www.ajbrui.org

Afr. J. Biomed. Res. Vol. 20 (May, 2017); 121- 126

Full Length Research Paper

Comparative Diagnosis of *Plasmodium Falciparum* Malaria with a Histidine-Rich Protein 2 Rapid Diagnostic Test and Light Microscopy in a Hospital Setting in Katsina-Ala Nigeria

T.F. Ikpa,[1*] N. Ikpa,[1] and V.U. Obisike[1]

[1]*Department of Biological Sciences University of Agriculture Makurdi, PMB 2373, Makurdi, Benue State, Nigeria.*

ABSTRACT

Malaria diagnosis with the rapid diagnostic tests (RDTs) is recommended for malaria endemic countries. However, some brands of the malaria RDTs currently in circulation may not satisfy the minimum requirement for diagnosis and clinical management of falciparum malaria. This study compared the performance of a histidine-rich protein 2 CareStart™ (Pf) malaria RDT, with light microscopy in the diagnosis of *Plasmodium falciparum* in a hospital setting in Katsina-Ala, Nigeria. A total of 723 hospital patients; 411 symptomatic and 312 asymptomatic were screened with CareStart™ (Pf) malaria RDT and light microscopy for the diagnosis of *P. falciparum* malaria. Using light microscopy as the reference test, the results indicated that the prevalence of *P. falciparum* was 25.9% by CareStart RDT and 23.2% by light microscopy. The level of the two tests concordance was outstanding in symptomatic malaria: kappa (k) = 0.92 (95% CI = 0.87 – 0.96) $p < 0.001$, and substantial in asymptomatic malaria: k = 0.70 (95% CI = 0.60 – 0.79) $p < 0.001$. Sensitivity = 86.8%; 95.7%, while specificity = 91.5%; 97.0% in asymptomatic and symptomatic malaria respectively. Similarly, positive predictive value = 67.6%; 92.4%, while negative predictive value = 97.1% and 98.3% in the same group of patients. These results suggest that CareStart™ (Pf) malaria RDT is suitable for the diagnosis of *P. falciparum* malaria. However, it has a tendency to overestimate malaria prevalence, albeit having a reduced probability of detecting truly positive *P. falciparum* infections in a hospital setting.

Keywords: *malaria diagnosis, microscopy, rapid diagnostic test, histidine-rich protein 2*

*Author for correspondence: *E-mail:* terwasefsi@yahoo.com

Received: July, 2016; Accepted: February, 2017

Abstracted by:
Bioline International, African Journals online (AJOL), Index Copernicus, African Index Medicus (WHO), Excerpta medica (EMBASE), CAB Abstracts, SCOPUS, Global Health Abstracts, Asian Science Index, Index Veterinarius

INTRODUCTION

Malaria is a vector borne parasitic protozoan infection that is caused by five known Plasmodium species in human populations (Antinori et al., 2012). The disease is currently estimated to cause 214 million cases; range (149 – 303) million, while the number of deaths due to malaria is estimated at 438 000; range (236 000 – 635 000). This is in spite of concerted efforts to fight malaria, in the last 15 years period that has drastically reduced its human burden (WHO 2015a). Some of the evolving new strategies to combat and perhaps eliminate malaria in regions where it is possible have led to the introduction of artemisinin combination therapies (ACTs) for the treatment of the disease (WHO 2010), and the rapid diagnostic tests (RDTs) for rapid detection of the malaria parasites before treatment is effected (WHO 2015b).

Prior to the introduction of RDTs, malaria diagnosis at the health centres in malaria endemic areas relied mainly on microscopic examination and detection of the parasites (Wongsrichanalai *et al*., 2007). To date, the microscopy procedure is still the 'gold standard' for malaria diagnosis (WHO 2009). However, microscopy diagnosis requires the acquisition of some technical skills by well trained, and competent microscopists whose competence needs to be frequently re-evaluated for quality assurance to effectively diagnose malaria (Wongsrichanalai et al., 2007, WHO 2009). The practical reality that is glaring is the fact that the required technical knowhow for microscopy diagnosis is not readily available in times of need, particularly in malaria endemic rural areas where the greatest burden of malaria is frequently felt. Equally lacking is a constant source of electricity required to power the light microscopes (LM) during slides examination for malaria parasites.

The introduction of malaria RDTs was intended to overcome these aforementioned challenges associated with malaria diagnoses in disease endemic areas (Aidoo 2013). Among many other objectives, the use of RDTs in malaria diagnosis is intended to promote evidenced based treatment, which was previously based on presumptive diagnosis (Chandramohan et al., 2002, Talisuna 2007). Presently the production of malaria RDT brands has soared to a level that there are over 200 malaria RDTs now available for malaria diagnoses (WHO 2015b). However, because of the heterogeneous performance response of the different brands of RDTs available for malaria diagnosis, the WHO (2016) has reviewed the minimum performance criteria recommended for the selection and use of malaria RDTs. These requirements include a panel detection score of at least 75% against *P. falciparum* at 200 parasites/µL of blood in all samples, for the detection of *P. falciparum* in all transmission settings. The same requirement applies for *P. vivax* detection in all transmission settings. In addition, less than 10% false positive rate, and less than 5% invalid rate for the specific RDT performance is recommended (WHO 2015b, 2016).

The CareStart™ Pf malaria RDT is a histidine - rich protein 2 (HRP2) based RDT brand that specifically detects *P. falciparum* malaria using the presence of HRP2; a water soluble antigen that is produced by all developmental stages of *P. falciparum* parasites in the blood (Rock et al., 1987). In the round 6 product testing of malaria RDTs, the CareStart™ Pf malaria RDT brand also met the WHO minimum requirements for malaria diagnosis (WHO 2015b, 2016). The test kit is currently being used for malaria diagnosis in Nigeria, but with varying levels of sensitivities reported in different parts of the country (Sheyin and Bigwan 2013, Brown and Azike 2014, Abdulkadir et al., 2015). The wide performance variation of this brand of malaria RDT, reported in Nigeria for malaria diagnoses in recent times, prompted us to evaluate its performance in a hospital setting. Thus we compared the *P. falciparum* malaria diagnostic performance of CareStart™ Pf malaria RDT, with LM as a reference test, in a hospital setting at Katsina-Ala Benue State, an endemic area of *P. falciparum* malaria in Nigeria.

MATERIALS AND METHODS

Study area: the study was conducted at Katsina-Ala. located at Latitude 7^0 10' 0" N and Longitude 9^0 17' 0" E, in Benue State, north central Nigeria. Samples were collected from the General Hospital Katsina-Ala from February to October 2015.

Ethical consideration

The study was conducted in line with international ethical guidelines for biomedical research involving human subjects (CIOMS 2002). Ethical approval was granted by the local ethics committee of the General Hospital Katsina-Ala. Subjects were recruited if they gave written informed consent when requested. In the case of minors, consent was sought and obtained from their parents or guardian.

Study subjects: subjects with a fever, or other clinical symptoms of malaria, with axillary temperature ≥ 37.5 ^{o}C, who attended the General Hospital Katsina-Ala, and gave written informed consent were enrolled to participate in the study, n = 411. In addition, asymptomatic subjects with normal body temperature, without evidence of fever, who attended the hospital were also enrolled, n = 312. There were no exclusion criteria. A total of n = 723 subjects that gave informed consent to participate in the study, during the period of sampling were enrolled.

Blood sample collection and malaria diagnosis: rapid diagnostic test. From each of the enrolled subject, about 5 µl of the finger prick blood was collected using a rubber pipette. The blood was dropped into the sample area of CareStart™ Pf HRP2 malaria rapid diagnostic test cassette (Access Bio Inc. Somerset NJ USA). This was followed by the addition of two drops of a buffer, supplied with the kit in the wicking area of the RDT test pad. The RDT cassette was placed on a flat surface for 20 minutes before it was examined for the result of the test. A positive test indicated a band in 'T' (test line) and another one in 'C' (control line) labels on the cassette. A negative test showed only one band in 'C'. While a band in 'T' without one in 'C' or no band appearing in both 'T' and 'C' indicated an invalid test (WHO 2015b).

Microscopy diagnosis: from each subject tested for the rapid diagnostic test, a single drop of the finger prick blood was dropped on a grease free microscopy slide to prepare a thick blood film. The thick film was stained with 10% Giemsa stain for 10 minutes, and examined on a microscope using X100 oil immersion objective (WHO 2009). Two independent microscopists, each blinded to the slide readings of one another scored the thick films as either positive or negative slides. All the slides with disagreement were resolved by a third independent microscopist. Also asexual parasite density/µl of blood, of the malaria positive slides were computed by counting the number of asexual parasites per 200 white blood cell (WBCs) multiplied by 40 (WHO 2009).

Statistical analysis: the test result of each malaria diagnosis by the RDT was compared with the corresponding diagnosis by LM which was the reference test. Values of kappa (k) and their 95% confidence interval (CI) were each determined for the three categories of patients, namely all subjects, symptomatic, and asymptomatic subjects. The resulting k values were interpreted according to Landis and Koch (1977). In order to determine the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of the RDT, the individual's test results were classified as true positive (TP), true negative (TN), false positive (FP) and false negative (FN) using reference microscopy. The sensitivity of RDT; that is the probability that a truly *P. falciparum* infected individual will test positive by RDT was estimated as = (TP/TP+FN). Specificity or the probability that a truly uninfected individual will test negative by the RDT = (TN/TN + FP). In addition, we determined the PPV or the probability that those testing positive by RDT were truly infected = (TP/TP + FP), and the NPV or the probability that those testing negative by RDT were truly uninfected = (TN/TN + FN). In each case, the 95% confidence interval (95% CI) of the respective probabilities was also calculated in each of the different subject categories (Banoo et al., 2010).

RESULTS

The mean age of all the patients diagnosed was 23.7 ± 9.5 years, range, 1 – 60 years. The prevalence of *P. falciparum* malaria among all the diagnosed hospital patients was 23.2% by light microscopy (n = 723). The corresponding prevalence by CareStart^{TM} Pf malaria RDT was 25.9%. In symptomatic subjects alone, LM prevalence slightly increased to 28.0%, while the RDT prevalence also increased to 29.0% (n = 411). However, among the asymptomatic subjects, there was a decrease in LM prevalence to 17.0%, while RDT prevalence equally reduced in the same category to 21.8% (n = 312). There was a significant agreement between LM and the RDT (k = 0.84, (95% CI = 0.79 – 0.88), p < 0.001), for all the subjects diagnosed. A similar significant agreement was obtained in both the symptomatic and asymptomatic subjects (p < 0.001, Table 1). By comparing the RDT to the reference test LM, it was observed that the percentage of false negatives were 1.7%, 2.2% and 2.9% in symptomatic subjects, all subjects, and asymptomatic subjects (Table 1). In contrast, false positives were 7.6%, 16.6% and 32.4% respectively in the corresponding subjects categories. The total cases of invalid tests were small, and constituted a mere 1.24% (9/723). The sensitivity of the CareStart^{TM} Pf malaria RDT in the symptomatic hospital subjects was = 95.7% and higher than asymptomatic patients which was = 86.8%. Similarly, the specificity of the RDT kit was higher in the symptomatic subjects = 97.0%, and lower in the asymptomatic subjects = 91.5%. A similar trend was also observed in the estimated PPV of the RDT kit = 92.4% and 67.6%; and the NPV = 98.3% and 97.1% of symptomatic and asymptomatic hospital subjects respectively (Table 2). The mean parasite density among asymptomatic subjects was 758 ± 174 asexual parasites/µl of blood (Figure 1). In this group, 17% (53/312) of the sampled patients in the hospital environment had detectable peripheral parasitaemia by LM, although they had no apparent symptoms of malaria.

Table 1: Comparative diagnosis of malaria with CareStart RDT and reference Microscopy

Category	CareStart RDT (%)	Reference microscopy (%) positive	Reference microscopy (%) negative	Total	Kappa (k) (95% CI)	*p* - value
All subjects (n = 723)	positive	156 (83.4)	31 (16.6)	187	0.84 (0.79 -0.88)	< 0.001
	Negative	12 (2.2)	524 (97.8)	536		
Symptomatic (n = 411)	positive	110 (92.4)	9 (7.6)	119	0.92 (0.87 – 0.96)	< 0.001
	negative	5 (1.7)	287 (98.3)	292		
Asymptomatic (n = 312)	positive	46 (67.6)	22 (32.4)	68	0.70 (0.60 – 0.79)	< 0.001
	negative	7 (2.9)	237 (97.1)	244		

Table 2:: Sensitivity, specificity, positive predictive value (PPV) and negative predictive value NPV) of CareStart RDT

Category	% Sensitivity (95% CI)	% Specificity (95% CI)	% PPV (95% CI)	% NPV (95% CI)
All subjects (n = 723)	92.9 (89.0 – 96.8)	94.4 (92.5 – 96.3)	83.4 (78.1 – 88.7)	97.8 (96.6 – 99.0)
Symptomatic (n = 411)	95.7 (92.0 – 99.4)	97.0 (95.1 – 98.9)	92.4 (87.6 – 97.2)	98.3 (96.8 – 99.8)
Asymptomatic (n = 312)	86.8 (77.7 – 95.9)	91.5 (88.1 – 94.9)	67.6 (56.5 – 78.7)	97.1 (95.0 – 99.2)

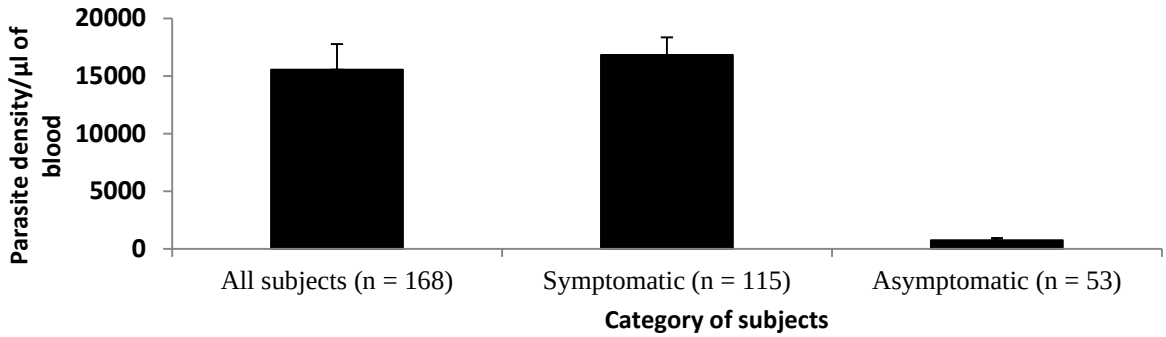

Figure 1
Mean parasite density of subjects ± standard error

DISCUSSION

The comparative diagnosis of *P. falciparum* malaria by CareStart™ Pf malaria RDT and LM showed a significant agreement ($p < 0.001$) between the two methods. The level of agreement between the two tests was outstanding (Landis and Koch 1977) in all the subjects ($k = 0.84$) as well as symptomatic subjects ($k = 0.92$). It was equally substantial in asymptomatic malaria patients ($k = 0.70$). This demonstrates that the CareStart™ Pf malaria RDT can be a very useful tool for malaria diagnosis in a hospital setting. It may be even more useful, particularly in rural health facilities in malaria endemic areas. Specifically, our findings suggest that the CareStart™ Pf RDT had slightly higher estimates of the prevalence of *P. falciparum* malaria among each category of the hospital patients in the present study. This over estimation was probably the product of the varying number of false positives, which arose among the different subjects groups. For instance, the 32.4% of false positives within the asymptomatic malaria category could be due to many probable factors (Maltha et al., 2013). Among them, the persistence of the HRP2 antigen in the blood, despite the absence of detectable parasitaemia appears to be a reasonable explanation.

The scenario may arise if treatment with antimalarial drugs, just prior to sampling had successfully reduced blood parasitaemia to a level that it was impossible to detect by microscopy. However, the remnants of circulating HRP2 antigen were enough to cause a sensitive RDT kit to test positive. Those remnants of HRP2 would be those that were produced in the presence of parasitaemia, but had not completely decayed from the blood stream, following parasites removal by the antimalarial drugs (Maltha et al., 2014). One implication of a high number of false positives in any test is that, it will lead to patients not suffering from malaria being treated with antimalarial drugs. This negates the very essence for the use of malaria RDTs in evidence based treatment of malaria (Bisoffi et al., 2012). It will also raise the cost of malaria treatment, by wasting antimalarial drugs on patients that were not infected with malaria parasites.

It must also be appreciated that matured asexual stages of *P. falciparum* parasites, which are sequestered produce HRP2 (Dondorp et al., 2005, Mouatcho and Goldring 2013). Since sequestered parasites do not circulate in the peripheral blood, they will not be detected by microscopy examination. Their reluctance to circulate albeit producing the HRP2 in the blood stream will ensure that microscopy test will be negative, if asexual forms of the parasite were removed by drugs. Whereas, RDT will be positive assuming the matured sequestered forms of the parasites remained intact. In this case, true positive cases will be misclassified as false positives, thus increasing their number in a sample. A situation like this couldn't be ruled out in a hospital setting. It has been shown that serum rheumatoid factor, cross reaction and other parasitic diseases may also raise the number of false positive during tests with HRP2 malaria RDTs (Maltha et al., 2013).

The overall cases of false negative tests due to CareStart™ Pf malaria RDT in this study were 2.2%. Some recent studies in south America and India have observed that the presence of false negatives in HRP2 based RDTs were due to polymorphism in the HRP2 gene, leading to deficient production of the HRP2 antigen (Gamboa et al., 2010; Kumar et al., 2013, Akinyi et al., 2013, Baldeviano et al. 2015). Although sequence variation in the PfHRP2 gene among global parasites, including many variants in Nigeria did not affect HRP2 based RDTs performance (Baker et al., 2010), the deletion of the HRP2 gene from parasites populations affected the performance of RDTs (Gamboa et al., 2010; Kumar et al., 2013, Akinyi et al., 2013, Baldeviano et al. 2015). This gene deletion has not been investigated in Nigeria. There will be a need to ascertain if such gene deletion is common in Nigeria, and correlates with false negative RDTs in the country. Low parasite densities may lead to false positives (Maltha et al., 2013) but in our finding this factor may not apply. This is because the least mean parasite load of 758 ± 174 asexual parasites/µl of blood, found in asymptomatic malaria category was 3 times more than the recommended minimum of 200 asexual parasites/µl of blood needed for RDT's detection of malaria parasites (WHO 2015b).

One certain implication of a false negative diagnosis is that, it poses a serious danger to the life of the patient being tested, as the patient may be denied appropriate treatment with antimalarial drugs despite being infected with *P. falciparum* parasites. One potential reason for the observed false negatives could be due to the prozone effect (Gillet et al., 2009). This phenomenon has been demonstrated in the laboratory (Luchavez et al., 2011) and documented in the field (Gillet et al., 2011). Also a recent demonstration that circulating anti-HRP2 antibodies interfere with antigen detection by malaria RDTs (Ho et al., 2014) could be yet another plausible reason for the false negative RDTs, seen in the present study. Thus a more comprehensive study that investigates all three possible causes of false positive RDTs is needed to elucidate the relationship that exist among HRP2 gene deletion, the prozone effect, circulating levels of anti-HRP2 antibodies, and false negative RDTs.

The sensitivity of 95.7% in symptomatic subjects was up to the recommended minimum requirement for selection and use of malaria RDT (WHO 2015b). In asymptomatic malaria subjects however, the sensitivity reduced to 86.8%, but was higher than the 78.4% sensitivity reported by Sheyin and Bigwan (2013) in Zaria Nigeria, when a similar brand of the RDT was used. The general performance indicators of the CareStart™ Pf malaria RDT kit reported in Nigeria when LM was used as the reference test have shown divergent and perhaps not very encouraging results. For instance, in Zaria Northern Nigeria, the sensitivity was 78.4% with specificity of 97.6% in hospital patients (Sheyin and Bigwan 2013). However, in Zamfara, also in northern Nigeria, a very low sensitivity of 40.3% and a specificity of 89.6% were reported in children < 5 years (AbdulKadir et al., 2015). Furthermore, another poor sensitivity of 37.7% and 89.0% Specificity was reported in Port-Harcourt southern Nigeria (Brown and Azike 2014). The last two studies in Zamfara and Port-Harcourt suggests that CareStart™ Pf malaria RDT alone may not be a useful tool for malaria diagnosis in these areas. Our findings do not agree with these studies. It must be noted that extreme environmental factors such as temperature and humidity affect RDTs performance (Chiodini et al., 2007, Murray et al., 2008) and the environmental conditions in Zamfara and Port-Harcourt represent these two extremes. Also end users errors may lead

to poor conduct, and poor interpretation of the results such as ignoring specific volumes of blood and buffer applied to the test, not recognizing faint test lines and invalid tests, all of which may lead to a poor sensitivity of an RDT (Maltha et al., 2013) that cannot be attributed to the test kit.

Although, in a large country like Nigeria, just as there are extreme environmental conditions in different regions, so also different geographical locations may produce varied responses to a HRP2 based RDTs. Perhaps, unknown to us, the heterogeneous sensitivities of HRP2 RDTs in Nigeria could be due to the distinct genetic composition of circulating *P. falciparum* parasites prevalent in a particular region. Which is known to affect the performance of HRP2 based RDTs elsewhere due to deficiency in HRP2 production as a result of gene deletion (Gamboa et al., 2010; Kumar et al., 2013, Akinyi et al., 2013, Baldeviano et al. 2015).

The positive predictive value of the test kit among asymptomatic malaria subjects was as low as 67.8%. This could be attributed to the high number of false positive in the asymptomatic malaria group which were almost half of the true positive individuals diagnosed by the test kit. It could be that in the hospital setting in which the study was conducted, majority of the false positive individuals were probably treated with antimalarial drugs. The drugs could have cleared peripheral parasitaemia, but left measurable quantities of HRP2 in the blood stream that lingered on to yield false positive test observed in this study. Moreover, the mean asexual parasite density of 758 ± 174 parasites/µl of blood observed in the asymptomatic subjects, that tested positive to the CareStart™ Pf malaria RDT tends to support the suggestion that the asymptomatic malaria subject were probably relieved of malaria symptoms by antimalarial drugs, just before they participated in the study.

The negative predictive value of CareStart™ Pf RDT was comparably higher (> 97%) in all subject categories than the other three parameters that were determined in this study. This shows the test kit as having the required ability to exclude truly uninfected *P. falciparum* malaria subjects that could be treated with ACTs, due to the presence of febrile illness. This is common in a *P. falciparum* endemic area like Nigeria (Isiguzo et al., 2014). Such ability to discriminate between febrile illnesses due to *P. falciparum* malaria and those not due to *P. falciparum* malaria may allow quick devotion of time and laboratory resources towards identifying other pathogens that may be responsible for the febrile illness in the absence of malaria.

One major limitation of this study was the inability of the study to unequivocally establish whether subjects that were apparently asymptomatic of malaria were or had been on active antimalarial drugs just prior to being tested by the RDT kit. Was this done, a better picture of performance ability of the CareStart™ Pf malaria RDT kit would have been obtained in the hospital environment. Thus in future evaluation of HRP2 based malaria RDTs, or similar malaria test kits in any setting, it may be reasonable to independently verify if participating subjects had recently been treated with antimalarial drugs. This is because, such treatment can alter the performance response of HRP2 based RDTs (Maltha et al., 2014) which normally diagnose *P. falciparum*, the most virulent species of human malaria.

REFERENCES

Abdulkadir I, Rufai HA, Ochapa SO, Malam MS, Garba BI, Oloko AGY, George II. (2015). Malaria rapid diagnostic test in children: the Zamfara Nigeria experience. *Nigerian Medical Journal*, 56(4): 276 – 282.

Aidoo M. (2013).Factoring quality laboratory diagnosis into the malaria control agenda for sub-Saharan Africa. *American Journal of Tropical Medicine and Hygiene*, 89(3): 403 - 406.

Akinyi S, Hayden T, Gamboa D, Torres K, Bendezu J, Abdallah JF, Griffing SM, Quezada WM, Arrospide N, De Oliveira AM, Lucas C, Magill AJ, Bacon DJ, Barnwell JW, Udhayakumar V. (2013). Multiple genetic origins of histidine-rich protein 2 gene deletion in *Plasmodium falciparum* parasites from Peru. Sci Rep. 3: 2797

Antinori S, Galimberti L, Millazo L, Corbellino L. (2012). Biology of human malaria Plasmodia including *Plasmodium Knowlesi*. *Mediterreanean Journal of Haematology and Infectious Diseases*, 4(1): e2012013, Doi 10.4084/MJHID.2012.013

Baker J, Ho MF, Pelecanos A, Gatton M, Chen N, Abdullah S, Arbetini A, Ariey F, Barnwell J, Bell D, Cunningham J, Djalle D, Echeverry DF, Gamboa D, Hii J, Kyaw MP, Luchavez J, Membi C, Menrad D, Murrillo C, Nhem S, Ogutu B, Onyor B, Oyibo W, Wang SQ, McCarthy J, Cheng Q. (2010). Global sequence variation in the histidine-rich proteins 2 and 3 of *Plasmodium falciparum*: implications for the performance of malaria rapid diagnostic tests. *Malaria Journal*, 9: 129.

Banoo S. Bell D, Bossuyt P, Herring A, Mabey D, Poole F, Smith PG, Sriram N, Wongsrichanalai C, Linke R, O'Brien R, Perkins M, Cunningham J, Matsoso P, Nathanson CM, Olliaro P, Peeling RW, Ramsay A. (2010). Evaluation of diagnosticsfor infectious diseases: general principles. *Nature Rewiews Microbiology*, *S*16 – *S*28. Doi 10.1038/nrmicro1523

Baldeviano GC, Okoth SA, Arrospide N, Gonzalez RV, Sɑ̃nchez JF, Macedo S, Conde S, Tapia LL, Sales C, gamboa D, Harrera Y, Edgel KA, Udhayakumar V, Lescano AG. (2015). Molecular epidemiology of *Plasmodium falciparum* malaria outbreak,Tumbes, Peru 2010 – 2012. *Emerging Infectious Diseases*, 21(5): 797 – 803.

Bisoffi Z, Gobbi F, Buonfrate D, Van den Ende J. (2012). Diagnosis of malaria infection with or without disease. *Mediterranean Journal of Hematology and Infectious Diseases*, 4(1): e2012036, Doi 10.4084/MJHID.2012.036

Brown H, Azike CA. (2014). A comparative study on malaria rapid diagnostic kit and conventional Giemsa stained blood film microscopy. *International Journal of Science and Research*, 3(12): 1433 – 1435.

Chandramohan D, Jaffar S, Greenwood B. (2002). Use of clinical algorithms for diagnosing malaria.*Tropical Medicine and International Health*, 7(1): 45 52.

Chiodini PL, Bowers K, Jorgenssen P, Barnwell JW, Grady KK, Luchavez J, Moody AH, Cenizal A, Bell D. (2007). The heat stability of Plasmodium lactate dehydrogenase – based and histidine – rich protein 2 – based malaria rapid diagnostic tests. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 101: 331 – 337.

Council of International Organizations of Medical Sciences (CIOMS). (2002). *International ethical guidelines for*

biomedical research involving human subjects. Prepared by the Council of International Organizations of Medical Sciences (CIOMS) in collaboration with the World Health Organization (WHO) Geneva, 2002.

Dondorp AM, Desakorn V, Pongtavornpinyo W, Sahassananda D, Silamut K, Chotivanich K, Newton PN, Pitisuttithum P, Smithyman AM, White NJ, Day NP. (2005). Estimation of total parasite biomass in acute falciparum malaria and plasma PfHRP2. *PLoS Medicine*, 2(8): e204

Gamboa D, Ho MF, Bendezu J, Torres K, Chiodini PL, Barnwell JW, Incardona S, Perkins M, Bell D, McCarthy J, Cheng Q. (2010). A large proportion of *P. falciparum* isolates in the Amazon region of Peru lack pfhrp2 and pfhrp3: implications for malaria rapid diagnostic tests. *PLoS One*, 5: e80

Gillet P,Mori M, Van Esbroeck M, Van den Ende J, Jacobs J. (2009). Assessment of the prozone effect in malaria rapid diagnostic tests. *Malaria Journal*, 8: 271.

Gillet P, Scheirlinck A, Stokx J, Weggheleire AD, Chaúque HS, Canhanga ODJV, Tadeu BBT, Mosse CDD, Tiago A, Mabunda S, Bruggeman C, Bottieau E, Jacobs J. (2011). Prozone in malaria rapid diagnostics tests: how many cases are missed? *Malaria Journal*, 10: 166.

Ho MF, Baker J, Lee N, Luchavez J, Ariey F, Nhem S, Oyibo W, Bell D, González I, Chiodini P, Gatton ML, Cheng Q, McCarthy JS. (2014). Circulating antibodies against *Plasmodium falciparum* histidine-rich protein 2 interfere with antigen detection by rapid diagnostic tests. *Malaria Journal*, 13: 480.

Isiguzo C, Anyanti J, Ujuju C, Nwokoko E, De la Cruz A, Schatzkin E, Modrek S, Montagu D, Liu J. (2014). Treatment of malaria from formal and informal drug vendors in Nigeria. *PloS One*, 9(10): e110361

Kumar N, Pande V, Bhatt RM, Shah NK, Mishra N, Srivastava B, Velecha N, Anvikar AR. (2013). Genetic deletion of HRP2 and HRP3 in Indian Plasmodium falciparum population and false negative malaria rapid diagnostic test. *Acta Tropica*, 125(1): 119 – 121.

Landis JR, Koch GG. (1977). Measurement of observer agreement to categorical data. *Biometrics*, 33: 159 – 174

Luchavez J, Baker J, Alcantara S, Belizario Jr. V, Cheng Q, McCarthy JS, Bell D. (2011). Laboratory demonstration of a prozone-like effect in HRP2-detecting malaria rapid diagnostics tests: implications for clinical management. *Malaria Journal*, 10: 286.

Maltha J, Gillet P, Jacob J. (2013). Malaria rapid diagnostic tests in endemic settings. *Clinical Microbiology and Infection*, 19(5): 399 – 407.

Maltha J, Guiraud I, Lompo D, Kaborė B, Gillet P, Van Geet C, Tinto H, Jacobs J. (2014). Accuracy of PfHRP2 versus Pf-pLDH antigens detection by malaria rapid diagnostic tests in hospitalized children in seasonal hyper endemic malaria transmission areas in Burkina Faso. *Malaria Journal*, 13: 20.

Mouatcho JC, Goldring JPD. (2013). Malaria rapid diagnostic tests: challenges and prospects. *Journal of Medical Microbiology*, 62: 1491 – 1505.

Murray CK, Grasser RA, Jr. Magill AJ, Miller RS. (2008). Update on rapid diagnostic testing for malaria. *Clinical Microbiology Reviews*, 21: 97 – 110.

Rock EP, Marsh K, Saul AL, Wellems TE, Taylor DW, Maloy WL, Howard RJ. (1987). A comparative analysis of Plasmodium falciparum histidine-rich proteins HRP-I, HRP-II, and HRP-III in malaria parasites of diverse origins. *Parasitology*, 95(Pt2): 209-227.

Sheyin Z, Bigwan IE. (2013). Comparison of CareStart HRP2 rapid malaria test with light microscopy for guiding patients treatment of fever in Nigeria endemic areas. *Journal and Medicine and Medical Sciences*, 4(9): 353 – 356.

Talisuna AO. (2007). Diagnosis and treatment of malaria. *BMJ*, 334(7590): 375 – 376.

Wongsrichanalai C, Barcus MJ, Muths S, Sutamihadja A, Wernsdorfer WH. (2009). A review of malaria diagnostic tools. Microscopy and rapid diagnostic test. *American Journal of Tropical Medicine and Hygiene*. 77*(Suppl 6):* 119 – 127.

World Health Organization. (2009). Malaria microscopy quality assurance manual version 1. World Health Organization.

World Health Organization. (2010). *Guidelines for the treatment of malaria 2nd Edition.* World Health Organization. ISBN 978 924 154792 5.

World Health Organization. (2015a). *World malaria report 2015*. World Health Organization. ISBN 978 924 156515 8.

World Health Organization (2015b). *Malaria rapid diagnostic test performance – results of WHO product testing of malaria RDTs round 6 (2014 – 2015).*

World Health Organization. (2016). *Global malaria programme information note on the recommended selection criteria for procurement of malaria rapid diagnostic test.* WHO/HTM/GMP/2015.12.rev1.