Original Article

Evaluation of Diagnostic Accuracy of Rapid Diagnostic Test for Malaria Diagnosis among Febrile Children in Calabar, Nigeria

Anthony Achizie Iwuafor, Okokon Ita Ita, Godwin Ibitham Ogban, Ubong A. Udoh, Chimereze Anthony Amajor[1]

Departments of Medical Microbiology and Parasitology and [1]Paediatrics, Faculty of Medicine, College of Medical Sciences, University of Calabar, Calabar, Cross River State, Nigeria

Abstract

Background: The WHO recommends that all cases of suspected malaria should undergo parasitological test. Currently, the parasitological test comprises the rapid diagnostic test (RDT) or the microscopy. The performance of RDT in relation to microscopy is yet to be fully comprehended. **Objectives:** This study evaluated the diagnostic accuracy of RDT as against the diagnosis provided by microscopy in detecting malaria parasites among febrile under-5 children. **Design:** The study was a cross-sectional hospital-based design. **Materials and Methods:** Capillary blood samples were collected from 167 children who came to the hospital with a history of fever over a period of 6 months. The Paracheck-Pf RDT kit was used and its performance was compared with the gold standard, microscopy using thick film. **Results:** The prevalence of malaria infection was 41.9%. On comparing RDT with microscopy (microscopy assumed to be 100% sensitive and specific), RDT had a sensitivity of 51.4% and a specificity of 73.2%. The false-positive rate was 26.8% whereas the false-negative rate was 48.6%. The positive predictive value was 58.1% whereas the negative predictive value (NPV) was 67.6%. The RDT also had a positive likelihood ratio (LR) of 1.92 and a negative LR of 0.67. The RDT test accuracy was 64.1%. **Conclusion:** Malaria prevalence among febrile children was found to be high. The findings also suggest that inconsistencies in the performance of RDT kits may arise from many extraneous factors, and as such, they should not be used as a stand-alone test kit except a prior batch/lot validation test was carried on them.

Keywords: Diagnostic accuracy, malaria diagnosis, rapid diagnostic test, test evaluation

Introduction

The African continent bears the greatest burden of malaria, contributing 90% of the world's malaria cases and 91% of malaria deaths globally. Nigeria, Africa's most populous country, accounts for 27% of malaria cases and 24% of malaria deaths globally in 2016, making that Nigeria accounts for more cases and deaths than any other country in the world.[1]

The WHO recommends that all cases of suspected malaria should have parasitological test, supported by a quality assurance program to confirm the diagnosis of malaria.[2] Currently, the parasitological test comprises rapid diagnostic test (RDT) or microscopy. Newer tests like the nucleic acid amplification-based tests such as loop-mediated isothermal amplification or polymerase chain reaction have a limited role in the management of clinical malaria.

Light microscopy referred to as "gold standard" is the standard method for laboratory diagnosis of malaria.[3] The microscopic technique involves collecting a finger-prick blood sample, preparing a thick and, in some occasions, a thin smear, staining the smear, usually with Giemsa, and examining with a microscope. RDTs are immunochromatographic test methods based on the detection of malaria parasite antigen in lysed blood. It usually involves the use of nitrocellulose test strip bearing monoclonal antibodies directed against a specific parasite antigen – the target antigen. The tests are relatively easy and fast to perform, mostly lasting for 15 min or less. Different antigens are targeted by the various kinds of RDTs available in the market today. Some of these antigens are the histidine-rich protein 2 (HRP-2), parasite lactate

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Address for correspondence: Dr. Anthony Achizie Iwuafor, Department of Medical Microbiology and Parasitology, Faculty of Medicine, College of Medical Sciences, University of Calabar, Calabar, Cross River, Nigeria.
E-mail: achiton@yahoo.com

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dehydrogenase (pLDH), and aldolase.[4-8] Each of these two parasitological tests (microscopy or RDT) has its strengths and weaknesses.[9] For instance, in determining "parasite density," RDTs give only a positive or negative result while microscopy gives parasite density; as such, RDT is not recommended for follow-up of admitted patients to monitor response to treatment. Malaria RDT again is not recommended in clinical setting where there is a need to confirm malaria case with persisting fever despite administration of antimalarial drug. This is because, whereas microscopy will give negative result as soon as the parasite is cleared from the patient's blood, RDT will detect persisting antigens even after parasite clearance. In many resource-poor countries, electricity supply is not available in communities and villages, thus hampering the use of microscopy for malaria diagnosis which needs a reliable electricity supply. RDTs, on the other hand, do not require electricity, so they are the only option. The turnaround time for parasitological diagnosis ordinarily should be available under 2 h.[2] However, in centers with high workload, for example, outpatient units of hospitals, the use of microscopy alone is not recommended since it requires more time to perform than RDT. In this setting, because of the delay in getting result needed to make clinical decision, patients' treatment is delayed or they are treated based on clinical signs/symptoms which are not specific, with propensity to lead to overtreatment.[10] Considerable level of competence and training is needed by the health workers for optimal performance in microscopy. Health workers with inadequate training in laboratory skills or with poor supervision should preferably use RDTs which are comparatively easier to use than microscopy. Furthermore, microscopy as a test is not reliable at low-density parasitemia, i.e., <50 parasites/μl; it is not also useful when there is sequestration of parasites into visceral organs.[11-13]

The use of parallel testing with RDT and microcopy for malaria diagnosis is not recommended. In a situation of persistent suspicion of malaria after a particular test has been done, carrying out a second test with entirely different working principle is admonished.[10]

Test performance accuracy is very critical in the choice of diagnostic tool or method for screening or diagnosis of any disease condition. Apart from the qualities of an "optimal rapid diagnostic test" as described by Murray *et al*.[14] which include: being easily learned by users, use of simple technology, having results that are easy to interpret both by the patients and the health-care worker who ordered it, functioning without need for electricity, not requiring refrigeration, and being rapid, they also must be valid and provide consistent reproducible results.[15] Test accuracy shows the diagnostic strength of the association between the predictor variable (in this case, RDT result) and outcome variable (disease) as measured against a "gold standard" test.[15] Diagnostic accuracy relates to the ability of a test to differentiate the patients and the healthy cases correctly.[16,17] Certain measures of diagnostic accuracy such as sensitivity, specificity, predictive values, likelihood ratios (LRs), area under the ROC curve, and odds ratio are used to evaluate a test. The two parasitological diagnostic tests, microscopy and RDTs, have reported varying sensitivities and specificities on different clinical settings and transmission status.[18]

In this study, the diagnostic accuracy of a Paracheck-Pf, a RDT kit when compared with the diagnosis provided by microscopy (gold standard) in detecting malaria parasites among febrile under-5-year-old children attending clinics in a tertiary hospital in Calabar, Nigeria, was evaluated. Paracheck-Pf was chosen based on summary results of the WHO Malaria RDT Product Testing rounds and also for being the candidate RDT used in 2010 Nigeria Malaria Indicator Survey.[8,19]

Materials and Methods

Study design and setting

The study was hospital-based cross-sectional design. It was undertaken between November 2012 and December 2013, to determine the sensitivity and specificity of RDT Paracheck-Pf in comparison with Giemsa-stained thick smear for diagnosis of malaria in Calabar city. This study was carried out in the pediatric wards of a tertiary health facility in Calabar, Nigeria. The two wards were children's emergency room and children's ward. Calabar is divided administratively into two local government areas – Calabar Municipal and Calabar South. The geographical location is latitude 4° 57' 32.15" N and longitude 8° 19' 37.02" E. It has a land mass of 406 km^2 and a population of 371,022 according to 2006 census.[20] According to Koppen climate classification, Calabar displays a tropical monsoon climate characterized by a lengthy wet season that spans 10 months and a short dry season of 2 months.[21] Throughout the year, temperatures in Calabar are relatively constant, ranging from 25°C to 28°C. The annual average rainfall in Calabar is little below 3000 ml.

Study participants

Participants included in the study were under-5-year-old children, either admitted in the children's ward or attending any clinic on outpatient basis. The patients were clinically evaluated and those suspected of having malaria (regardless of intake of antimalarial drugs or not) were selected to undergo testing by microscopy and RDT after an informed written consent has been signed by the parents/caregivers and assent obtained from the participants. A total number of 270 participants were enrolled in the study.

Ethical considerations

Approval of this study was obtained from the Health Research and Ethics Committee of the University of Calabar Teaching Hospital, Calabar, Nigeria (UCTH/HREC/33/106). We obtained written informed consent from the patients' caregivers. Caregivers who declined consent or participants who declined assent were excluded from the study.

Sample size

The sample size was determined using this formula for sample size calculation [$N = (Z)^2 p\,(1\text{-}p)/e^2$].[22] The sample size was 270. We assumed that malaria prevalence among under-5-year-old

children by microscopy/RDT in Calabar city is 20.0%,[23] using a confidence interval (CI) of 95%, 5% marginal error, and nonresponse rate of 10%.

Data collection

Data collection procedures

A convenient sampling method was used, to recruit up to the calculated sample size. As many of the respondents who gave consent each of the clinic day within the study period were enrolled until the target sample size was obtained. A pretested structured questionnaire was used to elicit information from caregivers. The information obtained included background characteristics of the respondents and clinical details elicited from the patients. The interviewers were selected based on their ability to understand the major languages spoken in Calabar (English and Efik languages) because, at some occasions, interpretations were done in the language of the caregiver.

Sample collection and processing

Using finger-pricking method, fresh capillary blood samples were collected aseptically from each participant as documented by Cheesbrough.[24] The blood sample was then quickly processed with Paracheck-Pf RDT kit (Orchid Biomedical Systems, India) according to the manufacturers' instructions.[25] In carrying out the test, a drop of the fresh blood sample was dropped into sample well "A," followed by immediate blotting. Afterward, six drops of the clearing buffer were then added into well "B," and the setup was allowed to stand undisturbed for 15 min [Figure 1]. Results were read at the end of 15 min. The test was negative when only one pink band appeared in the control window but positive if, in addition to the control band, there was also a pink-colored band in the test window. When no bands appeared on the strip device, test results were considered invalid or inconclusive, and the tests were repeated, ensuring that test procedure was followed more strictly. One hundred and sixty-seven RDTs were correlated with microscopy. Thick blood films were prepared at the pediatric clinics and transported to the pediatric side laboratory for staining. The thick films were stained with 10% Giemsa stain for 10 min as documented by Cheesbrough.[24] The slides were examined for parasite detection. Each Giemsa smear test was considered positive if at least one parasite was found per 100 high-power fields; else, it was considered as a negative test result. Quality control check was instituted by ensuring that both positive and negative slides were cross-checked by other trained microscopists.

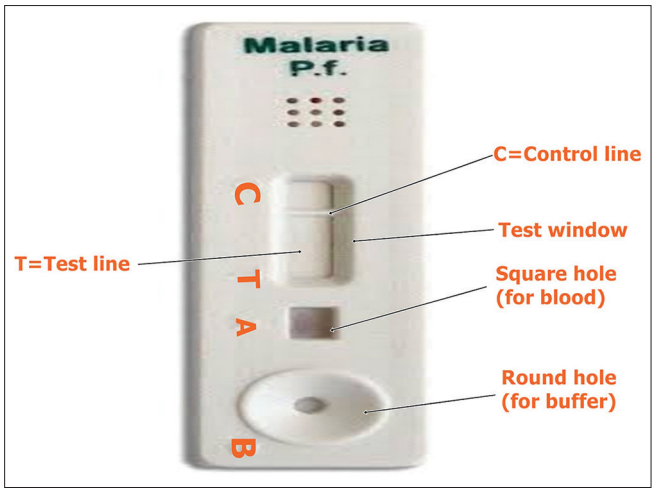

Figure 1: Paracheck-Pf rapid diagnostic test kit

Statistical analysis

Data were analyzed using diagnostic test calculator software version 2010042101 (free software available under the Clarified Artistic License).[26] RDT performance was calculated and compared with matched thick-film microscopy results with exact 95% CIs for sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), positive LR (PLR), and negative LR (NLR). Calculations were based on cross-tabulation (contingency table) tables as shown below [Table 1]. Overall accuracy was calculated using this formula: overall accuracy = (TP + TN)/(TP + FP + FN + TN), where TP = true positive, TN = true negative, FN = false negative, and FP = false positive.

Results

This study recruited 270 patients of age 1 month to 59 months. Twelve patients were excluded due to incomplete data. Two hundred and fifty-eight patients were tested using Paracheck-Pf RDT, while 167 patients were tested using thick-film microscopy. Because of 91 patients who did not have matching microscopic tests, these patients' results were excluded from the analysis [Figure 2]. The actual patients' results included for analysis were 167. A total of 70 (41.9%) were found to be positive by microscopy and 62 (37.1%) by RDT. Thirty-four of the 70 positive results by microscopy were negative (false-negative rate of 48.6%), while 26 of the 97 negative thick-film microscopic results were positive (false-positive rate of 26.8%).

On comparing the performance of the Paracheck-Pf RDT test with the results of the thick-film microscopy [Table 2], the RDT test sensitivity was 51.4%, the specificity was 73.2%, and the PPV and NPV were 58.1% and 67.6%, respectively. The PLRs were 1.92 (95% CI: 1.2862–2.8622) with an odds ratio of 0.7, indicating that about 1 in 1.7 positive test had positive parasitemia. On the other hand, the NLR was 0.66 (95% CI: 0.5176–0.8507) with an odds ratio of 0.5, meaning that 1 in

Table 1: 2×2 table (contingency table)

RDT	Microscopy (gold standard)		Total
	Positive parasitemia	Negative parasitemia	
Positive	TP=36	FP=26	62
Negative	FN=34	TN=71	105
Total	70	97	167

The diagnostic test calculator operates based on this table. RDT – Rapid diagnostic test; TP – True positive; TN – True negative; FN – False negative; FP – False positive

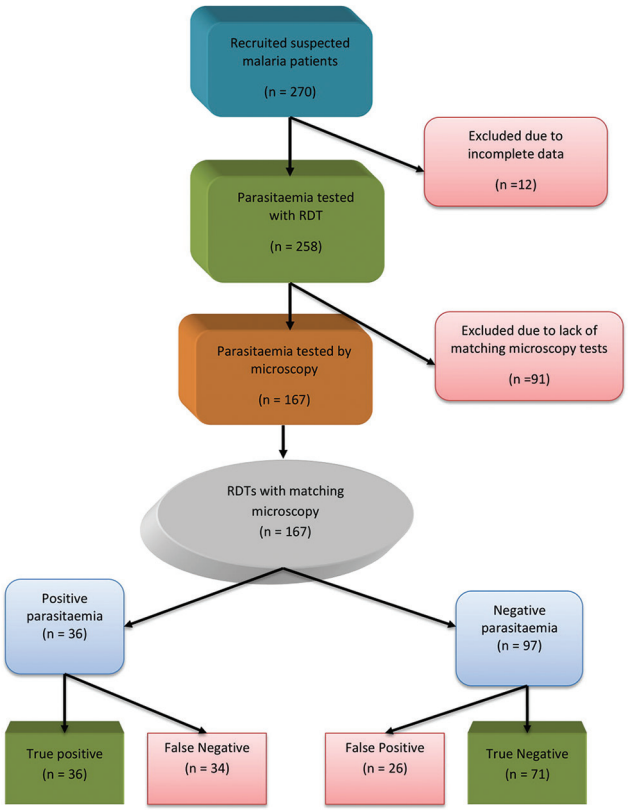

Figure 2: Study flow diagram showing patient enrollment and diagnosis performed

1.5 negative RDT test was well (had no parasites). The overall RDT test accuracy was 64.1%.

Discussion

This study shows a malaria prevalence of 41.9% among febrile children in Calabar. This finding shows that febrile illness is still a high predictor symptom for malaria infection among children in Nigeria. This prevalence rate is lower than the finding in a similar study in Nigeria[27] but higher than that in another study.[28] Calabar is in Nigeria; hence, it is among the malaria-endemic areas of Africa. The malaria prevalence rate found in our study agreed very well with the average prevalence rate of 42% found among under-5-year-old children in Nigeria's National Malaria Indicator Survey of 2010.[19]

Accurate diagnosis is of utmost importance to good malaria case management, whether the test is RDT or microscopy based. Due to high diagnostic performance capabilities of quality-assured RDT and microscopy in detecting clinical malaria, their relatively low cost, and availability, they have been considered the diagnostic tools of choice for the confirmation and management of suspected clinical malaria even in areas of low transmission.[29] The joint WHO-FIND-CDC-TDR Malaria RDT Evaluation program which has gone up to 6 test rounds offers quality standard panels to assist RDT product developers come up with RDTs with high accuracy. Currently, the WHO recommended

Table 2: Overall sensitivity, specificity, predictive values, and likelihood ratios of rapid diagnostic test using microscopy as the standard

	Estimated values (%)	Percentage CI
Prevalence	41.9	0.3441-0.4978
Sensitivity	51.4	0.39274-0.6343
Specificity	73.2	0.6308-0.8145
PPV	58.1	0.4488-0.7025
NPV	67.6	0.5769-0.7623
PLR	1.92	1.2862-2.8622
NLR	0.66	0.5176-0.8507

PPV – Positive predictive value; NPV – Negative predictive value; PLR – Positive likelihood ratio; NLR – Negative likelihood ration; CI – Confidence interval

selection criteria for procurement of RDT for member states' usage, which include panel detection score (PDS) of ≥75% in low transmission areas at 200 parasites/μL, <10% false-positive rate, and <5% invalid rate in all transmission areas.[30] PDS as a terminology is a composite index of test positivity as well as of inter-related and inter-lot consistency and is not a measure of clinical sensitivity.[31] PDS and RDT sensitivity are related, and variations in factors such as parasite densities, antigen expression, and condition of transportation and store can either higher or lower one against the other.[32] In this study, we tried to evaluate the diagnostic accuracy of the Paracheck-Pf, an RDT that performed relatively well in the WHO Malaria RDT Product Testing rounds of 2008–2011 as against microscopy which has been dubbed "gold standard."[8] RDT sensitivity and specificity were among diagnostic parameters we measured. Our study reveals a sensitivity of 51.4% and a specificity of 73.2%. The sensitivity rate in this study is low compared to similar other studies.[33-35] The expectation is that any quality-assured RDT used for diagnosis of malaria should have both sensitivity and specificity above 90%.[36] The clinical sensitivity of an RDT to detect malaria parasite is dependent to a great extent on the parasite density of the index population, and hence, sensitivity differs among populations of varying transmission levels.[8] At low parasite densities of ≤200 parasites/μL, sensitivity of various RDTs dwindles, while at high parasite densities (≥2000 parasites/μL), sensitivities of most of the RDTs become similar.[8] Although we did not determine parasite densities in this study, Calabar is a recognized high malaria transmission area, so one would have expected a high RDT sensitivity. Possible explanation for the poor performance of the RDT used in our study could be improper storage, transport, and handling of the RDT, from the production to the usage point. Although the RDTs that have passed the WHO minimum criteria for procurement are regularly re-evaluated, the WHO cannot guarantee that they will continue to meet the performance criteria as they did at the time of evaluation. Stability of RDTs is affected in areas with temperatures above 30°C. In Nigeria, temperature could be as high as 47°C. The HRP-2-based RDT sensitivity can be reduced greatly or even fails completely to detect *Plasmodium falciparum* in areas where the parasites fail to express HRP-2

proteins.[37] Interestingly, the WHO has hinted that in most settings, HRP-2/HRP-3 gene deletions in parasites are not likely cause of false-negative results with RDTs.[38] It behooves the local biomedical researchers to find the prevalent rate of the PFHRP-2/PFHRP-3 gene mutations in their localities. Some other studies have given other reasons contributing to the low sensitivity of RDT kits as prozone effect and variation in antigen structure.[39,40] The prozone phenomenon is known to limit antigen–antibody interaction-based assays. In patients with high parasite densities, the activation of the antigen–antibody complex can be prevented, ultimately resulting in false negative, especially in HRP-2-based RDTs.[33,41] This low sensitivity, which of course means high false-negative rate (48.6%), has grave implications on the health of patients, especially under-5-year-old children and pregnant women. Misdiagnosis of malaria in these two groups can easily lead to complications of malaria. Our study also gave a very high false-positive rate of 26.8% as against the WHO recommended <10% value. This high false-positive rate reflects poor specificity of the RDT test (73.2%). One of the causes of loss in specificity is improper interpretation of result. This was seriously guided against by ensuring that the readings were done carefully and correctly. A possible cause for the loss of specificity in this study might be that most of the patients had been treated with antimalarial drugs before coming to the hospital. The self-medication might have suppressed or cleared the parasites leaving only the HRP-2 antigen circulating in the blood.[42] False-positive RDT results also result from cross-reactivity with rheumatoid factor in patients' blood though the effect is reduced using IgM instead of IgG.[43,44] Occasionally, cross-reactivity with heterophile antibodies can as well take place.[45]

PPV which is calculated as (TP/(TP + FP) is used to define the probability of having the disease/state of interest in a subject with positive result. PPV, therefore, represents the proportion of patients with positive test result in total of subjects with positive result. Conversely, NPV describes the absence of a disease in a subject with a negative test result. Therefore, NPV represents the proportion of subjects without the disease with a negative test result in total of subjects with negative test results, calculated as (TN/TN + FN).[17] In our study, PPV and NPV are, respectively, 58.1% and 67.6%. These values are lower than the values obtained in similar studies elsewhere but similar to one by Anagu *et al*.[34,46] The predictive values of the RDT in our study can only give a slightly greater chance than average that a negative result is actually negative and a positive result means that a patient has malaria infection.

LR is a very important parameter for measuring diagnostic accuracy. It is the ratio of the expected test result in subjects with a certain disease to the subjects with the disease. LR functions in linking the pretest and the posttest probability of a disease in a given patient.[47] PLR, calculated thus (sensitivity/[1 − specificity]), is the best indicator for ruling-in diagnosis, while NLR ([1 − sensitivity]/specificity) is a good indicator for ruling-out diagnosis. In our study, PLR was 1.92 while the NLR was 0.67. Good diagnostic tests should have PLR of > 10 and NLR of < 0.1.[17] LR is dependent on the prevalence of the disease condition.

There are, however, limitations to this study. First, we did not calculate the malaria parasite density, and hence, we were not able to correlate the relationship between parasite density and sensitivity. Second, the RDT we used could only detect *P. falciparum* though other *Plasmodium* species contribute less than 5% of malaria infection in Nigeria; this could have led to missing out those few other species. The third limitation is that only a particular brand of RDT kit (Paracheck-Pf) was employed in the study. It would have been more revealing if more than one HRP-2-based and possibly other antigen-based kits were tested and their performances compared together. Finally, not all the participants recruited into the study were matched for both microscopy and RDT testing, and the excluded patients would have added to make the data more robust and increase the study validity.

Conclusion

This study revealed a high prevalence of malaria among febrile children that attended the tertiary health institution in Calabar. It was also observed that interpreting test results without gold standard can be challenging. The use of RDTs in the diagnosis of malaria infection offers an easy-to-use, low-cost, and rapid testing alternative; however, the performance of these kits easily wanes owing to a number of factors ranging from manufacture, poor storage, and handling to usage and interpretation by end users. Based on the findings of this study, it would be very necessary to carry out batch/lot validation of the RDTs before proceeding to use them on a massive scale for diagnosis in any health center.

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Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. World Health Organization. World Malaria Day 2018: Ready to Beat Malaria. WHO Media; 2018. Available from: http://www.who.int/malaria/media/world-malaria-day-2018/en/. [Last accessed on 2018 Jul 31].
2. World Health Organization. Guidelines for the Treatment of Malaria. 3rd ed. Geneva, Switzerland: WHO Press; 2015. p. 27-30.
3. World Health Organization. New Perspectives: Malaria Diagnosis. Report of joint WHO/USAID Informal Consultation. World Health Organization; 25-27 October, 1999. Available from: http://www.who.int/tdr/publications/documents/malaria-diagnosis.pdf. [Last accessed on 2018 Jul 31].
4. Howard RJ, Uni S, Aikawa M, Aley SB, Leech JH, Lew AM. Secretion of a malarial histidine-rich protein (Pf HRP II) from *Plasmodium falciparum*-infected erythrocytes. J Cell Biol 1986;103:1269-77.
5. Piper R, Wentworth L, Cooke A, Houze S, Chiodini P, Makler M. A capture diagnostic for assay for malaria using *Plasmodium* lactate dehydrogenase (pLDH). Am J Trop Med Hyg 1999;60:109-18.
6. Hurdayal R, Achilonu I, Choveaux D, Coetzer TH, Dean Goldring JP. Anti-peptide antibodies differentiate between plasmodial lactate dehydrogenases. Peptides 2010;31:525-32.
7. Mayxay M, Pukrittayakamee S, Newton PN, White NJ. Mixed-species malaria infections in humans. Trends Parasitol 2004;20:233-40.

8. World Health Organization. Malaria Rapid Diagnostic Test Performance: Summary Results of WHO Malaria RDT Product Testing: Rounds 1-3 (2008-2011). Geneva: World Health Organisation; 2011. Available from: http://www2.wpro.who.int/NR/rdonlyres/005E574B-FFCD-484C-BEDE-C580324AC2CF/0/RDTMalariaRd3_Summary_FINAL11Oct.pdf. [Last accessed on 2018 Jul 31].
9. World Health Organization. Universal Access to Malaria Diagnostic Testing: An Operational Manual. Revised ed. Geneva, Switzerland: WHO Press; 2013.
10. Nigerian National Malaria Elimination Program. National Guidelines for Diagnosis and Treatment of Malaria. 3rd. ed. Abuja, Nigeria: Federal Ministry of Health; 2015.
11. Bell DR, Wilson DW, Martin LB. False-positive results of a *Plasmodium falciparum* histidine-rich protein 2-detecting malaria rapid diagnostic test due to high sensitivity in a community with fluctuating low parasite density. Am J Trop Med Hyg 2005;73:199-203.
12. Kilian AH, Metzger WG, Mutschelknauss EJ, Kabagambe G, Langi P, Korte R. Reliability of malaria microscopy in epidemiological studies: Results of quality control. Trop Med Int Health 2000;5:3-8.
13. Leke RF, Djokam RR, Mbu R, Leke RJ, Fogako J, Megnekou R. Detection of the *Plasmodium falciparum* antigen histidine-rich protein 2 in blood of pregnant women: Implications for diagnosing placental malaria. J Clin Microbiol 1999;37:2992-6.
14. Murray CK, Gasser RA Jr., Magill AJ, Miller RS. Update on rapid diagnostic testing for malaria. Clin Microbiol Rev 2008;21:97-110.
15. Fransisca L, Kusnanto JH, Satoto TB, Sebayang B, Supriyanto, Andriyan E, *et al.* Comparison of rapid diagnostic test plasmotec malaria-3, microscopy, and quantitative real-time PCR for diagnoses of *Plasmodium falciparum* and *Plasmodium vivax* infections in Mimika regency, Papua, Indonesia. Malar J 2015;14:103.
16. Baratloo A, Hosseini M, Negida A, El Ashal G. Part 1: Simple definition and calculation of accuracy, sensitivity and specificity. Emerg (Tehran) 2015;3:48-9.
17. Šimundić AM. Measures of diagnostic accuracy: Basic definitions. Med Biol Sci 2008;19:1-9. Available from: http://www.ifcc.org/ifccfiles/docs/190404200805.pdf. [Last accessed on 2018 Aug 01].
18. Lo E, Zhou G, Oo W, Afrane Y, Githeko A, Yan G. Low parasitemia in submicroscopic infections significantly impacts malaria diagnostic sensitivity in the highlands of Western Kenya. PLoS One 2015;10:e0121763.
19. National Malaria Control Programme, National Population Commission, and ICF International. Nigeria Malaria Indicator Survey 2010. Abuja, Nigeria; 2012. Available from: https://dhsprogram.com/pubs/pdf/MIS8/MIS8.pdf. [Last accessed on 2018 Aug 01].
20. National Population Commission. National and State Population and Housing Tables: 2006 Census Priority Tables. Vol. 1. National Population Commission; 2010. Available from: http://www.population.gov.ng/index.php/publications/138-national-and-state-population-and-housing-tables-2006-census-priority-tables-vol-1. [Last accessed on 2018 Aug 03].
21. Wikipedia. Koppen Climate Classification; 2017. Available from: https://en.wikipedia.org/wiki/Köppen_climate_classification. [Last accessed on 2018 Aug 01].
22. Daniel W, Cross C. Biostatistics : A Foundation for Analysis in the Health Sciences. 10th ed. New York: Wiley; 2013.
23. Onyiri N. Estimating malaria burden in Nigeria: A geostatistical modelling approach. Geospat Health 2015;10:306.
24. Cheesbrough M. District Laboratory Practice in Tropical Countries. Part 1. 2nd. ed. New York, USA: Cambridge University Press; 2006. p. 245-7.
25. How to Use a Rapid Diagnostic Test (RDT): A Guide for Training at a Village and Clinic Level (Modified for Training in the Use of the Paracheck Pf Device Rapid Test for *P. falciparum* Malaria (Ver. 3) (30301025); 2010. Available from: https://www.finddx.org/wp-content/uploads/2016/03/paracheck_pf_manual_rev1_032612.pdf. [Last accessed on 2018 Aug 01].
26. Schwartz A. Diagnostic Test Calculator – Version 2010042101: available Under the Clarified Artistic License; 2006. Available from: http://araw.mede.uic.edu/cgi-bin/testcalc.pl. [Last accessed on 2018 Aug 03].
27. Nwaorgu OC, Orajaka BN. Prevalence of malaria among children 1 – 10 years old in communities in Awka North local government area, Anambra state South East Nigeria. Int Multidiscip J 2011;5:264-81. Available from: http://www.ajol.info. [Last accessed on 2018 Aug 03].
28. Nmadu P, Peter E, Alexander P, Koggie Z, Maikenti I. The prevalence of malaria in children between the ages 2-15 visiting Gwarinpa general hospital Life-Camp, Abuja, Nigeria. J Health Sci 2015;5:47-51.
29. World Health Organization. WHO Evidence Review Group on Malaria Diagnosis in Low Transmission Settings: Malaria Policy Advisory Committee Meeting Report. Geneva: World Health Organization; 2014.
30. World Health Organization. Recommended Selection Criteria for Procurement of Malaria Rapid Diagnostic Tests: Information Note. Geneva: World Health Organization; 2016. Available from: https://www.finddx.org/wp-content/uploads/2016/04/WHO-information-note-rdt-selection-criteria-March2016.pdf. [Last accessed on 2018 Aug 03].
31. World Health Organization. WHO Selection Criteria for Procuring Malaria RDTs: Working Paper for Discussion by the Malaria Policy Advisory Committee. Geneva: World Health Organization; 2012.
32. Panel Detection Score (PDS) and its Relationship to Sensitivity in the Field. Availablefrom: https://www.finddx.org/malaria/interactive-guide/pds/. [Last accessed on 2018 Aug 10].
33. Ameh J, Ahmad RM, Ekeh N, Linga P, Mangoro Z, Imam AU, *et al*. Laboratory diagnosis of malaria : Comparing giemsa stained thick blood films with rapid diagnostic test (RDT) in an endemic setting in North-West Nigeria. J Med Lab Diagn 2012;3:10-5.
34. Anagu OL, Ikegbunam MN, Unachukwu CK, Ogwaluonye UC, Esimone CO. Comparison of microscopic determination and rapid diagnostic tests (rdts) in the detection of *Plasmodium* infection. Adv Microbiol 2015;5:604-9.
35. Sheyin Z, Bigwan I. Comparison of CARE START HRP2 rapid malaria test with light microscopy for guiding patient's treatment of fever in Nigeria endemic areas. J Med Med Sci 2013;4:353-6.
36. World Health Organization. Malaria Rapid Diagnosis: Making It Work. Manila: World Health Organization; 2003.
37. Gamboa D, Ho MF, Bendezu J, Torres K, Chiodini PL, Barnwell JW, *et al.* 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 2010;5:e8091.
38. World Health Organization. Global Malaria Programme. False-Negative RDT Results and Implications of New Reports of *P. falciparum* Histidine-Rich Protein 2/3 Gene Deletions. Geneva: World Health Organization; 2016.
39. Lee N, Baker J, Andrews KT, Gatton ML, Bell D, Cheng Q, *et al.* Effect of sequence variation in *Plasmodium falciparum* histidine – Rich protein 2 on binding of specific monoclonal antibodies: Implications for rapid diagnostic tests for malaria. J Clin Microbiol 2006;44:2773-8.
40. Luchavez J, Baker J, Alcantara S, Belizario V Jr., Cheng Q, McCarthy JS, *et al.* Laboratory demonstration of a prozone-like effect in HRP2-detecting malaria rapid diagnostic tests: Implications for clinical management. Malar J 2011;10:286.
41. Gillet P, Mori M, Van Esbroeck M, Van den Ende J, Jacobs J. Assessment of the prozone effect in malaria rapid diagnostic tests. Malar J 2009;8:271.
42. Chinkhumba J, Skarbinski J, Chilima B, Campbell C, Ewing V, San Joaquin M, *et al.* 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. Malar J 2010;9:209.
43. Wongsrichanalai C, Barcus MJ, Muth S, Sutamihardja A, Wernsdorfer WH. A review of malaria diagnostic tools: Microscopy and rapid diagnostic test (RDT). Am J Trop Med Hyg 2007;77:119-27.
44. Grobusch MP, Alpermann U, Schwenke S, Jelinek T, Warhurst DC. False-positive rapid tests for malaria in patients with rheumatoid factor. Lancet 1999;353:297.
45. Moody AH, Chiodini PL. Non-microscopic method for malaria diagnosis using optiMAL IT, a second-generation dipstick for malaria pLDH antigen detection. Br J Biomed Sci 2002;59:228-31.
46. Mahende C, Ngasala B, Lusingu J, Yong TS, Lushino P, Lemnge M, *et al.* Performance of rapid diagnostic test, blood-film microscopy and PCR for the diagnosis of malaria infection among febrile children from Korogwe district, Tanzania. Malar J 2016;15:391.
47. Deeks JJ, Altman DG. Diagnostic tests 4: Likelihood ratios. BMJ 2004;329:168-9.