ejpmr, 2016,3(6), 116-122

SJIF Impact Factor 3.628

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

COMPARATIVE EVALUATION OF THE DIAGNOSTIC ACCURACIES OF THREE RAPID MALARIA TESTING KITS

Ifeanyi T. Nzekwe[1]*, Chukwuma O. Agubata[2], Samuel C. Umeigbo[1], Chigozie E. Utazi[3], Charles O. Esimone[4], Ifeanyi E. Okoye [1]

[1]Department of Pharmaceutics and Pharmaceutical Technology, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria.

[2]Department of Pharmaceutical Technology and Industrial Pharmacy, University of Nigeria, Nsukka, Enugu State, Nigeria.

[3]Department of Statistics, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria.

[4]Department of Pharmaceutical Microbiology and Biotechnology, Nnamdi Azikiwe University, Awka, Nigeria.

***Corresponding Author: Ifeanyi T. Nzekwe**

Department of Pharmaceutics and Pharmaceutical Technology, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria.

Article Received on 08/04/2016 **Article Revised on 28/04/2016** **Article Accepted on 18/05/2016**

ABSTRACT

The rapid testing kits used in malaria detection in Nigeria are deemed by some health professionals to have the same diagnostic efficiency as microscopy and some have advocated their use in non-invasive testing. This study aims at evaluating the comparative diagnostic efficiencies of three commercially available kits in relation to microscopy, using invasive and non-invasive methods. Matched blood and saliva from 50 symptomatic patients were tested for malaria using three rapid tests, with microscopy as gold standard. Data were analyzed using Cochran's Q and McNemar's tests. Out of the three brands tested, only one was in agreement with microscopy when blood was used as specimen, with the other two differing at 5% confidence interval. Saliva-based testing demonstrated high specificity, while blood-based testing demonstrated higher sensitivity and accuracy and there was no correlation with blood-based testing for all the three brands ($p < 0.05$). There were no statistical differences between the outcomes of saliva-based testing for the three tests ($p > 0.05$). Two-third of the RDTs tested were significantly inferior to thick smear microscopy, with blood used as test specimen. Also, testing with saliva gave significantly lower sensitivity than blood-based testing for each RDT. Rapid testing kits should not be solely used as confirmatory test for malaria and saliva should not be substituted for blood.

KEYWORDS: Sensitivity, malaria, detection, rapid, infectious disease.

INTRODUCTION

Malaria constitutes one of the gravest health challenges facing Nigeria and indeed Africa.[1, 2] The need for an effective management of drugs occasions a need for accurate confirmatory diagnosis since indiscriminate prescription is a serious factor in the development of resistance. Indiscriminate prescription arises from self medication and also clinical diagnosis, which lacks specificity.[3] To buttress this, it has been reported for instance that in India where about 100 million slides were tested on clinical suspicion of malaria, less than 2% were positive for malaria parasites.[4]

The most impactful tests for disease testing in developing countries are those with fairly high sensitivity and specificity, and which have little or no infrastructure requirement.[5] Though many tests abound for malaria detection, rapid diagnostic tests (RDTs) presenting as simple dipsticks or cassettes are rapid, user-friendly, relatively affordable and deliverable to remote testing locations. These advantages notwithstanding, they suffer several handicaps, such as inability to detect all species or to quantify parasitaemia, and limited sensitivity and specificity. In addition, many rapid diagnostic tests rely on blood as specimen and this carries increased risks of needle injuries, microbial inoculation and disease. Cultural and religious adherences are sometimes also an obstacle. Non-invasive systems using saliva would find greater use than blood based tests in the periphery of the health-care delivery system.[6] At present, saliva finds use as a diagnostic test fluid for the evaluation of humoral immunity to infectious agents such as hepatitis A, HIV, measles, mumps and rubella viruses.[7] However, in malaria, the failure of rapid diagnostic tests (RDTs) to detect the parasite in saliva, despite high parasitemia and positive RDT in matching whole-blood samples from the same patients, makes the use of saliva a little illusory. This could hinge on differences in biomarker concentration.[8]

Malaria RDTs using whole saliva were reported to have sensitivity lower than the WHO requirement of 95% sensitivity at parasite densities of 100 per µL.[9]

The aim of this study was to evaluate the sensitivity and specificity indices of some commercially available RDTs which have found increasing use in health facilities in Awka, Anambra State, Nigeria. Since these tests were designed as self-tests operable by all users according to manufacturers' protocols, the data could help in the regulation of rapid testing kits and also inform patient and physician choices in low infrastructure locations.

METHODOLOGY

Materials

Three imported histidine-rich protein 2 (HRP-2)-based malaria testing kits were procured from local pharmacies in Awka, Anambra State. In order to protect trade interests, the brands have been labelled as A, B and C. All reagents used in the tests were of high analytical grades.

Study site

This study was conducted between July and September, 2013 at the laboratory facilities of the Anambra State University Teaching Hospital, Amaku, Anambra State. Ethical clearance was obtained from the Ethics Committee of the Nnamdi Azikiwe University Teaching Hospital, Nnewi, Anambra State, Nigeria with reference no: NAUTH/CS/66/VOL.4/84. The study was conducted in strict adherence to ethical best practices.

Study population

Consecutive patients attending the outpatient Department of Anambra State University Teaching Hospital, Amaku, Awka, with clinical suspicion of malaria and who were sent for laboratory examination. Patients who were clinically suspected of other concomitant conditions, as evidenced by the Laboratory tests requested, were excluded. The target patients included adults as well as children equal to or above 7 months of age. Oral informed consent was obtained from all patients, or their guardians. Matched samples of blood and saliva were collected before commencement of drug treatment. All tests were prospectively performed. Sample size was limited to 50 due to difficulties in obtaining patients' consent for collection of both blood and saliva, but this number was enough for the purposes of the non-parametric statistical tests used.

Rapid diagnostic testing with blood

All tests based on the rapid testing kits were performed on all patients by a trained personnel involved in the project. The sterile lancet was used to prick the patient's finger in order to obtain a large drop of blood. The drop was collected in a small plastic bulb pipette, then transferred and stored in an EDTA sample bottle labelled appropriately. Ten microliters (10 µl) of the blood was inserted into the sample well of each of the different diagnostic kits and 2 - 4 drops of the assay buffer were added. The kits were kept at room temperature and the result was read after 20 min. These steps were repeated using saliva and also the other test kits and for the other 49 patients.

Rapid diagnostic testing with saliva

Matched saliva samples were collected from the same patients and used as specimen for each of the RDTs without further treatment. A volume of 10 µl was used in the tests, which were performed after about 10 minutes of the blood test. These steps were repeated with the other test kits, for the other 49 patients.

Thick blood smear microscopy

Microscopy was used as a reference test and was blindly performed by a competent microscopist who was not aware of the results from the rapid test kits. Microscopic examination was done approximately four (4) hours after the rapid testing with blood. A 10% giemsa solution was prepared by diluting 45 ml of buffer solution (pH 7.1) and 5 ml undiluted giemsa stain. A small drop of the patient's blood was added to the centre of the slide and spread gently on it, then allowed to air-dry. The dried slide was placed into a coupling jar containing the giemsa solution with the slide facing downwards for 10 min. The stain was washed off immediately with clean water and allowed to air-dry. After drying, a drop of immersion oil was applied to the film and the object viewed by the microscopist under the light microscope using the 100 x objective lens. Parasite density was graded as mild (0-3/hpf), moderate (4 to 10/hpf) and severe (> 10/hpf). To declare a sample as negative, up to 100 fields were examined and where microscopy and RDT tests differed (microscopy being negative), the sample was read by a second microscopist. Parasitaemia (per microliter) was calculated by relating the number of asexual cells per 200 white blood cells to the standard leucocyte count of 8000.

Data analyses

Diagnostic performance was calculated using accuracy, sensitivity and specificity parameters as well as negative and positive predictive values. Specimen-dependent differences (intra-test) and brand-dependent differences for each specimen (inter-test) were analysed using McNemar's tests and Cochran's Q test for matched samples nonparametric procedures for dichotomous outcomes. In the analyses, the positive and negative results were assigned arbitrary numerical values of 1 and 0, respectively.

RESULTS

Patients' profiles

Fifty patients with suspected *Plasmodium falciparum* infection, a history of fever and clinical suspicion of malaria were recruited into the study. The median age of the patients was 34 yrs, while the interquartile range was 23-49.5 yrs. Eight of the patients were male while forty-two were female. Thirty-one patients were 40 years or younger while twenty-nine were above 40 years. Fifty

four per cent of patients were positive while forty six were negative after carefully conducted thick film microscopy. A flow chart (Figure 1) shows the recruitment process and test steps and also the proportion of positive and negative patients for each test. A further illustration of the distribution of malaria-positive patients by age and sex is presented in Table 1. The age distribution shows that relatively smaller number of under-thirteen children had malaria in comparison with older patients. Ninety-six per cent of the patients had mild malaria (0 to 3/ hpf) while only 4% (2 patients) had moderate malaria (4 to 10/ hpf). The latter patients were both adults.

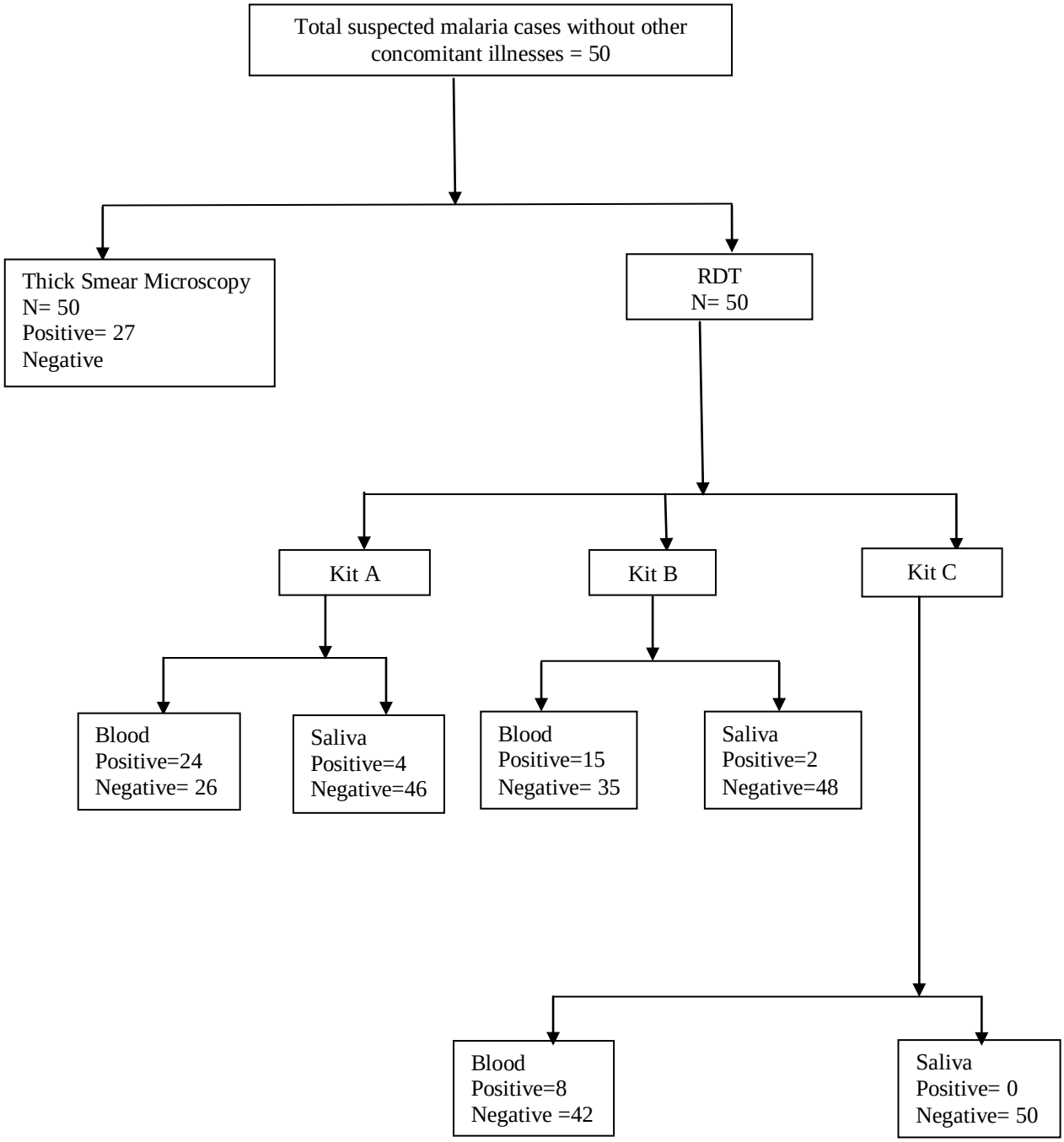

Figure 1: A flow chart indicating the testing procedure in the evaluation of three rapid test kits sold in Awka, Anambra State, Nigeria, between July and September, 2013

Table 1: Age and Sex distribution of malaria patients tested in Awka, Anambra State, Nigeria, between July and September, 2013

Variable	Percentage of total	Fraction Positive (%)	Fraction Negative (%)
Thick smear microscopy	-	54	46
Age			
≤ 13	6 (3/50)	33 (1/3)	67 (2/3)
>13	94 (47/50)	55 (26/47)	45 (21/47)
Sex			
Females	82 (41/50)	54 (22/41)	46 (19/41)
Males	18 (9/50)	56 (5/9)	44 (4/9)
Parasitaemia			
Mild (0-3/hpf)	96 (48/50)		
Moderate to severe (≥4/hpf)	4 (2/50)		

RDT performance indices

The performance characteristics of each RDT brand were computed using thick blood smear microscopy as the gold standard. These characteristics included sensitivity and specificity, as well as positive and negative predictive values and the values obtained are displayed in Table 2. No indeterminate results were encountered. The performance characteristics of interest, namely, sensitivity, specificity, positive predictive value, negative predictive value and accuracy of the tests were calculated using the relationships in Equations 1 to 5.

Sensitivity = [TP/ (TP + FN)] × 100 (1)

Where TP equals number of true positives; FN equals number of false negatives

Specificity is given as:

Specificity = [TN/ (TN + FP)] × 100 (2)

TN equals number of true negatives; FP equals number of false positives

Positive predictive value is given as:

PPV= [TP/ (TP+FP)] x 100 (3)

where FP equals number of false positives; TP equals no of true positives

Negative predictive value is given as:

NPV= [TN/ (TN+FN)] x 100 (4)

where TN equals number of true negatives; FN equals number of false negatives

Table 2: Performance characteristics of three rapid test kits for malaria in Anambra State, Nigeria between July and September, 2013, in comparison to blood smear microscopy.

Brand*	A		B		C	
Specimen	Blood	Oral fluid	Blood	Oral fluid	Blood	Oral fluid
Sensitivity (%)	55.6	14.8	31.0	7.4	29.6	0.0
Specificity (%)	60.9	100.0	71.4	100.0	100.0	100.0
PPV (%)	62.5	100.0	60.0	100.0	100.0	0.0
NPP (%)	53.8	50.0	42.9	48.0	54.8	56.0
Accuracy (%)	58.0	54.0	48.0	50.0	62.0	46.0

* The brands have been coded to protect trade interests.
PPV= positive predictive value
NPP= negative predictive value
Total number of patients is 50

The accuracy/efficiency is the percentage of test results correctly identified by the test. It is given by:

Accuracy= [(TP +TN)/ (TP+TN+FP+FN)] x 100 (5)

The statistical summaries are presented in Tables 3 and 4. All RDT-based tests with saliva differed from the corresponding tests with blood for the same kit. With blood as specimen, one of the kits was in agreement with thick smear microscopy (Brand A), while two differed significantly.

Table 3: Statistical summary (p-values) for McNemar's tests for three rapid malaria test kits sold in Anambra State, Nigeria, between July and September, 2013, with microscopy as gold standard

Brand	Blood vs saliva both used in RDT	Inference	Blood as RDT specimen vs thick smear microscopy	Inference
A	<0.01	Not equally effective	0.664	Equally effective
B	0.001	Not equally effective	0.023	Not equally effective
C	0.008	Not equally effective	<0.01	Not equally effective

p-values of <0.05 considered signifcant

Table 4: Statistical summary for Cochran's Q tests for three rapid malaria test kits sold in Anambra State, Nigeria, between July and September, 2013 using blood and saliva as specimens

RDT type	Cochran's Q Test statistic	P-value	Inference
Blood-based RDT for three brands	21.444	0.000	Equally effective
Saliva-based RDT for three brands	4.000	0.135	Not equally effective

p-values of <0.05 considered signifcant

DISCUSSION

This study was constrained by lack of PCR machine and also reluctance on the part of patients to donate saliva and blood, especially saliva. As such, thick smear microscopy was used as gold standard and sample size was relatively small. An additional reason for the use of thick smear microscopy is that it is a common reference tool for confirmatory testing in most resource-limited settings where PCR would be untenable. According to our findings, only about 54% of the patients presenting for test on clinical suspicion of malaria were positive for malaria. If treatment was initiated for malaria without confirmatory diagnosis, 46% of patients would have been placed needlessly on anti-malaria drugs. This means that there is need for review of the prevalence burden often quoted for malaria, much of which derives from clinical suspicion. Age distribution shows that relatively smaller number of under-13 patients have malaria, in comparison to older patients. This and the fact that the two cases of moderate parasitaemia occurred in older patients are an early pointer to the gains of the campaign for insecticidal mosquito nets, which has targeted children in preference.

The three kits are HRP2-based third generation immunochromatographic assays specific for *Plasmodium falciparu*m, the dominant species in Nigeria. To have the required impact in the management of malaria in developing countries, diagnostics requiring no infrastructure like rapid test kits should have sensitivity and specificity benchmarks of 90%,[5] or 95% if reliant on infrastructure.[9] As seen in Table 2, none of the tests attained this specification with blood as specimen even when comparably high specificity values were recorded with saliva for all the kits. This high specificity (100% in most cases) means that any patient in whom the saliva test was positive for malaria also had positive thick smear results. All tests with saliva gave low sensitivity results. For instance, in this study, RDT failed to detect parasite antigen in some whole-saliva samples, despite moderately high parasitemia (≥ 1000/μL blood) and positive RDT result in matching whole-blood samples. Thus, notwithstanding the high specificity, saliva alone has no diagnostic utility as far as these brands are concerned. Saliva showed higher specificity in relation to blood possibly because blood contains numerous plasma proteins which are involved in non-specific reactions, giving rise to false positives. The results obtained here are partly in agreement with the values reported by Wilson *et al*.,[10] who reported that in 30 patients with positive thick film, the sensitivity for *P. falciparum* detection was 43% with saliva, but with the medium having a specificity of 100%.

From the statistical summaries, blood and saliva are simply not equally effective as specimens for RDTs and so the use of saliva should be discouraged. The fact that only one of the tests (A) was in agreement with thick smear microscopy when blood was used as specimen means that a simple majority of test kits in the market may be inadequate for use in confirming malaria disease.

The authors share a concern about these results. Rapid test kits have gained wide acceptance in Nigeria and government-owned hospitals are using RDTs as confirmatory tests for malaria. Even though this practice is believed to hold the potential to significantly reduce the malaria scourge and prevent indiscriminate prescription of anti-malaria drugs, the poor regulation of the brands marketed on the Nigerian market may leave the populace at the mercy of inferior brands and poor designs of rapid testing kits. However, the poor performance encountered with the kits could be due to other reasons such polymorphisms in the HRP-2 molecule,[11, 12, 13] or gene deletions[14] leading to inability to elaborate the protein. Both could result in false

negative results which could easily be detected with PCR. False negative results could also stem from high parasite densities, resulting from prozone effects.[15, 16, 17] On the other hand, false positive results could result from occurrence in blood of rheumatoid factor,[18, 19] or from HRP-2 residues in blood after the clearance of parasitaemia.[20] Poor storage conditions may also be a factor here because the whole country suffered from frequent power outages in the period of test, which may have made room temperature and humidity regulation impossible.

CONCLUSION

There is need for the Nigerian government to introduce greater regulation of rapid testing kits. Microscopy, though requiring high expertise, should be retained as a confirmatory test, where possible. Even though health professionals recognize the need for non-invasive testing, substitution of saliva for blood should be discouraged until such a time when kits sensitive enough to work with the rather low levels of biomarker in saliva are developed. The limitations of RDTs including the prozone phenomenon and the possibility of occurrence of false negative and false positive results should be factored in at all times. Further studies can be done to investigate the impact of storage conditions, since room temperature regulation remains quite challenging in Nigeria to this day due to poor power supply. In this era of nanotechnology, research to improve the features of diagnostics for resource-limited settings, particularly researches to make tests less invasive and also more robust, stable, sensitive and specific, are urgently needed.

Conflict of interests

The authors declare no conflict of interest.

REFERENCES

1. Santos-Magalhães NS, Mosqueira VCF. Nanotechnology applied to the treatment of malaria. Adv Drug Deliv Rev, 2010; 62: 560-575.
2. World Health Organization (2015) World Malaria Report 2014 Summary. Geneva: World Health Organization
3. Perkins MD, Bell DR. Working without a blindfold: the critical role of diagnostics in malaria control. Malar J, 2008; 7 Suppl 1: S5.
4. World Health Organization (2008) World Malaria Report. Geneva: World Health Organization.
5. Urdea M, Penny LA, Olmsted SS, Giovanni MY, Kaspar P, Shepherd A, Wilson P, Dahi CA, Buchsbaum S, Moeller G, Hay Burgess DC (2009) Requirements for high impact diagnostics in the developing world. Available at: http://www.nature.com/diagnostics. Accessed: 1 December 2014.
6. Piper R, Lebras J, Wentworth L, Hunt-Cooke A, Houze S, Chiodini P, Markler M Immunocapture diagnostic assays for malaria using Plasmodium lactate dehydrogenase (pLDH). Am J Trop Med Hyg, 1999; 60: 109-118.
7. Oba IT, Spina AM, Saraceni CP, Lemos MF, Senhoras R, Moreira RC, Granato CF Detection of hepatitis A antibodies by ELISA using saliva as clinical samples. Rev Inst Med Trop Sao Paulo, 2000; 42: 197-200.
8. Nwakanma DC, Gomez-Escobar N, Walther M, Crozier S, Dubovsky F, Malkin E, Locke E, Conway D Quantitative detection of *Plasmodium falciparum* DNA in saliva, blood and urine. J Infect Dis, 2009; 199: 1567-1574.
9. Bell D, Peeling RW. Evaluation of rapid diagnostic tests: malaria. Nat Rev Microbiol, 2006; 4: S34–S38.
10. Wilson NO, Adjei AA, Anderson W, Baidoo S, Stiles JK. Short Report: Detection of *Plasmodium falciparum* Histidine-rich protein II in saliva of malaria patients. Am J Trop Med Hyg, 2008; 78: 733-735.
11. Deme AB, Park DJ, Bei AK, Sarr O, Badiane AS, Gueye PEO, Ahouidi A, Omar Ndir O, Mboup S, Wirth DF, Ndiaye D, Volkman SK. Analysis of pfhrp2 genetic diversity in Senegal and implications for use of rapid diagnostic tests, 2014. doi: 10.1186/1475-2875-13-34.
12. Wurtz N, Fall B, Bui K, Pascual A, Fall5 M, Camara C, Diatta B, Fall KB, Mbaye PS, Diémé Y, Bercion , Wade B, Briolant S, Pradines B. Pfhrp2 and pfhrp3 polymorphisms in Plasmodium falciparum isolates from Dakar, Senegal: impact on rapid malaria diagnostic tests. Malar J, 2013; 12: 34. doi: 10.1186/1475-2875-12-34.
13. Mariette N, Barnadas C, Bouchier C, Tichit M, Menard D. Country-wide assessment of the genetic polymorphism in *Plasmodium falciparum* and *Plasmodium vivax* antigens detected with rapid diagnostic tests for malaria. Malar J, 2008; 7: 219. doi:10.1186/1475-2875-7-219.
14. Mouatcho JC, Goldring JP. Malaria rapid diagnostic tests: challenges and prospects. J Med Microbiol, 2013; 62: 1491-1505.
15. Santos L, Pereira NR, Andrade P, Dias PF,, Alves CL, Abreu C, Serrão R, Ribeiro M, Sarmento A. Prozone-like phenomenon in travellers with fatal malaria: report of two cases. J Infect Dev Ctries, 2015; 9(3): 321-324.
16. Gillet P, Mori M, Van Esbroeck M, Van den Ende J, Jacobs J. Assessment of the prozone effect in malaria rapid diagnostic tests. 2009. doi:10.1186/1475-2875-8-271.
17. Ratsimbasoa A, Fanazava L, Radrianjafy R, Ramilijaona J, Rafanomezantsoa H, Ménard D. Short Report: Evaluation of Two New Immunochromatographic Assays for Diagnosis of Malaria. Am J Trop Med Hyg, 2008; 79(5): 670–672.
18. Lee JH, Jang JW, Cho CH, Kim JY, Han ET, Yun SG, Lim CS. False-Positive Results for Rapid Diagnostic Tests for Malaria in Patients with Rheumatoid Factor. J Clin Microbiol, 2014; 52(10): 3784–3787.

19. Woyessa A, Deressa W, Ali A, Lindtjørn B. Evaluation of CareStart™ malaria Pf/Pv combo test for Plasmodium falciparum and Plasmodium vivax malaria diagnosis in Butajira area, south-central Ethiopia. Malar J, 2013; 12: 218. doi: 10.1186/1475-2875-12-218.
20. Mtove G, Nadjm B, Amos B, Hendriksen IC, Muro F, Reyburn H. Use of an HRP2-based rapid diagnostic test to guide treatment of children admitted to hospital in a malaria-endemic area of north-east Tanzania. Trop Med Int Health, 2011; 16(5): 545-550.