International Journal of Innovation and Applied Studies
ISSN 2028-9324 Vol. 9 No. 2 Nov. 2014, pp. 642-647

http://www.ijias.issr-journals.org/

Performance of Hrp2 Based Malaria Rapid Diagnostic Test (Sd-Bioline) in Whole Blood and Saliva in Patient's in Lagos State

Judith Anurika Elendu and Wellington Oyibo

Department of Medical Microbiology and Parasitology, College of Medicine, University of Lagos, Nigeria

ABSTRACT: The study assessed the performance of SD Bioline Histidine Rich Protein 2 (HRP2) based rapid diagnostic test in blood and saliva samples in order to assess the usefulness of saliva as an alternative sample to blood in malaria diagnosis. A total of 1026 blood samples and 186 saliva samples (total RDT positive sample) were collected from patients who attended the clinic, and fell within the inclusion criteria at St Mathew's Hospital, Amukoko, Lagos State.
Microscopy and rapid diagnostic test were carried out on the whole blood samples and saliva, the RDTs tested demonstrated a high sensitivity 96.7% for SD Bioline, and a high specificity 98.7% for SD Bioline at a 95% confidence interval(p=0.445). Saliva samples demonstrated a low sensitivity of 12.7% for SD Bioline whilst a high specificity of 99.9% for SD Bioline HRP-2 RDT.
There was no significant difference between microscopy and SD Bioline HRP 2(χ^2, P=0.05) and as such it is a good diagnostic tools for malaria, while saliva serves as a promising possible means of non-invasive technique in malaria diagnosis.

KEYWORDS: Malaria, Histidine rich protein 2, sensitivity, specificity, diagnostic tool, microscopy.

1 INTRODUCTION

Malaria rapid diagnostic tests are a group of commercially available tests, that allows the rapid diagnosis of malaria by, laboratory technicians, those who are unskilled in traditional laboratory techniques for malaria diagnosis or, in situations where such equipments are unavailable, especially in endemic countries, where malaria is a major cause of morbidity and mortality (1) with a prevalence of 225 million and a death rate of about 730 thousand (18).

Prompt diagnosis and treatment are vital keys to address morbidity and mortality due to malaria (18). A major obstacle to effective malaria control is the lack of affordable and accurate malaria diagnostics and treatment, which has led to misuse, and abuse of anti-malaria drugs and the development of drug resistance in parasites. Microscopic examination of blood smears is the gold standard for *Plasmodium* specie detection and is currently being augmented with antigen rapid diagnostic test (RDT) and PCR-for blood. However, microscopy does need well qualified and supervised laboratory workers, maintenance of the microscope and regular supply with consumables, conditions which are difficult to sustain in developing an under developed countries (12).

Malaria rapid diagnostic test (MRDT), detect parasite within a threshold of 100 parasites/µl of blood. They are easy to perform and do not require extensive training or equipment (15). Malaria antigens currently targeted by RDTs are the histidine rich protein 2(HRP2), Parasite Lactate Dehydrogenase (PLDH) (8) and Aldolase. RDTs are currently being used to detect antigens of *Plasmodium* species in blood, serum, plasma, etc, to supplement microscopic evaluation of blood smears, to manage tropical febrile disease (11).

However, there is continued negative impact to malaria diagnosis due to; inaccurate microscopic evaluation of blood smears, which have resulted in misdiagnosis and misclassification of malaria severity (8), blood taboos and increased risk of accidental infections (albeit minor) due to needle pricks. In non specialized laboratories, microscopic evaluation of blood smears is slow and may lead to late diagnosis and treatment, which contributes to high mortality rates (7).

Corresponding Author: Judith Anurika Elendu

One alternative is the use of saliva, which has been in use in the surveillance of vaccine preventable diseases such as measles, mumps, rubella and for individual diagnosis of human immune virus (HIV/AIDS) by detecting antibodies against the target pathogens (14,13,5). *Plasmodium falciparum* HRP2 antigen has been detected in erythrocytes, serum, plasma, cerebrospinal fluid and even urine and saliva (4, 13). Saliva has also been shown to contain high levels of immunoglobulin gamma (IgG) and immunoglobulin miu (IgM) (3).

Saliva collection, is a non invasive method, as it does not require the use of sharp objects, it is easy to collect and store, and could be used to access communities with blood taboos. It will be more ethically acceptable for repeat sampling of the same individual (13) especially children and pregnant women who are most susceptible to malaria (8, 17).

Thus, the objectives of this research are to evaluate the performance of HRP2 RDT as a diagnostic tool, in saliva and whole blood, and determine the usefulness of saliva in malaria diagnosis.

2 MATERIALS AND METHOD

2.1 STUDY AREA

The study was conducted during November 2010 – May 2011 at St Mathew's primary health centre/ hospital, Amukoko, in Apapa / Iganmu Local Council Development Area in Ajeromi Ifelodun Local Government Area, in Lagos State, Nigeria, West Africa.

2.2 STUDY PARTICIPANT

Case report forms were used to recruit participants at the hospital, after obtaining informed written consent from individuals, parents/ guardians of children below 18 who presented with clinical symptoms of malaria such as fever on site or within the past three days, body pain/ joint pain, head ache, vomiting, loss of appetite, weakness, e.t.c. ranging from children below 5 years of age to adults. The exclusion criteria were those with other complaints unrelated to malaria and complicated malaria.

2.3 SAMPLE COLLECTION

Venous blood (approximately 3ml) was collected from each patient and placed into a properly labelled (with patient's identity number) EDTA container, for malaria diagnosis by 2 methods: microscopy and RDT; immediate slide containing both a thick smear and a thin smear was provided (according to the world health organization standard) to the clinical staff for immediate staining and observation, while the remaining blood samples was transported to the microscopy laboratory at the Department of Medical Microbiology and Parasitology, College of Medicine, Idi-Araba for further testing.

Saliva samples (approximately 1 ml) were collected by spitting, in properly labelled sterile bottles, from patients who were positive for malaria by rapid diagnostic test, done immediately, at the site of collection; A total of 1026 whole blood samples and 186 saliva samples (total number of positive sample by rapid diagnostic test) were collected.

2.4 SAMPLE PROCESSING

2.4.1 MALARIA RAPID DIAGNOSTIC TESTS (RDT)

Malaria diagnosis by commercial RDT SD Bioline Malaria Ag (Standard Diagnostic Incorporation, Hagal-Dong, Korea) ® catalogue no 082043 and 082065 with expiry date march, 2012 and September, 2012 respectively; was performed on all blood samples using the methods/ procedure described by the manufacturer.

In saliva samples, no further processing was done; the saliva was properly mixed and the same MRDT kits were used (SD Bioline Malaria Ag (HRP2)) following the manufacturer's instruction. All RDT kits were stored as directed by the manufacturer and underwent quality assurance testing at the World Health Organization quality assurance testing center at the Department of Medical Microbiology and Parasitology ,College of Medicine ,Idi-Araba.

2.4.2 RESULT INTERPRETATION

The presence of control line and test lines indicated a positive result for *P. falciparum* while, the presence of only the control line indicated a negative result. The absence of a control line was interpreted as invalid and the test was repeated.

Positivity of the samples were graded as 1+, 2+, and 3+, depending on the intensity of the test line when compared to the control line.

2.4.3 MICROSCOPIC EXAMINATIONS

Two slides were prepared designated read and archive for the study purpose, excluding the one done at the study site for the clinical staff; each slide having both a thick and thin blood smear and stained with 3% giemsa stock solution, for 45 minutes according to study protocol (WHO, 2010). Slides were examined independently by two microscopist (A and B) who were blinded to each other's interpretations, as well as, to the results of the Rapid Diagnostic Tests. One hundred thick film oil immersion high power fields were examined before a slide was interpreted as negative for malaria. The interpretation was determined to be positive if asexual *Plasmodium sp* stages were observed. The presence of gametocytes in the absence of asexual parasite forms was interpreted as a negative result for the purpose of assay evaluation.

In cases were *P. falciparum* was observed during microscopic examination the number of asexual forms with a concomitant enumeration of white blood cells (WBC).If after 200 WBCs were counted,10 or more asexual parasite stages were counted then the total number of asexual parasites were recorded. If malaria parasites were present but numbered fewer than 10 parasites per 200 WBCs, then the microscopists continued to examine the smear counting asexual stage parasites and WBCs until at least 500 WBCs had been counted.

For all positive smears, the number of asexual parasites counted was multiplied by the patient's total WBC count, and the resulting value divided by the number of WBCs counted during the microscopic examination. The final result was a calculated parasitemia expressed as the number of asexual stage parasites per microliter of whole blood.

2.5 VALIDATION AND CONTROL

2.5.1 BLINDING OF RESULTS

While performing the RDTs, I and reader 2 were blinded to each of the other measurements collected during the study. Care was taken to ensure that technicians using the rapid diagnostic device were blinded to patient histories and examinations, WBC determination and patient demographics. In all cases the results of the RDTs (SD Bioline HRP2) were determined prior to diagnostic microscopy with strict blinding between the rapid test results and technicians performing the microscopy

2.5.2 MICROSCOPY

The independent readings for read slide, determined by the microscopists were compared for concordance in three areas : i) agreement about the presence of asexual forms of plasmodium ii) agreement about the species of *Plasmodium* when present and iii) agreement on the calculated level of parasitemia within a factor of 2. When all three conditions for concordance are met, the mean parasitemia value from the two independent readings was recorded as the true diagnostic outcome. In events of discordant value between the microscopists for any of the three criteria cited, a third senior microscopist, examined both study slides (read and archive) using the same procedure sited above. The cumulative findings of the senior microscopist for both slides were then considered the true diagnostic outcome for the specimen without regard to the previous result of the previous two microscopists (19).

2.6 DATA ANALYSIS AND ETHICAL APPROVAL

This study was approved by the Ethics and Experimental Committee of the College of Medicine of the University of Lagos, Lagos, Nigeria. Data were entered and verified using the Microsoft excel format and analysed using SPSS version. Over all agreement of reliability of RDT readings was calculated. Using microscopy as the reference, Proportions were assessed for statistical significance using the Pearson chi-square test. A p- value <0.05 was considered significant.

3 RESULTS

Of the 1026 patients sampled during the study, 402 (39.2%) of them were males while, 624 (61%) were females; with a mean age of 25.6 (SD=16.7, range 0.1- 80 years).

181 of the study sample (17.6%) {95% CI 15.4-20.1%) were positive for malaria by microscopy with *Plasmodium falciparum* being the most predominant specie (97.8%) and 2.2% represented other species of which *P.malariae* consist of 25%. The trophozoite stage was the most frequent (93.1%) while gametocytes represented 6.0%.

186 (18.1%) whole blood samples were positive for malaria by SD Bioline RDT. 24 out of 186 (12.9%) saliva samples were positive for malaria by SD Bioline RDT.

The performance of the individual test (SD Bioline) varied in blood and saliva (table 1)

Table1: Performance of sd bioline using blood and saliva

Blood				Saliva			
SD Bioline	**Microscopy**		**Total**	**SD Bioline**	**Microscopy**		**Total**
	Negative	**Positive**			**Negative**	**Positive**	**(n=1026)**
	No (%)	**No (%)**	**No (%)**		**No (%)**	**No (%)**	**No (%)**
Negative	834(81.3)	6(0.6)	840(81.9)	**Negative**	844(82.3)	158(15.4)	1002(97.7)
Positive	11(1.1)	175(17.1)	186(18.1)	**Positive**	1(0.1)	23(2.2)	24(2.3)
Total	845(82.4)	181(17.6)	1026(100)	**Total**	845(82.4)	181(17.6)	1026(100)

The comparative performance characteristics of SD Bioline HRP2 in blood and saliva showed a sensitivity of (96.7% and12.7%), a specificity of 98.7%, 99.9%, a positive predictive value of 94.1% and 95.8% and a negative predictive value of 99.3% and 84.2% respectively at a 95% confidence interval (p=0.445).

226 (22.0%) of the 1026 samples were diagnosed as febrile (>37.5^{o}C), with a mean temperature of 36.9^{o}C (SD 0.96, range 33.6^{o}C - 41.0^{o}C), of which 59(26.1%) were positive by microscopy, 60(26.5%) were positive for malaria by SD Bioline for blood, 12 (5.3%) were positive for saliva by SD Bioline.

Age group 10-19, showed the highest prevalence {45(24.9%), 45(24.2%)} in blood by microscopy, and SD Bioline HRP2 respectively but the difference was not significant, whereas, the highest prevalence {9(37.5%)} in saliva samples was seen in age group 20-29 by SD Bioline HRP2.

Parasitemia levels ranged from 3 to 217,135, with a mean of 9,316.6. SD Bioline RDT proved highly sensitive at low levels of parasitemia as sensitivity in detecting *P. falciparum* was 95.1% when parasite density between 1-200 parasites/µl of blood in SD Bioline RDTs. A sensitivity of 9.8% was observed in saliva samples at a low parasite density; which increased as parasite density increased, although this difference was not statistically significant (p=0.3852).

4 DISCUSSION

Over time, alternative tests for malaria diagnosis have been developed in order to support the performance of microscopy and to facilitate prompt and accurate diagnosis and timely intervention.

This study demonstrated a low prevalence (17.7%); SD Bioline HRP2 demonstrated a high sensitivity (96.7%,) and specificity (98.7%,) in blood, respectively; which is in agreement with previous studies (9, 2) and the WHO standard for all RDTs (18).

The false negative observed by SD Bioline RDT was attributed to very low parasite density and presence of other *Plasmodium species*, while the false positive was attributed to cross reaction with auto antibodies /rheumatoid factors, or persistence of circulating HRP2 antigen either due to sequestration or incomplete treatment. Blood specificity was high, showing that the test is reliable in detecting the absence of *Plasmodium falciparum* antigen which agreed with past studies (6, 16), issues concerning over diagnosis and waste of therapeutic drugs (ACT) did not arise.

SD Bioline HRP2 RDT also demonstrated high frequency (95.8%) at detecting *Plasmodium falciparum* antigen at low parasitemia.

Evaluation of saliva as a possible means of diagnosis with HRP2 based RDTs has revealed a high specificity (99.5%), a very low sensitivity (9.4%), a high negative predictive value (83.7%) and a positive predictive value (100%) SD Bioline HRP2 RDT; which re affirms the results gotten from other research (2, 13) with slight deviation. Saliva is able to detect the absence of *Plasmodium falciparum* effectively, thus reducing the possibility of false positive result.

Low sensitivity of the HRP2 RDT (SD Bioline) in saliva was due to limitations of the commercially available kit used, which is designed to detect higher levels of *Pf*HRP2 in whole blood or plasma, than is found in saliva. However, the result showed that the antigen can be detected in saliva, which is a non invasive method.

Saliva is not yet a reliable alternative in the diagnosis of *Plasmodium falciparum,* but is still a potential alternative and as such, the development of a test kit that is highly sensitive to detect lower levels of the antigen present in saliva and standardization of the sample collection and processing will be a more appropriate approach to malaria diagnostic and in epidemiological surveys.

SD Bioline HRP2 RDTs is a good diagnostic tool for malaria diagnosis as it augments malaria microscopy and ensures prompt diagnosis, and timely treatment; thereby reducing the morbidity and mortality associated with malaria.

ACKNOWLEDGEMENT

We would like to thank the staff of St Mathew's Catholic Hospital Amukoko, Lagos State. All the standard diagnostic RDTs used were supplied by Codix Pharm Limited.

REFERENCES

[1] Bell, D, Peeling, R. W, (2003). Evaluation of Rapid Diagnostic Tests: Malaria. *Nature Review Microbiology 4(9):* 34-38.

[2] Buppan, P, Putaporntip, C, Pattanawong, U, Seethamchai, S, Jongwutuwes, 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*:72-76.

[3] Estevez, P. T, Judith, S, Nwakanma, D. C, Sheila, W, David, J. C , Chris, J. D, (2011). Human Saliva as a Source of Antimalaria Antibodies to Examine Population Exposure to *Plasmodium falciparum. Malaria Journal 10*:104-112.

[4] Genton, B, Paget, S, Beck, H, P, Gibson, N, Alpers, M. P, Hii, J, (1998). Diagnosis of *Plasmodium falciparum* Infection Using Para Sight ®- F Test in Blood and Urine of Papua , New Guinean Children. *Southeast Asian Journal of Tropical Medical Public Health 29*:35-40.

[5] Grenade, T. C, Phillips, S. K, Parek, B, Gomez, P, Kitson-Piggott, W, Oleander, H, Mahabir, B, Les, W, Lee-Thomas, S, (1998). Detection of Antibodies of Human Immunodeficiency Virus Type 1 in Oral Fluids: A Large Scale Evaluation of Immunoassay Performance. *Clinical Diagnostic Laboratory and Immunology 5*:171-175.

[6] Jobiba, C, Jacek, S, Ben, C, Carl, C, Victoria, E, Miguel, S .J, John, S, Doreen, A, Don, M, (2010). Comparative Field Performance and Adherence to Test Results of Four Malaria Rapid Diagnostic Tests Among Febrile Patients More Than Five Years of Age in Blantyre Malawi. *Malaria Journal 9*:209.

[7] Kain, K. C, Harrington, M. A, Tennyson, S, Keystone, J. S, (1998). Imported Malaria: Prospective Analysis of Problems in Diagnosis and Management. *Clinical Infectious Diseases 27*:142-149.

[8] Makler, M, Palmer, C. J, Ager, A.l, (1998). A Review of Practical Techniques for the Diagnosis of Malaria. *Annals of Tropical Medical Parasitology 92*:419-433.

[9] Maltha, J, Phillipe, G, Emmanuel, B, Lieselote, C, Marjan, V. E, Jan, J, (2010). Evaluation of Rapid Diagnostic Test (Care Start Tm Malaria HRP-2 / PLDH (*Pf*/Pan) Combo Test) for the Diagnosis of Malaria in a Reference Setting. *Malaria Journal 9*:171.

[10] Maltha, J, Philippe, G, Lieselotte, C, Emmanuel, B, Marjan, V. E, Cathrien, B, Jan, J, (2011). Evaluation of the Rapid Diagnostic Test SD FK 40 (*Pf*-PLDH/Pan-PLDH) for the Diagnosis of Malaria in a Non Endemic Setting. *Malaria Journal 10*:7-18.

[11] Moody, A, Hunt-Cooke, A, Gabbett, E, Chiodini, P, (2000). Performance of the Optimal Malaria Antigen Capture Dipstick for Malaria Diagnosis and Treatment Monitoring at the Hospital for Tropical Diseases. *London British Journal of Hematology 109(4):*891-894.

[12] Murray, C. K, Gasser, R .A .Jr, Magill, A. J, Miller, R. S, (2008). Update on Rapid Diagnostic Testing for Malaria. *Clinical Microbiology Reviews 21(1*): 97-110.

[13] Nana, W. O, Andrew, A. A, Winston, A, Stella, B, Jonathan, K. S, (2008). Short Report: Detection of *Plasmodium falciparum* Histidine Rich Protein II in Saliva of Malaria Patients. American *Journal of Tropical Medical Hygiene 78(5):* 733-735.

[14] Nokes, D .J, Enquselassie, F, Nigeria, W, Vyse, A. J, Cohen, B .J, Brown, D. W, Cuts, F. T, (2001). Has Oral Fluid the Potential to Replace Serum for the Evaluation of Population Immunity Levels? A Study of Measles, Rubella and Hepatitis B in Rural Ethiopia. *Bulletin World Health Organization 30*:1076-1079.

[15] Singh, N, Saxena, A, Sharma, V.P, (2002). Usefulness of an Inexpensive Paracheck Test in Detecting Asymptomatic Infectious Reservoir of *Plasmodium falciparum* During Dry Season in an Inaccessible Terrain in Central India. *Journal of Infection 45(3):*165-168.

[16] Wanna, C, Thane, W, Ronnatrai, R, Kesara, N. B, (2011). Evaluation of Rapid Diagnostics for *Plasmodium falciparum* and *P.vivax* in Make Sot Malaria Endemic Thailand. *Korean Journal of Parasitology 4 (91)* :33-38.

[17] Wilson, N. O, Adjei, A. A, Anderson, W, Baidoo, S, Stiles, J.K, (2008). Detection of Plasmodium falciparum Histidine –Rich Protein II in Saliva of Malaria Patients. .*American Journal of Tropical Medical Hygiene .78*:733-735.

[18] World Health Organization (2010). Malaria Case Management: Operation Manual. Geneva, Switzerland.

[19] World Health Organization (2010).Malaria Diagnosis: WHO Guidelines and Their Implementations. Geneva, Switzerland.