorescent microscopy which uses acridine orange (AO) flourochrome coated on capillary tube to stain plasmodium DNA. When combined with centrifugation of blood sample it simplifies detection, as the parasites concentrate into specific, easy-to-locate layers in the tube. Also, the test concentrates a relatively large volume of blood (55-65μl), thus providing benefits in cases of low parasite density. The preparation and evaluation of the test takes about eight minutes, which is far less than the time it takes to prepare and read a slide for conventional microscopy. IT is an important technology platform to improve the inherent challenges for conventional microscopy in the era of pre-elimination and elimination of malaria. However, QBC does not provide quantitative information and also it is not suited for species identification. The overall purpose of these improvements is in fact to enhance visual detection of malaria parasites from blood smears to advance parasite detection in settings of low transmission. Interestingly, one cannot be an absolute substitute of another as each technology has got its own advantages and disadvantages as well as differential candidacy for applicability in pre-elimination and elimination settings to detect low parasitemia including asymptomatic reservoir persons (Table 3).

CONCLUSION

Although, conventional microscopic examination of peripheral thick and thin blood smears remains the gold standard for malaria diagnosis it requires a trained microscopist and its sensitivity and specificity depend highly on technical expertise. However it is relatively inexpensive compared with recent technical advances. RDTs are simple to perform and are convenient for large number of sample and applicable to many remote settings where electricity, technical expertise and other resources are limited. However, they are relatively costly, not suitable for diagnosis of early malaria and need continuous quality check and are not as specific as microscopy. PCR based approaches are highly convenient, specific and sensitive, though these parameters greatly vary according to the molecular-biological technical modifications. They are particularly suited for the detection of low density parasitemias and diagnosis of asymptomatic malaria in this era of elimination. Even though, these methods are often meant for research laboratories; they can be developed in a field friendly and time saving formats. The developments field applicable, high throughput PCR methods for point of care services are urgently needed to address malaria elimination. biosensors have emerged as a reliable diagnostic technique for malaria due to many inherent advantages set by their tenet including reusability, quantitative nature, enhanced signals at low sample volume and better storage and operational stability compared to RDTs. Advanced microscopes and cell phone based visualization method are also under continuous improvements for the diagnosis of malaria. Indeed there is no a perfect technology platform that

works in all circumstances and therefore, choice of malaria diagnostic method depends on the goal of malaria control program, the level of malaria endemicity, the urgency of diagnosis and availability of resources among other factors.

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