Review Article

Rapid Diagnosis of Malaria

Clinton K. Murray and Jason W. Bennett

Infectious Disease Service, Brooke Army Medical Center, 3851 Roger Brooke Drive, Fort Sam Houston, TX 78234, USA

Correspondence should be addressed to Clinton K. Murray, clinton.murray@amedd.army.mil

Malaria's global impact is expansive and includes the extremes of the healthcare system ranging from international travelers returning to nonendemic regions with tertiary referral medical care to residents in hyperendemic regions without access to medical care. Implementation of prompt and accurate diagnosis is needed to curb the expanding global impact of malaria associated with ever-increasing antimalarial drug resistance. Traditionally, malaria is diagnosed using clinical criteria and/or light microscopy even though both strategies are clearly inadequate in many healthcare settings. Hand held immunochromatographic rapid diagnostic tests (RDTs) have been recognized as an ideal alternative method for diagnosing malaria. Numerous malaria RDTs have been developed and are widely available; however, an assortment of issues related to these products have become apparent. This review provides a summary of RDT including effectiveness and strategies to select the ideal RDT in varying healthcare settings.

1. Introduction

According to the World Malaria Report released by the World Health Organization (WHO) in 2008, there were 247 million malaria cases among 3.3 billion people at risk in 2006 from 109 countries resulting in an estimated 881,000 deaths. These deaths were primarily in Africa (91%) and in children under 5 years of age (85%) [1]. Effective management of malaria per the WHO has focused on long-lasting insecticidal nets, indoor residual spraying of insecticide, intermittent preventive therapy in pregnancy, and artemisinin-based combination therapy (ACT). Fundamental to improving the care of patients infected with malaria is prompt and accurate diagnosis in order to prevent excess morbidity and mortality while avoiding unnecessary use of antimalarial agents and minimizing the spread of resistance to antimalarial drugs. Diagnostic strategies need to be effective not only in resource-limited areas where malaria has a substantial burden on society but also in developed countries where expertise in the diagnosis of malaria is frequently lacking [2, 3].

Historical strategies to diagnose malaria emphasize clinical diagnostic algorithms, light microscopy, and empiric therapy. The accuracy of clinical diagnosis, the most commonly employed method, is poor, even in countries with high incidence rates of malaria due to overlapping clinical symptoms with other tropical diseases and the fact that coinfections can occur [4–11]. In an era when chloroquine therapy was widely effective and the primary antimalarial agent used around the world, definitive diagnosis was not necessary. Today, given the paucity of effective antimalarial agents with widespread use of ACT, an increased emphasis on diagnosis is necessary. This is especially true now that artesunate failures have been noted [12]. Microscopy remains the gold standard for detection of malaria parasitemia as it can provide information on both the species of parasite and parasite density of infection [13]. However, the procedure is labor-intensive and time-consuming, requiring substantial training and expertise due to fleeting skills [14–26]. These problems are magnified in nonendemic regions where light microscopy to diagnose malaria is infrequently performed, resulting in missed diagnosis, misidentification of *Plasmodium* species, and therapeutic delays [19]. Methods using advances in technology have been evaluated as alternatives to light microscopy. While these methods have varying strengths and weaknesses, they are limited by equipment, supplies, expertise, cost, time,

applicability in acute infection, and/or availability [13, 27].

New immunochromatographic rapid diagnostic tests (RDTs) for malaria were introduced in the early 1990s but have suffered from numerous problems as recently reviewed [13, 27–31]. Strategies to improve the impact of malaria RDT have included The Special Programme for Research and Training in Tropical Diseases which has introduced principles for development and evaluation of diagnostic tests for infectious diseases [32]. The WHO has also undertaken a malaria RDT evaluation program to improve the overall impact of these tests (http://www.wpro.who.int/sites/rdt/who_rdt_evaluation/ accessed 19 January 2009)

Requirements for malaria RDT vary based on malaria local epidemiology and the goals of a malaria control program; focusing on performance and operational characteristics (Table 1) [33, 34]. Expectations of an ideal malaria RDT are minimal operator training, ease of platform with minimal steps, reproducibility of results, rapid availability of results (<20 minutes), and low cost. Ideally, the test should be able to detect recrudescence or relapse and clearance of parasitemia if therapeutic monitoring is necessary (Table 1). The WHO has recommended a minimum standard of 95% sensitivity at parasite densities of 100/uL [33]. In Sub-Saharan Africa, malaria RDT needs a high sensitivity for *Plasmodium falciparum*, but specificity is required to avert inflated estimates of the burden of malaria, misperceptions of inadequate therapeutic responses when fever is due to other illnesses, and unnecessary drug pressure. High specificity and the ability to detect *nonfalciparum Plasmodium* species are necessary in low-incidence regions due to low rates of malaria, and the lower virulence of these species is allowing for repeat testing. The quality of manufacturing and reproducibility of the test results are critical. In addition, field conditions require the RDT to be stable under extremes of temperatures and humidity during use and storage.

2. Malaria Rapid Diagnostic Tests

Malaria RDT employ lateral flow immunochromatographic technology similar to rapid pregnancy tests. In these assays, the clinical sample migrates as a liquid across the surface of a nitrocellulose membrane by capillary action [13, 27, 33, 34]. For a given targeted parasite antigen, two sets of monoclonal or polyclonal antibodies are used, a capture antibody and a detection antibody. Monoclonal antibodies in contrast to polyclonal antibodies can be very specific but less sensitive. Also, the source of antigen used (purified native protein, recombinant proteins, or peptides) can make significant differences in the performance characteristics of RDT.

The malaria antigens currently used as diagnostic targets are either specific to a *Plasmodium* species or are conserved across all four of the human malaria parasites. Falciparum-specific monoclonals include histidine-rich protein-2 (HRP-2) and *P. falciparum* lactate dehydrogenase (pfLDH) [32, 33]. Targets conserved across all human malaria have been identified on lactate dehydrogenase (pLDH) and aldolase enzymes [35–38].

HRP-2 is a *P. falciparum*-specific water-soluble protein, localized in the parasite cytoplasm and on the surface membrane of infected erythrocyte. It is present on protrusions, known as knobs, thought to account for sequestration of the trophozoites and schizonts in postcapillary venules. There is increasing concentration of HRP-2 as the parasite advances from ring stage to trophozoite, and it readily diffuses into the plasma [39, 40]. HRP-2 is predominately found in the asexual stages, but it is also found in young *P. falciparum* gametocytes. This possibly allows detection at lower parasitemias and at detectable levels 28 days after clinical presentation, well after resolution of symptoms and apparent clearance of parasites from patients [41–44]. Therefore, this antigen has not yet proven valuable in monitoring response to therapy. Mutants can escape recognition by monoclonal antibodies and may be responsible for false negative tests [45, 46]. An assessment of HRP-2 from nineteen countries revealed that only 84% of *P. falciparum* could be detected.

Another antigen target to detect sexual and asexual stage malaria parasites is Plasmodium lactose dehydrogenase (pLDH), which is the final enzyme in the malaria parasite's glycolytic pathway. Monoclonal antibodies against pLDH can target all human malaria species or can specifically differentiate *P. falciparum* or *P. vivax* [36]. Aldolase, another key enzyme in the glycosis pathway conserved across all malaria parasites, can be used as a universal antigen target [47, 48]. Other antigens have been recognized as possible components of future diagnostic tests, but evaluations of *P. ovale*- or *P. malariae*-specific antigens have not been widely tested [38, 49, 50]. Aldolase and pLDH rapidly fall to undetectable levels after initiation of effective therapy, however they are expressed in gametocytes, as does HRP-2, which may allow detection of *P. falciparum* after the clinical infection is cleared [51].

2.1. Available Malaria Rapid Diagnostic Tests. Numerous reviews have highlighted the rapid turnover of commercially available products and varying quality control issues in manufacture and product stability [13, 27–29, 52]. Articles in peer-reviewed journals of independent evaluations have not existed for many products. In addition, there are numerous methodological flaws associated with many of the published evaluations limiting the ability to compare malaria RDT [13, 27]. The WHO has listed online RDT manufacturers and distributors. To be included in these summaries requires evidence of good manufacturing practices as documented by either compliance with ISO 13485:2003 or 21 CFR 820 from the US Food and Drug Administration. Overall it appears that RDTs using HRP-2 are generally more sensitive than falciparum-specific pLDH for diagnosing infections caused by *P. falciparum* when using RDT. However, data assessing the utility of pLDH and aldolase in nonfalciparum infections is limited.

Currently the BinaxNOW® Malaria test kit is the only US FDA approved kit. It is based upon the HRP-2 and aldolase antigens (Binax, INC., Inverness Medical

TABLE 1: Ideal requirements for a malaria rapid diagnostic test (RDT).

Common requirements		
Rapid results (<20 minutes)		
Easy to use with minimal training and simple instructions		
Environmentally stable device (heat, humidity, air movement, lighting) during use and storage		
Reproducible results including quality manufacturing		
Detect below parasite density of <100 parasites/μL with appropriate specificity		
Region-specific requirements		
	Sub-Saharan Africa	Other malaria endemic areas	Malaria free countries
P. falciparum-specific	+++	−	−
Detects all human malaria	+	+++	+++
Plasmodium species specific	+	+++	+++
Able to detect mixed infections	+	+++	+++
High sensitivity (< 50 parasites/μL)	−	+++	+++
High specificity	−	+++	+++
Semi-quantitative	−	−	+++
Able to monitor response to therapy	+	+++ (if drug-resistant *P. falciparum*)	+
Assay specifications:			
ICH GMP	−	−	+++
Stable to 40°C	+++	+++	−
Long shelf life	+++	+++	+++
Point-of-care use (CLIA waived)	−	−	+++
Cost	< $1 per test	$1–3 per test	<microscopy (∼ $20/test)

+++ (high priority), + (low priority), − (not necessary), International Conference on Harmonization (ICH); Good Manufacturing Practices (GMP); Clinical Laboratory Improvement Amendment (CLIA).

Professional Diagnostic, Scarborough, Me, USA). One large trial revealed an overall sensitivity of 82% [44]. The overall specificity for *P. falciparum* was 94% [44]. Again, using the BinaxNOW Malaria test kit, a second trial primarily assessed the utility of finger-stick versus venipuncture obtained blood samples [53]. The finger-stick technique revealed an overall sensitivity of 100% for *P. falciparum* and 83% for *P. vivax*. Venipuncture produced similar results to fingerstick for the detection of *P. falciparum* and *P. vivax* (Table 2). According to the package insert, the overall sensitivity and specificity are 95% and 94% for *P. falciparum*, 69% and 100% for *P. vivax*, respectively (Table 2). The sensitivity for detecting *P. malariae* was 44% (7 of 16 positive samples) and 50% for *P. ovale* (1 of 2 positive samples), although these numbers were too small to determine reliable sensitivity and specificity. Although the kit is not approved for diagnosing mixed *P. falciparum* and *P. vivax* infections, the sensitivity was 94%. No clear data exists for using RDT to detect a recently described species of malaria, *P. knowlesi*, that has been reported to infect humans [54].

Overall, the BinaxNOW® Malaria test kit meets many of the needs required of an ideal malaria diagnostic platform; however, it has also a number of limitations including the FDA indications comment that this kit is only for use in laboratories that have or can acquire blood samples containing *P. falciparum* for use as a positive control. It is also approved for use in the evaluation of symptomatic patients, with negative results requiring confirmation by thick and thin smears. Therefore individual clinicians or

patients themselves might not have rapid results to initiate immediate therapy. Other limitations include the kit's ability to detect viable and nonviable malaria organisms, including gametocytes and sequestered *P. falciparum* parasites. In some settings, such as pregnancy, this is possibly advantageous; however, this prohibits monitoring the level of parasitemia, which is often used in management decisions. Additionally this prevents the monitoring of therapeutic response as antigens persist after elimination of the parasite. Finally, positive rheumatoid factor has been associated with false positive results. Overall, many of these limitations also plague other RDT [13, 27].

2.2. Applicability of Malaria Rapid Diagnostic Tests. It has been estimated that 16 million RDTs were delivered in 2006 of which 10.8 million were in Africa and 2.8 million in India [1]. Malaria RDTs are used at almost every level of the healthcare system. Most of the data supports using these devices in settings where trained personnel perform the assay in targeted adult patient populations presenting with a febrile illness. There is limited data in children [44]. Among pregnant women, *P. falciparum* malaria is associated with placental sequestration of parasites that can reduce the sensitivity of microscopic diagnosis. In this clinical scenario, the detection of HRP-2 might improve diagnostic capability as this antigen is recovered peripherally [55]. However, the relevance of persistent HRP-2 antigen for up to a month after therapy is unclear in this setting. Overall, the ideal setting for these devices would include use by village workers

TABLE 2: Performance characteristics of the BinaxNOW® malaria kit for *Plasmodium falciparum* and *P. vivax*(data obtained from package insert).

Parasitemia level (per vL)	*Plasmodium falciparum* Percent sensitivity (95% confidence interval)	*Plasmodium vivas* Percent sensitivity (95% confidence interval) %
>5000	99.7% (98%–100%)	93.5% (91%–96%)
1000–5000	99.2% (96%–100%)	81.0% (76%–85%)
500–1000	92.6% (76%–99%)	47.4% (36%–59%)
100–500	89.2% (75%–97%)	23.6% (17%–31%)
0–100	53.9% (37%–70%)	6.2% (3%–12%)
Overall	95.3% (93%–97%)	68.9% (66%–72%)
Specificity	94.2% (93%–95%)	99.8% (99%–100%)
Venous versus fingerstick samples	100% (96–100%) versus 98.8% (94–100%)	81.6% (74–87%) versus 80.6% (73–87%)
Specificity	94.7% (93–96%) versus 90.4% (88-92%)	99.7% (99–100%) versus 99.5% (99–100%)

without formal medical laboratory training, or travelers for self-diagnosis and treatment; however, there is conflicting evidence on the utility of RDT in these settings [56–59]. The diagnosis of *P. falciparum* has been made on convalescent serologic (day 14–21 after febrile illness) or post-mortem assessments, all supporting possible diagnosis after initiation of empiric therapy [13, 60].

Other possible indications for malaria RDT could include malaria prevalence surveys; however, they are insensitive for use in asymptomatic screening and high throughput detection cannot be achieved with individually packaged RDT [17, 61–64]. Currently the American Red Cross does not screen blood donation units for malaria, instead deferring donations based upon exposure risks.

2.3. Selection of Malaria Rapid Diagnostic Tests. Selection of specific malaria RDT is based upon region specific criteria including the expected health benefit, implementation plans, monitoring process, and cost with a focus on expected species of infection, level of parasitemia, and treatment paradigms. Parasitological confirmation of the diagnosis of malaria is recommended in all cases except for children under 5 years of age residing in areas of high prevalence of *P. falciparum*. It is unclear if the risk of not treating false-negative tests outweighs the benefits of empiric therapy.

The WHO has typically outlined 3 broad zones for selecting devices. Zone 1 occurs primarily in Sub-Saharan Africa and in lowland Papua New Guinea where infections occur with *P. falciparum* only or where nonfalciparum species occur as coinfections with *P. falciparum*. The HRP-2-based kits are probably best in this region because of overall improved antigen detection for *P. falciparum*. Zone 2 occurs in endemic areas of Asia, the Americas, and in isolated areas in Africa specifically the Ethiopian highlands, where falciparum and nonfalciparum malaria typically cocirculate. RDT in these regions will need to distinguish between falciparum and nonfalciparum infections. Zone 3 contains areas with nonfalciparum malaria only; including the *P. vivax* areas of East Asia and Central America. Here, RDT should focus on *P. vivax*-specific or pan-Plasmodium specific antigen detection without a need to detect or differentiate falciparum. Even in *P. falciparum* predominate regions, it

is possible for 1–10% of patients to be coinfected including cases requiring anti-relapse therapy with primaquine [65].

Although the major burden of malaria is in endemic countries, malarious regions are frequent destinations of the roughly 900 million yearly international travelers (www.unwto.org/index.php, accessed 24 January 2009). These travelers might require management of their malaria infection while abroad or upon returning home; however, laboratory personnel in nonendemic regions often lack experience or expertise in microscopic diagnosis of malaria [66]. Based upon a large meta-analysis of malaria RDT use in travelers, RDT may be an effective adjunct to microscopy in centers without substantial expertise in tropical medicine [52, 67]. However, expert microscopy is still needed for species identification and confirmation. Strategies need to be developed to determine how best to evaluate and field malaria RDT for use in nonendemic regions and for travelers who will be on holiday for prolonged periods of time in highly endemic regions.

3. Conclusions

Malaria is a life-threatening infection impacting the most developed countries of the world along with regions of the world lacking basic healthcare infrastructure. Increasing burden of disease, emerging antimalarial drug resistance, and broad implementation of ACT are placing greater emphasis on rapid and accurate diagnosis of patients infected with malaria. Given the difficulty performing microscopy, especially in endemic areas, alternative diagnostic strategies are needed. A highly effective RDT could avert over 100.000 malaria related deaths and about 400 million unnecessary treatments [68]. In addition, it is likely that RDTs will be cost-effective due to improved treatment and health outcomes for febrile disease not due to malaria along with cost savings associated with antimalarial drugs [69]. Although there is now an FDA approved malaria RDT, RDTs have limitations to include the inability to detect mixed infections, all species of *Plasmodium*, and infections at low concentrations of parasites, along with an inability to monitor response to therapy. In addition, in the case of a negative result, microscopy is still recommended. Therefore

RDTs do not eliminate the need to obtain thick and thin smears, and maintaining expertise in microscopy is still a global priority until a new gold-standard is developed. However, malaria RDTs are ushering in a new era of diagnosis to improve the overall global healthcare system.

Disclaimer

The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or reflecting the views of the US Department of the Army, the US Department of Defense, or the US government. The authors are employees of the US government and this work was performed as part of their official duties. As such, there is no copyright to be transferred.

References

[1] WHO, World Malaria Report, 2008.

[2] D. Bell, C. Wongsrichanalai, and J. W. Barnwell, "Ensuring quality and access for malaria diagnosis: how can it be achieved?" *Nature Reviews Microbiology*, vol. 4, no. 9, supplement, pp. 682–695, 2006.

[3] H. Reyburn, H. Mbakilwa, R. Mwangi, et al., "Rapid diagnostic tests compared with malaria microscopy for guiding outpatient treatment of febrile illness in Tanzania: randomised trial," *British Medical Journal*, vol. 334, no. 7590, p. 403, 2007.

[4] D. Chandramohan, S. Jaffar, and B. Greenwood, "Use of clinical algorithms for diagnosing malaria," *Tropical Medicine & International Health*, vol. 7, no. 1, pp. 45–52, 2002.

[5] J. A. Berkley, K. Maitland, I. Mwangi, et al., "Use of clinical syndromes to target antibiotic prescribing in seriously ill children in malaria endemic area: observational study," *British Medical Journal*, vol. 330, no. 7498, pp. 995–999, 2005.

[6] R. P. Peters, E. E. Zijlstra, M. J. Schijffelen, et al., "A prospective study of bloodstream infections as cause of fever in Malawi: clinical predictors and implications for management," *Tropical Medicine & International Health*, vol. 9, no. 8, pp. 928–934, 2004.

[7] C. Wongsrichanalai, C. K. Murray, M. Gray, et al., "Co-infection with malaria and leptospirosis," *The American Journal of Tropical Medicine and Hygiene*, vol. 68, no. 5, pp. 583–585, 2003.

[8] J. R. M. Armstrong-Schellenberg, T. Smith, P. L. Alonso, and R. J. Hayes, "What is clinical malaria? Finding case definitions for field research in highly endemic areas," *Parasitology Today*, vol. 10, no. 11, pp. 439–442, 1994.

[9] C. Luxemburger, F. Nosten, D. E. Kyle, L. Kiricharoen, T. Chongsuphajaisiddhi, and N. J. White, "Clinical features cannot predict a diagnosis of malaria or differentiate the infecting species in children living in an area of low transmission," *Transactions of the Royal Society of Tropical Medicine and Hygiene*, vol. 92, no. 1, pp. 45–49, 1998.

[10] D. H. Hamer, M. Ndhlovu, D. Zurovac, et al., "Improved diagnostic testing and malaria treatment practices in Zambia," *The Journal of the American Medical Association*, vol. 297, no. 20, pp. 2227–2231, 2007.

[11] V. D'Acremont, C. Lengeler, H. Mshinda, D. Mtasiwa, M. Tanner, and B. Genton, "Time to move from presumptive malaria treatment to laboratory-confirmed diagnosis and treatment in African children with fever," *PLoS Medicine*, vol. 6, no. 1, article e252, pp. 1–3, 2009.

[12] A. P. Alker, P. Lim, R. Sem, et al., "Pfmdr1 and in vivo resistance to artesunate-mefloquine in falciparum malaria on the Cambodian-Thai border," *The American Journal of Tropical Medicine and Hygiene*, vol. 76, no. 4, pp. 641–647, 2007.

[13] C. K. Murray, R. A. Gasser Jr., A. J. Magill, and R. S. Miller, "Update on rapid diagnostic testing for malaria," *Clinical Microbiology Reviews*, vol. 21, no. 1, pp. 97–110, 2008.

[14] D. C. Warhurst and J. E. Williams, "ACP broadsheet no 148. July 1996. Laboratory diagnosis of malaria," *Journal of Clinical Pathology*, vol. 49, no. 7, pp. 533–538, 1996.

[15] L. M. Milne, M. S. Kyi, P. L. Chiodini, and D. C. Warhurst, "Accuracy of routine laboratory diagnosis of malaria in the United Kingdom," *Journal of Clinical Pathology*, vol. 47, no. 8, pp. 740–742, 1994.

[16] F. E. McKenzie, J. Sirichaisinthop, R. S. Miller, R. A. Gasser Jr., and C. Wongsrichanalai, "Dependence of malaria detection and species diagnosis by microscopy on parasite density," *The American Journal of Tropical Medicine and Hygiene*, vol. 69, no. 4, pp. 372–376, 2003.

[17] R. E. Coleman, J. Sattabongkot, S. Promstaporm, et al., "Comparison of PCR and microscopy for the detection of asymptomatic malaria in a *Plasmodium falciparum/vivax* endemic area in Thailand," *Malaria Journal*, vol. 5, article 121, pp. 1–7, 2006.

[18] D. Zurovac, B. Midia, S. A. Ochola, M. English, and R. W. Snow, "Microscopy and outpatient malaria case management among older children and adults in Kenya," *Tropical Medicine & International Health*, vol. 11, no. 4, pp. 432–440, 2006.

[19] K. C. Kain, M. A. Harrington, S. Tennyson, and J. S. Keystone, "Imported malaria: prospective analysis of problems in diagnosis and management," *Clinical Infectious Diseases*, vol. 27, no. 1, pp. 142–149, 1998.

[20] L. Barat, J. Chipipa, M. Kolczak, and T. Sukwa, "Does the availability of blood slide microscopy for malaria at health centers improve the management of persons with fever in Zambia?" *The American Journal of Tropical Medicine and Hygiene*, vol. 60, no. 6, pp. 1024–1030, 1999.

[21] M. Zhou, Q. Liu, C. Wongsrichanalai, et al., "High prevalence of *Plasmodium malariae* and *Plasmodium ovale* in malaria patients along the Thai-Myanmar border, as revealed by acridine orange staining and PCR-based diagnoses," *Tropical Medicine & International Health*, vol. 3, no. 4, pp. 304–312, 1998.

[22] D. N. Durrheim, P. J. Becker, and K. Billinghurst, "Diagnostic disagreement—the lessons learnt from malaria diagnosis in Mpumalanga," *South African Medical Journal*, vol. 87, no. 8, p. 1016, 1997.

[23] N. W. Stow, J. K. Torrens, and J. Walker, "An assessment of the accuracy of clinical diagnosis, local microscopy and a rapid immunochromatographic card test in comparison with expert microscopy in the diagnosis of malaria in rural Kenya," *Transactions of the Royal Society of Tropical Medicine and Hygiene*, vol. 93, no. 5, pp. 519–520, 1999.

[24] S. P. Kachur, E. Nicolas, V. Jean-François, et al., "Prevalence of malaria parasitemia and accuracy of microscopic diagnosis in Haiti, October 1995," *Revista Panamericana de Salud Pública*, vol. 3, no. 1, pp. 35–39, 1998.

[25] A. H. D. Kilian, W. G. Metzger, E. J. Mutschelknauss, et al., "Reliability of malaria microscopy in epidemiological studies: results of quality control," *Tropical Medicine & International Health*, vol. 5, no. 1, pp. 3–8, 2000.

[26] D. Payne, "Use and limitations of light microscopy for diagnosing malaria at the primary health care level," *Bulletin*

of the World Health Organization, vol. 66, no. 5, pp. 621–626, 1988.

[27] C. Wongsrichanalai, M. J. Barcus, S. Muth, A. Sutamihardja, and W. A. Wernsdorfer, "A review of malaria diagnostic tools: microscopy and rapid diagnostic test (RDT)," *The American Society of Tropical Medicine and Hygiene*, vol. 77, no. 6, supplement, pp. 119–127, 2007.

[28] A. Moody, "Rapid diagnostic tests for malaria parasites," *Clinical Microbiology Reviews*, vol. 15, no. 1, pp. 66–78, 2002.

[29] C. K. Murray, D. Bell, R. A. Gasser Jr., and C. Wongsrichanalai, "Rapid diagnostic testing for malaria," *Tropical Medicine & International Health*, vol. 8, no. 10, pp. 876–883, 2003.

[30] C. J. Shiff, Z. Premji, and J. N. Minjas, "The rapid manual *Para*Sight®-F test : a new diagnostic tool for *Plasmodium falciparum* infection," *Transactions of the Royal Society of Tropical Medicine and Hygiene*, vol. 87, no. 6, pp. 646–648, 1993.

[31] D. P. Mason, F. Kawamoto, K. Lin, A. Laboonchai, and C. Wongsrichanalai, "A comparison of two rapid field immunochromatographic tests to expert microscopy in the diagnosis of malaria," *Acta Tropica*, vol. 82, no. 1, pp. 51–59, 2002.

[32] S. Banoo, D. Bell, P. Bossuyt, et al., "Evaluation of diagnostic tests for infectious diseases: general principles," *Nature Reviews Microbiology*, vol. 4, no. 12, supplement, pp. S20–S32, 2006.

[33] D. Bell and R. W. Peeling, "Evaluation of rapid diagnostic tests: malaria," *Nature Reviews Microbiology*, vol. 4, no. 9, supplement, pp. S34–S38, 2006.

[34] WHO, "New perspectives: malaria diagnosis," Report of a Joint WHO/USAID Informal Consultation, World Health Organization, Geneva, Switzerland, October 1999.

[35] M. T. Makler and D. J. Hinrichs, "Measurement of the lactate dehydrogenase activity of *Plasmodium falciparum* as an assessment of parasitemia," *The American Journal of Tropical Medicine and Hygiene*, vol. 48, no. 2, pp. 205–210, 1993.

[36] M. T. Makler, R. C. Piper, and W. K. Milhous, "Lactate dehydrogenase and the diagnosis of malaria," *Parasitology Today*, vol. 14, no. 9, pp. 376–377, 1998.

[37] W. M. Brown, C. A. Yowell, A. Hoard, et al., "Comparative structural analysis and kinetic properties of lactate dehydrogenases from the four species of human malarial parasites," *Biochemistry*, vol. 43, no. 20, pp. 6219–6229, 2004.

[38] J. R. Forney, C. Wongsrichanalai, A. J. Magill, et al., "Devices for rapid diagnosis of malaria: evaluation of prototype assays that detect *Plasmodium falciparum* histidine-rich protein 2 and a *Plasmodium vivax*-specific antigen," *Journal of Clinical Microbiology*, vol. 41, no. 6, pp. 2358–2366, 2003.

[39] R. J. Howard, S. Uni, M. Aikawa, et al., "Secretion of a malarial histidine-rich protein (Pf HRP II) from *Plasmodium falciparum*-infected erythrocytes," *The Journal of Cell Biology*, vol. 103, no. 4, pp. 1269–1277, 1986.

[40] E. P. Rock, K. Marsh, A. J. Saul, et al., "Comparative analysis of the *Plasmodium falciparum* histidine-rich proteins HRP-I, HRP-II and HRP-III in malaria parasites of diverse origin," *Parasitology*, vol. 95, part 2, pp. 209–227, 1987.

[41] 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?" *Malaria Journal*, vol. 6, article 58, pp. 1–6, 2007.

[42] J. Karbwang, O. Tasanor, T. Kanda, et al., "*Para*Sight™-F test for the detection of treatment failure in multidrug resistant *Plasmodium falciparum* malaria," *Transactions of the Royal*

Society of Tropical Medicine and Hygiene, vol. 90, no. 5, pp. 513–515, 1996.

[43] J. Richter, K. Göbels, I. Müller-Stöyer, B. Hoppenheit, and D, Häussinger, "Co-reactivity of plasmodial histidine-rich protein 2 and aldolase on a combined immuno-chromographic-malaria dipstick (ICT) as a potential semi-quantitative marker of high *Plasmodium falciparum* parasitaemia," *Parasitology Research*, vol. 94, no. 5, pp. 384–385, 2004.

[44] R. A. Gasser Jr., A. J. Magill, T. K. Ruebush II, et al., "Malaria diagnosis: performance of NOW® ICT malaria in a large scale field trial," in *Proceedings of the 54th Annual Meeting of the American Society of Tropical Medicine and Hygiene*, Washington, DC, USA, December 2005.

[45] N. Lee, J. Baker, K. T. Andrews, et al., "Effect of sequence variation in *Plasmodium falciparum* histidine-rich protein 2 on binding of specific monoclonal antibodies: implications for rapid diagnostic tests for malaria," *Journal of Clinical Microbiology*, vol. 44, no. 8, pp. 2773–2778, 2006.

[46] J. Baker, J. McCarthy, M. Gatton, et al., "Genetic diversity of *Plasmodium falciparum* histidine-rich protein 2 (PfHRP2) and its effect on the performance of PfHRP2-based rapid diagnostic tests," *The Journal of Infectious Diseases*, vol. 192, no. 5, pp. 870–877, 2005.

[47] N. Lee, J. Baker, D. Bell, J. McCarthy, and Q. Cheng, "Assessing the genetic diversity of the aldolase genes of *Plasmodium falciparum* and *Plasmodium vivax* and its potential effect on performance of aldolase-detecting rapid diagnostic tests," *Journal of Clinical Microbiology*, vol. 44, no. 12, pp. 4547–4549, 2006.

[48] G. L. Genrich, J. Guarner, C. D. Paddock, et al., "Fatal malaria infection in travelers: novel immunohistochemical assays for the detection of *Plasmodium falciparum* in tissues and implications for pathogenesis," *The American Journal of Tropical Medicine and Hygiene*, vol. 76, no. 2, pp. 251–259, 2007.

[49] I. B. Suh, H. K. Choi, S. W. Lee, et al., "Reactivity of sera from cases of *Plasmodium vivax* malaria towards three recombinant antigens based on the surface proteins of the parasite," *Annals of Tropical Medicine and Parasitology*, vol. 97, no. 5, pp. 481–487, 2003.

[50] A. K. Nyame, Z. S. Kawar, and R. D. Cummings, "Antigenic glycans in parasitic infections: implications for vaccines and diagnostics," *Archives of Biochemistry and Biophysics*, vol. 426, no. 2, pp. 182–200, 2004.

[51] I. Mueller, I. Betuela, M. Ginny, J. C. Reeder, and B. Genton, "The sensitivity of the OptiMAL rapid diagnostic test to the presence of *Plasmodium falciparum* gametocytes compromises its ability to monitor treatment outcomes in an area of Papua New Guinea in which malaria is endemic," *Journal of Clinical Microbiology*, vol. 45, no. 2, pp. 627–630, 2007.

[52] A. Marx, D. Pewsner, M. Egger, et al., "Meta-analysis: accuracy of rapid tests for malaria in travelers returning from endemic areas," *The American Journal of Tropical Medicine and Hygiene*, vol. 142, no. 10, pp. 836–846, 2005.

[53] R. S. Miller, N. Uthaimongkol, M. F. Fukuda, et al., "Comparison of performance characteristics of the Binax NOW® Malaria test using venous and fingerstick samples," in *Proceedings of the 55th Annual Meeting of the American Society of Tropical Medicine and Hygiene*, Atlanta, Ga, USA, November 2006.

[54] T. F. McCutchan, R. C. Piper, and M. T. Makler, "Use of malaria rapid diagnostic test to identify *Plasmodium knowlesi*

infection," *Emerging Infectious Diseases*, vol. 14, no. 11, pp. 1750–1752, 2008.

[55] R. F. Leke, R. R. Djokam, R. Mbu, et al., "Detection of the *Plasmodium falciparum* antigen histidine-rich protein 2 in blood of pregnant women: implications for diagnosing placental malaria," *Journal of Clinical Microbiology*, vol. 37, no. 9, pp. 2992–2996, 1999.

[56] M. Trachsler, P. Schlagenhauf, and R. Steffen, "Feasibility of a rapid dipstick antigen-capture assay for self-testing of travellers' malaria," *Tropical Medicine & International Health*, vol. 4, no. 6, pp. 442–447, 1999.

[57] S. A. Harvey, L. Jennings, M. Chinyama, F. Masaninga, K. Mulholland, and D. R. Bell, "Improving community health worker use of malaria rapid diagnostic tests in Zambia: package instructions, job aid and job aid-plus-training," *Malaria Journal*, vol. 7, article 160, pp. 1–12, 2008.

[58] T. Endeshaw, T. Gebre, J. Ngondi, et al., "Evaluation of light microscopy and rapid diagnostic test for the detection of malaria under operational field conditions: a household survey in Ethiopia," *Malaria Journal*, vol. 7, article 118, pp. 1–7, 2008.

[59] A. H. Roukens, J. Berg, A. Barbey, and L. G. Visser, "Performance of self-diagnosis and standby treatment of malaria in international oilfield service employees in the field," *Malaria Journal*, vol. 7, article 128, pp. 1–8, 2008.

[60] R. L. Miller, S. Ikram, G. J. Armelagos, et al., "Diagnosis of *Plasmodium falciparum* infections in mummies using the rapid manual *Para*Sight-F test," *Transactions of the Royal Society of Tropical Medicine and Hygiene*, vol. 88, no. 1, pp. 31–32, 1994.

[61] S. A. Shekalaghe, J. T. Bousema, K. K. Kunei, et al., "Submicroscopic *Plasmodium falciparum* gametocyte carriage is common in an area of low and seasonal transmission in Tanzania," *Tropical Medicine & International Health*, vol. 12, no. 4, pp. 547–553, 2007.

[62] P. Mens, N. Spieker, S. Omar, M. Heijnen, H. Schallig, and P. A. Kager, "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," *Tropical Medicine & International Health*, vol. 12, no. 2, pp. 238–244, 2007.

[63] R. E. Coleman, N. Maneechai, N. Rachaphaew, et al., "Comparison of field and expert laboratory microscopy for active surveillance for asymptomatic *Plasmodium falciparum* and *Plasmodium vivax* in western Thailand," *The American Journal of Tropical Medicine and Hygiene*, vol. 67, no. 2, pp. 141–144, 2002.

[64] A. D. Kitchen and P. L. Chiodini, "Malaria and blood transfusion," *Vox Sanguinis*, vol. 90, no. 2, pp. 77–84, 2006.

[65] G. Snounou and N. J. White, "The co-existence of *Plasmodium*: sidelights from falciparum and vivax malaria in Thailand," *Trends in Parasitology*, vol. 20, no. 7, pp. 333–339, 2004.

[66] B. Susi, T. Whitman, D. L. Blazes, T. H. Burgess, G. J. Martin, and D. Freilich, "Rapid diagnostic test for *Plasmodium falciparum* in 32 Marines medically evacuated from liberia with a febrile illness," *Annals of Internal Medicine*, vol. 142, no. 6, pp. 476–477, 2005.

[67] W. M. Stauffer, A. M. Newberry, C. P. Cartwright, et al., "Evaluation of malaria screening in newly arrived refugees to the United States by microscopy and rapid antigen capture enzyme assay," *Pediatric Infectious Disease Journal*, vol. 25, no. 10, pp. 948–950, 2006.

[68] M. E. Rafael, T. Taylor, A. Magil, Y.-W. Lim, F. Girosi, and R. Allan, "Reducing the burden of childhood malaria in Africa: the role of improved," *Nature*, vol. 444, supplement 1, pp. 39–48, 2006.

[69] S. Shillcutt, C. Morel, C. Goodman, et al., "Cost-effectiveness of malaria diagnostic methods in sub-Saharan Africa in an era of combination therapy," *Bulletin of the World Health Organization*, vol. 86, no. 2, pp. 101–110, 2008.