Malaria Diagnosis: Current Approaches and Future Prospects

Onile-ere Olabode, Openibo John and Olasehinde Grace
Department of Biological Sciences,
Covenant University,
Ota, Nigeria

Abstract- **Scaled up efforts by a consortia of organisations in the diagnosis, treatment and prevention of malaria have led to a significant reduction in the overall malaria mortality and morbidity in the past few years. Malaria has, nonetheless, remained one of the world's most burdensome diseases with the over 214 million cases and 438,000 deaths recorded in 2015 (2.68% of global DALYs). This burden is unevenly domiciled in sub-Saharan Africa where 89% of all cases and 91% of all deaths occurred. These figures however, only represent a fraction of the actual global burden of Malaria as surveillance fails to cover most cases in sub-Saharan Africa where the majority of malaria endemic regions lack facilities for diagnosis, case management and active surveillance. The emergence of drug resistant strains of the *Plasmodium* species prompted WHO to recommend a confirmatory diagnosis of each case of Malaria before treatment. The workability of this recommendation however, begs to be questioned as the majority of all malaria diagnosis is done via Clinical diagnosis; which lacks precision, is still the major form of diagnosis in many malaria endemic regions, and contributes to the over-diagnosis of malaria and subsequent under-diagnosis of other febrile illnesses. Of higher import is the risk of the emergence of drug resistant species due to the unregulated antimalarial use caused by inaccurate clinical diagnosis. Microscopy, which is the gold standard of malaria diagnosis, and the Rapid Diagnostic Tests (RDT) for malaria antigens have proven to be very useful in the diagnosis of malaria giving high levels of specificity and sensitivity. They however have the downside of having relatively high limits of detection, invasiveness, being labour intensive and expensive in the light of the low income countries where malaria is endemic. More sophisticated tools such as those that employ nucleic acid techniques (Polymerase Chain Reaction and Gene probes) are not field deployable and are mostly applied for research purposes. This necessitates the need for new diagnostic approaches that are suited to the conditions found in malaria endemic regions. A range of novel diagnostic tools with a do-it-yourself approach, leveraging on previously untapped diagnostic material such as urine are currently being assessed. These novel tools promise great results if successful. This review presents an overview of current diagnostic methods, the prospects in malaria diagnostics and finally makes an effort to recommend what an ideal malaria diagnostic tool should be made up of, all the while focusing on sub-Saharan Africa.**

Keywords- Malaria, Diagnosis, sub-Saharan Africa

I. INTRODUCTION

The end of the Millennium Development Goals (MDGs) necessitated the switch to Sustainable Development Goals (SDGs). A great deal of progress was made during the 15 years ofMDGs as it relates to malaria. The world saw a 37% and 60% reduction in malaria incidence and mortality with increased coverage of key interventions such as Insecticide Treated Nets, preventive treatment in pregnancy and increased use of rapid diagnostic tests[1], there is however a great deal of work to be done as there are still many issues threatening the new goal of eradicating malaria by 2030.

The WHO in its recent strategy forelimination has identified malaria diagnosis as a major factor in getting to zero come 2030 as prompt and accurate diagnosis is the mainstay for effective disease management and surveillance[2]. Currently, malaria diagnosis rests majorly on the Microscopic detection of parasites and Rapid Diagnostic tests (RDT)[3]. These two tools have proven really effective in times past but may fall short in playing their role towards malaria elimination viz-a-viz their shortcomings.

A. Objectives

This review considered the status of currently used diagnostic methods as well as prospective tools in delivering the information which will help inform policy in a bid to eliminating malaria in sub Saharan Africa where the bulk of the burden lies.

B. Methods

In this review, keywords such as 'malaria', 'diagnosis', 'issues' and 'prospects' were put into the google scholar databases to find relevant papers. Papers with a focus outside sub-Saharan Africa or falciparum malaria were excluded from this review.

II. MALARIA LIFE CYCLE & DISTRIBUTION

The malaria life cycle is summarized in Figure 1. It typically begins when an infected female Anopheles mosquito takes a blood meal injecting sporozoites into the human bloodstream. These sporozoites travel to the liver, infecting hepatocytes to initiate the pre-erythrocytic phase. About 2 weeks after, the hepatocytes rupture, releasing merozoites which go on to infect erythrocytes, initiating the erythrocytic phase of the infection which is responsible for the symptoms

Copyright © 2016 by Covenant University Press

observed during malaria infection. This phase is also marked by the breakdown of haemoglobin into an insoluble malaria pigment of diagnostic importance called haemozoin. Merozoites become ring-like trophozoites, which form the basis for malaria diagnosis via microscopy. Subsequently, trophozoites divide repetitively to give schizonts which go on to develop into merozoites. The new merozoites burst out of the erythrocytes and infect other erythrocytes. Some merozoites, however develop into male and female gametocytes which lay dormant until picked up by a mosquito during a blood meal. The gametocytes fuse to form a zygote in the stomach of the insect vector after which the zygote develops into sporozoites which travel to the salivary gland from where they are injected into a human during the next blood meal starting the cycle again[4].

Malaria symptoms include fever, headache, chills and vomiting and in severe cases severe anaemia, respiratory distress in relation to metabolic acidosis, or cerebral malaria[5].

Malaria is currently endemic in most of sub-Saharan Africa (Fig. 1).There were 214 million cases of malaria and 438 000 deaths globally with about 90% of all global cases occurring in the sub-Saharan region[3]. Of particular risk are children and pregnant women who suffer the most from the scourge of malaria.

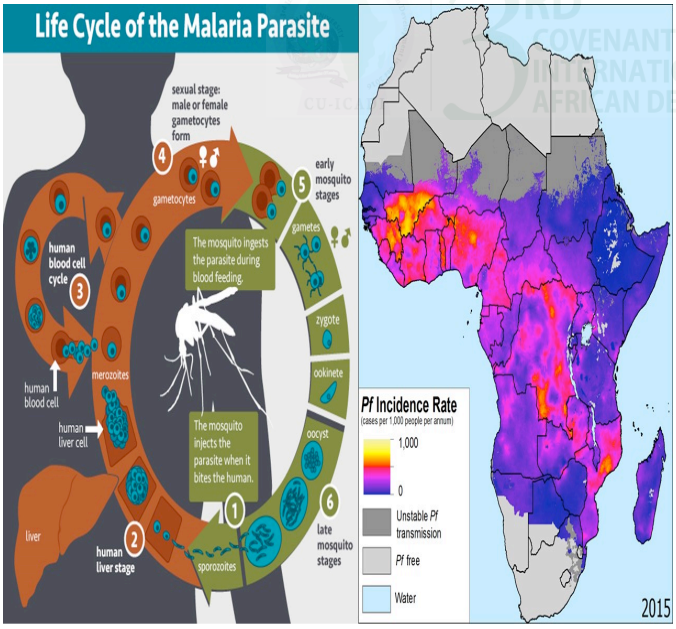

Figure 1: Life cycle of malaria (right), *P. falciparum* incidence in 2015 [6]

III. MALARIA DIAGNOSTIC TARGETS

Malaria diagnosis has been predominantly invasive, utilizing whole blood as its primary diagnostic material. Recent efforts, however have been given to the application other non-invasive materials such as saliva and urine as diagnostic materials in malaria diagnosis[7]–[10].Malaria diagnosis targets whole cells, such as infected Red blood cells (iRBCs) and leukocytes that have ingested parasites or detectable analytes such as parasite antigens, haemozoin crystals and nucleic acids. These targets have effective thus far in malaria diagnosis but not without their individual limitations. The ability and accuracy at which these targets are detected is dependent on the technology used and such factors as reagents, operator proficiency, sample quality and volume. Whole cell targets are especially useful as they give current information on the state of the parasite in the human body as such, they inform treatment and intervention decisions.

Antigenic targets of malaria diagnosis include the *Plasmodium falciparum* histidine rich protein-II (HRPII), lactate dehydrogenase(pLDH) and aldolase. Aldolase is not expressed by*P.falciparum*as such it won't be considered here. HRPII is a water soluble protein antigen expressed by *P.falciparum* trophozoites and localized in several cell compartments. The parasite releases a substantial amount of the protein into its host's blood stream making it detectable in blood stream[11], [12], cerebrospinal fluid and urine[13]. HRPII however persists in the blood stream for up to two weeks[14] after parasite must have been cleared greatly limiting its diagnostic efficacy as a diagnostic target. This persistence has the implication of contributing to the over diagnosis and subsequent over treatment of malaria[15], [16]. pLDH isis the last enzyme in the glycolytic pathway essential for ATP generation. As opposed to HRPII, the amount of pLDH in the blood stream reduces as parasites clear from the blood stream as such it can be used to monitor treatment outcome[17].

The most suitable diagnostic targets, however would be one that accurately tells the current condition of infection viz-a-viz parasite biomass and species identification

IV. CURRENT METHODS IN MALARIA DIAGNOSIS

A. ClinicalDiagnosis

Clinical diagnosis, also known as presumptive diagnosis, is the most practiced method of diagnosing malaria as it is very cheap and rapid[18]. It is based on the signs and symptoms presented by the patient. These signs and symptoms are often non-specific and include fever, headache, dizziness, cold, nausea, pruritus and anorexia [19]. Clinical diagnosis however possess many problems due to the overlap of malaria symptoms with those of other common infections found in endemic regions such as bacterial infections. This overlap of symptoms with other diseases greatly reduces the specificity of clinical diagnosis ergo contributing to the over diagnosis and over treatment of malaria as shown in various studies with one reporting as high as 83% over diagnosis and over treatment of malaria in children in the age group of 1-5 years of age[20], [21]

Clinical diagnosis is most useful in areas where there is no laboratory support which is often the case in many areas in

Copyright © 2016 by Covenant University Press

sub-Saharan Africa, it is especially useful, and has been recommended by the integrated child management initiative(IMCI), in treating children, 5 years and below, in these areas as malaria infections can quickly become fatal for this age group[19], [21]–[23].

The sensitivity of clinical diagnosis is determined by factors such as endemicity, season and age group with accuracy of clinical diagnosis increasing with prevalence and endemicity[19], [21].

The combination of clinical diagnosis with other parasite based methods greatly increases the accuracy of malaria diagnosis.

B. Microscopy

Microscopic examination of Giemsa-stained blood smears remains gold standard for the diagnosis of malaria.Diagnosis is done by the examination of Giemsa stained thin and/or thick blood smear. When performed under optimal conditions by a competent microscopist, microscopy can detect as low as 5 iRBCs, however its sensitivity can go as low as 200iRBCs when conditions are not optimal [24], [25].

Microscopic examination of blood smears allows for the diagnosis of infection in symptomatic and asymptomatic persons depending on the skill of the microscopist, parasitemia and the number of high powered fields observed[26], [27]. It also has the advantage being both qualitative (species, stage identification) and quantitative hence it's preferred use in the monitoring of patient response to treatment. Microscopy, however falls short in that it requires expertise for accurate results, maintenance costs, need for constant supply of electricity and others as summarized in table 1.Sampling preparation also greatly affects the sensitivity of this test as the quality of the film, duration of staining and quality of stain affect the ability to visualize the different stages of the parasite [18].

Recent advances in technology have given rise to new ways of manipulating blood in a bid to concentrate cells before analyses. The exposure of blood samples to a magnetic fields helps concentrate iRBC as a result of the presence of haemozoin[28], [29]. This increases the sensitivity of the test by as much as 100 fold. Other methods to increase sensitivity include the centrifugation of heparinized blood to concentrate the different stages of parasite growth before making a film[30] however, its application in Africa is not well documented.

The advent of power packs and solar batteries have made microscopy more field deployable hence increasing its efficacy in resource poor areas. Nonetheless, there is still a dearth of experienced mciroscopists in the sub-Saharan African region making microscopy a less effective method in these parts [31].

C. ImmunochromatographicRapidDiagnosticTests

The Rapid Diagnostic Test (RDT) is a very effective tool in malaria diagnosis and forms the mainstay of diagnosis in many resource poor areas where there is no access to a laboratory. It is especially useful as it requires no electricity, infrastructure, minimal sample preparation, technical expertise and interpretation of results is relatively easy.

The RDT detects malaria antigens in 5-15 µL of blood using monoclonal antibodies, impregnated on a test strip, specific for the targeted antigens, in an immunochromatographic assay. Test results are interpreted by the absence or presence of a coloured line on the strip and can be obtained in 5-20mins.RDTs detects three plasmodial antigens; *P.falciparum* histidine rich protein II (PfHRPII), *Plasmodium* lactate dehydrogenase (pLDH) and aldolase [17], [32]. Most RDT products incorporate two of these three antigens in a bid to distinguish falciparum from non-falciparum infections.

HRPII antigen is specific for *P.falciparum* and is a major constituent of RDTs in sub-Saharan Africa, it however has the shortcoming of persisting for up to 2 weeks after parasite must have cleared from the blood stream as such it is not effective in monitoring treatment[33], [34]. There have been reports of false negatives from HRPIIRDT kits as a result of a mutated or deleted HRPII gene [35]. It is recommended that regions with more than 10% prevalence of HRPII deletion should seek alternatives such as microscopy for the diagnosis of*P.falciparum* malaria[34]. The presence of the rheumatoid factor has been shown to give false negative results due to a cross reactivity between the factor and HRPII[36], [37]. These issues greatly undermine the suitability of HRPII as an ideal antigen for the diagnosis of malaria as such RDTs containing just HRPII are not advisable for use.pLDH on the other hand is highly effective for monitoring treatment outcomes as it reflects the current status of parasites in the blood stream [17], [38]. pLDH is however not specific to *P.falciparum* hence its combination with HRPII on RDT kits.

RDTs have a detection limit as low as 200-2000 iRBCs/µL[32] depending on the quality of the RDT. The sensitivity of RDTs are affected by such factors as storage conditions, temperature, and time of assay.One pertinent issue militating against the effectiveness of RDTs in sub-Saharan Africa is compliance to manufacturer's instructions and to results. A meta-analysis of health workers compliance showed that compliance to negative results was low contributing to over treatment of malaria [39]

RDTs for sub-Saharan Africa should be able to withstand the conditions found therein. RDTs nonetheless have the unique advantage of being field deployable and is especially effective in resource poor regions.

Copyright © 2016 by Covenant University Press

D. NucleicAcidTechniques

Tests that target nucleic acids have proven to be one of the most specific and sensitive methods with the ability to detect parasitemia of 0.005 iRBC/μL [34]. They especially come in handy in detecting sub-microscopic infections which greatly threaten the elimination strategies that abound. Nucleic acid techniques are not yet field deployable as they are expensive, require expert personnel and specialized equipment as such, their use is limited to clinical research in population diversity studies[40] and monitoring of drug resistance[41].

Nucleic acid techniques target gene sequences such as the 18S ribosomal RNA gene sequences[42], *P.falciparum*stevor multigene family[43], mitochondrial DNA, and telomere-associated repetitive element-2[44]. Nucleic acid techniques include the Polymerase Chain Reaction and its various methods (real time PCR. Nested PCR) which use specific primers to make many copies of a small amount of template DNA, followed by post-PCR methods such as gel electrophoresis. The PCR has the advantages of being able to process many samples at once.

Another nucleic acid technique is the Loop-mediated isothermal amplification (LAMP) technique. This technique has the potential to make nucleic acid strategies field deployable, as it requires lesser instrumentation than PCR and is easy to use. LAMP in contrast to PCR is an isothermal technique requiring a constant temperature for amplification. The amount of DNA produced in LAMP rivals that of PCR as LAMP using two to three sets of primers. Amplification is detected by turbidimetry due to an increasing quantity of magnesium pyrophosphate and as such can be followed in real time[45]. Some studies have reported the technique's ability to detect SMI and sensitivities that surpass that of RDT and rival that found in PCR[45]–[47]. LAMP is easy, sensitive and cheaper than PCR, it however has the downside of the need for cold storage of reagents [18].

V. PROSPECTSINMALARIADIAGNOSIS

Recent efforts in malaria diagnosis have been targeted towards utilizing other non-invasive diagnostic materials in a bid to making testing procedure less painful for most. Saliva[7], [8], [48] and urine[9], [13] have been assessed as diagnostic material with promising results reported thus far.

A cheap urine based RDT with the ability to deliver results in 25 minutes, is currently undergoing evaluation and has shown positive reports with a reported limit of detection of 125parasites/μL[13] which is well within the range recommended by the WHO [32]. If successful, this new tool could finally usher in the era of non-invasive diagnosis of malaria. Some studies have also shown the detection of parasite DNA in saliva and urine howbeit in smaller quantities than those found in the blood[9]. They have however be shown to be suitable alternatives to blood where blood samples are not available [49]–[51].

TABLE 1: Summary of currently used diagnostic techniques

	Advantages	Disadvantages
Clinical Diagnosis	•Useful in resource poor areas •Particularly useful in preventing infant mortality in resource poor regions	• Sensitivity depends on endemicity • Leads to over diagnosis and subsequent over treatment.
Microscopy	•Low Tech, simple and inexpensive •Field Deployable •Identifies parasite itself •Useful in monitoring treatment outcome	• Requires expertise • Labour intensive • Low throughput • Rigorous sample preparation • Requires maintenance
RDT	•Fielddeployable •Easy to use •Requires no expertise •Can detect *P.falciparum* •Zero to minimal sample preparation required.	• Quality control is necessary and expensive • Environmental condition sensitive • Antigen can persist beyond infection • Can't be used to monitor treatment outcome
Nucleic Acid Techniques	•Highly sensitive •Ability to detect SMI •Can be used to investigate population diversity and distinguish between new and recrudescent infections •High throughput	• Time consuming • Expensive • Highly skilled personnel required • Requires provisions to avoid cross contamination • Generally not field deployable

Other strategies seek to exploit the electromagnetic and physical properties of haemozoin crystals formed during the malaria life cycle [28], [52], [53]. Haemozoin based detection strategies have the added advantage of giving adequate diagnosis during sequestration of parasites. They may however not be field deployable as some strategies do not detect haemozoin in early ring stage parasites [54] while most other techniques require specialized equipment and personnel.

Another emerging diagnostic tool are the aptamers. Aptamers are single-stranded oligonucleotides made *in-vitro* which possess a specific three-dimensional structure depending on its sequence [55]. Aptamers interact specifically with their targets by binding to them and altering their activity. Aptamers targeting pLDH have been designed and tested with promising results[56], [57]. It is expected that this technology will be perfected and field deployed in the nearest future.

VI. CONCLUSION

The conventional microscopic examination of blood films remain the gold standard as it is cheap and offers sensitivity unrivalled by any other field deployable method.RDTs are convenient but could lead to over diagnosis and over treatment. Nucleic acid techniques offer the best sensitivities, they are however expensive, out of reach to most and are limited to clinical research.Getting to zero would require

 Copyright © 2016 by Covenant University Press

diagnostic tools that are non-invasivehighly sensitive, robust and suited to the conditions found in the endemic regions.

REFERENCES

[1] WHO (World Health Organization), "Health in 2015: from MDGs, Millennium Development Goals to SDGs, Sustainable Development Goals.," Geneva, 2015.

[2] WHO, "Global technical strategy for malaria 2016-2030," Geneva, 2015.

[3] WHO, "World Malaria Report," Geneva, 2015.

[4] L. H. Bannister and I. W. Sherman, "*Plasmodium*," in *Encyclopedia of Life Sciences*, no. December, Chichester, UK: John Wiley & Sons, Ltd, 2009.

[5] WHO (World Health Organization), "Malaria," 2016. [Online]. Available: http://www.who.int/mediacentre/factsheets/fs094/en/. [Accessed: 25-Mar-2016].

[6] S. Bhatt, D. J. Weiss, E. Cameron, D. Bisanzio, B. Mappin, U. Dalrymple, K. E. Battle, C. L. Moyes, A. Henry, P. A. Eckhoff, E. A. Wenger, O. Briet, M. A. Penny, T. A. Smith, A. Bennett, J. Yukich, T. P. Eisele, J. T. Griffin, C. A. Fergus, M. Lynch, F. Lindgren, J. M. Cohen, C. L. J. Murray, D. L. Smith, S. I. Hay, R. E. Cibulskis, and P. W. Gething, "The effect of malaria control on Plasmodium falciparum in Africa between 2000 and 2015," *Nature*, vol. 526, no. 7572, pp. 207–211, Sep. 2015.

[7] G. O. Gbotosho, C. T. Happi, O. Folarin, O. Keyamo, A. Sowunmi, and A. M. J. Oduola, "Rapid Detection of Lactate Dehydrogenase and Genotyping of Plasmodium falciparum in Saliva of Children with Acute Uncomplicated Malaria," *Am. J. Trop. Med. Hyg.*, vol. 83, no. 3, pp. 496–501, 2010.

[8] D. Visanuvimol, "Saliva as a proven , non-invasive sample type for molecular malaria testing and surveillance using OMNIgene ® • DISCOVER at ambient temperatures," no. 2, pp. 9–11, 2014.

[9] D. C. Nwakanma, N. Gomez-Escobar, M. Walther, S. Crozier, F. Dubovsky, E. Malkin, E. Locke, and D. J. Conway, "Quantitative detection of Plasmodium falciparum DNA in saliva, blood, and urine.," *J. Infect. Dis.*, vol. 199, no. 11, pp. 1567–1574, 2009.

[10] O. J. Pooe, A. Shonhai, and S. Mharakurwa, "Optimization of a polymerase chain reaction based assay for the detection of malaria in human saliva samples," *Malar. J.*, vol. 9, no. Suppl 2, p. P38, 2010.

[11] M. E. Parra, C. B. Evans, and D. W. Taylor, "Identification of Plasmodium falciparum histidine-rich protein 2 in the plasma of humans with malaria," *J. Clin. Microbiol.*, vol. 29, no. 8, pp. 1629–1634, Aug. 1991.

[12] R. J. Howard, S. Uni, M. Aikawa, S. B. Aley, J. H. Leech, A. M. Lew, T. E. Wellems, J. Rener, and D. W. Taylor, "Secretion of a malarial histidine-rich protein (Pf HRP II) from Plasmodium falciparum-infected erythrocytes," *J. Cell Biol.*, vol. 103, no. 4, pp. 1269–1277, Oct. 1986.

[13] T. Oguonu, E. Shu, B. U. Ezeonwu, B. Lige, A. Derrick, R. E. Umeh, and E. Agbo, "The performance evaluation of a urine malaria test (UMT) kit for the diagnosis of malaria in individuals with fever in south-east Nigeria: cross-sectional analytical study.," *Malar. J.*, vol. 13, no. 1, p. 403, 2014.

[14] M. Mayxay, S. Pukrittayakamee, K. Chotivanich, S. Looareesuwan, and N. J. White, "Persistence of Plasmodium falciparum HRP-2 in successfully treated acute falciparum malaria," *Trans. R. Soc. Trop. Med. Hyg.*, vol. 95, no. 2, pp. 179–182, Jan. 2001.

[15] A. L. Kilauzi, J. G. Tshikuka, M. G. Mjungu, R. Matchaba-Hove, R. Tapera, N. S. Magafu, and J. J. Muyembe, "SD Bioline malaria antigen Pf (HRP-2/pLHD) for assessing efficacy of artemisinin combination therapy against Plasmodium falciparum in pedriatric patients in the Democratic Republic of the Congo," *Pan Afr. Med. J.*, vol. 22, pp. 1–7, 2015.

[16] T. D. Swarthout, H. Counihan, R. K. K. Senga, and I. van den Broek, "Paracheck-Pf accuracy and recently treated Plasmodium falciparum infections: is there a risk of over-diagnosis?," *Malar J*, vol. 6, p. 58, 2007.

[17] M. H. Nyunt, M. P. Kyaw, K. K. Win, K. M. Myint, and K. M. Nyunt, "Field evaluation of HRP2 and pan pLDH-based immunochromatographic assay in therapeutic monitoring of uncomplicated falciparum malaria in Myanmar.," *Malar. J.*, vol. 12, p. 123, Jan. 2013.

[18] N. Tangpukdee, C. Duangdee, P. Wilairatana, and S. Krudsood, "Malaria diagnosis: A brief review," *Korean J. Parasitol.*, vol. 47, no. 2, pp. 93–102, 2009.

[19] D. Chandramohan, S. Jaffar, and B. Greenwood, "Use of clinical algorithms for diagnosing malaria," *Trop. Med. Int. Heal.*, vol. 7, no. 1, pp. 45–52, 2002.

[20] O. O. Oladosu and W. a. Oyibo, "Overdiagnosis and Overtreatment of Malaria in Children That Presented with Fever in Lagos, Nigeria," *ISRN Infect. Dis.*, vol. 2013, pp. 1–6, 2013.

[21] T. W. Mwangi, M. Mohammed, H. Dayo, R. W. Snow, and K. Marsh, "Clinical algorithms for malaria diagnosis lack utility among people of different age groups," *Trop. Med. Int. Heal.*, vol. 10, no. 6, pp. 530–536, Jun. 2005.

[22] A. Nicoll, "Integrated management of childhood illness in resource-poor countries: an initiative from the World Health Organization.," *Trans. R. Soc. Trop. Med. Hyg.*, vol. 94, no. 1, pp. 9–11, Jan. 2000.

[23] WHO (World Health Organization), "New Perspectives. Report of a Joint WHO/USAID Informal Consultation, October 25-27, 2000.," Geneva, 2000.

[24] F. Joanny, S. J. Löhr, T. Engleitner, B. Lell, and B. Mordmüller, "Limit of blank and limit of detection of Plasmodium falciparum thick blood smear microscopy in a routine setting in Central Africa.," *Malar. J.*, vol. 13, no. 1, p. 234, 2014.

[25] C. Ohrt, W. P. O'Meara, S. Remich, P. McEvoy, B. Ogutu, R. Mtalib, and J. S. Odera, "Pilot assessment of the sensitivity of the malaria thin film.," *Malar. J.*, vol. 7, no. 1, p. 22, Jan. 2008.

[26] C. Ohrt, Purnomo, M. A. Sutamihardja, D. Tang, and K. C. Kain, "Impact of microscopy error on estimates of protective efficacy in malaria-prevention trials.," *J. Infect. Dis.*, vol. 186, no. 4, pp. 540–546, 2002.

[27] S. C. Murphy, J. P. Shott, S. Parikh, P. Etter, W. R. Prescott, and V. A. Stewart, "Review article: Malaria diagnostics in clinical trials," *Am. J. Trop. Med. Hyg.*, vol. 89, no. 5, pp. 824–839, 2013.

[28] S. Karl, M. David, L. Moore, B. T. Grimberg, P. Michon, I. Mueller, M. Zborowski, and P. a Zimmerman, "Enhanced detection of gametocytes by magnetic deposition microscopy predicts higher potential for Plasmodium falciparum transmission.," *Malar. J.*, vol. 7, p. 66, 2008.

[29] P. A. Zimmerman, J. M. Thomson, H. Fujioka, W. E. Collins, and M. Zborowski, "Diagnosis of malaria by magnetic deposition microscopy," *Am. J. Trop. Med. Hyg.*, vol. 74, no. 4, pp. 568–572, Apr. 2006.

[30] M. J. Pinto, S. R. Rodrigues, R. Desouza, and M. P. Verenkar, "Usefulness of quantitative buffy coat blood parasite detection system in diagnosis of malaria.," *Indian J. Med. Microbiol.*, vol. 19, no. 4, pp. 219–21, Jan. 2001.

[31] P. Mukadi, V. Lejon, B. Barbé, P. Gillet, C. Nyembo, A. Lukuka, J. Likwela, C. Lumbala, J. Mbaruku, W. Vander Veken, D. Mumba, P. Lutumba, J.-J. Muyembe, and J. Jacobs, "Performance of microscopy for the diagnosis of malaria and human African trypanosomiasis by diagnostic laboratories in the Democratic Republic of the Congo: results of a nation-wide external quality assessment," *PLoS One*, vol. 11, no. 1, p. e0146450, Jan. 2016.

[32] WHO, "Malaria Rapid Diagnostic Test Performance. Summary results of WHO product testing of malaria RDTs: Round 1-5 (2008-2013)," Geneva, 2012.

[33] J. C. Mouatcho and J. P. Dean Goldring, "Malaria rapid diagnostic tests: Challenges and prospects," *J. Med. Microbiol.*, vol. 62, no. PART10, pp. 1491–1505, 2013.

[34] P. A. Zimmerman and R. E. Howes, "Malaria diagnosis for malaria elimination.," *Curr. Opin. Infect. Dis.*, vol. 28, no. 5, pp. 446–454, 2015.

[35] O. A. Koita, O. K. Doumbo, A. Ouattara, L. K. Tall, A. Konar??, M. Diakit??, M. Diallo, I. Sagara, G. L. Masinde, S. N. Doumbo, A. Dolo, A. Tounkara, I. Traor??, and D. J. Krogstad, "False-negative rapid diagnostic tests for malaria and deletion of the histidine-rich repeat region of the hrp2 gene," *Am. J. Trop. Med. Hyg.*, vol. 86, no. 2, pp. 194–198, Feb. 2012.

[36] J. Iqbal, A. Sher, and A. Rab, "Plasmodium falciparum histidine-rich protein 2-based immunocapture diagnostic assay for malaria: Cross-reactivity with rheumatoid factors," *J. Clin. Microbiol.*, vol. 38, no. 3, pp. 1184–1186, Mar. 2000.

[37] J.-H. Lee, J. W. Jang, C. H. Cho, J. Y. Kim, E. T. Han, S. G. Yun, and C. S. Lim, "False-Positive Results for Rapid Diagnostic Tests for

Copyright © 2016 by Covenant University Press

Malaria in Patients with Rheumatoid Factor," *J. Clin. Microbiol.*, vol. 52, no. 10, pp. 3784–3787, 2014.

[38] H. Hopkins, W. Kambale, M. R. Kamya, S. G. Staedke, G. Dorsey, and P. J. Rosenthal, "Comparison of HRP2- and pLDH-based rapid diagnostic tests for malaria with longitudinal follow-up in Kampala, Uganda," *Am. J. Trop. Med. Hyg.*, vol. 76, no. 6, pp. 1092–1097, Jun. 2007.

[39] A. N. Kabaghe, B. J. Visser, R. Spijker, K. S. Phiri, M. P. Grobusch, and M. van Vugt, "Health workers' compliance to rapid diagnostic tests (RDTs) to guide malaria treatment: a systematic review and meta-analysis," *Malar. J.*, vol. 15, no. 1, p. 163, Dec. 2016.

[40] C. T. Happi, G. O. Gbotosho, A. Sowunmi, C. O. Falade, D. O. Akinboye, L. Gerena, D. E. Kyle, W. Milhous, D. F. Wirth, and A. M. J. Oduola, "Molecular analysis of Plasmodium falciparum recrudescent malaria infections in children treated with chloroquine in Nigeria," *Am. J. Trop. Med. Hyg.*, vol. 70, no. 1, pp. 20–26, 2004.

[41] O. A. Folarin, G. O. Gbotosho, A. Sowunmi, O. O. Olorunsogo, A. M. J. Oduola, and T. C. Happi, "Chloroquine Resistant Plasmodium falciparum in Nigeria: Relationship between pfcrt and pfmdr1 Polymorphisms, In-Vitro Resistance and Treatment Outcome.," *Open Trop. Med. J.*, vol. 1, no. 1, pp. 74–82, Oct. 2008.

[42] M. Rougemont, M. Van Saanen, R. Sahli, H. P. Hinrikson, J. Bille, and K. Jaton, "Detection of four Plasmodium species in blood from humans by 18S rRNA gene subunit-based and species-specific real-time PCR assays," *J. Clin. Microbiol.*, vol. 42, no. 12, pp. 5636–5643, Dec. 2004.

[43] M. Niang, Y. Y. Xue, and P. R. Preiser, "The Plasmodium falciparum STEVOR multigene family mediates antigenic variation of the infected erythrocyte," *PLoS Pathog.*, vol. 5, no. 2, p. e1000307, Feb. 2009.

[44] N. Hofmann, F. Mwingira, S. Shekalaghe, L. J. Robinson, I. Mueller, and I. Felger, "Ultra-Sensitive Detection of Plasmodium falciparum by Amplification of Multi-Copy Subtelomeric Targets," *PLoS Med.*, vol. 12, no. 3, p. e1001788, Mar. 2015.

[45] J. Cook, B. Aydin-Schmidt, I. J. González, D. Bell, E. Edlund, M. H. Nassor, M. Msellem, A. Ali, A. K. Abass, A. Mårtensson, and A. Björkman, "Loop-mediated isothermal amplification (LAMP) for point-of-care detection of asymptomatic low-density malaria parasite carriers in Zanzibar," *Malar. J.*, vol. 14, no. 1, p. 43, Jan. 2015.

[46] M. Sema, A. Alemu, A. Bayih, S. Getie, G. Getnet, D. Guelig, R. Burton, P. LaBarre, and D. R. Pillai, "Evaluation of non-instrumented nucleic acid amplification by loop-mediated isothermal amplification (NINA-LAMP) for the diagnosis of malaria in Northwest Ethiopia," *Malar. J.*, vol. 14, no. 1, p. 44, Jan. 2015.

[47] D. H. Paris, M. Imwong, A. M. Faiz, M. Hasan, E. Bin Yunus, K. Silamut, S. J. Lee, N. P. J. Day, and A. M. Dondorp, "Loop-mediated isothermal PCR (LAMP) for the diagnosis of falciparum malaria," *Am. J. Trop. Med. Hyg.*, vol. 77, no. 5, pp. 972–976, Nov. 2007.

[48] P. Buppan, C. Putaporntip, U. Pattanawong, S. Seethamchai, and S. Jongwutiwes, "Comparative detection of Plasmodium vivax and Plasmodium falciparum DNA in saliva and urine samples from symptomatic malaria patients in a low endemic area," *Malar. J.*, vol. 9, no. 1, p. 72, 2010.

[49] O. J. Pooe, A. Shonhai, and S. Mharakurwa, "A PCR screen for malaria carrier infections using human saliva samples," *African J. Microbiol. Res.*, vol. 5, no. 28, pp. 5120–5126, Nov. 2011.

[50] C. Putaporntip, P. Buppan, and S. Jongwutiwes, "Improved performance with saliva and urine as alternative DNA sources for malaria diagnosis by mitochondrial DNA-based PCR assays," *Clin. Microbiol. Infect.*, vol. 17, no. 10, pp. 1484–1491, Oct. 2011.

[51] Y. Jian-hai and X. Zhi-gui, "Noninvasive sampling of saliva as an alternative way for malaria diagnosis: a systematic review," *Sci. J. ...*, vol. 2013, Oct. 2013.

[52] J. L. Burnett, J. L. Carns, and R. Richards-Kortum, "In vivo microscopy of hemozoin: towards a needle free diagnostic for malaria.," *Biomed. Opt. Express*, vol. 6, no. 9, pp. 3462–74, 2015.

[53] A. Orbán, Á. Butykai, A. Molnár, Z. Pröhle, G. Fülöp, T. Zelles, W. Forsyth, D. Hill, I. Müller, L. Schofield, M. Rebelo, T. Hänscheid, S. Karl, and I. Kézsmárki, "Evaluation of a novel magneto-optical method for the detection of malaria parasites.," *PLoS One*, vol. 9, no. 5, p. e96981, May 2014.

[54] C. Delahunt, M. P. Horning, B. K. Wilson, J. L. Proctor, and M. C. Hegg, "Limitations of haemozoin-based diagnosis of Plasmodium falciparum using dark-field microscopy.," *Malar. J.*, vol. 13, no. 1, p. 147, 2014.

[55] M. Moreno and V. M. González, "Advances on aptamers targeting Plasmodium and trypanosomatids.," *Curr. Med. Chem.*, vol. 18, no. 32, pp. 5003–10, Jan. 2011.

[56] M. Godonoga, T.-Y. Lin, A. Oshima, K. Sumitomo, M. S. L. Tang, Y.-W. Cheung, A. B. Kinghorn, R. M. Dirkzwager, C. Zhou, A. Kuzuya, J. A. Tanner, and J. G. Heddle, "A DNA aptamer recognising a malaria protein biomarker can function as part of a DNA origami assembly.," *Sci. Rep.*, vol. 6, p. 21266, Jan. 2016.

[57] S. Lee, K.-M. Song, W. Jeon, H. Jo, Y.-B. Shim, and C. Ban, "A highly sensitive aptasensor towards Plasmodium lactate dehydrogenase for the diagnosis of malaria.," *Biosens. Bioelectron.*, vol. 35, no. 1, pp. 291–6, May 2012.

 Copyright © 2016 by Covenant University Press