Songklanakarin J. Sci. Technol.
36 (6), 633-641, Nov. - Dec. 2014

SJST SONGKLANAKARIN JOURNAL OF SCIENCE AND TECHNOLOGY
http://www.sjst.psu.ac.th

Review Article

Malaria detection using non-blood samples

Kwannan Nantavisai*

Department of Microbiology, Faculty of Medicine,
Srinakharinwirot University, Watthana, Bangkok, 10110 Thailand.

Received: 2 July 2014; Accepted: 26 October 2014

Abstract

Malaria is still a major problem in many parts of the world. Accurate diagnosis, crucial in disease control, currently relies on the microscopic detection of parasites in blood samples. This technique is invasive, increases risk of blood-borne disease transmission, and is uncomfortable for the patient. Non-invasive approaches to detect antibodies against malaria, malarial antigens, and malarial DNA in non-blood samples, i.e. saliva, urine and buccal mucosa, have been developed to overcome these problems. This review summarizes the techniques that have been used to detect malaria in non-blood samples, their sensitivities and specificities as well as the factors influencing them. The provided information may be useful for further development of highly efficient non-invasive malaria detection methods.

Keywords: noninvasive, saliva, urine, buccal mucosa, malaria diagnosis

1. Introduction

Malaria remains a public health concern in many parts of the world. The world malaria report 2013 released by World Health Organization (WHO) reported that around 207 million people were infected and about 627,000 people died of the disease in 2012 (WHO, 2013). The causative agent of the disease is obligate intraerythrocytic protozoa of the genus *Plasmodium*. Malaria in human was previously known to be caused by four species of *Plasmodium* (*P. falciparum*, *P. vivax*, *P. malariae* and *P. ovale*). Recently, a simian malaria parasite, *P. knowlesi*, is considered to be the fifth species of *Plasmodium* that can cause malaria in human since a large number of human cases infected with *P. knowlesi* were described in Malaysia and in many countries in Southeast Asia including Thailand (Jongwutiwes *et al*., 2004; Singh and Daneshvar, 2013). Among human malaria parasites, however, *P. falciparum* is the species responsible for the most severe form of the disease. It can cause fatal complications in malaria patients such as cerebral malaria, anemia, metabolic acidosis, acute renal failure, respiratory distress, and multiple organ failure (Trampuz *et al*., 2003). In contrast to historical observations that infection with *P. vivax* rarely develops into severe and lethal disease, recent reports show that severe and sometimes fatal outcome associated with *P. vivax* infection has become more frequent in some regions (White *et al*., 2014). In addition, *P. vivax* possesses a dormant stage known as the hypnozoite which can cause relapse, and the incidence of *P. vivax* infection in certain endemic areas, e.g. Thailand, has increased and become equal to that of *P. falciparum* (Muhamad *et al*., 2011). Thus, *P. vivax* infection should be considered to be as important as *P. falciparum* infection. Comparing with *P. falciparum* and *P. vivax*, infection with *P. malariae* and *P. ovale* occurs infrequently, and the disease is generally benign with a low parasitemia due to the preference of the parasites to selectively invade erythrocytes (e.g. *P. ovale* invades only young erythrocytes) (Mueller *et al*., 2007).

A key factor to control malaria is the early and accurate diagnosis as it is crucial to manage infected individuals effectively, avoiding unnecessary presumptive treatment.

* Corresponding author.
Email address: kwannan@yahoo.com

Diagnosis of malaria is based on detection of the malarial antigens/products or the parasite itself in patient blood. The techniques commonly used for malaria diagnosis include microscopy, rapid diagnostic tests (RDTs) and polymerase chain reactions (PCRs) (Cordray and Richards-Kortum, 2012). Microscopic examination of blood film stained with Giemsa, Wright's, or Field's stain remains the gold standard for malaria diagnosis. This technique is simple, cheap, and useful to determine the species and density of parasites. However, a skillful technician is required to interpret the slides, particularly when identifying parasites at low density or in mixed malarial infection (Tangpukdee *et al*., 2009). Another caveat of microscopic examination is the low sensitivity at low parasite levels. Although an experienced microscopist can detect parasites at the density of ~50 parasites/µl (Moody, 2002), routine laboratory diagnosis detects at a density higher than ~500 parasites/µl (Milne *et al*., 1994).

To improve malaria diagnosis, an alternative method such as rapid diagnostic tests (RDTs) has been developed. RDTs detect malarial antigens in blood migrating across a membrane containing specific anti-malaria antibodies (Moody, 2002). This method is quick, easy to perform, and does not require any specific equipment (Bell *et al*., 2006). RDTs have a limit of detection of >100 parasites/µl, and display 80-95% sensitivity and 85% specificity when consider microscopy as the gold standard (Bell, 2004; Endeshaw *et al*., 2008; Ochola *et al*., 2006; Ratnawati *et al*., 2008; Wongsrichanalai *et al*., 2007). Nonetheless, the performance of RDTs from different manufacturers and lots vary widely (WHO, 2012). Furthermore, RDTs may give false-positive results due to antigens circulating for up to two weeks in the patient blood after the infection has been cleared (Mayxay *et al*., 2001). An important shortcoming of this technique is that it does not provide information about the markers of drug resistance or the level of infection (Waitumbi *et al*., 2011).

PCR is considered to be one of the most specific and sensitive malaria diagnostic methods especially in patient with low parasitemia (Morassin *et al*., 2002). Its sensitivity is shown to be superior to that of RDTs or conventional microscopic examination (Coleman *et al*., 2006) with a detection limit of 0.5-5 parasites/µl. PCR also has the ability to detect mixed infection and drug-resistant parasites (Cordray and Richards-Kortum, 2012). Although this technique has shown great specificity and sensitivity in malaria detection, it may be unsuitable to use in remote rural area because of its high cost and complex methodologies which need trained technicians and specific equipment (Tangpukdee *et al*., 2009).

Although the malaria diagnostic methods described above show great benefits, the major disadvantage is that they require a blood sample to detect the parasite. Blood drawing requires trained personnel who are not always available in remote malaria endemic area. This approach increases the risks of needle injuries and accidental infection from diseases such as HIV/AIDs. The cultural objection of considering blood withdrawal as taboo, the fear of small children and some adults from blood collection, and the requirement of repeated sampling during post-treatment follow-up may lead to poor compliance of patients (Wilson *et al*., 2008). These considerations call for the development of non-invasive diagnostic tools using materials other than blood as the source for malaria detection. Since the publications regarding detection malaria parasites in non-blood samples mainly focus on *P. falciparum* and *P. vivax*, this review describes only these two species.

2. Non-blood samples for malaria detection

2.1 Saliva

Whole saliva consists of secretions from salivary glands, bronchial and nasal secretions, serum and blood derivatives from oral wounds, bacterial and bacterial products, viruses and fungi, epithelial cells, and other cellular components (Kaufman and Lamster, 2002). Recently, many researchers have become interested in using saliva as material to evaluate physiological and pathological conditions in human since it can be collected non-invasively by health-care staff with limited training and no special equipment is needed.

Saliva contains several components that may help to detect systemic diseases, for example, cardiovascular disease (Floriano *et al*., 2009), renal disease (Arregger *et al*., 2008), autoimmune disease (Hu *et al*., 2007) and infectious diseases including malaria (Sutherland and Hallett, 2009). The utilization of saliva for malaria diagnosis has focused on detection of host antibodies against malaria parasites, malarial antigens or malarial nucleic acid in patient saliva by using enzyme-linked immunosorbent assay (ELISA), rapid diagnostic tests (RDTs) and polymerase chain reaction (PCR) (Table 1).

Even though the precise route leading malarial antigens to appear in the saliva is still unknown, malarial products such as *P. falciparum* histidine-rich protein II (*Pf*HRP II) or *P. falciparum* lactate dehydrogenase (pLDH) released upon schizont rupture into circulation may reach to saliva via pericellular ultrafiltration from the surrounding vasculature (Fung *et al*., 2012). Some studies have successfully detected either malarial antigens or antibodies in saliva. Wilson *et al*. (2008) detected *Pf*HRP II in whole saliva at 43% sensitivity, yet the study by Fung *et al*. (2012) achieved sensitivity of 100%. The difference in their sensitivities is probably affected by the ELISA product used and sample preparation. The former study used a commercially available ELISA kit designed to detect higher level of *Pf*HRP II in blood or plasma, while the latter study used a custom chemiluminescent ELISA. In addition, the latter study used saliva samples stabilized with protease inhibitors to prevent target protein from degradation (Fung *et al*., 2012; Wilson *et al*., 2008). Using a RDT to detect pLDH, Gbotosho *et al*. (2010) reported a sensitivity of 77.9% in whole saliva and 48.4% in saliva supernatant. It seems that centrifugation of saliva samples results in the sedimentation of parasite proteins. Hence, concentration of parasite antigen detectable in supernatant is

Table 1. Summary of sensitivity and specificity of methods based on human saliva

Authors	Type of sample	Methods	Target molecules	Sample size	Sensitivity (%)	Specificity (%)	Reference standard method
Wilson *et al*. 2008	Saliva	ELISA	*Pf*HRP II	40	43	100	Microscopy
Chidi *et al*. 2010	Saliva	ELISA	Antibodies to whole, asexual stage *P. falciparum* antigens	53	100	100	ELISA dried blood spots antibodies
Estevez *et al*. 2011	Saliva	ELISA	IgG against *P. falciparum* $MSP\text{-}1_{19}$	253	64-77	91-100	ELISA (plasma antibodies)
			IgG against *P. falciparum* AMA-1		47-67	90-97	
Fung *et al*. 2012	Saliva	ELISA	*Pf*HRP II	24	100	100	Microscopy
Gbotosho *et al*. 2010	Saliva	Optimal-IT dipsticks	pLDH	78 (whole saliva) 72 (supernatant of spun saliva)	77.9 (whole saliva) 48.4 (supernatant of spun saliva)	100	Microscopy
		Nested PCR	*Pfcrt* K76T	37	91	50	
Mharakurwa *et al*. 2006	Saliva	Nested PCR	*Pfmsp2*	51	-	-	Microscopy
			Pfdhfr				
Nwakanma *et al*. 2009	Saliva	Nested PCR	SSUrRNA	386	73	97	Microcopy
A-Elgayoum *et al*. 2010	Saliva	Nested PCR	*msp1*/*msp2*	21	NA	NA	NA
Buppan *et al*. 2010	Saliva	Nested PCR	SSUrRNA	120	74.1 (P.f)84 (P.v)	100	Microcopy
Putaporntip *et al*. 2011	Saliva	Nested PCR	*Cytb*	157	74.2 (P.f) 79.2 (P.v)	100 (P.f) 98.7 (P.v)	Nested PCR targeting *Cytb* of blood
Pooe *et al*. 2011	Saliva	Nested PCR	*Pfdhfr*	45	94.12	97.3	Nested PCR targeting *Pfdhfr* of blood
Singh *et al*. 2013	Saliva	Nested PCR	SSUrRNA	89	93.5 (P.v)	94.1 (P.v)	Microscopy
		LAMP			76.3 (P.v)	94.1 (P.v)	
Kast *et al*. 2013	Saliva	QT-NASBA	SSU-rRNA/*Pfs16*-mRNA/*Pfs25*-mRNA	15	NA	NA	NA

Table 1. Summary of sensitivity and specificity of methods based on human saliva (continued)

Authors	Type of sample	Methods	Target molecules	Sample size	Sensitivity (%)	Specificity (%)	Reference standard method
Singh *et al.* 2014	Saliva	Nested PCR	SSUrRNA	223	100 (P.f) 86.36 (P.v)	100 (P.f) 98.46 (P.v)	Microscopy
		Singleplex PCR	CRS		100 (P.f) 79.55 (P.v)	100 (P.f) 98.46 (P.v)	
		Multiplex PCR			71.43 (P.f) 70.45 (P.v)	100 (P.f) 99.23 (P.v)	
Najafabadi *et al.* 2014	Saliva	Nested PCR	SSUrRNA	108	92.2	97.4	Microscopy
		LAMP			48.5	100	

Note: AMA: apical membrane antigen; CRS: species-specific consensus repetitive sequences; *Cytb*: mitochondrial cytochrome *b*; ELISA: enzyme-linked immunosorbent assay; LAMP: loop-mediated isothermal amplification; $MSP\text{-}1_{19}$: merozoite surface protein-1; PCR: polymerase chain reaction; *Pfdhfr*: *Plasmodium falciparum* dihydrofolate reductase; *Pf*HRP II: *Plasmodium falciparum* histidine-rich protein II; pLDH: *Plasmodium falciparum* lactate dehydrogenase; QT-NASBA: quantitative nucleic acid sequence based amplification; SSU rRNA: small subunit rRNA; P.f: *Plasmodium falciparum*; P.v: *Plasmodium vivax*. NA: not available

low (Gbotosho *et al*., 2010). Saliva is also rich in serum IgA and IgG. Recent researches showed a correlation between malarial antibodies level in saliva and in plasma making saliva likely to be useful for community surveillance (Chidi *et al*., 2011; Estevez *et al*., 2011).

Even if the detection of malarial antigens/antibodies provides benefits, it may show false-positive results because of antigen/antibodies remaining after the infection has been cleared. In addition, it lacks of ability to test for the drug resistant markers or to quantify the level of infection. Molecular methods based on DNA amplification are sensitive, offer the ability to detect species/drug resistant markers, and are able to quantify the level of infection. Such methods have been developed to detect malaria in saliva (Cordray and Richards-Kortum, 2012).

The sensitivity of molecular methods for malaria detection in saliva was shown to be affected by several factors i.e. DNA extraction methods, size of target gene, sample fraction, and sample preservation. By applying nested PCR to amplify *Pfmsp*2 and *Pfdhfr* in saliva samples from *P. falciparum* infected individuals, Mharakurwa *et al*. (2006) found that amplicon yields significantly depended on DNA extraction method. A commercial Qiagen kit extraction of saliva had 2.6x better amplification success than the Chelex approach. The size of the amplicon also influenced PCR amplification in saliva samples. Among the primer sets used in the study, the *Pfdhfr* U1-4 primer set which amplify the shortest amplicon fragment get the highest amplification success. This is consistent with a study conducted in Zambia which found that short amplicon primers were more sensitive compared to long amplicon primers in amplifying *P. falciparum* DNA (Pooe *et al*., 2011). Low amplification sensitivity when amplified by long amplicon primers may suggest the degradation of parasite DNA in saliva (Mharakurwa *et al*., 2006; Pooe *et al*., 2011). The copy number of gene also affects the success of PCR amplification. Nested PCR targeting mitochondrial cytochrome b (*Cytb*) which has a copy number ranging from 30-100 per parasite showed 16% and 39.8% more sensitive in malaria detection than nested PCR targeting SSU-rRNA and microscopy, respectively (Putaporntip *et al*., 2011). Recently, the high copy species-specific consensus repetitive sequences (CRS) which presents 14 copies in *P.vivax* and 41 copies in *P. falciparum* were used to detect *P. falciparum* and *P.vivax* in saliva samples. The one-step CRS PCR assay displayed almost 80% sensitivity compared with microscopy (Singh *et al*., 2014).

Apart from DNA extraction method, size of amplicon, and gene copy number, parasite density seems to impact the success of amplification (Mharakurwa *et al*., 2006). The study in Gambia found that the sensitivity of nested PCR increased from 73% to 82% in samples with a parasite density of $\geq$1000 parasites/µl (Nwakanma *et al*., 2009). Although the study of Buppan *et al*. (2010) found that the positive rates of nested PCR of saliva samples increased with parasite density for *P. falciparum*, this finding however was not found in the results from nested PCR of *P. vivax* saliva samples.

The proper preservation of clinical specimens and the fraction of saliva used are also key factors influencing the success of the amplification. Saliva preserved in ethanol gave more than twice the yield of positive results than saliva kept on ice (Buppan *et al*., 2010). It has been reported that the pellet fraction is more suitable to use than supernatant fraction of spun saliva. Nested PCR in pellet of spun saliva displayed 91% sensitivity compared with microscopic examination (Gbotosho *et al*., 2010), and DNA amplified from pellet fraction revealed higher amplification success than DNA isolated from the soluble fraction of saliva (Pooe *et al*., 2011). The volume of the sample may also be important for the success of the test. The study of Najafabadi *et al*. (2014) using two-fold volume of saliva used in the studies of Mharakurwa *et al*. (2006) and Buppan *et al*. (2010) yield higher sensitivity of nested PCR in saliva samples.

The current PCR-based methods are costly and need specific equipment run by trained personnel to perform, making them impractical for routine diagnosis of malaria in remote areas. Thus, a new molecular method, loop-mediated isothermal amplification (LAMP), was designed to circumvent these problems. LAMP is a rapid, inexpensive, sensitive, specific, and simple technique. It can be performed at a single temperature, and the result can be observed with the naked eyes making it suitable for use in field setting (Han, 2013). LAMP for malaria detection in non-blood samples has been assessed in some studies. A study conducted in India using LAMP to detect malaria in saliva showed a sensitivity of 76.3% (Singh *et al*., 2013) while another study conducted in Iran reported a sensitivity of LAMP in saliva at only 48.5% despite preserving saliva in ethanol (Najafabadi *et al*., 2014). As saliva contains several microbes, microbial DNases, and other constituents which may degrade the DNA template and inhibit the amplification, rinsing the patient mouth with water before saliva collecting in the former study may have reduced contamination from other substances resulting in increased sensitivity (Singh *et al*., 2013).

Although malarial DNA in saliva samples has been successfully detected, the biological processes by which the parasite DNA reaches into saliva are unknown. DNA from lysed parasites may passively enter saliva via the serum or within phagosomes of macrophages through intraoral bleeding or the gingival cervicular fluid (Sutherland and Hallett, 2009). The precise mechanisms by which traces of malarial DNA appears in saliva need to be further investigated.

2.2 Urine

Urine is another sample which has been assessed for non-invasive malaria detection. Previous studies suggested that both malarial antigens and antibodies are possibly released into the urine during malaria infection. An ultrastructural pathological study of renal tissue from *P. falciparum* patients revealed the presence of parasitized erythrocytes sequestered in glomerular and tubulointerstitial vessels (Nguansangiam *et al*., 2007) and also immune complexes including IgG, C3, and malarial antigens (Das, 2008). These results along with proteinuria reported in patients infected with *P. falciparum* lead to the idea that parasite antigens and antibodies may be excreted into urine. Antisera raised against urine from *P. falciparum* patients showed positive results with *P. falciparum* parasites in indirect immunofluorescence test and immunoprecipitated extracts of parasites metabolic. These results suggest that a variety of malarial antigens are released into urine (Valle *et al*., 1991). Moreover, dot-blot and western blot assay also revealed the presence of malarial antigens in urine of patients infected with *P. vivax*. It should be noted however that the antigens can still be detected in patients who no longer had detectable parasitemia after treatment (Militao *et al*., 1993).

The molecular detection of malarial DNA in urine seems less sensitive than in saliva ranging from approximately 30% to 70% sensitivity (Table 2). This is probably due to the less amount of DNA template presence in urine than in saliva. Quantitative real-time PCR analysis showed that the mean quantity of parasite DNA in blood was ~600-fold and ~2500-fold that in concurrent saliva and urine, respectively (Nwakanma *et al*., 2009). The sensitivity may also depend on the particular PCR technique used as LAMP showed a sensitivity only 30% compared with nested PCR. This possibly indicates that the amount of parasite DNA in urine is below the minimum detection limit of LAMP, which has been reported at 30±5 parasites/μl while nested PCR has been reported at 3±5 parasites/μl (Chen *et al*., 2010; Lu *et al*., 2012). The gene copy number may also affect the sensitivity of using urine as found in saliva. In the study of Nwakanma *et al*. (2009) and Buppan *et al*. (2010), nested PCR of SSU-rRNA showed approximately 30% sensitivity whereas the study of Putaporntip *et al*. (2011) amplifying higher copy number *cytb* showed higher sensitivity at around 50%. The success of amplification also depends on the volume of urine samples used. By using five-fold the urine volume used in the study of Nwakanma *et al*. (2009) and Buppan *et al*. (2010), Najafabadi *et al*. (2014) showed a higher sensitivity of PCR amplifying SSU-rRNA. Other factors impacting the sensitivity of gene amplification in saliva samples such as parasite density, DNA extraction method, size of amplicon, and sample preservation also seem to affect the sensitivity in urine samples.

2.3 Buccal mucosa

Buccal mucosa, fast-replaced tissues, has also been used for malaria detection. In a study applying nested PCR to target *msp1* and *msp2* genes, the researchers found that the specific genes were successfully amplified in buccal mucosa and were comparable to saliva and urine. They did not however find the association of the PCR positive rate with parasite density, patient age, or body temperature (A-Elgayoum *et al*., 2010). Another study using real-time quantitative nucleic acid sequence based amplification (QT-NASBA) also showed successful RNA amplification in buccal mucosa samples. Interestingly, the authors found that

Table 2. Summary of sensitivity and specificity of methods based on human urine and buccal mucosa

Authors	Type of sample	Methods	Target molecules	Sample size	Sensitivity (%)	Specificity (%)	Reference standard method
Valle *et al*. 1991	Urine	IFA / Immuno precipitation	*Plasmodium* antigens	30	NA	NA	NA
Militao *et al*. 1993	Urine	Dot-blot Western-blot	*Plasmodium* antigens	50	NA	NA	NA
Mharakurwa *et al*. 2006	Urine	Nested PCR	*Pfmsp2* *Pfdhfr*	51	-	-	Microscopy
Nwakanma *et al*. 2009	Urine	Nested PCR	SSUrRNA	386	32	98	Microcopy
Buppan *et al*. 2010	Urine	Nested PCR	SSUrRNA	120	44.4 (P.f) 34 (P.v)	100	Microcopy
Putaporntip *et al*. 2011	Urine	Nested PCR	*Cytb*	157	55.1 (P.f) 53.3 (P.v)	100 (P.f) 97.5 (P.v)	Nested PCR targeting *Cytb* of blood
Najafabadi *et al*. 2014	Urine	Nested PCR / LAMP	SSUrRNA	108	73.3 / 30	100 / 100	Microscopy
A-Elgayoum *et al*. 2010	Urine / Buccal mucosa	Nested PCR	*msp1*/*msp2*	21	NA	NA	NA
Kast *et al*. 2013	Urine / Buccal mucosa	QT-NASBA	SSU-rRNA/*Pfs16* -mRNA/*Pfs25*-mRNA	15	NA	NA	NA

Note: *Cytb*: mitochondrial cytochrome *b*; IFA: indirect immunofluorescence; LAMP: loop-mediated isothermal amplification; PCR: polymerase chain reaction; *Pfdhfr*: *Plasmodium falciparum* dihydrofolate reductase; QT-NASBA: quantitative nucleic acid sequence based amplification; SSU rRNA: small subunit rRNA; P.f: *Plasmodium falciparum*; P.v: *Plasmodium vivax*.
NA: not available

the mucosa spread on filter paper displayed four times better than fresh frozen mucosal swab (Kast *et al*., 2013). The processes leading to the presence of parasite DNA in the buccal mucosa are unknown. It is proposed that mucus membrane including buccal mucosa or epithelial lining of the oral cavity act as the filters for the passage of parasite constituents into the saliva. Thus, the traces of parasite DNA can be found in these tissues (A-Elgayoum *et al*., 2010).

3. Conclusions

Detection of malaria by using non-invasive samples including saliva, urine, and buccal mucosa has been developed to reduce the risks of blood drawing and improve patient compliance. The utility of non-blood samples would prove to be beneficial in antimalarial drug and vaccine trials which require repeated sampling from volunteers. In addition, combining non-blood samples with molecular methods might be as beneficial as blood-based conventional microscopy in determining parasite density during post-treatment follow-up.

Among the non-blood samples described in this review, saliva is most likely to be suitable for malaria detection. However, detection of antigens/antibodies in saliva could give false-positive results due to the antigens/antibodies circulating after the infection has ended, and it does not provide information about infection level and drug resistant markers. Thus, molecular methods which offer the ability to quantify infection level and detect drug resistance markers have been developed to solve these problems. It is quite obvious that nested PCR is the most sensitive and specific method among the techniques used to detect malaria in saliva as well as in urine. However, an improvement in the sensitivity of PCR in non-blood samples including the DNA extraction method, sample preservation, primer set and fraction/volume of sample used is needed before this can become a tool with real utility.

Acknowledgement

The author thanks Dr. Alfredo Villarroel, Faculty of Medicine, Srinakharinwirot University for editing the English language.

References

A-Elgayoum, S., El-Rayah el, A., and Giha, H.A. 2010. Towards a noninvasive approach to malaria diagnosis: detection of parasite DNA in body secretions and surface mucosa. Journal of Molecular Microbiology and Biotechnology. 18, 148-55.

Arregger, A.L., Cardoso, E.M., Tumilasci, O., and Contreras, L.N. 2008. Diagnostic value of salivary cortisol in end stage renal disease. Steroids. 73, 77-82.

Bell, D. 2004. The use of Malaria Rapid Diagnostic Tests. World Health Organization, Geneva, Switzerland.

Bell, D., Wongsrichanalai, C., and Barnwell, J.W. 2006. Ensuring quality and access for malaria diagnosis: how can it be achieved? Nature Reviews Microbiology. 4, 682-95.

Buppan, P., Putaporntip, C., Pattanawong, U., Seethamchai, S., and Jongwutiwes, S. 2010. Comparative detection of *Plasmodium vivax* and *Plasmodium falciparum* DNA in saliva and urine samples from symptomatic malaria patients in a low endemic area. Malaria Journal. 9, 72.

Chen, J.H., Lu, F., Lim, C.S., Kim, J.Y., Ahn, H.J., Suh, I.B., Takeo, S., Tsuboi, T., Sattabongkot, J., and Han, E.T. 2010. Detection of *Plasmodium vivax* infection in the Republic of Korea by loop-mediated isothermal amplification (LAMP). Acta Tropica. 113, 61-5.

Chidi, A.P., Chishimba, S., Kobayashi, T., Hamapumbu, H., Mharakurwa, S., Thuma, P.E., and Moss, W.J. 2011. Validation of oral fluid samples to monitor serological changes to *Plasmodium falciparum*: an observational study in southern Zambia. Malaria Journal. 10, 162.

Coleman, R.E., Sattabongkot, J., Promstaporm, S., Maneechai, N., Tippayachai, B., Kengluecha, A., Rachapaew, N., Zollner, G., Miller, R.S., Vaughan, J.A., Thimasarn, K., and Khuntirat, B. 2006. Comparison of PCR and microscopy for the detection of asymptomatic malaria in a *Plasmodium falciparum/vivax* endemic area in Thailand. Malaria Journal. 5, 121.

Cordray, M.S., and Richards-Kortum, R.R. 2012. Emerging nucleic acid-based tests for point-of-care detection of malaria. American Journal of Tropical Medicine and Hygiene. 87, 223-30.

Das, B.S. 2008. Renal failure in malaria. Journal of Vector Borne Diseases. 45, 83-97.

Endeshaw, T., Gebre, T., Ngondi, J., Graves, P.M., Shargie, E.B., Ejigsemahu, Y., Ayele, B., Yohannes, G., Teferi, T., Messele, A., Zerihun, M., Genet, A., Mosher, A.W., Emerson, P.M., and Richards, F.O. 2008. Evaluation of light microscopy and rapid diagnostic test for the detection of malaria under operational field conditions: a household survey in Ethiopia. Malaria Journal. 7, 118.

Estevez, P.T., Satoguina, J., Nwakanma, D.C., West, S., Conway, D.J., and Drakeley, C.J. 2011. Human saliva as a source of anti-malarial antibodies to examine population exposure to *Plasmodium falciparum*. Malaria Journal. 10, 104.

Floriano, P.N., Christodoulides, N., Miller, C.S., Ebersole, J.L., Spertus, J., Rose, B.G., Kinane, D.F., Novak, M.J., Steinhubl, S., Acosta, S., Mohanty, S., Dharshan, P., Yeh, C.K., Redding, S., Furmaga, W., and McDevitt, J.T. 2009. Use of saliva-based nano-biochip tests for acute myocardial infarction at the point of care: a feasibility study. Clinical Chemistry. 55, 1530-8.

Fung, A.O., Damoiseaux, R., Grundeen, S., Panes, J.L., Horton, D.H., Judy, J.W., and Moore, T.B. 2012. Quantitative detection of *Pf*HRP2 in saliva of malaria patients in the Philippines. Malaria Journal. 11, 175.

Gbotosho, G.O., Happi, C.T., Folarin, O., Keyamo, O., Sowunmi, A., and Oduola, A.M., 2010. Rapid detection of lactate dehydrogenase and genotyping of *Plasmodium falciparum* in saliva of children with acute uncomplicated malaria. American Journal of Tropical Medicine and Hygiene. 83, 496-501.

Han, E.T. 2013. Loop-mediated isothermal amplification test for the molecular diagnosis of malaria. Expert Review of Molecular Diagnostics. 13, 205-18.

Hu, S., Wang, J., Meijer, J., Ieong, S., Xie, Y., Yu, T., Zhou, H., Henry, S., Vissink, A., Pijpe, J., Kallenberg, C., Elashoff, D., Loo, J.A., and Wong, D.T. 2007. Salivary proteomic and genomic biomarkers for primary Sjogren's syndrome. Arthritis and Rheumatism. 56, 3588-600.

Jongwutiwes, S., Putaporntip, C., Iwasaki, T., Sata, T., Kanbara, H., 2004. Naturally acquired *Plasmodium knowlesi* malaria in human, Thailand. Emerging Infectious Diseases. 10, 2211-3.

Kast, K., Berens-Riha, N., Zeynudin, A., Abduselam, N., Eshetu, T., Loscher, T., Wieser, A., Shock, J., and Pritsch, M., 2013. Evaluation of *Plasmodium falciparum* gametocyte detection in different patient material. Malaria Journal. 12, 438.

Kaufman, E., and Lamster, I.B. 2002. The diagnostic applications of saliva—a review. Critical Reviews in Oral Biology and Medicine. 13, 197-212.

Lu, F., Gao, Q., Zhou, H., Cao, J., Wang, W., Lim, C.S., Na, S., Tsuboi, T., and Han, E.T. 2012. Molecular test for *vivax* malaria with loop-mediated isothermal amplification method in central China. Parasitology Research. 110, 2439-44.

Mayxay, M., Pukrittayakamee, S., Chotivanich, K., Looareesuwan, S., and White, N.J. 2001. Persistence of *Plasmodium falciparum* HRP-2 in successfully treated acute falciparum malaria. Transactions of the Royal Society of Tropical Medicine and Hygiene. 95, 179-82.

Mharakurwa, S., Simoloka, C., Thuma, P.E., Shiff, C.J., and Sullivan, D.J. 2006. PCR detection of *Plasmodium falciparum* in human urine and saliva samples. Malaria Journal. 5, 103.

Militao, D.N., Camargo, L.M., and Katzin, A.M. 1993. Detection of antigens in the urine of patients with acute *Plasmodium vivax* malaria. Experimental Parasitology. 76, 115-20.

Milne, L.M., Kyi, M.S., Chiodini, P.L., and Warhurst, D.C. 1994. Accuracy of routine laboratory diagnosis of malaria in the United Kingdom. Journal of Clinical Pathology. 47, 740-2.

Moody, A., 2002. Rapid diagnostic tests for malaria parasites. Clinical Microbiology Reviews. 15, 66-78.

Morassin, B., Fabre, R., Berry, A., and Magnaval, J.F. 2002. One year's experience with the polymerase chain reaction as a routine method for the diagnosis of imported malaria. American Journal of Tropical Medicine and Hygiene. 66, 503-8.

Mueller, I., Zimmerman, P.A., and Reeder, J.C. 2007. *Plasmodium malariae* and *Plasmodium ovale*—the "bashful" malaria parasites. Trends in Parasitology. 23, 278-83.

Muhamad, P., Ruengweerayut, R., Chacharoenkul, W., Rungsihirunrat, K., and Na-Bangchang, K. 2011. Monitoring of clinical efficacy and in vitro sensitivity of *Plasmodium vivax* to chloroquine in area along Thai Myanmar border during 2009-2010. Malaria Journal. 10, 44.

Najafabadi, G., Oormazdi, H., Akhlaghi, L., Meamar, A., Nateghpour, M., Farivar, L., and Razmjou, E. 2014. Detection of *Plasmodium vivax* and *Plasmodium falciparum* DNA in human saliva and urine: Loop-mediated isothermal amplification for malaria diagnosis. Acta Tropica. 136, 44-49.

Nguansangiam, S., Day, N.P., Hien, T.T., Mai, N.T., Chaisri, U., Riganti, M., Dondorp, A.M., Lee, S.J., Phu, N.H., Turner, G.D., White, N.J., Ferguson, D.J., and Pongponratn, E., 2007. A quantitative ultrastructural study of renal pathology in fatal *Plasmodium falciparum* malaria. Tropical Medicine and International Health. 12, 1037-50.

Nwakanma, D.C., Gomez-Escobar, N., Walther, M., Crozier, S., Dubovsky, F., Malkin, E., Locke, E., and Conway, D.J. 2009. Quantitative detection of *Plasmodium falciparum* DNA in saliva, blood, and urine. Journal of Infectious Diseases. 199, 1567-74.

Ochola, L.B., Vounatsou, P., Smith, T., Mabaso, M.L., and Newton, C.R. 2006. The reliability of diagnostic techniques in the diagnosis and management of malaria in the absence of a gold standard. Lancet Infectious Diseases. 6, 582-8.

Pooe, O.J., Shonhai, A., and Mharakurwa, S. 2011. A PCR screen for malaria carrier infections using human saliva samples. African Journal of Microbiology Research. 5, 5120-5126.

Putaporntip, C., Buppan, P., and Jongwutiwes, S. 2011. Improved performance with saliva and urine as alternative DNA sources for malaria diagnosis by mitochondrial DNA-based PCR assays. Clinical Microbiology and Infection. 17, 1484-91.

Ratnawati, Hatta, M., and Smits, H.L. 2008. Point-of-care testing for malaria outbreak management. Transactions of the Royal Society of Tropical Medicine and Hygiene. 102, 699-704.

Singh, B., and Daneshvar, C. 2013. Human infections and detection of *Plasmodium knowlesi*. Clinical Microbiology Reviews. 26, 165-84.

Singh, R., Savargaonkar, D., Bhatt, R., and Valecha, N. 2013. Rapid detection of *Plasmodium vivax* in saliva and blood using loop mediated isothermal amplification (LAMP) assay. Journal of Infection. 67, 245-7.

Singh, R., Singh, D.P., Gupta, R., Savargaonkar, D., Singh, O.P., Nanda, N., Bhatt, R.M., and Valecha, N. 2014. Comparison of three PCR-based assays for the non-invasive diagnosis of malaria: detection of *Plasmodium* parasites in blood and saliva. European Journal of Clinical Microbiology and Infectious Diseases.

Sutherland, C.J., and Hallett, R. 2009. Detecting malaria parasites outside the blood. Journal of Infectious Diseases. 199, 1561-3.

Tangpukdee, N., Duangdee, C., Wilairatana, P., and Krudsood, S. 2009. Malaria diagnosis: a brief review. Korean Journal of Parasitology. 47, 93-102.

Trampuz, A., Jereb, M., Muzlovic, I., and Prabhu, R.M. 2003. Clinical review: Severe malaria. Critical Care. 7, 315-23.

Valle, M., Quakyi, I., Amuesi, J., Quaye, J., Nkrumah, F., and Taylor, D. 1991. Detection of antigens and antibodies in the urine of humans with *Plasmodium falciparum* malaria. Journal of Clinical Microbiology. 29, 1236-1242.

Waitumbi, J.N., Gerlach, J., Afonina, I., Anyona, S.B., Koros, J.N., Siangla, J., Ankoudinova, I., Singhal, M., Watts, K., Polhemus, M.E., Vermeulen, N.M., Mahoney, W., Steele, M., and Domingo, G.J. 2011. Malaria prevalence defined by microscopy, antigen detection, DNA amplification and total nucleic acid amplification in a malaria-endemic region during the peak malaria transmission season. Tropical Medicine and International Health. 16, 786-93.

White, N.J., Pukrittayakamee, S., Hien, T.T., Faiz, M.A., Mokuolu, O.A., and Dondorp, A.M. 2014. Malaria Lancet. 383, 723-35.

WHO, 2012. Malaria Rapid Diagnostic Test Performance. Results of WHO product testing of malaria RDTs: Round 4 (2012). World Health Organization, Geneva, Switzerland

WHO, 2013. World Malaria Report 2013. World Health Organization, Geneva, Switzerland

Wilson, N.O., Adjei, A.A., Anderson, W., Baidoo, S., and Stiles, J.K. 2008. Detection of *Plasmodium falciparum* histidine-rich protein II in saliva of malaria patients. American Journal of Tropical Medicine and Hygiene. 78, 733-5.

Wongsrichanalai, C., Barcus, M.J., Muth, S., Sutamihardja, A., and Wernsdorfer, W.H. 2007. A review of malaria diagnostic tools: microscopy and rapid diagnostic test (RDT). American Journal of Tropical Medicine and Hygiene. 77, 119-27.