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Chapter

Point-of-Care Strategies Applied to Malaria Diagnosis

Alexandre Dias Tavares Costa, Anna Caroline Campos Aguiar, Angelina Moraes Silva and Dhelio Batista Pereira

Abstract

Rapid and specific diagnosis of malaria remains one of the main strategies to fight the disease. The diagnosis is made primarily by the simple and low-cost thick drop technique, considered the gold standard test. However, the requirement for good quality microscopes and well-trained personnel often lead to inaccurate diagnosis, especially in cases of mixed infections or low parasitemia. Although PCR-based tests can help in these situations, this technique requires large and sensitive equipments, being unsuitable for point of care (POC) settings. A myriad of POC diagnostic tests have being developed in the last years, relying on molecular methods but also on novel strategies. New platforms, miniaturization techniques, and multiplexing possibilities promise great potential to improve disease diagnostics through fast and accurate detection of cases, even at remote places. Here, we will address the main POC strategies developed for the diagnosis of malaria, highlighting their strengths and weakness as POC applications.

Keywords: point-of-care, diagnosis, malaria

1. Introduction

Malaria is one of the deadliest diseases of poverty. It is estimated that malaria causes 228 million illnesses and 405 thousands deaths each year. Among the sick, children aged under 5 years are the most vulnerable group affected by malaria; in 2018, they accounted for 67% (272 000) of all malaria deaths worldwide [1].

In many countries where malaria is endemic, a lack of access to adequate diagnostic services leads to poor health outcomes for fever patients, as well as poor surveillance of infections and outbreaks, and treatment monitoring [2].

To make matters worse, the appearance of antimalarial resistant parasites including artemisinin derivatives pose a major public health threat [3]. In addition, drugs such as the artemisinin-derivatives are more expensive, leading to an increased demand for patient evaluation by accurate diagnostic tests before treatment [4–6].

Therefore, it has grown in the last years a general agreement that new diagnostic tests are needed for remote areas in malaria-endemic countries. However, the new tests must show improved performance over existing techniques, so that adequate distribution of anti-malarial drugs can effectively target the disease and its outbreaks, contributing to the reduction of generation of drug-resistant parasite strains [7].

In malaria-endemic countries, the major hurdle for widespread access to malaria diagnostics is the limited health care infrastructure [8, 9]. According to the World Health Organization (WHO) guidelines, the useful diagnostic tool at the point of care (POC) is defined by some characteristics. It should be low cost, deliver sensitive and accurate results in as little time as possible, run on a portable instrument (ideally, should be instrument-free), require minimal external power, require minimal training before use, and not require refrigerated reagent storage and transportation. These guidelines are collectively known by the acronym ASSURED (affordable, sensitive, specific, user-friendly, rapid and robust, equipment-free, and deliverable to end-users) [10]. In addition to those requirements, an ideal POC malaria diagnostic device should determine which species is infecting the patient, to establish the level of parasitemia, and be able to detect mixed or low-level infections.

Current POC tests for malaria include the smear microscopy and immunochromatographic rapid tests (RDTs). However, more sensitive and specific techniques based on nucleic acid amplification tests (NAAT) have been praised as the best choice for a successful malaria POC diagnostic test. In this work, we will review the status of the diagnostic technologies that have been used for malaria detection at POC conditions, discussing their main advantages and disadvantages in the POC context.

2. Currently available POC tests

2.1 Smear microscopy

Microscopy remains the gold standard for malaria diagnosis in most endemic areas. This technique allows the identification of different malaria-causing parasites (*P. falciparum, P. vivax*, *P. malariae* and *P. ovale*), their various parasite stages, including gametocytes, and the quantification of parasite density to monitor response to treatment [11].

There are two variations of the microscopy technique, the thick drop and the blood smear, both use the Giemsa dye in their preparations and are performed with sample of peripheral blood. Thick droop is made by placing a few drops of blood on a glass slide, allowing the blood to dry, and then lysing the blood (usually with water) before staining. The blood smear is made from a thin layer of cells and are fixed with methanol before reading. The thick drop allows the identification of lower parasitemias, by concentrating the parasites. The blood smear technique is more sensitive in speciating the parasites, however it does not allow the identification of low parasitemias [12].

This technique has the great advantage of being cheap (costs approximately $ 0.20 per sample), fast (approximately 1 hour between collection and the result, if performed by a skilled laboratory technicians), and does not need sophisticated equipment. The number of patients tested by microscopic examination increased an increase of 165 million tests in 2010 [13] to more than 208 million testes in 2017[1]. The global total is dominated by India. The sensitivity of the optical microscopy technique using the thick drop method is50–500 parasites/μL, however, many factors may interfere with the results found in the thick drop technique, such as the quality of the microscope, the quality of the available staining reagents, and the skill of the technician. Several studies have shown that the sensitivity of the

[1] https://www.who.int/malaria/areas/diagnosis/microscopy/en/, accessed on February, 17th, 2021.

microscopy technique may be lower in several areas of transmission depending on the quality of the examination and the expertise of the microscopists, which can increase the number of false negative results [14, 15].

2.2 Rapid diagnostic tests (RDTs)

Rapid diagnostic test (RDT) is a quick diagnostic approach to detect malaria among malaria-suspected patients and rule out malaria among individuals without malaria. RDTs detect parasite-specific antigens in a drop of fresh blood through lateral flow immunochromatography using antibodies to detect one or several antigens [16] (**Figure 1**).

The RDTs detect a single species (either *P. falciparum* or *P. vivax*), some detect multiple species (*P. falciparum*, *P. vivax*, *P. malariae* and *P. ovale*) and some further distinguish between *P. falciparum* and non-*P. falciparum* infection, or between specific species. The most commonly used antibodies react to histidine-rich protein-2 (HRP2), aldolase and *Plasmodium* lactate dehydrogenase (pLDH). HRP-2 is a marker for *P. falciparum*, while pLDH antibodies can be specific for *P. falciparum*, or *P. vivax*. Aldolase antibodies are pan-specific, detecting all types of malaria parasite but not differentiating between them [17].

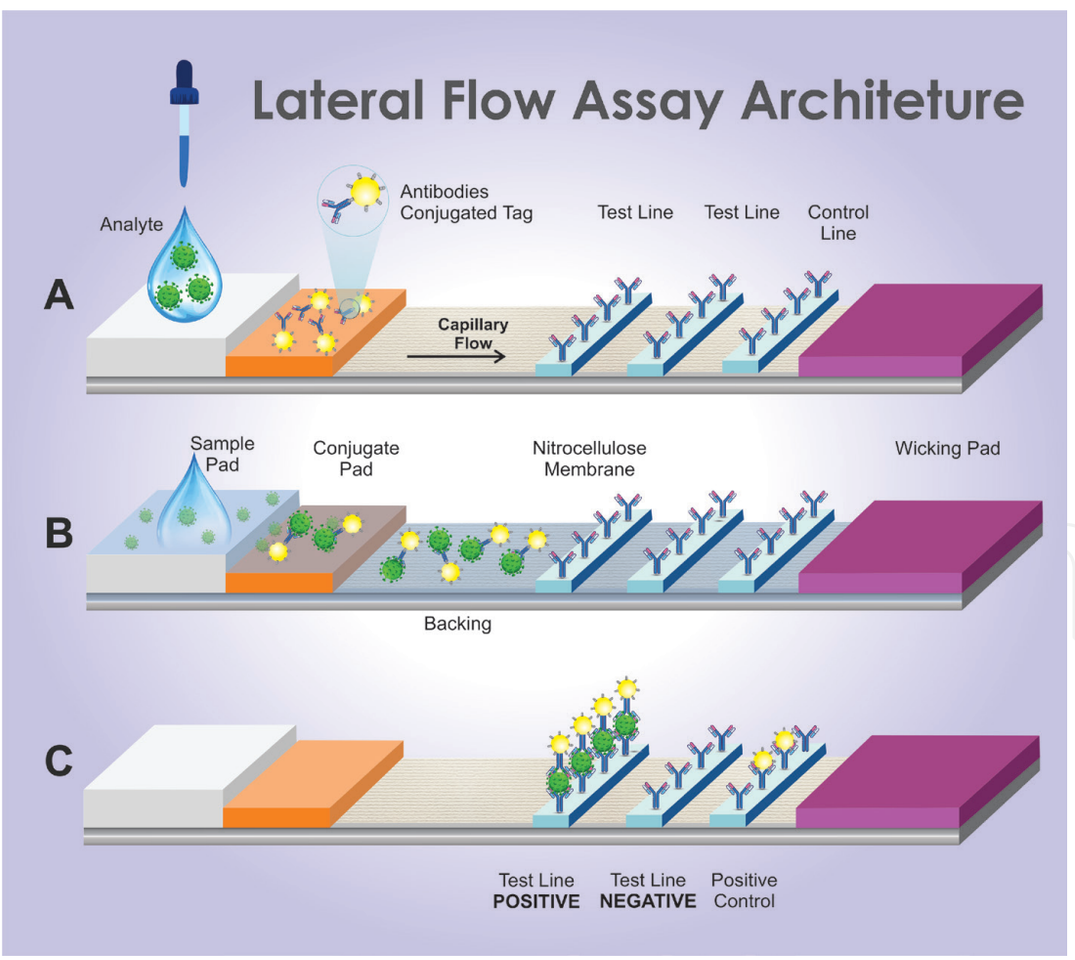

Figure 1.
Lateral flow assay architecture. Samples containing the analyte flow through the nitrocellulose membrane by capillary flow (panel A), carrying reporter antibodies (labeled with gold, latex or a fluorophore) until the mixture interacts with the test line (containing antibodies that bind the analyte of interest) and the control line (containing anti-IgG antibodies that bind to human IgG molecules) (panel B). If the control line shows a positive reaction, it is a valid test. If the test line shows a positive reaction, it is a positive sample for the specific analyte (panel C).

Product name	Manufacturer	Panel detection score				False positive rates (%)				Meets WHO performance criteria?
		200 parasites/μL		2000 parasites/μL		200 parasites/μL		2000 parasites/μL		
		Pf	Pv	Pf	Pv	Pf	Pv	Pf	Pv	
						non-Pf infection	Pf-infection	non-Pf infection	Pf-infection	
Paracheck Pf - Rapid Test for *P. falciparum* Malaria Device (Ver. 3)	Orchid Biomedical Systems – Tulip Diagnostics (P) Ltd	94.0	NA	100.0	NA	NA	1.4	NA	4.3	Yes
One Step test for Malaria Pf/Pan Ag MERISCREEN Malaria Pf/Pan Ag	Meril Diagnostics Pvt. Ltd	73.0	NA	99.0	NA	NA	0.7	NA	0.0	No
Parascreen - Rapid test for Malaria Pan/Pf	Zephyr Biomedicals – Tulip Diagnostics (P) Ltd	91.0	94.3	100.0	97.1	0.0	0.7	0.0	1.4	Yes
FalciVax - Rapid test for Malaria Pv/Pf	Zephyr Biomedicals – Tulip Diagnostics (P) Ltd.	95.0	100.0	100.0	100.0	0.8	0.0	0.0	0.0	Yes
STANDARD Q Malaria Pf/Pv Ag Tes	SD Biosensor, Inc	85.0	100.0	99.0	100.0	0.5	0.0	0.5	0.0	Yes
STANDARD Q Malaria Pf Pan Ag Test	SD Biosensor, Inc.	88.0	100.0	99.0	100.0	0.0	0.0	0.5	0.0	Yes
STANDARD Q Malaria Pf Ag Test	SD Biosensor, Inc.	87.0	NA	99.0	NA	NA	0.0	NA	0.0	Yes
First Response Malaria Antigen *P. falciparum* (HRP2) Card Test	Premier Medical Corporation Limited	95.0	NA	100.0	NA	NA	0.7	NA	0.0	Yes
First Response Malaria Ag. pLDH/ HRP2 Combo Card Test	Premier Medical Corporation Limited	85.0	73.0	100.0	100.0	0.3	0.0	0.0	0.0	No
First Response Malaria Ag. Pf/Pv Card Test	Premier Medical Corporation Limited	94.0	100.0	100.0	100.0	0.8	0.7	0.5	0.0	Yes

Product name	Manufacturer	Panel detection score				False positive rates (%)				Meets WHO performance criteria?
		200 parasites/μL		2000 parasites/μL		200 parasites/μL		2000 parasites/μL		
		Pf	Pv	Pf	Pv	Pf	Pv	Pf	Pv	
						non-Pf infection	Pf-infection	non-Pf infection	Pf-infection	
One Step test for Malaria Pf/Pv Ag MERISCREEN Malaria Pf/Pv Ag	Meril Diagnostics Pvt. Ltd	78.0	85.7	100.0	100.0	0.5	0.7	0.0	1.4	Yes
ParaHIT Ver. 1.0 Rapid Test for *P. falciparum* Malaria Device	ARKRAY Healthcare Pvt. Ltd	77.0	NA	100,0	NA	NA	0.0	NA	0.0	Yes

NA = not applied; Pf = Plasmodium falciparum; Pv = Plasmodium vivax.

Table 1.
Malaria RDT phase 2 performances in wild type clinical samples containing P. falciparum *and* P. vivax *at low (200) and high (2000) parasite density (parasites/*μL*) and clean-negative samples. Data modified from [18].*

	Microscopy	RDT	Nucleic Acid
Limit of detection (parasitemia)	50 parasites/μL	> 100 parasites/μL	0.5–5 parasites/μL
Time to perform	60 min	15–20 min	2 hours
Cost	$0.20	<$1 to $5	$1.50 –$20
Need for technical training	Yes	No	Yes
Appropriate for remote field testing	Yes	Yes	Not yet

Some data were compiled from [29, 60].

Table 2.
Comparison of characteristics from currently commercially available malaria diagnostic methods.

The RDT test is highly sensitive and specific, it is easy to perform, simple to interpret and the results can be read in 15–30 min. These make it suitable for community-level health facilitates in rural areas and other endemic situations where equipment and professional microscopists are not accessible. However, they have a limited shelf life, and need to be kept dry and away from extremes of temperature. They may also fail to detect malaria in cases where there are low levels of parasites in the blood, and false positives are possible due to cross reactions or gametocytemia (infection with the sexual stage of the parasite only).

A brief comparison of WHO pre-qualified RDTs is presented in **Table 2**. WHO, in collaboration with Special Programme for Research and Training in Tropical Diseases (TDR), FIND, the United States Centers for Disease Control and Prevention (CDC) and other partners, established a protocol to measure the quality of malaria RDTs that are designed to diagnose *P. falciparum*-only through detection of histidine rich protein 2 (HRP2) and those designed to diagnose and distinguish *P. falciparum* and *P. vivax* or non-falciparum malaria. The protocol assesses the sensitivity and specificity of RDTs against specimen banks consisting of recombinant antigens, culture-derived parasites, wild-type parasites, and parasite-negative blood samples, as well as the stability of RDTs at various temperatures and usage conditions. The protocol also describes aspects of the RDTs that affect ease of use in the field, and methods for recording all information in specific databases. Up-to-data information on WHO pre-qualification policies and protocols can be found at WHO's website[2]. **Table 1** shows the malaria RDT performances in wild type *P. falciparum* and *P. vivax* clinical samples containing low (200 parasites /μL) and high (2000 parasites /μL) parasite density (parasites/μL) and clean-negative samples, an obligatory step of the WHO standardized tests [18]. The following criteria must be met for a product to be pre-qualified: (i) For the detection of *P. falciparum* in all transmission settings, the panel detection score should be at least 75% at 200 parasites/μL; (ii) For the detection of *P. vivax* in all transmission settings, the panel detection score should be at least 75% at 200 parasites/μL; (iii) The false positive rate should be less than 10%; (iv) The invalid rate should be less than 5%.

3. Nucleic acid amplification-based tests (NAAT)

NAAT have the potential to overcome several hurdles for malaria diagnostic at POC, more specifically related to the detection of low limits of infection, as well as the ability to discriminate the species and quantify the parasitemia.

[2] https://www.who.int/diagnostics_laboratory/evaluations/en/, accessed on February, 17th, 2021.

3.1 Polymerase chain reaction (PCR)

As the most known nucleic acid amplification technique, PCR has long been used for malaria diagnostic in laboratory settings. PCR-based malaria assays for laboratory use are found in many variations: conventional, nested, hydrolysis probe, digital, or high resolution melting [19–21].

As expected, there is a clear correlation between the level of PCR detection and the parasitemia of a given sample ([22] and **Figure 2**). The lower the parasitemia, the higher the detection cycle above the baseline/threshold (a value known as C_t). Conversely, the higher the parasitemia, the lower the C_t. **Figure 1** shows the detection of different amounts of parasites using two instruments: a lab-based and a portable, hand held thermocycler, the Q3-Plus [23, 24]. The linear regressions show that there is no difference in performance between both instruments using Spearman correlation, with r^2 ranging from 0.73 to 0.80, showing the viability of this portable platform for POC malaria diagnosis.

PCR is more sensitive than both microscopy and immunochromatographic tests. PCR has been found to be especially useful for identifying low-level infections often missed by other techniques, showing a detection limit in the range of 0.5–5 parasites per μL of sample. PCR can detect multiple targets in the same reaction without losing sensitivity and specificity and can be easily parallelized in high throughput instruments [23, 25–28]. The higher sensitivity is achieved by targeting multi copy genomic regions, such as 18S ribosomal RNA gene, which might be present in up to 1000 copies per genome and contains enough similarity across the genus *Plasmodium* and yet contains enough specificity to discriminate the species [23, 25–28].

PCR has been coupled with lateral flow strips to create a simple and easy-to-use detection method for the amplified products, called nucleic acid lateral flow immunoassay (NALFIA) [29, 30]. It combines the specificity of the PCR amplification with the simplicity of lateral flow strips, using DNA capture and recognition sequences and antibody-based colorimetric methods to visualize the targets on the nitrocellulose membrane. NALFIA has been evaluated under field condition

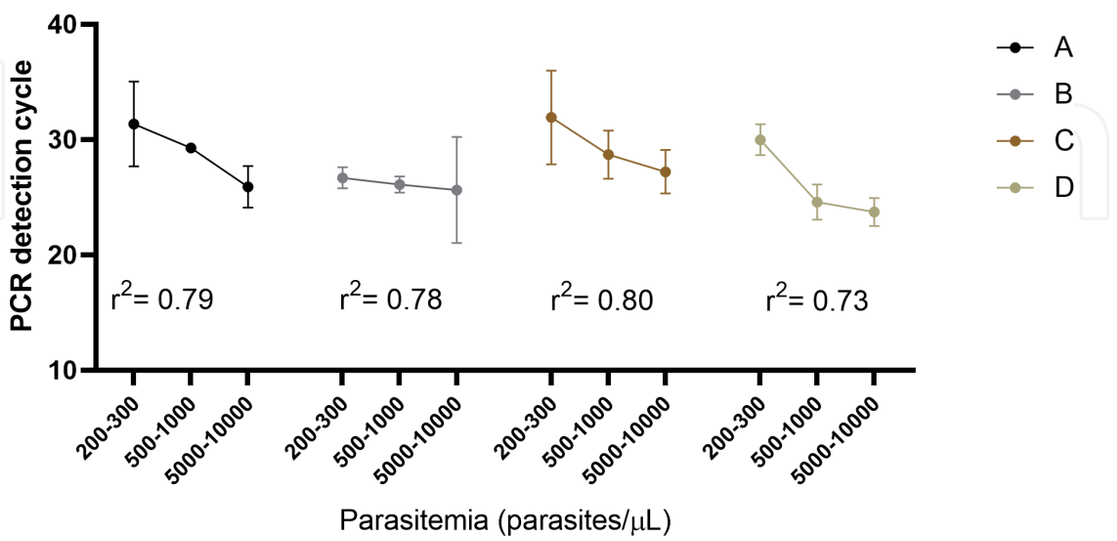

Figure 2.
Spearman correlation between PCR detection cycle and parasitemia estimated by optical microscopy. Detection of P. falciparum *or* P. vivax *DNA in human blood samples is plotted against the parasitemia estimated by optical microscopy. Samples were evaluated with two PCR instruments, the benchtop ABI7500 (applied Biosystems, Thermo fisher, USA) and the portable Q3-plus (ST microelectronics, Italy). Panels a and C show the detection of* P. falciparum *and* P. vivax *with the ABI7500. Panels B and D show the detection of* P. falciparum *and* P. vivax *with the Q3-plus.*

in Kenya, yielding results as sensitive and specific as laboratory PCR and a limit of detection of less than 3 parasites/μL [29].

However, in general, PCR reagents must be stored and transported at freezing temperatures (−20 °C), which is one of the main factors impairing its widespread use at POC settings all over the world and partially explaining why there are so many few available options for POC use for detection of infectious diseases. Some companies that have developed complete systems that use PCR, either conventional or nested, and melting curve analysis to detect targets of interest in POC conditions. However, neither has a solution targeted to malaria.

Much effort has been done to minimize or eliminate the requirement for freezing temperature for storage and transportation of PCR reagents. Even though some progress was indeed achieved, no commercial product is yet available. Rampazzo and colleagues [23] showed a ready-to-use qPCR that can be stored in the reaction vessel at room temperature for up to 28 days without losing performance. Iglesias and coworkers [31] showed similar results using a nested PCR for malaria detection, providing a comparison with microscopy and rapid diagnostic tests. Kamau and colleagues also reported a ready-to-use multiplex PCR for malaria detection [32]. Taylor and coworkers [33] developed a portable PCR instrument that performed 12 reactions in parallel, using lyophilized reagents and high resolution melting to detect and differentiate *Plasmodium* parasites, however with low sensitivity. Sun and colleagues [34] were able to pre-store the reagents in a portable device for rapid detection of *Campylobacter* sp. DNA. However, despite the availability of these technologies, we are unaware of a commercial product for malaria diagnosis that make use of them.

3.2 Isothermal amplification methods

Loop-mediated isothermal amplification (LAMP) is the most common isothermal amplification technique, although other methods have been developed in the last years. Other common isothermal methods, developed after the pioneering technique of the rolling circle amplification [35], are recombinase polymerase amplification (RPA), nucleic acid sequence-based amplification (NASBA), helicase-dependent amplification (HDA), strand displacement amplification (SDA), and even transcription mediated amplification (TMA) [36–42].

LAMP uses a complex set of four primers that bind to the region of interest and its boundaries and, after the initial amplification, create a secondary stem-and-loop structure that serves as a binding site constantly open for a new set of primers to anneal and keep the amplification happening [41, 43]. Amplification by LAMP involves two main repetitive steps of elongation by loop primers: the self-elongation step, where the template is elongated from the stem loop structure that was formed at the 3′ end, and the elongation step, where the formation of the new PCR product actually happens, polymerizing from the primers. That way, the end product is not a single band, but a series of concatamers of different sizes, all containing an amplified sequence of the target. Usually LAMP is performed at 55–65 °C by the enzyme Bst, from *Bacillus stearothermophilus*, and can use either RNA or DNA as templates. The requirement for a single stable temperature is a positive feature for a POC assay [41, 43].

Yamamura and colleagues have developed a rapid diagnostic solution for malaria by using DNA extraction through FTA paper, combined with a LAMP assay and melting curve analysis [44]. Although the solution was analyzed with real samples, the analyses were performed in the lab and not at the POC, so the protocol remains promising until tested in field conditions.

A complete technological solution able to extract DNA, amplify and detect specific DNA sequences from malaria parasites was published [43, 45]. The presented an origami device that vertically processed the sample coupled with a microfluidic lateral flow LAMP amplification and detection platform. The platform was able to detect the presence of the malaria parasite in 98% of infected individuals, with a better performance than standard POC tests, such as optical microscopy and commercial rapid immunochromatographic tests, which detected the parasite in 86 and 83% of the cases, respectively [43, 45].

A high throughput LAMP assay for malaria detection has been shown to work in field conditions, with a clinically relevant sensitivity and, importantly, low cost per analysis [46]. It needs to be evaluated on asymptomatic patients, but it remains one of the most promising candidates for a true POC test.

LAMP has also been coupled with a MiniION sequencer, being able to amplify a genomic target and then differentiate all five *Plasmodium* species. The procedure is sensitive enough to determine the C580Y mutation of *P. falciparum*, which confers protection against artemisinin and is an emerging threat for malaria eradication efforts. Although the procedure is relatively long because it is necessary to prepare the sequencing library, it is very versatile and shows great potential for POC settings [47, 48].

Detection of PCR-amplified products by lateral flow strips has been used for detection of malaria parasites in field samples [29]. In that work, DNA was extracted using a commercial kit. However, considering the difficulties of DNA extraction in the field, we believe that LAMP is the most suitable amplification technique to be conjugated with lateral flow strips as a feasible tool to be implemented in POC settings, and it should be further evaluated. Indeed, a meta-analysis of diagnostic accuracy of LAMP methods revealed that it is robust for diagnosing malaria, both in symptomatic and asymptomatic patients [49].

LAMP has also been used for detection of malaria parasites with a simple 1:1000 sample dilution, thus bypassing the hurdles of nucleic acid extraction in the field [50]. Although the protocol is fast and showed excellent detection limits, it has not been tested with real samples in field conditions, and therefore its positive features remain to be confirmed.

Nucleic acid sequence-based amplification (NASBA) is an isothermal reaction that continuously cycle between the reverse transcriptase activity that copies an RNA sequence into a cDNA, and the activity of a polymerase for subsequent amplification. NASBA generates a high number of copies of the target per cycle, usually reaching detectable concentrations of the product faster than other isothermal methods [42].

NASBA has been used to estimate the prevalence in asymptomatic migrants [51], to estimate gametocytes density [52] and has been shown to have significant correlation with quantitative PCR [53]. Because of its great potential for detecting low-level infections, NASBA has been proposed to be a good alternative to microscopy, especially in low prevalence areas [29, 37]. However, NASBA has not been tested in field conditions, so its performance remains to be evaluated.

4. Microfluidics and other new technologies

In recent year, biological research questions have become the center of convergence of several scientific disciplines, such as physics and engineering, joining the early partner chemistry.

Microfluidics is a general term for engineering techniques that leverage on physics forces at the micro scale to enable the miniaturization, simplification and

automation of complicated analytical processes, while consuming less reagents, minimizing waste, and requiring less supporting instrumentation. Working at the microscale, microfluidic devices are portable, easy-to-use, self-contained, and low-cost diagnostic devices that allow the precise manipulation of minute amounts of liquids to be manipulated by miniaturized structures, such as micropumps, micromixers, microtweezers, and microvalves. Most attractive features of microfluidic technology for POC applications are the low volume of reagents required, faster reaction times, and compact/portable platforms [54, 55].

Microfluidics can be divided in conventional and paper-based assays. Conventional microfluidics focuses on miniaturizing regular laboratory protocols, with a special emphasis on integration of all necessary elements for the diagnostic reaction into stand-alone systems [54, 56]. In conventional microfluidic systems, liquid control can be exerted by acoustic, mechanical, magnetic, as well as capillary and centrifugal forces. Paper-based systems, on the other hand, rely mostly on the power of capillary forces, passively controlling liquid flow via hydrophilic and hydrophobic interactions. However, paper-based assays can integrate sample preparation and pre-concentration more easily than conventional microfluidic systems [56].

Mao and colleagues developed a portable multiplex microfluidic array system, which used LAMP to simultaneously amplify and detect malaria-related *Anopheles* and *Plasmodium* species. The system used a simple color change to discriminate positive from negative results [57].

Among the microfluidic devices, the centrifugal microfluidic device or lab-on-a-disc (LOAD) has advanced remarkably due to simple operation by rotation, allowing for total integration of protocol steps, and high-throughput capability. LOAD devices have been extensively used for molecular diagnostic assays [58]. Among many targets and applications, one stands out relative to our goals in this text. Choi and colleagues developed a platform consisting of a disposable centrifugal disc and a compact instrument able to perform real time PCR for malaria diagnosis with a relevant limit of detection [59]. Although this and other platforms showed great promise towards POC applications, they have not been tested in field conditions [58].

4.1 DNA-based capture molecules

Aptamers, also known as 'chemical antibodies', are short single-stranded oligonucleotides, either DNA- or RNA-based, obtained from synthetic libraries by a process called 'systematic evolution of ligands by exponential enrichment (SELEX)', that fold into distinct tertiary structures and are able to bind to the target with high affinity and specificity. Aptamer-coated surfaces, such as beads, sensors, or micro channels have been used to capture malaria parasites, which then can be detected by several techniques.

Fraser and colleagues developed a portable biosensor with a colorimetric approach to detect *P. falciparum* LDH in a device with three separate microfluidic chambers, obtaining high sensitivity and specificity [60]. Aptamers against other proteins, such as pGDH, PfEMP1, of HMGB1, have also been test in laboratory conditions but still largely remain as a promising approach for use in malaria-diagnosing POC assays [55].

4.2 Non-invasive samples and detection methods

Since blood collection is an invasive technique, other samples have been evaluated for their diagnostic capacity, in particular saliva and urine [61]. Although there

has been some tests in the field using saliva for serological evaluation of samples with reasonable success, no nucleic acid amplification protocol has been used in conjunction with non-invasive samples in field application to help diagnose malaria [61]. In addition, PCR sensitivity in these samples is lower than other POC methods, impairing their current use as samples in molecular POC tests.

Infected or healthy red blood cells (RBCs) have different paramagnetic behaviors, likely due to the presence of hemozoin crystallites in iRBCs [62], and thus can be separated using magnetic fields, taking advantage of the miniaturization of magnetic resonance relaxometry (MRR) [63].

In fact, iRBCs can be enriched by a variety of methods, such as margination [64], dielectrophoresis [65], or magnetic methods [66] [67]. Margination is a separation method based on the deformability of RBCs, where microfluidic forces within the microchannels allow for the stiffer malaria iRBCs to segregate towards the device's sidewalls [62–64, 68]. Dielectrophoresis is a force exerted on any particle when it is subjected to a non-uniform electric field, and particle manipulation is dependent on physical properties of the medium and particle electrical properties, on particle size and shape as well as frequency of electric field [69]. In contrast to dielectrophoresis, microfluidic margination offers the convenience of not needing external electrical and magnetic fields for iRBCs separation. However, both dielectrophoresis as well as magnetic methods have been used for development of diagnostic methods for malaria infection [70]. Magnetic resonance relaxometry has also recently emerged as a very attractive technique to detect ring stage parasites, using a portable permanent magnet, with very encouraging results [62, 68]. However, although promising, dielectrophoresis, magnetic resonance, or margination have not been used in POC devices for malaria diagnosis.

Banoth and colleagues developed a portable device that measures optical absorbance at 405 nm of single RBCs flowing through microfluidic channels. Variation in optical absorbance is then used to discriminate infected from health RBCs from other cellular components present in few microliters of whole blood [71]. Nam and coworkers produced a device with microfluidic channels decorated with a magnetic wire where iRBCs can be separated from hRBCs due to the paramagnetic properties of hemozoin with very high efficiencies [72].

Some malaria diagnostic approaches try to avoid sample collection and processing altogether, such as infrared spectroscopy. Fourier transform infrared (FTIR) spectra are acquired directly from the biological sample without the need for any signal enhancer, such as dyes or reagents highlighting the targets of interest [73, 74]. The acquired FTIR spectra are representative of the molecular composition of the sample, and multivariate data analysis can be used to uncover changes in the FTIR spectra produced by cellular and biochemical changes induced by the presence of a specific pathogen [75, 76]. A work describing the use of this technique for malaria diagnosis employed Attenuated Total Reflection Fourier transform infrared (ATR-FTIR) to detect gametocytes at a level 100 times lower than microscopy-based testing [77]. Heraud and colleagues evaluated this technique using portable infrared spectrometers at four regional clinics in Thailand, comparing against laboratory-based qPCR. The analysis of 318 patients resulted in a high degree of sensitivity and specificity, supporting further testing in POC settings [78]. Recently, Mwanga and colleagues extended previous work [77] and provided the first demonstration that infrared spectroscopy could be coupled with supervised machine learning to accurately diagnose malaria in human dried blood spots [79]. Although more extensive field-testing must be performed, FTIR spectroscopy is indeed a promising method for point of care diagnostic of malaria.

5. The nucleic acid extraction problem

Sample preparation has always been a challenge for molecular assays as well as a bottleneck in translating complex molecular based tests to easy-to-use point of care products. Several prototype devices have been proposed and have shown good results in laboratory settings [80].

For nucleic acid testing, Govindarajan and colleagues reported a low cost μPAD for POC extraction of bacterial DNA from raw viscous samples using microfluidic origami. As demonstrated, *Escherichia coli* with a bacterial load as low as 33 CFU/mL was reliably extracted from pig mucin (simulating sputum) and subsequently detected [81].

The testing of clinical samples for nucleic acids has also been performed for infectious disease diagnosis in resource-limited environments, for example, to detect Ebola virus from extracted RNA or other infectious diseases [82, 83].

A complete technological solution able to extract DNA, amplify and detect specific DNA sequences from malaria parasites was published [43, 45].

Gan and colleagues developed a plastic microfluidic device that integrated a filter disc for DNA extraction from samples as distinct as whole blood, dried blood on various paper substrates, buccal swabs, saliva, and even cigarette ends. The device produced DNA suitable in quantity and quality for several downstream applications such as sequencing, SNP evaluation, and PCR [84].

Rodriguez and colleagues integrated nucleic acid extraction with isothermal amplification and detection by lateral flow on a foldable paper-based device able to detect RNA from Influenza A (H1N1) in less than one hour [85].

Kastania and coworkers present a polymeric microfluidic chip capable of purifying DNA through solid phase extraction that can be used as a stand-alone device or integrated in a lab-on-chip platform. The microfluidic channels were randomly roughened in the micro-nano scale with oxygen plasma, thereby creating high surface area as well as high density of carboxyl groups (–COOH). The –COOH groups together with an optimized buffer are able to bind DNA on the microchannel surface. DNA was the washed away by changing the solution biochemical properties. DNA extracted by this device was evaluated by conventional PCR, yielding satisfactory results [86].

These are just some examples. For an in-depth analysis of the issues of nucleic acid extraction possibilities and problems, the reader is directed to excellent reviews on the subject [80, 87–90].

Currently, magnetic beads and solid-phase extraction are the prime choices for nucleic acid extraction in POC devices, even though neither is yet ready for large-scale application [80, 91, 92]. Although both methods rely on the use of chaotropic agents for cell lysis and release the nucleic acids from structural proteins, washing steps are more efficient in beads-based methods in POC prototypes. However, some simplified protocols do not use beads or membranes and rely purely on both chemical and mechanical methods to denature cell membranes as well as scaffold proteins to release nucleic acids in adequate amounts and purity for diagnosis tests [80].

As shown above, despite extensive advances, no extraction method has made its way to the market of POC diagnostic solutions, and this is a major obstacle for nucleic acid-based assays. Although promising, integration of sample preparation, nucleic acid extraction, amplification and detection of genomic targets into microfluidic devices has not yet achieve the maturity to have impacts on the diagnosis of malaria in field settings. This is clear when one considers that FTA cards are primarily used for sample transportation from the field to the lab, instead as part of a complete kit combining their sample preparation and nucleic acid capabilities

with the portability, not to mention sensitivity and specificity, of some of the techniques discussed in the prior sections of this work. We are not aware of such commercial kit.

A less-spoken but nonetheless quite important challenge of POC devices is waste disposal. Proper discard of biological waste generated by POC tests is a matter of concern, and open air burning might be the only option in some circumstances. However, the situation is more complex, since some chemical waste require special treatment before disposal, such as guanidine thiocyanate used in nucleic acid extraction protocols [93]. Developers need to take this issue in consideration when developing the assays and user-friendly tests.

6. Advantages and disadvantages for POC settings

An absolute requirement for identification and treatment of all parasite carriers, both symptomatic and asymptomatic, is inherent to all malaria elimination programs. Such identification implies that an active search for asymptomatic patients must be performed in POC conditions: since these patients will not look for medical attention, the medical team must find them. In addition, all techniques described in the present text have intrinsic advantages and disadvantages for application in POC settings, and thus each will fulfill a different aspect of the diagnostic needs, some better than others. **Table 2** presents a brief comparison between the different techniques used.

Microscopy, for example, requires few and inexpensive equipments, can be operated under virtually any environment conditions and within a few hours of collecting the blood, the microscopy test can provide valuable information. However, microscopy requires a very skilled professional for appropriate reading of the slides, which poses a problem due to the relative scarce availability. Furthermore, a review work found out that microscopy underestimates *P. falciparum* prevalence by 50% when compared to PCR, the gap being even more significant in low transmission areas [94, 95]. Likewise, Cheng and colleagues described that, on average, 69.5% of infections by *P. vivax* in areas of low transmission are detected only by PCR [94, 96]. Besides, microscopy results are only as reliable as the laboratories performing the tests. In non-endemic areas where the number of cases is low, the laboratorian does not perform this test regularly, and may not be maintaining optimal proficiency.

RDTs, on the other hand, do not require a skilled professional and also does not require expensive instruments. In fact, RDTs can be used with the naked eye, which makes it the perfect choice for remote and hard-to-reach areas. Although they identify *P. falciparum*, specifically, RDTs have no species-specific capacity to identify all five malaria species and cannot provide information on developmental stages. The RDTs rely on antibody detection of parasite antigens, which are constantly under selective pressure and evolve over time, resulting in tests with lower affinities and, consequently, lower sensitivity and specificity [97]. It is important to mention that false-positive results are associated with persistence of PfHRP2 in peripheral blood, cross reactivity against human rheumatoid factor, and other infectious diseases and false-negative results are associated with deletions of pfhrp2 and pfhrp3 genes [98].

PCR is very sensitive and specific, as well as a very robust and well understood technique. Although PCR is more sensitive and specific than microscopy or RDTs, it has limited use as a POC diagnostic tool because of its proneness to contamination, relative expensive reagents, delicate instruments, the need for a stable power source and skilled workers [99–101]. If a portable and robust PCR instrument is developed

and is loaded with a malaria detecting reaction as it has been recently done [23, 33], then PCR might have a place in POC settings.

Compared to PCR, LAMP has the advantage of not requiring a complex and sensitive instrument, using less energy and time to achieve a sensitive target detection. However, LAMP is prone to contamination and production of false positive results from non-target amplifications, which decreases the specificity. If these technological hurdles are solved, LAMP is the most promising nucleic acid amplification technique for use in POC settings. NASBA, a sensitive and specific amplification technique, also does not require a complex thermocycler. However, it is high cost, prone to contamination and thus production of false positives, and requires a more extensive sample preparation than LAMP. Although equipment-free, NASBA is still far from POC applications.

Non-invasive methods such as infrared detection of hemozoin are very promising due to the good results in field tests and low cost of the instrument. Together with LAMP, it remains one of the most promising techniques for POC malaria diagnosis.

Finally, yet importantly, microfluidic techniques should collaborate with any nucleic acid amplification technique to make feasible a robust, sensitive and specific malaria POC diagnostic assay. Microfluidics have the capability of overcoming most of the obstacles of sample preparation and adequate amplification and detection of genomic targets. We believe that microfluidics will be in the center of a malaria POC diagnostic assay within a few years.

7. Costs and cost effectiveness

When one considers the production of the tests and its associated accessories such as required instruments (microscope, thermal cycler, thermal block, to name a few) in the cost analysis, microscopy is the cheapest in the long term, after the cost of the microscope is diluted over the years, and qPCR is the most expensive. The average cost for a microscopy slide test is in the range of $0.20, while RDTs typically have a production cost of $0.50 to $2.00 per test, and nucleic acid amplification techniques harbor a production cost ranging from <$1–$5 for a LAMP assay up to $10–$20 per qPCR or NASBA assay (**Table 2**). Non-invasive methods have a negligible cost per assay, but its overall cost is expected to be as high as qPCR tests due to instrument costs [30, 61].

Costs associated with the different available tests must be considered not solely in terms of production costs. Cost evaluation models must also include variables such as DALY, patient's waiting time, cost acceptance (or 'willingness to pay') and, perhaps most importantly, adherence to the test result by patients, pharmacists, and health care providers, which is ultimately related to the perceived reliability of the test.

As discussed in the previous sections, microscopy and RDTs are the only current diagnostic tools adapted for use in resource-limited settings. Although samples can be transported to centralized laboratories to be evaluated by nucleic acid amplification techniques, turn-around time are typically too long in rural areas of developing countries, defeating the purpose of point of care testing.

Some works have explored cost effectiveness of available malaria diagnostic options for low-resource areas: presumptive, standard microscopy, and RDTs. Shillcut and colleagues used a decision tree model and probabilistic sensitivity analysis to evaluate costs and effects of RDT-positive patients and RDT-negative non-malaria febrile patients [5]. The authors found that the threshold level at which RDTs are more cost-effective than presumptive treatment is intrinsically related to

the disease prevalence in the area. A health care provider can be 95% certain that RDTs are cost-effective relative to presumptive treatment at any prevalence below 62%, while there is a 95% certainty that RDTs are not cost-effective if the prevalence if higher than 90%. Microscopy would be cost-effective with 95% certainty relative to presumptive treatment if prevalence were below 41%, and would not be effective at prevalence rates above 83%. Finally, Shillcut and colleagues determined that RDTs are more than 85% likely to be cost-effective relative to microscopy at any level of disease prevalence [5]. Although the authors clearly show that RDTs are cost-effective, little is known about the impact of the facility of use and adherence to therapy that easy-to-use diagnostic tests might have on the patients [5, 102]. Similar results were observed in a deeper analysis using data from urban and rural areas of Nigeria [103], Kenya [104], and the Brazilian Extra-Amazon Region [105].

However, a recent study showed that patients are not always willing to pay for RDTs [106]. Since subsidies for artemisinin combination therapies (ACTs) already exist, it has been argued that similar subsidies could incentivize patients and health care providers to choose RDTs instead of presumptive treatment [107, 108]. Under that assumption, Bath and colleagues used a more complete decision tree and showed that subsidized RDTs could promote increased use of first-line antimalarials. Therefore, RDTs cost-effectiveness relative to microscopy or presumptive treatment would be true also in high prevalence areas, where a greater proportion of patients would benefit from increased first-line antimalarials use [108].

8. Conclusion

In summary, there are different methods can be employed for the diagnosis of human malaria. While microscopy remains the gold standard, being the mainstay of parasite-based diagnosis, its characteristics are inadequate for ensuring good sensitivity and specificity of malaria diagnosis in the field, adversely affecting health outcomes and optimal use of resources. An acceptable microscopy service is one that is both cost-effective and provides results that are consistently accurate and sufficiently timely to have a direct impact on treatment. This requires a comprehensive and functioning quality assurance program that is lacking in most malaria-endemic countries. RDT tests, which require little expertise to use and are quality-assured from the factory, are a good alternative for malaria diagnosis in remote areas. However, RDTs have no species-specific capacity to identify all five malaria species, and the parasite can present different mutations that hinder this diagnosis by increasing false-negative results. Nuclei acid-based tests, on the contrary, are specific, sensitive, and flexible to be adapted into a POC assay. Nucleic acid-based POC tests have the potential to reduce inappropriate use of anti malarials in endemic regions, bypassing the time and expertise required for microscopic analysis; however, they are more expensive and have not yet been truly adapted for use in remote areas. However, significant advances have been made to facilitate the use of POC methods, so we firmly believe that, within the coming years, interdisciplinary partnership will certainly blend innovative knowledge in biology, chemistry and physics to overcome the major hurdles impairing widespread use of nucleic acid POC tests.

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References

[1] Global Malaria Programme: WHO Global. World malaria report 2019. WHO Regional Office for Africa. 2019.

[2] WHO. World Malaria Report. World Health Organization. 2016. doi:10.4135/9781452276151.n221

[3] Uwimana A, Legrand E, Stokes BH, Ndikumana J-LM, Warsame M, Umulisa N, et al. Emergence and clonal expansion of in vitro artemisinin-resistant *Plasmodium falciparum* kelch13 R561H mutant parasites in Rwanda. Nat Med. 2020;26: 1602-1608. doi:10.1038/s41591-020-1005-2

[4] Das LK, Jambulingam P, Sadanandane C. Impact of community-based presumptive chloroquine treatment of fever cases on malaria morbidity and mortality in a tribal area in Orissa State, India. Malar J. 2008;7: 75. doi:10.1186/1475-2875-7-75

[5] Shillcutt S, Morel C, Goodman C, Coleman P, Bell D, Whitty C, et al. Cost-effectiveness of malaria diagnostic methods in sub-Saharan Africa in an era of combination therapy. Bull World Health Organ. 2008;86: 101-110. doi:10.2471/BLT.07.042259

[6] UNICEF, UNAIDS, World Health Organization, Médicins sans Frontières, Management Sciences for Health, Population Services International. Sources and Prices of Selected Products for the Prevention, Diagnosis and Treatment ofMalaria. Geneva: World Health Organization; 2004.

[7] Drakeley C, Reyburn H. Out with the old, in with the new: the utility of rapid diagnostic tests for malaria diagnosis in Africa. Trans R Soc Trop Med Hyg. 2009;103: 333-337. doi:10.1016/j.trstmh.2008.10.003

[8] Breman J, Alilio M, White N. Defining and Defeating the Intolerable Burden of Malaria III: Progress and Perspectives. Am Soc Trop Med Hyg. 2007;77.

[9] Snow RW, Guerra CA, Noor AM, Myint HY, Hay SI. The global distribution of clinical episodes of *Plasmodium falciparum* malaria. Nature. 2005;434: 214-217. doi:10.1038/nature03342

[10] Meeting MPAC. WHO technical consultation on research requirements to support policy recommendations on highly sensitive point-ofcare diagnostics for *P. falciparum* malaria. Geneva; 2018.

[11] Mathison BA, Pritt BS. Update on Malaria Diagnostics and Test Utilization. Kraft CS, editor. J Clin Microbiol. 2017;55: 2009-2017. doi:10.1128/JCM.02562-16

[12] World Health Organization. Basic malaria microscopy – Part I: Learner's guide. 2nd ed. Geneva, Switzerland: World Health Organization; 2010.

[13] WHO Global Malaria Programme. World Malaria Report 2010. Geneva, Switzerland; 2011.

[14] Berzosa P, de Lucio A, Romay-Barja M, Herrador Z, González V, García L, et al. Comparison of three diagnostic methods (microscopy, RDT, and PCR) for the detection of malaria parasites in representative samples from Equatorial Guinea. Malar J. 2018;17: 333. doi:10.1186/s12936-018-2481-4

[15] Thimasarn K, Miller RS, Maneechai N, Sattabongkot J, Soyseng V, Rachaphaew N, et al. Comparison of field and expert laboratory microscopy for active surveillance for asymptomatic *Plasmodium falciparum* and *Plasmodium vivax* in western Thailand. Am J Trop Med Hyg. 2002;67: 141-144. doi:10.4269/ajtmh.2002.67.141

[16] WHO. Malaria Rapid Diagnostic Test Performance - results of WHO product testing of malaria RDTs: Round 1 (2008). Malaria Rapid Diagnostic Test Performance - Summary results of WHO product testing of malaria RDTs: round 1-7 (2008-2016). Geneva, Switzerland; 2017.

[17] Ling X-X, Jin J-J, Zhu G-D, Wang W-M, Cao Y-Y, Yang M-M, et al. Cost-effectiveness analysis of malaria rapid diagnostic tests: a systematic review. Infect Dis Poverty. 2019;8: 104. doi:10.1186/s40249-019-0615-8

[18] WHO, FIND, CDC. Malaria rapid diagnostic test performance. Results of WHO product testing of malaria RDTs: round 8 (2016-2018). Geneva, Switzerland; 2018.

[19] Murillo E, Muskus C, Agudelo LA, Vélez ID, Ruiz-Lopez F. A new high-resolution melting analysis for the detection and identification of Plasmodium in human and Anopheles vectors of malaria. Sci Rep. 2019;9: 1674. doi:10.1038/s41598-018-36515-9

[20] Roth JM, Korevaar DA, Leeflang MMG, Mens PF. Molecular malaria diagnostics: A systematic review and meta-analysis. Crit Rev Clin Lab Sci. 2016;53: 87-105. doi:10.3109/10408363.2015.1084991

[21] Koepfli C, Nguitragool W, Hofmann NE, Robinson LJ, Ome-Kaius M, Sattabongkot J, et al. Sensitive and accurate quantification of human malaria parasites using droplet digital PCR (ddPCR). Sci Rep. 2016;6: 39183. doi:10.1038/srep39183

[22] Ballard E, Wang CYT, Hien TT, Tong NT, Marquart L, Pava Z, et al. A validation study of microscopy versus quantitative PCR for measuring *Plasmodium falciparum* parasitemia. Trop Med Health. 2019;47: 49. doi:10.1186/s41182-019-0176-3

[23] Rampazzo RCP, Graziani AC, Leite KK, Surdi JA, Biondo CA, Costa MLN, et al. Proof of Concept for a Portable Platform for Molecular Diagnosis of Tropical Diseases. J Mol Diagnostics. 2019;21: 839-851. doi:10.1016/j.jmoldx.2019.04.008

[24] Cereda M, Cocci A, Cucchi D, Raia L, Pirola D, Bruno L, et al. Q3: A Compact Device for Quick, High Precision qPCR. Sensors. 2018;18: 2583. doi:10.3390/s18082583

[25] Rougemont M, Saanen M Van, Sahli R, Hinrikson HP, Bille J, Jaton K. Detection of Four Plasmodium Species in Blood from Humans by 18S rRNA Gene Subunit-Based and Species-Specific Real-Time PCR Assays. 2004;42: 5636-5643. doi:10.1128/JCM.42.12.5636

[26] Kamau E, Alemayehu S, Feghali KC, Saunders D, Ockenhouse CF. Multiplex qPCR for Detection and Absolute Quantification of Malaria. PLoS One. 2013;8. doi:10.1371/journal.pone.0071539

[27] Alemayehu S, Feghali KC, Cowden J, Komisar J, Ockenhouse CF, Kamau E. Comparative evaluation of published real-time PCR assays for the detection of malaria following MIQE guidelines. Malar J. 2013;12: 277. doi:10.1186/1475-2875-12-277

[28] Kamau E, Tolbert LS, Kortepeter L, Pratt M, Nyakoe N, Muringo L, et al. Development of a highly sensitive genus-specific quantitative reverse transcriptase real-time PCR assay for detection and quantitation of plasmodium by amplifying RNA and DNA of the 18S rRNA genes. J Clin Microbiol. 2011;49: 2946-2953. doi:10.1128/JCM.00276-11

[29] Mens P, Spieker N, Omar S, Heijnen M, Schallig H, Kager PA. Is molecular biology the best alternative for diagnosis of malaria to microscopy?

A comparison between microscopy, antigen detection and molecular tests in rural Kenya and urban Tanzania. Trop Med Int Heal. 2006; 061127095204005-??? doi:10.1111/j.1365-3156.2006.01779.x

[30] Cordray MS, Richards-Kortum RR. Emerging nucleic acid-based tests for point-of-care detection of malaria. Am J Trop Med Hyg. 2012;87: 223-30. doi:10.4269/ajtmh.2012.11-0685

[31] Iglesias N, Subirats M, Trevisi P, Ramírez-Olivencia G, Castán P, Puente S, et al. Performance of a new gelled nested PCR test for the diagnosis of imported malaria: comparison with microscopy, rapid diagnostic test, and real-time PCR. Parasitol Res. 2014;113: 2587-91. doi:10.1007/s00436-014-3911-z

[32] Kamau E, Alemayehu S, Feghali KC, Juma DW, Blackstone GM, Marion WR, et al. Sample-ready multiplex qPCR assay for detection of malaria. Malar J. 2014;13: 158. doi:10.1186/1475-2875-13-158

[33] Taylor BJ, Howell A, Martin KA, Manage DP, Gordy W, Campbell SD, et al. A lab-on-chip for malaria diagnosis and surveillance. Malar J. 2014;13: 179. doi:10.1186/1475-2875-13-179

[34] Sun Y, Høgberg J, Christine T, Florian L, Monsalve LG, Rodriguez S, et al. Pre-storage of gelified reagents in a lab-on-a-foil system for rapid nucleic acid analysis. Lab Chip. 2013;13: 1509-14. doi:10.1039/c2lc41386h

[35] Ali MM, Li F, Zhang Z, Zhang K, Kang D-K, Ankrum JA, et al. Rolling circle amplification: a versatile tool for chemical biology, materials science and medicine. Chem Soc Rev. 2014;43: 3324-41. doi:10.1039/c3cs60439j

[36] Li J, Macdonald J, von Stetten F. Review: a comprehensive summary of a decade development of the recombinase polymerase amplification. Analyst. 2018;144: 31-67. doi:10.1039/c8an01621f

[37] Oriero EC, Jacobs J, Van Geertruyden J-P, Nwakanma D, D'Alessandro U. Molecular-based isothermal tests for field diagnosis of malaria and their potential contribution to malaria elimination. J Antimicrob Chemother. 2014;70: 2-13. doi:10.1093/jac/dku343

[38] Vincent M, Xu Y, Kong H. Helicase-dependent isothermal DNA amplification. EMBO Rep. 2004;5: 795-800. doi:10.1038/sj.embor.7400200

[39] Toley BJ, Covelli I, Belousov Y, Ramachandran S, Kline E, Scarr N, et al. Isothermal strand displacement amplification (iSDA): a rapid and sensitive method of nucleic acid amplification for point-of-care diagnosis. Analyst. 2015;140: 7540-9. doi:10.1039/c5an01632k

[40] Comanor L, Anderson F, Ghany M, Perrillo R, Heathcote EJ, Sherlock C, et al. Transcription-mediated amplification is more sensitive than conventional PCR-based assays for detecting residual serum HCV RNA at end of treatment. Am J Gastroenterol. 2001;96: 2968-72. doi:10.1111/j.1572-0241.2001.04669.x

[41] Becherer L, Borst N, Bakheit M, Frischmann S, Zengerle R, von Stetten F. Loop-mediated isothermal amplification (LAMP) – review and classification of methods for sequence-specific detection. Anal Methods. 2020;12: 717-746. doi:10.1039/C9AY02246E

[42] Compton J. Nucleic acid sequence-based amplification. Nature. 1991;350: 91-92. doi:10.1038/350091a0

[43] Reboud J, Xu G, Garrett A, Adriko M, Yang Z, Tukahebwa EM, et al. Paper-based microfluidics for DNA diagnostics of malaria in low resource underserved rural communities. Proc Natl Acad Sci. 2019;116: 4834-4842. doi:10.1073/pnas.1812296116

[44] Yamamura M, Makimura K, Ota Y. Evaluation of a new rapid molecular diagnostic system for *Plasmodium falciparum* combined with DNA filter paper, loop-mediated isothermal amplification, and melting curve analysis. Jpn J Infect Dis. 2009;62: 20-25.

[45] Xu G, Nolder D, Reboud J, Oguike MC, van Schalkwyk DA, Sutherland CJ, et al. Paper-Origami-Based Multiplexed Malaria Diagnostics from Whole Blood. Angew Chemie Int Ed. 2016;55: 15250-15253. doi:10.1002/anie.201606060

[46] Britton S, Cheng Q, Sutherland CJ, McCarthy JS. A simple, high-throughput, colourimetric, field applicable loop-mediated isothermal amplification (HtLAMP) assay for malaria elimination. Malar J. 2015;14: 335. doi:10.1186/s12936-015-0848-3

[47] Imai K, Tarumoto N, Misawa K, Runtuwene LR, Sakai J, Hayashida K, et al. A novel diagnostic method for malaria using loop-mediated isothermal amplification (LAMP) and MinION™ nanopore sequencer. BMC Infect Dis. 2017;17: 621. doi:10.1186/s12879-017-2718-9

[48] Imai K, Tarumoto N, Runtuwene LR, Sakai J, Hayashida K, Eshita Y, et al. An innovative diagnostic technology for the codon mutation C580Y in kelch13 of *Plasmodium falciparum* with MinION nanopore sequencer. Malar J. 2018;17: 217. doi:10.1186/s12936-018-2362-x

[49] Picot S, Cucherat M, Bienvenu A-L. Systematic review and meta-analysis of diagnostic accuracy of loop-mediated isothermal amplification (LAMP) methods compared with microscopy, polymerase chain reaction and rapid diagnostic tests for malaria diagnosis. Int J Infect Dis. 2020;98: 408-419. doi:10.1016/j.ijid.2020.07.009

[50] Modak SS, Barber CA, Geva E, Abrams WR, Malamud D, Ongagna YSY. Rapid Point-of-Care Isothermal Amplification Assay for the Detection of Malaria without Nucleic Acid Purification. Infect Dis Res Treat. 2016;9: IDRT.S32162. doi:10.4137/IDRT.S32162

[51] Marangi M, Di Tullio R, Mens PF, Martinelli D, Fazio V, Angarano G, et al. Prevalence of Plasmodium spp. in malaria asymptomatic African migrants assessed by nucleic acid sequence based amplification. Malar J. 2009;8: 12. doi:10.1186/1475-2875-8-12

[52] Pett H, Gonçalves BP, Dicko A, Nébié I, Tiono AB, Lanke K, et al. Comparison of molecular quantification of *Plasmodium falciparum* gametocytes by Pfs25 qRT-PCR and QT-NASBA in relation to mosquito infectivity. Malar J. 2016;15: 539. doi:10.1186/s12936-016-1584-z

[53] Schneider P, Wolters L, Schoone G, Schallig H, Sillekens P, Hermsen R, et al. Real-time nucleic acid sequence-based amplification is more convenient than real-time PCR for quantification of *Plasmodium falciparum*. J Clin Microbiol. 2005;43: 402-5. doi:10.1128/JCM.43.1.402-405.2005

[54] Pham NM, Karlen W, Beck H-P, Delamarche E. Malaria and the 'last' parasite: how can technology help? Malar J. 2018;17: 260. doi:10.1186/s12936-018-2408-0

[55] Gitta B, Kilian N. Diagnosis of Malaria Parasites Plasmodium spp. in Endemic Areas: Current Strategies for an Ancient Disease. BioEssays. 2020;42: 1-12. doi:10.1002/bies.201900138

[56] Naik P, Jaitpal S, Paul D. The Resurgence of Paperfluidics: A new technology for cell, DNA, and blood analysis. IEEE Nanotechnol Mag. 2020;14: 35-45. doi:10.1109/MNANO.2020.2966063

[57] Mao R, Ge G, Wang Z, Hao R, Zhang G, Yang Z, et al. A multiplex

microfluidic loop-mediated isothermal amplification array for detection of malaria-related parasites and vectors. Acta Trop. 2018;178: 86-92. doi:10.1016/j.actatropica.2017.10.025

[58] Nguyen H Van, Nguyen VD, Nguyen HQ, Chau THT, Lee EY, Seo TS. Nucleic acid diagnostics on the total integrated lab-on-a-disc for point-of-care testing. Biosens Bioelectron. 2019;141: 111466. doi:10.1016/j.bios.2019.111466

[59] Choi G, Prince T, Miao J, Cui L, Guan W. Sample-to-answer palm-sized nucleic acid testing device towards low-cost malaria mass screening. Biosens Bioelectron. 2018;115: 83-90. doi:10.1016/j.bios.2018.05.019

[60] Fraser LA, Kinghorn AB, Dirkzwager RM, Liang S, Cheung Y-W, Lim B, et al. A portable microfluidic Aptamer-Tethered Enzyme Capture (APTEC) biosensor for malaria diagnosis. Biosens Bioelectron. 2018;100: 591-596. doi:10.1016/j.bios.2017.10.001

[61] Mbanefo A, Kumar N. Evaluation of Malaria Diagnostic Methods as a Key for Successful Control and Elimination Programs. Trop Med Infect Dis. 2020;5: 102. doi:10.3390/tropicalmed5020102

[62] Peng WK, Kong TF, Ng CS, Chen L, Huang Y, Bhagat AAS, et al. Micromagnetic resonance relaxometry for rapid label-free malaria diagnosis. Nat Med. 2014;20: 1069-1073. doi:10.1038/nm.3622

[63] Fook Kong T, Ye W, Peng WK, Wei Hou H, Marcos, Preiser PR, et al. Enhancing malaria diagnosis through microfluidic cell enrichment and magnetic resonance relaxometry detection. Sci Rep. 2015;5: 11425. doi:10.1038/srep11425

[64] Hou HW, Bhagat AAS, Lin Chong AG, Mao P, Wei Tan KS, Han J, et al. Deformability based cell margination—A simple microfluidic design for malaria-infected erythrocyte separation. Lab Chip. 2010;10: 2605. doi:10.1039/c003873c

[65] Gascoyne P, Mahidol C, Ruchirawat M, Satayavivad J, Watcharasit P, Becker FF. Microsample preparation by dielectrophoresis: isolation of malaria. Lab Chip. 2002;2: 70. doi:10.1039/b110990c

[66] ZIMMERMAN PA, FUJIOKA H, THOMSON JM, ZBOROWSKI M, COLLINS WE. DIAGNOSIS OF MALARIA BY MAGNETIC DEPOSITION MICROSCOPY. Am J Trop Med Hyg. 2006;74: 568-572. doi:10.4269/ajtmh.2006.74.568

[67] Ribaut C, Berry A, Chevalley S, Reybier K, Morlais I, Parzy D, et al. Concentration and purification by magnetic separation of the erythrocytic stages of all human Plasmodium species. Malar J. 2008;7: 45. doi:10.1186/1475-2875-7-45

[68] Thamarath SS, Xiong A, Lin P-H, Preiser PR, Han J. Enhancing the sensitivity of micro magnetic resonance relaxometry detection of low parasitemia *Plasmodium falciparum* in human blood. Sci Rep. 2019;9: 2555. doi:10.1038/s41598-019-38805-2

[69] Jubery TZ, Srivastava SK, Dutta P. Dielectrophoretic separation of bioparticles in microdevices: a review. Electrophoresis. 2014;35: 691-713. doi:10.1002/elps.201300424

[70] Kasetsirikul S, Buranapong J, Srituravanich W, Kaewthamasorn M, Pimpin A. The development of malaria diagnostic techniques: a review of the approaches with focus on dielectrophoretic and magnetophoretic methods. Malar J. 2016;15: 358. doi:10.1186/s12936-016-1400-9

[71] Banoth E, Kasula VK, Gorthi SS. Portable optofluidic absorption flow

analyzer for quantitative malaria diagnosis from whole blood. Appl Opt. 2016;55: 8637. doi:10.1364/AO.55.008637

[72] Nam J, Huang H, Lim H, Lim C, Shin S. Magnetic Separation of Malaria-Infected Red Blood Cells in Various Developmental Stages. Anal Chem. 2013;85: 7316-7323. doi:10.1021/ac4012057

[73] Baker MJ, Trevisan J, Bassan P, Bhargava R, Butler HJ, Dorling KM, et al. Using Fourier transform IR spectroscopy to analyze biological materials. Nat Protoc. 2014;9: 1771-1791. doi:10.1038/nprot.2014.110

[74] Perez-Guaita D, Marzec KM, Hudson A, Evans C, Chernenko T, Matthäus C, et al. Parasites under the Spotlight: Applications of Vibrational Spectroscopy to Malaria Research. Chem Rev. 2018;118: 5330-5358. doi:10.1021/acs.chemrev.7b00661

[75] Diem M, Griffiths PR, Chalmers JM. Vibrational Spectroscopy for Medical Diagnosis. Wiley Anal. Weinheim, Germany: Wiley-VCH Verlag GmbH & Co; 2008.

[76] Brereton RG. Chemometrics for Pattern Recognition. West Sussex, United Kingdom: John Wiley & Sons, Ltd; 2009.

[77] Khoshmanesh A, Dixon MWA, Kenny S, Tilley L, McNaughton D, Wood BR. Detection and Quantification of Early-Stage Malaria Parasites in Laboratory Infected Erythrocytes by Attenuated Total Reflectance Infrared Spectroscopy and Multivariate Analysis. Anal Chem. 2014;86: 4379-4386. doi:10.1021/ac500199x

[78] Heraud P, Chatchawal P, Wongwattanakul M, Tippayawat P, Doerig C, Jearanaikoon P, et al. Infrared spectroscopy coupled to cloud-based data management as a tool to diagnose malaria: a pilot study in a malaria-endemic country. Malar J. 2019;18: 348. doi:10.1186/s12936-019-2945-1

[79] Mwanga EP, Minja EG, Mrimi E, Jiménez MG, Swai JK, Abbasi S, et al. Detection of malaria parasites in dried human blood spots using mid-infrared spectroscopy and logistic regression analysis. Malar J. 2019;18: 341. doi:10.1186/s12936-019-2982-9

[80] Ali N, Rampazzo RDCP, Costa ADT, Krieger MA. Current Nucleic Acid Extraction Methods and Their Implications to Point-of-Care Diagnostics. Biomed Res Int. 2017;2017. doi:10.1155/2017/9306564

[81] Govindarajan a. V., Ramachandran S, Vigil GD, Yager P, Böhringer KF. A low cost point-of-care viscous sample preparation device for molecular diagnosis in the developing world; An example of microfluidic origami. Lab Chip. 2012;12: 174-181. doi:10.1039/c1lc20622b

[82] Magro L, Escadafal C, Garneret P, Jacquelin B, Kwasiborski A, Manuguerra J-C, et al. Paper microfluidics for nucleic acid amplification testing (NAAT) of infectious diseases. Lab Chip. 2017;17: 2347-2371. doi:10.1039/C7LC00013H

[83] Magro L, Jacquelin B, Escadafal C, Garneret P, Kwasiborski A, Manuguerra J-C, et al. Paper-based RNA detection and multiplexed analysis for Ebola virus diagnostics. Sci Rep. 2017;7: 1347. doi:10.1038/s41598-017-00758-9

[84] Gan W, Zhuang B, Zhang P, Han J, Li C-XX, Liu P. A filter paper-based microdevice for low-cost, rapid, and automated DNA extraction and amplification from diverse sample types. Lab Chip. 2014;14: 3719-3728. doi:10.1039/c4lc00686k

[85] Rodriguez NM, Linnes JC, Fan A, Ellenson CK, Pollock NR, Klapperich CM. Paper-Based RNA

Extraction, in Situ Isothermal Amplification, and Lateral Flow Detection for Low-Cost, Rapid Diagnosis of Influenza A (H1N1) from Clinical Specimens. Anal Chem. 2015;87: 7872-7879. doi:10.1021/acs.analchem.5b01594

[86] Kastania AS, Tsougeni K, Papadakis G, Gizeli E, Kokkoris G, Tserepi A, et al. Plasma micro-nanotextured polymeric micromixer for DNA purification with high efficiency and dynamic range. Anal Chim Acta. 2016;942: 58-67. doi:10.1016/j.aca.2016.09.007

[87] Huang QS, Mei QB, Shi JL, Xu F, Gong Y, Yang H, et al. Advances in paper-based sample pretreatment for point-of-care testing. Crit Rev Biotechnol. 2016;37: 411-428. doi:10.3109/07388551.2016.1164664

[88] Foudeh AM, Fatanat Didar T, Veres T, Tabrizian M. Microfluidic designs and techniques using lab-on-a-chip devices for pathogen detection for point-of-care diagnostics. Lab Chip. 2012;12: 3249-66. doi:10.1039/c2lc40630f

[89] Cui F, Rhee M, Singh A, Tripathi A. Microfluidic Sample Preparation for Medical Diagnostics. Annu Rev Biomed Eng. 2015;17: 267-286. doi:10.1146/annurev-bioeng-071114-040538

[90] Ibrahim F, Thio T, Faisal T, Neuman M. The Application of Biomedical Engineering Techniques to the Diagnosis and Management of Tropical Diseases: A Review. Sensors. 2015;15: 6947-6995. doi:10.3390/s150306947

[91] Azimi SM, Nixon G, Ahern J, Balachandran W. A magnetic bead-based DNA extraction and purification microfluidic device. Microfluid Nanofluidics. 2011;11: 157-165. doi:10.1007/s10404-011-0782-9

[92] Kim J, Johnson M, Hill P, Gale BK. Microfluidic sample preparation: cell lysis and nucleic acid purification. Integr Biol (Camb). 2009;1: 574-86. doi:10.1039/b905844c

[93] Wang S, Lifson MA, Inci F, Liang L-G, Sheng Y-F, Demirci U. Advances in addressing technical challenges of point-of-care diagnostics in resource-limited settings. Expert Rev Mol Diagn. 2016;16: 449-59. doi:10.1586/14737159.2016.1142877

[94] Britton S, Cheng Q, McCarthy JS. Novel molecular diagnostic tools for malaria elimination: a review of options from the point of view of high-throughput and applicability in resource limited settings. Malar J. 2016;15: 88. doi:10.1186/s12936-016-1158-0

[95] Okell LC, Ghani AC, Lyons E, Drakeley CJ. Submicroscopic Infection in *Plasmodium falciparum* –Endemic Populations: A Systematic Review and Meta-Analysis. J Infect Dis. 2009;200: 1509-1517. doi:10.1086/644781

[96] Cheng Q, Cunningham J, Gatton ML. Systematic Review of Sub-microscopic *P. vivax* Infections: Prevalence and Determining Factors. Rodrigues MM, editor. PLoS Negl Trop Dis. 2015;9: e3413. doi:10.1371/journal.pntd.0003413

[97] World Health Organization. Malaria rapid diagnostic test performance: results of WHO product testing of malaria RDTs: round 5 (2013). Geneva, Switzerland; 2014.

[98] Cheng Q, Gatton ML, Barnwell J, Chiodini P, McCarthy J, Bell D, et al. *Plasmodium falciparum* parasites lacking histidine-rich protein 2 and 3: a review and recommendations for accurate reporting. Malar J. 2014;13: 283. doi:10.1186/1475-2875-13-283

[99] Kralik P, Ricchi M. A basic guide to real time PCR in microbial diagnostics:

Definitions, parameters, and everything. Front Microbiol. 2017;8: 1-9. doi:10.3389/fmicb.2017.00108

[100] Gonçalves-de-Albuquerque SDC, Pessoa E Silva R, de Morais RCS, Trajano-Silva LAM, Régis-da-Silva CG, Brandão-Filho SP, et al. Tracking false-negative results in molecular diagnosis: proposal of a triplex-PCR based method for leishmaniasis diagnosis. J Venom Anim Toxins Incl Trop Dis. 2014;20: 16. doi:10.1186/1678-9199-20-16

[101] Lorenz TC. Polymerase Chain Reaction: Basic Protocol Plus Troubleshooting and Optimization Strategies. J Vis Exp. 2012. doi:10.3791/3998

[102] Loubiere S, Moatti J-P. Economic evaluation of point-of-care diagnostic technologies for infectious diseases. Clin Microbiol Infect. 2010;16: 1070-1076. doi:10.1111/j.1469-0691.2010.03280.x

[103] Uzochukwu BS, Obikeze EN, Onwujekwe OE, Onoka CA, Griffiths UK. Cost-effectiveness analysis of rapid diagnostic test, microscopy and syndromic approach in the diagnosis of malaria in Nigeria: implications for scaling-up deployment of ACT. Malar J. 2009;8: 265. doi:10.1186/1475-2875-8-265

[104] Zurovac D, Larson BA, Skarbinski J, Slutsker L, Snow RW, Hamel MJ. Modeling the financial and clinical implications of malaria rapid diagnostic tests in the case-management of older children and adults in Kenya. Am J Trop Med Hyg. 2008;78: 884-91.

[105] de Oliveira MRF, Giozza SP, Peixoto HM, Romero GA. Cost-effectiveness of diagnostic for malaria in Extra-Amazon Region, Brazil. Malar J. 2012;11: 390. doi:10.1186/1475-2875-11-390

[106] Ezennia IJ, Nduka SO, Ekwunife OI. Cost benefit analysis of malaria rapid diagnostic test: the perspective of Nigerian community pharmacists. Malar J. 2017;16: 7. doi:10.1186/s12936-016-1648-0

[107] Hansen KS, Lesner TH, Østerdal LP. Optimal price subsidies for appropriate malaria testing and treatment behaviour. Malar J. 2016;15: 534. doi:10.1186/s12936-016-1582-1

[108] Bath D, Goodman C, Yeung S. Modelling the cost-effectiveness of introducing subsidised malaria rapid diagnostic tests in the private retail sector in sub-Saharan Africa. BMJ Glob Heal. 2020;5: e002138. doi:10.1136/bmjgh-2019-002138