Review

## A sequential review on accuracy of detecting malaria Parasitemia in developing countries with large restriction on resources

### \*Osamuyimen Igbinosa<sup>1</sup>, Owen Igbinosa<sup>2</sup> Osasere Asowata, <sup>3</sup> Chenyi Jeffery<sup>4</sup>

<sup>1</sup>Drexel University College of Medicine/Saint Peter's University Hospital New Brunswick, New Jersey, USA <sup>2</sup>College of Health Sciences, University of Ghana Medical School, Korle-Bu, Ghana <sup>3</sup>Department of Optometry, University of Benin, Benin City, Nigeria <sup>4</sup>St. Martinus University, Brionplein 1, Otrobanda, Curacao, Netherlands Antilles

#### Accepted 10 August, 2010

Malaria is a leading cause of mortality worldwide, rapid and accurate diagnosis is fundamental to effective management and control of malaria. New technologies have immerged to circumvent limitations of the traditional diagnostic method. This review focuses on the quality issues with current malaria diagnostics that have been developed over time. Databases including MEDLINE, PubMed and Google beta were searched for literature pertaining to malaria diagnostics published between January 1970 and March 2009. The references of the retrieved articles were hand-searched for relevant studies. Reduction of malaria morbidity and drug resistance intensity, along with its associated economic losses require urgent upgrade of the quality of parasite-based diagnostic methods. An investment in anti-malarial drug development or malaria vaccine development should be accompanied by a parallel commitment to improve diagnostic tools and their availability to people living in resource-poor malaria endemic areas.

**Keywords:** Malaria, diagnosis microscopy, rapid diagnostic tests, polymerase chain reaction, mass spectrometry.

### INTRODUCTION

From time immemorial malaria has been a plague to human race especially to the population of sub-Saharan Africa. More than a third of the world's population (about two billion people) is estimated to carry parasites at any one time (Mishra el al., 1999). In Africa alone there are an estimated 200-450 million cases of fever in children infected with malaria parasites each year. Estimates for annual malaria mortality range from 0.5 to 3 million people (Breman, 2001). This wide range of 200 million reflects the lack of precision of current malaria statistics (Kilama, 2001). Malaria endemic countries are among the poorest in the world, where access of medical care is limited and lack facilities for laboratory diagnosis. As a result, malaria treatment is given on the basis of clinical diagnosis. In general clinical diagnosis is very inaccurate even in areas where malaria is a common cause of fever.

Rapid and accurate diagnosis is fundamental to effective management and rational malaria therapy which is essential to reduce mortality, prevent drug resistance and to save cost on alternative drugs. Confirmatory test before treatment is regaining the much needed attention partly influenced by the spread of drug resistance and requirement of more expensive drugs unaffordable to poor countries (Barnish et al., 2004). In this review we attempt to focus on different approaches that have developed over time for the diagnosis of malaria, in view to approach present characteristics such as cost, ease of performance and accuracy.

#### Malaria Diagnostic Technique

#### **Clinical diagnosis**

Clinical diagnosis which is the most commonly used, is the bases for self diagnosis and treatment. It is

<sup>\*</sup>Correspondence author: email: Uyi80@yahoo.com

inexpensive and requires no special equipment and supplies. It is based on patient's symptoms, physical findings at examination and a high suspicion index (Mwangi et al., 2005). Clinical diagnosis has only been feasible in rural areas at the periphery of the health care system where laboratory support to clinical diagnosis does not exist. However, the overlapping of malaria symptoms with other febrile illness and the subjective nature of fever impairs its specificity and therefore encourages the indiscriminate use of antimalarial drugs for managing febrile conditions in endemic areas. Although highly debatable, disease management based on clinical ground alone may be justified in some settings (Biritwum et al., 2005), (Ruebush et al., 1995).

The specificity of clinical diagnosis is 20-60% compared with microscopy (Olivar et al., 1991), (Van der et al., 1998). Accuracy varies with the level of endemicity, malaria season and age group. Studies of fever cases on population with different malaria attributable properties from Philipines, Sri lanka, Thailand, Tanzania, Chad Mali and Kenya have shown a wide range of percentages (40-80%) of malaria over-diagnosis (Mwangi et al., 2005). Attempts at improving the accuracy have lead to development of algorithms; no single clinical algorithm is a universal predictor (Chandramohan et al., 2001).

#### **Microscopic Diagnosis**

Gustav Giemsa in 1904 developed a mixture of methylene blue and eosin stain which has subsequently became the gold standard. Giemsa microscopy is regarded as the most suitable diagnostic instrument for malaria control because it is relatively inexpensive cost estimate for endemic countries range from about US\$ 0.12 - 0.40 per slide (Palmer et al., 1998). This method is able to differentiate malaria species and quantify parasites. The blood film technique has undergone very little improvement since its development in the early 1900s, both thick and thin films have been described. Giemsa stained thick blood film can detect an estimate of 4-20 parasite/mcl blood (Payne, 1988), (Bruce-Chwatt , 1984).

Microscopy has limitations; it requires well-trained competent microscopist, it is labor intensive, time consuming and requires rigorous maintainers of functional infrastructures also effective quality control and quality assurance. This variability plus the risk of untreated malaria has led clinicians to treat febrile conditions in patients without regard to the laboratory result (Othnigue et al., 2006), (McCutchan et al., 2008). Recently there has been a push in improving the current practice of microscopy, an on-line self test for competency in malaria microscopy is now available (Icke et al., 2005), also World Health Organization (WHO) training materials are widely available and frequently used even though an update is necessary.

#### Fluorescence microscopy

An attempt to enhance the detection of malaria parasites in blood films lead to development of fluorescence microscopy. Certain fluorescent dyes have an affinity for the nucleic acid in the parasite nucleus and will attach to the nuclei. When excited by UV light at an appropriate wavelength, the nucleus will fluoresce strongly. Two fluorochromes have frequently been used for this purpose. acridine orange (AO) and benzothiocarboxypurine (BCP), Rhodamine-123 has also been tried (Bruce-Chwatt , 1984). With experience, using workers methods involving fluorochrome compounds are able to detect parasites rapidly and accurately, however this will still pose a problem in certain areas of the world where fluorescence microscopes or adequate training in their use is not available.

#### Rapid Diagnostic Tests (RDTs)

The introduction of malaria rapid diagnostic devices in the early 1990s (Shiff et al., 1993) ushered in a new era of diagnosis that was expected to challenge the limitations of other diagnostic tests. RDTs are based on malaria antigen detection in a small amount of lysed blood, usually 5-15 µl by immunochromatographic assay with monoclonal antibodies directed against the target parasite antigen and impregnated on a test strip. Commercial test are manufactured with different combination of target antigens to suit local malaria epidemiology. Current target antigens include (i) Histidine- rich protein II (HRP-II) (Howard et al., 1986), it is a water soluble protein produced by trophozoites and gametocytes of P. falciparum (ii) Parasite lactate dehydrogenase (pLDH) (Makler et al., 1993), produced by asexual and sexual stages of malaria parasites. (iii) Plasmodium aldolase is an enzyme of the parasite glycolytic pathway expressed by the blood stages of P. falciparum as well as the non-fa1ciparum malaria parasites. Monoclonal antibodies against Plasmodium aldolase are pan-specific in their reaction. It has been used in a combined P. falciparum / P. vivax immunochromatographic test that target the pan-malarial antigen along with HRP-II (Makler et al., 1993). Most RDTs products are suitable for *P. falciparum* malaria diagnosis, some claim that they can effectively and rapidly diagnose P. vivax malaria (Lee et al., 2008). A new method has been recently been developed for detecting P. knowlesi (McCutchan e al., 2008).

Test procedures varies between kits, generally it involves collection of a finger-prick blood specimen using microcapillary tubes, mixing blood specimen with a buffer solution that contains hemolyzing compounds as well as a specific antibody that is labeled with a visually detectable marker. The labeled antigen-antibody complex migrates up the test strip by capillary action toward test specific reagent that has been pre-deposited during manufacture. Finally washing buffer is then added to remove heamoglobin and permit visualization of colored line on the strip. The result is obtained in less than 15 minutes, usually indicated as a colored line on a strip (World Health Organization, 2010). These tests require little training and are subjected to less investigator related variation than microscopy. They are easy to interpret, do not require capital investment, installations or electricity (Zurovac et al., 2006).

Sensitivity: The sensitivity of RDTs for the diagnosis of *P. falciparum* malaria is >90% at densities above 100 parasites per  $\mu$ l blood and the sensitivity decreases markedly below that level of parasite density (lvo et al., 2007) (Praveen et al., 2008). Some studies have achieved >95% sensitivity at parasitemia of approximately 500 parasites/ $\mu$ l, but this high parasitemia is seen in only a minority of patients (Bustos et al., 1999), (Piper et al., 1999).

*Limitations*: Current limitations of test sensitivity are compounded by the fact commercially available RDTs targeting HRP-II can detect only *P. falciparum* and do not differentiate non-*P. falciparum* species from each other. These kits can only detect portion of cases in areas where other *Plasmodium* species are co-endemic (Mason et al., 2002).

RDTs that target HRP-II of *P. falciparum* can give positive results for up to two weeks after malaria therapy and parasite clearance as confirmed by microscopy. This might yield confusing results in relation to the assessment of treatment failure or drug resistance (Playford et al., 2002). Gametocytes are not pathogenic and can persist after malaria therapy; therefore RDTs that detect antigens produced by gametocytes (such as pLDH) can give positive results in infections where only gametocytes are present. Such false positive RDT results can thus lead to erroneous interpretations and unnecessary exposing people to anti-malaria side effects (Laferi et al., 1997).

Cross-reactivity with rheumatoid factor causing false positive in earlier versions of RDTs tests has been known (Bartoloni et al., 1998). The use of IgM capture antibody instead of IgG, thus rendering more difficult binding by rheumatoid factor, was thought to be associated with lower percentages of false positivity in a certain brand of RDTs (Mishra et al., 1999). Although, WHO (Zurovac et al., 2006) report on malaria diagnosis claims that this problem has been corrected, some believed that rapid experts still any malaria immunochromatographic assay may cross-react with rheumatoid factor (Grobusch et al., 1999). A recent assessment of nine different product types using rheumatoid factor positive specimens

training materials are widely available and frequently suggested that some tend to give false positive results (approximately 30%) more frequently than the others (5–

10%) leading to a conclusion of an unexplained phenomenon (Craig et al., 2002).

Overall, RDTs is an important, rapid malaria-diagnostic tool for healthcare workers, the simplicity and reliability of RDTs have been improved for use in rural endemic areas (Erdman et al., 2008), however they are not quantitative or semi quantitative at best. The WHO is developing guidelines to ensure quality control (World Health Organization, 2010). It should be used in conjunction with other methods to confirm the results and monitor treatment.

#### Molecular Diagnosis

Parasite nucleic acids are detected using polymerase chain reaction (PCR). This technique is more accurate than microscopy. It is been considered a possible alternative to microscopy since low parasitemia and or unsual clinical symptoms can impair malaria diagnosis which are often caused by a prophylaxis not properly followed, or a partially efficient drug. Polymerase chain reaction (PCR) based methods have been a recent development in the molecular diagnosis of malaria (Snounou et al., 1993). Since 1990, several experimental assays have been reported that use various primers, and extraction and detection techniques (Barker et al., 1989), (Jaureguiberry et al., 1990), (Kain et al., 1991).

Several PCR assays have been developed for the diagnosis of malaria. The 18S rRNA gene has been used as a DNA target for the differentiation of plasmodial species by nested PCR (Snounou et al., 1993), (Tahar et al., 1997) and reverse transcription-PCR<sup>1</sup>. Other DNA targets such as the circumsporozoite protein gene (Sethabutr et al., 1992), (Tahar et al., 1997) have also been investigated for species-specific regions.

Several reports have shown that the PCR had a higher sensitivity than examination of thin blood smears, especially in cases with low parasitemia or mixed infections (Sethabutr et al., 1992), (Tahar et al., 1997). Conventional PCR assays, while demonstrating increased sensitivity and specificity, remain laborintensive, slow and prone to potential amplicon contamination problems. It requires specialized and costly equipment and reagents, as well as laboratory conditions that are often not available in the field. However, the time lag between sample collection, transportation, processing and dissemination of results back to the physician, limits the usefulness of PCR in routine clinical practice. Furthermore, in most areas with malaria transmission, factors such as limited financial resources, persistent subclinical parasitaemia and inadequate laboratory infrastructures in remote rural areas preclude PCR as a diagnostic method. Even in affluent, non-endemic countries, PCR is not a suitable method for routine use.

#### Antibody Detection by Serology

Anti-malarial antibodies can be detected by two major methods; (i) Antibody Detection-Indirect Fluorescent Antibody and Enzyme immunoassays (ii) Antigen Detection-Immunochromatographic. Immuno-Fluorescence Antibody Test (IFAT) is still regarded as the gold standard for malarial serology and until recently was the only validated method for detecting Plasmodiumspecific antibodies in blood banks<sup>10</sup>. Following infection with any of the four species of *Plasmodium*, specific antibodies are produced, in virtually all individuals, one or two weeks after initial infection and persist for three to six months after parasite clearance. These antibodies may persist for months or years in semi-immune patients in endemic countries where reinfection is frequent. However, in a non-immune patient, treated for a single infection, antibody levels fall more rapidly and may be undetectable by three to six months. Reinfection or relapse leads to a secondary response with a high and rapid rise in antibody titers (Garraud et al., 2003), (Seed et al., 2005).

Serological methods have three main uses (i) Screening blood donors involved in cases of transfusion-induced malaria when the donor's parasitemia may be below the detectable level of blood film examination (ii) Testing a patient with a febrile illness who is suspected of having malaria and from whom repeated blood smears are negative and (iii) Testing a patient who has been recently treated for malaria but in whom the diagnosis is guestioned (Garraud et al., 2003).

IFAT has sensitivity and specificity of 98% and 99.5% respectively (Sulzer et al., 1971). Serologic testing is not practical for routine diagnosis of acute malaria because of the time required for development of antibody and also the persistence of antibodies. It is time-consuming and difficult to automate. It requires fluorescence microscopy and trained technicians, making it operator-dependent and subjective, particularly for serum samples with low antibody titers (Candolfi et al., 2005). Additionally, the standardization of IFAT reagents and lack of manipulations makes it impossible to harmonize interlaboratory results. Moreover, the antigen is obtained by in vitro culture of P. falciparum and gives very good sensitivity for this species, but shows limited cross reactivity with other human pathogenic species.

# Automated Malaria Diagnosis Using Pigment Detection

Over a decade ago malaria pigment (hemozoin) was found to be birefringent and it was predicted that this property could be used to automate malaria diagnosis. Severe and fatal malaria is associated with the increased presence of malaria hemozoin in peripheral phagocytes (Amodu el al., 1998). Malaria pigment is produced by the malaria parasites during intraerythrocytic development as the end product of hemoglobin digestion. When the malaria infected red cells rupture, the parasites and hemozoin aggregates are released into the plasma, which is then ingested by circulating phagocytes.

A South African study was first to document the automated detection of leukocyte hemozoin (Scott et al., 2002). Experimental detection of malaria parasite based on abnormal cell clusters and small particles with DNA fluorescence and probably free malarial parasites using flow cytometry and mass spectrometry is currently under investigation. Future improvements in the equipment and technique can make this method deployable and useful.

#### Gold Standard

The advent of several new diagnostic methods for malaria diagnosis and proliferating field studies that evaluate their utility have made definition of a "gold standard" for malaria diagnosis is an issue that needs to be addressed. Traditionally microscopic examination of thin and thick blood films has long been used for assessing the outcomes of drug and vaccine trials and for serving as a reference standard in the evaluation of new tools for malaria diagnosis. However, microscopy even when performed by an expert has its limitations. There is no international agreement on the precise number of microscope fields to be scanned and gold standard studies may be confusing and misleading when readers try to compare these results with other studies, since methodologies and references standard vary significantly. RDTs is fast and easy to use, its reliability has been improved for use in rural endemic areas. Recent improvements in molecular diagnostic techniques pose the question of whether the polymerase chain reaction (PCR) should now become the reference standard for the detection of malaria parasites.

#### CONCLUSION AND RECOMMENDATION

Malaria is a leading cause of mortality worldwide and accurate diagnostic testing for malaria can potentially save lives. High quality, accurate, rapid and affordable diagnostic tools are urgently needed now that new antimalarial regimens, characterized by higher cost and increased toxicity, have been introduced more widely in response to emerging multi-drug resistance. Most malaria treatment is based on clinical diagnosis alone. Accuracy of a clinical diagnosis varies with the level of endemicity, malaria season, and age group and no single clinical algorithm has been shown to be a universal predictor (Mwangi et al., 2005). Only in children in hightransmission areas can clinical diagnosis determine the treatment decision (Zurovac et al.. 2006). (Chandramohan et al., 2001). In this situation, a majority

of the population is chronically parasitemic and malaria may be concomitant but not the responsible agent of the febrile illness. In most situations the clinical diagnosis would benefit from laboratory confirmation by microscopy or RDTs. Such situations include suspected cases of severe malaria, suspected treatment failures, disease management by private-sector health providers in urban areas and multi-drug resistance.

However, the microscope is a key tool in the integrated management of disease in resource poor settings. The optimal role and conditions for the use of RDTs in relation to microscopy remain to be determined. The WHO is currently developing quality control guidelines that will enhance the public confidence on RDTs, their cost has come closer to what most national malaria programmes can afford. PCR appears to be the more efficient than optical methods in the detection of longstanding infections but its cost has hampered progress. Assays based on the PCR can be used for unambiguous species identification and may complement or even replace light microscopy in developed countries. Experimental diagnostics using flow cytometry and mass spectrometry are currently under investigation.

The need for accurate diagnostic modalities is alarming; however existing technologies have not yet met the needs of the developing world. The health sectors in African countries have few means at their disposal, hoping to work round these difficulties WHO; the main funding agencies are now strongly encouraging the setting up of programmes to combat malaria with insecticide-impregnated bed nets. While this exercise is laudable, we believe that the way to combat malaria in especially in sub Saharan Africa with current available means are; (i) improvements in health services, and health education (ii) Rapid and accurate diagnosis in other to effect rational malaria therapy. The cost of improved malaria diagnosis will inevitably increase. However, such investment offers a more promising strategy to deal with increasing costs of therapy driven by drug resistance.

Finally the reduction of malaria morbidity and drug resistance intensity with its associated economic losses require urgent upgrade up of the quality of parasite-based diagnostic methods. Also an investment in anti-malarial drug development or malaria vaccine development should be accompanied by a parallel commitment to improve diagnostic tools and their availability to people living in resource-poor malaria endemic areas. Such investment offers a more promising strategy to deal with increasing costs of therapy driven by drug resistance.

#### REFERENCE

Abdullah NR, Furuta T, Taib R, Kita K, Kojima S, Wah MJ. Short report: development of a new diagnostic method for *Plasmodium falciparum* infection using a reverse transcriptase-polymerase chain reaction

Am. J. Trop. Med. Hyg. 1996; 54:162–163.

Intraleucocytic malaria pigment and clinical severity of malaria in children. Trans R Soc Trop Med & Hyg 1998; 92(1):54-56

- Barker RHJ, Suebsaeng L, Rooney W, Wirth DF. Detection of *Plasmodium falciparum* in human patients: a comparison of the DNA probe method to microscopic diagnosis. Am J Trop Med Hyg 1989 41: 266–272
- Barnish G, Bates I, Iboro J. Newer drug combinations for malaria. BMJ 2004; 328: 1511–1512
- Bartoloni A, Strohmeyer M, Sabatinelli G, Benucci M, Serni U, Paradisi F. False a positive ParaSight-F test for malaria in patients with rheumatoid factor. Trans R Soc Trop Med & Hyg 1998; 92: 33–34
- Biritwum RB, Welbeck J, Barnish G. Incidence and management of malaria in two communities of different socioeconomic level, in Accra, Ghana. Ann. Trop. Med. Parasitol. 2000; 94:771–778
- Breman JG. The ears of the hippopotamus: manifestations, determinants, and estimates of the malaria burden. Am J Trop Med Hyg 2001; 64: 1–11
- Bruce-Chwatt LJ. DNA probes for malaria diagnosis. Lancet 1984 1: 795
- Bustos DG, Olveda RM, Negishi M, Kurimura T. Evaluation of a new rapid diagnostic test "Determine Malaria Pf" against standard blood film, ICT Malaria P. f and ParaSight F. Japanese J. Trop. Med. & Hyg.1999; 27:(3) 417-425
- Candolfi E. Transfusion-transmitted malaria, preventive measures. Transfus Clin. Biol. 2005; 12:107-113
- Chandramohan D, Carneiro I, Kavishwar A, Brugha R, Desai V, Greenwood BA. clinical algorithm for the diagnosis of malaria: results of an evaluation in an area of low endemicity. Trop. Med. Int. Health 2001; 6: 505-510
- Chandramohan D, Jaffar S, Greenwood B. Use of clinical algorithms for diagnosing malaria. Trop. Med. Int. Health 2002; 7: 45–52
- Craig MH, Bredenkamp BL, Williams CHV. Field and laboratory comparative evaluation of ten rapid malaria diagnostic tests. Trans. Royal Soc. Trop. Med. & Hyg. 2002; 96: 258–265
- Erdman LK, Kain KC. Molecular diagnostic and surveillance tools for global malaria control. Travel Med Infect Dis 2008; 6: 82-99.
- World Health Organization. List of known commercially available antigen-detecting malaria RDTs. [Accessed July 24, 2010]. Available at: http://www.wpro.who.int/sites/rdt
- Garraud O, Mahanty S, Perraut R. Malaria-specific antibody subclasses in immune individuals: a key source of information for vaccine design. Trends Immunol. 2003; 24:30-35
- Grobusch MP, Alpermann U, Schwenke S, Jelinek T, Warhurst DC. False-positive rapid tests for malaria in patients with rheumatoid factor. The Lancet 1999; 353: 297
- Howard RJ, Uni S, Aikawa M, Aley SB, Leech JH, Lew AM, Wellems TE, Rener J Taylor DW. Secretion of a malarial histidine-rich protein (Pf HRP II) from *Plasmodium falciparu*m-infected erythrocytes. J. Cell Biol. 1986; 103:1269–1277
- Icke G, Davis R, McConnell W. Teaching health workers malaria diagnosis. PLoS Med 2005; 2:11
- Ivo M, Inoni B, Meza G, John R, Blaise G. 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. J. Clin. Microbiol. 2007; 45(2): 627–630
- Jaureguiberry G, Hatin I, d'Auriol L, Galibert G, PCR detection of *Plasmodium falciparum* by oligonucleotide probes. Mol. Cell Probes 1990; 4: 409–414.
- Kain KC, Lanar DE. Determination of genetic variation within *Plasmodium falciparum* by using enzymatically amplified DNA from filter paper disks impregnated with whole blood. J. Clin. Microbiol. 1991; 29: 1171–1174
- Kilama WL. The malaria burden and the need for research and capacity strengthening in Africa. Am. J. Trop. Med. Hyg. 2001; 64: i
- Laferi H, Kandel K, Pichler H. False positive dipstick test for malaria. New England J. Med. 1997; 337, 1635–1636
- Makler MT, Hinrichs DJ. Measurement of the lactate dehydrogenase activity of *Plasmodium falciparum* as an assessment of parasitaemia. Am. J. Trop. Med. Hyg. 1993; 48:205–210
- Marsh K. Malaria disaster in Africa. Lancet 1998; 352: 924-25
- Mason DP, Kawamoto F, Lin K, Laoboonchai A, Wongsrichanalai C. A

Amodu OK, Adeyemo AA, Olumese PE, Gbadegesin RA

comparison of two rapid field immunochromatographic tests to expert microscopy in the diagnosis of malaria. Acta Tropica 2002; 82, 51–59

- Mishra B, Samantaray JC, Kumar A Mirdha BR. Study of false positivity of two rapid antigen detection tests for diagnosis of *Plasmodium falciparum* malaria. J. Clin. Microbio. 1999; 37: 1233
- Mwangi TW, Mohammed M, Dayo H, Snow RW, Marsh K. Clinical algorithms for malaria diagnosis lack utility among people of different age groups. Trop. Med. Int. Health 2005. 10:530–536
- Mwangi TW, Mohammed M, Dayo H, Snow RW, Marsh K. Clinical algorithms for malaria diagnosis lack utility among people of different age groups. Trop Med. Int. Health 2005; 10: 530–536
- Olivar M, Develoux M, Chegou AA, Loutan L. Presumptive diagnosis of malaria results in a significant risk of mistreatment of children in urban. Sahel. Trans. R. Soc. Trop. Med. Hyg. 1991; 85:729–30
- Othnigue N, Wyss K, Tanner M, Genton B. Urban malaria in the Sahel: prevalence and seasonality of presumptive malaria and parasitaemia at primary care level in Chad. Trop. Med. Int. Health 2006; 11: 204– 210
- Palmer CJ, Lindo JF, Klaskala WI, Quesada JA, Kaminsky R, Baum MK, Ager AL. Evaluation of the OptiMAL test for rapid diagnosis of *Plasmodium vivax* and *Plasmodium falciparum* malaria. J. Clin. Microbiol. 1998; 36: 203–2004
- Payne D. Use and limitations of light microscopy for diagnosing malaria at the primary health care level. Bull World Health Organ. 1988; 66: 621–626
- Piper R, Lebras J, Wentworth L, Hunt-Cooke A, Houze S, Chiodini P, Makler M. Immunocapture diagnostic assays for malaria using *Plasmodium* lactate dehydrogenase (pLDH). Am. J. Trop. Med. & Hyg. 1999; 60:109–118
- Playford EG, Walker J, Evaluation of the ICT Malaria P.F/P.v and the OptiMal rapid diagnostic tests for malaria in febrile returned travellers. J. Clin. Microbiol. 2002; 40:4166–4171
- Praveen KB, Nipun S, Pushpendra, PS, Mrigendra PS, Manmohan S, Gyan C, Aditya PD, Neeru S. The usefulness of a new rapid diagnostic test, the First Response® Malaria Combo (pLDH/HRP2) card test, for malaria diagnosis in the forested belt of central India. Malar J. 2008; 7: 126
- Ruebush TK, Kern MK, Campbell CC, Oloo AJ. Self treatment of malaria in a rural area of western Kenya. Bull World Health Organ 1995 73: 229–236
- Scott CS, van Zyl D, Ho E, Meyersfeld DR, Ruivo L, Mendelow BV, Coetzer TL. Automated detection of WBC intracelluar malaria associated pigment (hemozoin) with Abbott Cell Dyn 3200 and Cell Dyn 3,700 analyzers: overview and results from South African Institute of Medical Research (SAIMR) II evaluation. Lab Hematol. (2002) 8:91-101
- Seed CR, Kitchen A, Davis TM. The current status and potential role of laboratory testing to prevent transfusion-transmitted malaria. Transfusion Med. Rev. 2005 19:229-240
- Sethabutr O, Brown AE, Panyim S, Kain KC, Webster HK, Echeverria P. Detection of *Plasmodium falciparum* by polymerase chain reaction in a field study. J. Infect. Dis. 1992; 166:145–148
- Shiff CJ, Premji Z. Minjas JN. The rapid manual *Para*Sight-F test. A new diagnostic tool for *Plasmodium falciparum* infection. Trans. Royal Soc. Trop. Med. & Hyg. 1993; 87: 646–648
- Snounou G, Viriyakosol S, Zhu XP, Jarra W, Pinheiro L, do Rosario VE, Thaithong S, Brown KN. High sensitivity of detection of human malaria parasites by the use of nested polymerase chain reaction. Mol. Biochem. Parasitol. 1993; 61: 315–320
- Snounou G. Detection and identification of the four malaria parasite species infecting humans by PCR amplification. Methds. Mol. Biol. 1996; 50:263–291
- Sulzer AJ, Mariann W. The Fluorescent Antibody Test for Malaria. Critical Reviews in Clinical Laboratory Sciences. 1971; 2: (4) 601-619
- Tahar R, Ringwald P, Basco LK. Diagnosis of *Plasmodium malariae* infection by the polymerase chain reaction. Trans. R. Soc. Trop. Med. & Hyg. 1997; 91:410–411.
- Van der HW, Premasiri DA, Wickremasinghe AR. Clinical diagnosis of uncomplicated malaria in Sri Lanka. Southeast Asian J. Trop. Med. & Public Health 1998; 29: 242–45

- World Health Organization. Malaria Diagnosis New Perspectives. Report of a Joint WHO/USAID Informal Consultation, Geneva: WHO 25-27, 2000.
- Zurovac D, Midia B, Ochola SA, English M, Snow RW. Microscopy and outpatient malaria case management among older children and adults in Kenya. Trop. Med. Int. Health 2006; 11: 432–440
- McCutchan TF, Piper RC, Makler MT. Use of malaria rapid diagnostic test to identify *Plasmodium knowlesi* infection. Emerg Infect Dis 2008; 14: 1750-1752.
- Lee SW, Jeon K, Jeon BR, Park I. Rapid diagnosis of vivax malaria by the SD Bioline Malaria Antigen test when thrombocytopenia is present. J Clin Microbiol 2008; 46: 939-942.