

Research Article


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EVALUATION OF MICROSCOPY AND RAPID DIAGNOSTIC TESTS FOR DIAGNOSING MALARIA AMONG FEBRILE SUBJECTS IN EZINIHITE LOCAL GOVERNMENT AREA, IMO STATE NIGERIAIrole-Eze, O.P.¹, Ukpai, O.M.¹, Ejike, B.U.², Nwadike, C.C.³, Egwu, O.¹, Okorocha, N.A.⁴ & Amaechi, E.C.⁵*¹Department of Zoology and Environmental Biology, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria.²Department of Biology/Microbiology, Abia State Polytechnic, Aba Abia State, Nigeria³Dept of Zoology, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria⁴Abia State University Teaching Hospital, Aba, Abia State, Nigeria.⁵Department of Zoology, University of Ilorin, Ilorin, Kwara State, Nigeria**Abstract**

Malaria is the leading cause of morbidity and mortality in Nigeria, the country accounting for the highest devastation worldwide. This study aimed at evaluating the performance of microscopy and rapid diagnostic tests (RDTs) for diagnosis of malaria. A total of 443 suspected *Plasmodium falciparum* infected patients in hospitals in selected communities of Ezinihitte Local Government Area south eastern Nigeria between January and June 2014 were examined by using microscopy and RDTs. Thick and thin blood smears made on clean slides from venipuncture blood collected from febrile subjects have been examined for the presence of malarial parasites. Serological diagnosis was performed using Carestart, SD Bioline and Antec kits. The overall prevalence of the three RDTs were Carestart pf (41.8%), SD Bioline pf (52.8%), and Antec pf/pv (25.3%) as against microscopy the standard (44.9%). The varied prevalence observed from the three kits did not differ statistically ($p > 0.05$). The females appeared to have more infected (42.4%) than the males (39.6%) with no significant difference between the diagnostic methods. The overall sensitivities of Carestart pf, SD Bioline pf and Antec pf/pv were 71.9%, 86.9% and 50.8% respectively while their corresponding specificities were 82.8%, 75.0%, and 95.5% respectively. SD Bioline HRP2 based test demonstrated a superior sensitivity compared to microscopy. Some RDTs can be useful alternatives to microscopy in the diagnosis of *P. falciparum* especially in resource limited communities.

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1. Introduction

Malaria is a serious health problem in most developing countries and is a major disease affecting people living in tropical and sub-tropical areas. The annual incidence of fever is over 270 million in these areas of which around 214 million are confirmed cases of malaria. Further there are about 3.2 billion people at severe risk of the infection. Death toll due to malaria has been reported to be around 438,000 in 2015 of 90% have been reported in the sub Saharan Africa region. Nigeria accounts for 26%-29% of the global total deaths due to malaria and 55% of estimated malaria cases (WHO, 2016). Malaria accounts for 60% of outpatient visits to hospitals, approximately 11% of maternal mortality and 30% child mortality, especially children below 5 years (Nigeria Malaria Indicator Survey, 2015).

Malaria presents a diagnostic challenge to laboratories in most developing countries where the infection is endemic (Adekunle *et al.*, 2014; Ubiaru *et al.*, 2018). The urgency and importance of obtaining results quickly from the examination of blood samples from patients with suspected acute malaria limits the use of the more sensitive methods for malaria diagnosis which are less amenable for routine laboratory use. The reasons for high morbidity and mortality in malaria in endemic areas are the inappropriate and inaccurate diagnosis, lack of experienced personnel at all levels of health care and inadequate information on prevention and control of the disease.

In malaria diagnosis, several factors in the manufacturing process and storage of rapid diagnostic tests (RDTs), affects the performance of these kits in addition to environmental conditions in different parts of the country (Ubiaru *et al.*, 2018). A comparison of these diagnostic procedures is worthwhile to determine

more rapid and accurate diagnostic procedures that give quick results consequently helping to bring down a drastic reduction in malaria morbidity and mortality. The present study aimed at comparing three RDTs for malaria with the routine microscopic detection identifying a quick and accurate diagnostic procedure for malaria detection and thereby improving the effectiveness of the malaria disease management in Ezinihitte Local Government Area of Imo State, southeastern Nigeria.

2. Material and Methods

2.1 Description of study area

The study was conducted in four communities in the Ezinihitte Local Government Area, Imo State, southeastern Nigeria located between Longitude 6°50'E and latitude 7°25'E (Ukpai *et al.*, 2017; Irole-Eze *et al.*, 2017) namely Umuevu, Umuawadu, Umuoma, and Umuekpeke. The communities are similar in many aspects, lying within same latitudes and longitudes, inhabitants being Igbos and the area experiencing two main climate regimes- a dry season falling in November to April and a rainy season between April and October (Ukpai *et al.*, 2017). The mean annual rainfall is between 1,500 and 2,800 mm per year and the relative humidity ranges between 77% and 86%. The temperature ranges from 22.2°C-24.1°C (minimum) to 29.1°C-32.1°C (maximum) (Chukwuocha & Dozie, 2011). The area being a rain forest zone favors breeding of malaria vector mosquito. The area experiences stable malaria transmission all year round with an entomological inoculation rate of 15.0 lying in an altitude of 182m above sea level. The inhabitants are mostly farmers and petty traders with few civil servants and artisans.

2.2 Ethical considerations

Ethical clearance for the protocol of the study was obtained from the Post Graduate Committee of the Department of Zoology and Environmental Biology, Michael Okpara University of Agriculture, Umudike prior to commencement of the work. Ethical approval was also obtained from the Health Department of Ezinihite Local Government Area. Meetings were conducted with local leaders and community members in all the study communities to create awareness on the objectives and procedures of the study by appropriately explaining in the local Igbo language for proper understanding. The participants were also informed that they have the option to withdraw from the study at any time without any consequences. Written and verbal consents were obtained from all the participants before commencing the study. For individuals less than 16 years of age the approval of parents or guardians was obtained on their behalf.

2.3 Collection of blood samples

Blood was collected aseptically by venipuncture using syringes from each volunteer by pricking the thumb with a scalpel needle and using the micropipette picking about 5µl of blood into the sample wells and adding 60µl of buffer into wells of the malaria RDTs for reading the test after 15 – 20min. About 4ml of blood samples were collected and stored in sterile EDTA bottles. The blood samples were also used for the detection of malaria parasites by microscopy. Analysis of the samples was carried out in the Parasitology laboratory of God First Medical Laboratory, Umuahia, Abia State.

2.4 Examination of malaria parasites by microscopy

Thin and thick blood smears were prepared on grease free slides according to the standard procedure (WHO,

2010). The slides were properly labeled to allow proper identification of each respondents result. The smears were air dried, Giemsa stained and air dried again and observed under the microscope with magnifications initially x40 and further on first then x100 (oil immersion). All slides with malaria parasites were recorded as positive and as negative if malaria parasites were absent.

2.5 Rapid diagnostic tests (RDTs) for malaria.

Three different RDT kits Carestart Malaria (Access Bio, USA); SD Bioline Malaria Antigen Pf (Standard Diagnostics, Inc, Korea) and Antec Pf/Pv Antigen. (Plasmatec Laboratory Products Ltd, United Kingdom) were used for diagnosing of malaria. The Carestart and SD Bioline are a two-band HRP2 detection antigen kits for *P. falciparum* malaria, while the Antec Pf/Pv is a three-band test HRP2 and pLDH antigen kit for detection of *P. falciparum* and non *falciparum* malaria. The Carestart and SD Bioline produce test bands along their lengths impregnated with monoclonal antibodies specific to HRP2 antigen for *falciparum* malaria, while Antec Pf/Pv produce tests bands along its length impregnated with monoclonal antibodies specific for HRP2 and PLDH for *P.falciparum* and non-*falciparum* malaria. Blood was collected by finger pricking and using the micropipette provided in the kit dropped; into each sample wells and subsequently adding buffers of each of the kit. The presence of only the control line indicates a negative test and the presence of both the test and control lines indicated positive test while absence of each of the lines or both was seen as unreactive. Each kit was properly labeled to prevent mis identification of respondents results.

2.6 Statistical data analysis

All data were collated, entered into SPSS version 16.0 for analysis. The performance of RDTs was analyzed using ANOVA. The sensitivity, specificity, and predictive values of each of the three malaria RDTs and microscopy were calculated keeping microscopy as the reference.

The sensitivity, specificity and predictive values of the malaria diagnostic methods were calculated using following formulae

$$\text{Sensitivity} = \frac{TP}{TP + FN} \times 100$$

$$\text{Specificity} = \frac{TN}{TN + FP} \times 100$$

$$\text{Positive Predictive Value (PPV)} = \frac{TP}{TP + FP} \times 100$$

$$\text{Negative Predictive Value (NPV)} = \frac{TN}{TN + FN} \times 100$$

Where TP = true positive, FP = false positive, TN = true negative, and FN = false negative. Sensitivity was defined as the probability that a truly infected individual will test positive and specificity as the probability that a truly uninfected individual will test negative (Ojurongbe *et al.*, 2013).

3. Results

3.1 Overall performance of the diagnostic tests

This study showed differences in malaria detection by each RDTs and microscopic diagnosis. The overall percentage prevalence recorded by three RDTs were Carestart pf (41.76%), SD Bioline pf (52.82%) and Antec Pf/Pv (25.28%) as

against microscopy (44.92%). All these were *falciparum* malaria. However, the varied prevalence of malaria detected by each diagnostic tests did not differ statistically ($p > 0.05$) (Table 1).

The overall detection percentage of malaria by all diagnostic tests was (41.19%) and it was- 39.59% in males and 42.37% in females. SD Bioline was more effective (52.82%) in diagnosing the infection both males (53.51%) and females (52.33%). The performances of these diagnostic criteria tests did not differ statistically with respect to gender ($p > 0.05$).

The results also revealed the sensitivity, specificity, positive predictive and negative predictive values of these RDT's, based on microscopy as the gold standard (Table 2) The overall sensitivities of Carestart Pf, SD Bioline Pf and Antec Pf / Pv were 71.9% , 86.9% and 50.8% respectively while their corresponding specificities were 82.8%, 75.0%, 95.5% respectively. The Positive Predictive Values (PPVs) were 77.3%, 73.9%, and 90.2% respectively while their corresponding Negative Predictive Values (NPVs) were 78.3%, 87.6% and 70.4% respectively (Table 2).

3.2 Age-related performances of the different RDTs and microscopy

The RDTs performed differently in different age groups. The effective has been more in younger age groups than the older age groups. Microscopy which was considered as the gold standard performed more effectively in four different age groups 40-49years (76.92%), 50-59% (59.57%), 30-39years (57.58%) and 60-69years (55.00%) while SD Bioline RDT performed more effectively in three age groups, <9years (69.01%), 10-19years (67.12%) and 30-39years (60.61%). Carestart and Antec were more effective in one age group each 10-19years (73.97%) and 40-49 years (41.03%) respectively. Antec was not as effective in determining infection as the

Table1. Overall performance of the diagnostic tests.

Gender	NE	No. positive (Microscopy)	No. positive (Carestart)	No. positive (SD bioline)	No. positive (Antec)	Mean %
Male	185	75(40.54%)	68(36.76%)	99(53.51%)	51(27.57%)	39.59%
Female	258	124(48.16%)	117(45.35%)	135(52.33%)	61(23.64%)	42.37%
Total	443	199(44.92%)	185(41.76%)	234(52.82%)	112(25.28%)	41.19%

NE – Number examined.

Table 2. Evaluation of RDTs using microscopy as standard for Sensitivity, Specificity, Positive Predictive Value and Negative Predictive Value.

RDTs (%)	Sensitivity	Specificity	Positive Predictive Value	Negative Predictive Value
Carestart	71.9	82.8	77.3	78.3
SDBioline	86.9	75.0	73.9	87.6
Antec	50.8	95.5	90.2	70.4

other RDTs and microscopy (Table 3). The age-related performances of the diagnostic tests differed statistically ($p>0.05$).

3.3 Performance of RDTs and microscopy among subjects in communities

The performances of RDTs and microscopy in the communities showed that microscopy was more effective in

detecting malaria parasites in Umuevu (68.0%) and Umuawada (66.0%) communities while SD Bioline was more effective in Umuoma (58.5%) and Umuekpeke (55.8%) communities (Table 4).

Table 3. Age-related performances of the different RDTs and Microscopy among the subjects.

AGE RANGE	N	Carestart	SD Bioline	Antec	MCP
(≤ 9)	71	28 (39.44%)	49 (69.01%)	11(15.49%)	1(1.41%)
(10-19)	73	54 (73.97%)	49(67.12%)	22(30.14%)	39(53.42%)
(20-29)	57	28 (49.12%)	30(52.63%)	16(28.07%)	28(49.12%)
(30-39)	33	14 (42.42%)	20(60.61%)	5(15.15%)	19(57.58%)
(40-49)	39	16 (41.03%)	21(53.85%)	16(41.03%)	30(76.92%)
(50-59)	47	15 (31.91%)	26(55.32%)	12(25.53%)	28(59.57%)
(60-69)	60	21 (35.00%)	24(40.00%)	19(31.67%)	33(55.00%)
(70-79)	53	8 (15.09%)	13(24.53%)	8(15.09%)	19(35.85%)
(80-89)	9	1 (11.11%)	2(22.22%)	3(33.33%)	2(22.22%)
(90-99)	1	0 (0%)	0(0%)	0(0%)	0(0%)
TOTAL	443	185(41.76%)	234(52.82%)	112(25.28%)	199(44.92%)

Table 4. The Effectiveness of the RDT'S and Microscopy in the four communities.

RDTs	Umuevu (N=100)		Umuawada (N=100)		Umuoma (N=123)		Umuekepeke (N=120)	
	N	%	N	%	N	%	N	%
Carestart	24	24	45	45	60	48.8	56	46.7
SD Bioline	45	45	50	50	72	58.5	67	55.8
Antec	27	27	22	22	34	27.6	29	24.2
MCP	68	68	66	66	36	29.3	29	24.2

4. Discussion

Irrespective of the type of rapid malaria screening strip used, the only measurement for evaluating the diagnostic value of the strip is determining the sensitivity and specificity using the gold standard (microscopy) as the basis of comparison (Tarazon *et al.*, 2004).The sensitivity of most of the reagent strips has been reported to

reduce with decrease in parasite density, suggesting that they are most useful in areas endemic for the disease (Tarazon *et al.*, 2004).Malaria antigens currently targeted by RDTs are HRP-2, parasite lactate hydrogenase (pLDH) and *Plasmodium* aldolase (pL-aldo). Moody (2002) demonstrated that *Plasmodium* species secrete these proteins allowing the possibility assessing the sensitivity

and specificity of RDTs. The sensitivity of SD Bioline in this study was found to be (86.9%) while its specificity was 75.0% for the diagnosis of *P. falciparum*. This findings is in sharp contrast with the observations of Agomo *et al.*, (2003) where SD Bioline's sensitivity and specificity was reported to be 54.84% and 42.9% respectively. The positive (PPV) and negative predictive values (NPV) around 68.0% are however at variance with the PPV of (73.9%) and NPV (87.6%) obtained in this study. This also varied with the work of Zacheaus *et al.*, (2007) who reported 47% sensitivity, 100% for specificity and PPV but with a similarity of 83.2% NPV. Difference in the detection of HRP-2 in the study area could have been the reason for the difference observed as compared to other settings. For Carestart the sensitivity, specificity, PPV and NPV were 71.9%, 82.8%, 77.3% and 78.3%. This compares well with similarity with the study of Sheyin and Bigwan, 2013 who reported a sensitivity of 78.4% with Carestart pf but differed with the specificity 97.6%, PPV 97.3% and NPV 80.1%. It also didn't agree with that of Sani *et al.*, 2013 who reported 90.2%, 95.4%, 93.0% and 93.4% for sensitivity, specificity, PPV and NPV. When compared to that of SD Bioline used in this study the percentages varied in sensitivity, specificity and NPV but showed similarity in PPV. This may imply that the RDTs when used for detecting *P. falciparum* such differences should not arise. These differences may have been due to differences stemming from company composition of RDTs and it is likely that symptomatic subjects used in their studies are likely to have higher parasite densities. Antec pf/pv RDT showed a very low sensitivity of 50.8% but higher specificity 95.5% and PPV 90.2% with 70.4% for NPV. This low sensitivity is similar to Zacheaus *et al.*, 2007 and Agomo *et al.*, 2003 who reported 47% and 54.84% sensitivities in their studies, also a high specificity and PPV of 95.5% and 90.2% reported in this study concurs with that of Zacheaus *et al.*, 2007 and an NPV of 70.5%, this is however at variance with that of Agomo

et al., 2003. Since the study was carried out in an endemic setting it was expected that the HRP2 test line should give a higher sensitivity but this wasn't so. The low sensitivity could mean a high rate of false negatives as confirmed by the report of previous authors that PLDH test perform less well than the HRP2 especially in low parasite densities (Abba *et al.*, 2011; Kattenberg *et al.*, 2012). However it has an advantage that it does not persist in the blood but clears about the same time as the parasites following treatment. The lack of antigen persistence after treatment makes the PLDH useful in predicting treatment failure unlike the Pf HRP2 that persists in the blood after parasite clearance.

The RDTs performed differently in different age groups. Microscopy was more effective in determining infection in four age groups namely 40-49 yrs (76.92%), 50-59 yrs (59.6%), 30-39 yrs (57.58%), 60-69 yrs (55.0%) and 10-19 years (53.42%). The lower performance of microscopy with younger age groups could be attributed to recurrent self-treatment at any attack of fever which could give false positives with the RDTs. SD Bioline performed more effectively in three age groups namely ≤ 9 years (69.0%), 10-19 yrs (67.12%) and 30-39 yrs (60.6%). Carestart and Antec were more effective in determining infection in one age group each namely 10-19 years (73.97%) and 80-89 years (33.31%) respectively. Antec was not as effective in determining infection as the other RDTs and microscopy. The differences in effectiveness of the diagnostic tests could be as a result of parasite densities in each person's blood as a result of level of exposure to the bites of infected mosquitoes and composition of the RDTs since they emanate from different manufacturers. The highest prevalence of infection with RDTs was in the age group of 10-19 yrs (73.97%) with Carestart and 40-49 years (76.92%) with microscopy. This might not be too different with the reports of Sheyin and Bigwan (2013) whose reports showed highest prevalence of infection in age group 0-20 years with

Carestart and microscopy. Increased exposure to mosquito bite due to the practice of improper utilization of long lasting insecticide nets might have led to the high prevalence recorded.

Gender related infective rates showed that females (42.37%) were more vulnerable than males (39.59%) for malaria a finding that is similar to that of Sheyin and Bigwan (2013). This could be attributed to the fact that the women and girls were exposed to mosquito bites because they are more involved in agricultural activities than their male counterparts. Females have lower red blood count which is associated with excessive loss of blood which occurs during menstruation making them more susceptible to malaria infection. This finding differs from that of Adekunle *et al.*, 2014 and Ubiaru *et al.*, 2018 who reported higher a prevalence in males than females. Performances between the communities showed that in Umuevu (68.0%) and Umuawada (66.0%) microscopy was more effective than the RDT's. The higher prevalence in the two communities could be that the inhabitants sought treatment in the patent medicine stores rather than going to the health centers because of the distance. This recourse to self-medication will lead to anti-malaria drug resistance that usually worsens their malaria burden (Brieger *et al.*, 2001, Amuta & Ikpa, 2005).

The lower performances of the RDTs could be due to delay in HRP2 surge after increased parasite density or *P. falciparum* with an HRP2 gene deletion or reduced HRP2 expression (Bell *et al.*, 2005) and such patients never give a positive result with these tests (Moody, 2002). Some studies have also reported reduced sensitivity of RDTs as a result of low parasitaemia (Ishengoma *et al.*, 2011). In Umuoma and Umuekpeke, RDTs performed better than microscopy and this may be as a result of nearness of these communities to the health center than the former. These communities visit the health centers and get diagnosed and treated thus, there may not be new

episodes of malaria infection but persistence of malaria antigen in the bloodstream will give a positive result with the RDTs. It is also likely that some of the subjects with false-positive results may have performed self-medication with antimalarial drugs during an attack of fever. However, it is unlikely that these factors account for the entire set of false-positive cases. It is more probable that most of the false-positive cases were true positives which were not detected by microscopy, due to sequestration limiting the number of circulating parasites at the time of blood collection or due to the parasitaemia being below the detection limit of approximately 50/ μ l by microscopy.

5. Conclusion

Malaria is the most important infectious diseases that present a diagnostic challenge to laboratories in most countries especially in poor resource settings. This study has shown that some RDTs can outperform expert microscopy in detecting *P. falciparum* and thus can offer a useful alternative to microscopy in situations where reliable microscopic diagnosis procedures are not available.

References

- Abba, K., Deeks, J.J., Olliaro, P.L., Naing, C.M., Jackson, S.M., Takwoingi, Y., Donegan, S., Garner, P. (2011) Rapid diagnostic tests for diagnosing uncomplicated *P. falciparum* malaria in endemic countries, *Cochrane Database Systematic Reviews* 7, CD008122.
- Adekunle, N.O., Sam-Wobo, S.O., Dedeke, G.A., Ojo, D.A., Abimbola, W.A., Adeleke, M.A., Surakat, O.A. (2014) Evaluation of Rapid Methods in malaria diagnosis from persons attending primary health facilities, Ogun State, Nigeria, *Nigeria Journal of Parasitology* 35 (1-2): 19-25.
- Agomo, P.U., Asianya, V.N., Akindele, S.K., Agomo, C.O., Akinyele, M.O., Adewole,

- T.A., Igbasi, U.T., Anyanwu, R.C., Egbuna K.N. (2003) Investigation of the efficacy of two rapid assessment techniques (Optimal 1 and SD Bioline) for diagnosis of malaria in rural areas of Nigeria, *African Journal of Clinical and Experimental Microbiology* **4**: 6-13
- Amuta, E.U., Ikpa, T.F. (2005) High prevalence of HIV infection, increased tendency to malaria burden and development of AIDS at the Federal Medical Centre, Makurdi, Nigeria, *The Nigerian Journal of Parasitology* **26**: 7-11.
- Bell, D.R., Wilson, D.W., Martin, B. (2005) 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, *American Journal of Tropical Medicine and Hygiene* **73**: 199-203.
- Brieger, M.C., Sesay, H.R., Adesina, H., Mosanya, M.F., Ogunlade, P.B., Ayodele J.O., Orisason S.A. (2001) Urban malaria treatment behavior in the context of low level malaria transmission in Lagos, Nigeria, *African Journal of Malaria Science* **30**: 7-15.
- Chukwuocha, U.M., Dozie I.N.S. (2011) Malaria transmission and morbidity patterns in holoendemic areas of Imo River Basin of Nigeria, *Biomed Central Research Notes* **4**: 514-520.
- Irole-Eze, O.P., Ukpai, O.M., Amaechi, E.C., Ejike, B.U., Nwadike, C.C. (2017) Impact of *Plasmodium falciparum* infection on some haematological parameters in a malaria endemic population, southeastern Nigeria, *Journal of Coastal Life Medicine* **5** (12): 547-549.
- Ishengoma, D., Francis, F., Mmbando, B., Lusingu, J., Magistrado, P., Alifrangis, M., Theander, T.G., Bygbjerg, I.C., Lemnge, M.M. (2011) Accuracy of malaria rapid diagnostic tests in community studies and their impact on treatment of malaria in an area with declining malaria burden in north-eastern Tanzania, *Malaria Journal* **10**: 176-183.
- Kattenberg, J.H., Christian, M., Tahita, I.A.J., Versteeg, H.T., Schallig, T. H.D.F.H., Mens, P.F. (2012) Antigen persistence of rapid diagnostic tests in pregnant women in Nanoro, Burkina Faso, and the implications for the diagnosis of malaria in pregnancy, *Tropical Medicine and International Health* **17** (5): 550-557.
- Moody, A. (2002) Rapid diagnostic tests for malaria parasites, *Clinical Microbiology Reviews* **15** (1): 66-78.
- Nigeria Malaria Indicator Survey (2015) Abuja, Nigeria
- Ojurongbe, O., Adegbosin, O.O., Taiwo, S.S., Alli, O.A.T., Olowe, O.A., Ojurongbe, T.A., Bolaji, O.S., Adeyeba, O.A. (2013). Assessment of Clinical Diagnosis, Microscopy, Rapid Diagnostic Tests, and Polymerase Chain Reaction in the Diagnosis of *Plasmodium falciparum* in Nigeria, *Malaria Research and Treatment*. Article ID 308069, p.5
- Sani, U.M., Jiya, N.M., Ahmed, H. (2013) Evaluation of a malaria rapid diagnostic test among febrile children in Sokoto state, Nigeria, *International journal of medicine and medical sciences* **3** (5): 434-440.
- Sheyin, Z., Bigwan, I. E. (2013) Comparison of Care start HRP2 rapid malaria test with light microscopy for guiding patient's treatment of fever in Nigerian endemic areas, *Journal of Medical Sciences* **4** (9): 353-356.
- Tarazon, A.S., Zerpa, L.S., Requena, D.M., Lianos-Cuentas, A., Magill, A. (2004) Evaluation of the rapid diagnostic test optimal for diagnosis of malaria due to *Plasmodium vivax*, *Brazilian Journal of Infectious Disease* **28**: 151-155.
- Ubiaru, P.C., Ukpai, O.M., Ohaeri, C.C. (2018) Evaluation of microscopy and rapid diagnostic tests in screening clinically suspected malaria patients in a

hospital setting in Umuahia, Nigeria, *Nigerian Journal of Parasitology* **39 (1)**: 35-41.

Ukpai, O.M., Irole-Eze, O.P., Amaechi, E.C., Ejike, B.U., Ejike, E.N., Okorocha, N.A. (2017) A study of Knowledge, Attitude, and practices regarding malaria among inhabitants of Ezinihitte Local Government Area, Imo State, Eastern Nigeria, *Manila Journal of Science* **10**: 65-74.

World Health Organization (2010) Malaria Rapid Diagnostic Test Performance: Results of WHO Product Testing of Malaria RDTs: Round 2(2009). Geneva:WorldhealthOrganization.<http://www.who.int/malaria/publications/atoz/9789241599467/en/index.html>. Assessed 5th June 2018

World Health Organization (2016) *World Malaria Report 2015*. Geneva: World Health Organization.