DIAGNOSTIC EFFICIENCY OF RAPID DIAGNOSIS TEST KITS IN MALARIA DIAGNOSIS – THE NIGERIAN STORY

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ABSTRACT

Malaria constitutes a significant global public health problem. WHO guidelines recommend actual prompt parasitological confirmation before therapy. Microscopy remains the gold standard for parasitological diagnosis but requires trained personnel and elaborate equipment, contributing to cases of presumptive diagnosis. The development of new malarial rapid test kits has contributed to solving this problem of presumptive diagnosis. These RDTs have gained increasing widespread application in Nigeria. WHO recommended RDT standards include a sensitivity of 95% for the detection of 100/μl of P. falciparum and 95% specificity. Diagnostic accuracy of these tests however varies based on geographical region. No current review of the literature has however been carried out to assess the diagnostic accuracy of RDTs in the Nigerian setting. A survey of the literature on RDT use in Nigeria identified twenty-six different comparative studies following a PubMed and Google search. These studies reported a range of sensitivity and specificity values, with mean values of 76.7% and 91.2% respectively. The sensitivity values in this study were similar to previous reports but specificity values were on average higher than sensitivity values. This finding differed from the generally accepted dogma of lower specificity of HRP2-based RDTs due to the ability of HRP2 to persist in the bloodstream even after clearance of the parasite. This study provides a summary of the current research on the use of RDT in malaria diagnosis in Nigeria. It highlights the increase in use of RDTs and points at a need for more standardized multisite studies to provide a better understanding of the effects of variables on diagnostic accuracy of these tests and better inform on policies.

BACKGROUND

Malaria is the leading cause of plasmodium infection in humans and a significant global public health problem. The World malaria report published by the World Health Organization (WHO) in 2013, estimates that malaria was responsible for 207 million clinical episodes and 627,000 deaths worldwide in 2012, with 482,000 of these in children below 5 years of age[1]. Significantly however, most of these deaths (90%) were found to occur in sub-Saharan Africa. Prompt and accurate diagnosis of malaria has been pegged as a cornerstone in the management of this scourge.

One of the basis of the target of WHO's initiative to achieve a 75% reduction in malarial incidence by 2015, is diagnostic testing to demonstrate the presence of the malaria parasite in suspected cases. WHO guidelines recommend that malarial therapy is preceded by actual prompt parasitological confirmation. A diagnosis that is both prompt and accurate would impact on disease outcome (reducing the incidence of both morbidity and mortality). This would also prevent the indiscriminate use of antimalarial agents, thereby ultimately influencing selection of resistance with the added benefit of saving cost. All these factors make a confirmatory diagnosis essential in the management of malaria.

The 2013 World malaria report observed a 37% to 61% increase in the proportion of presumptive cases of malaria in the public sector of the WHO Africa Region which were confirmed using a diagnostic method. Despite the general increase in number of presumptive malaria cases confirmed by diagnostic testing, the diagnosis of malaria in Nigeria is still based on a presumptive or clinical diagnosis. A WHO household survey between 2010 and 2012 on the proportion of febrile children who actually had their blood tested, reported that Nigeria had the second lowest proportion out of 14 African countries with proportions less than 20% and 10% for both the public and private sectors respectively.

Issue of over reporting

Due to the non-specific signs and symptoms of malaria, and an overlap with other tropical diseases[2], presumptive or clinical diagnosis generally leads to an over reporting of the prevalence of malaria[3], causing indiscriminate intake of antimalarial

drugs. The World Health Organization therefore recommends management of malarial cases based on parasitological diagnosis[1]. Microscopy remains the gold standard for parasitological diagnosis. This method has been found to have a high sensitivity, with the ability to detect densities of malaria parasite as low as 5 to 10 parasites/μl of blood[4] but it requires trained personnel and elaborate equipment. This lack of trained personnel, as well as irregular power supply[3], has been the predominant factor contributing to the presumptive diagnosis of malaria especially in rural settings.

Introduction of RDT

The development of new rapid test kits for the diagnosis of malaria infections has contributed to solving this problem of presumptive diagnosis. These malarial rapid diagnostic test (RDT) kits use immunochromatographic methods based on lateral flow strip technology. The RDTs detect malaria antigens in small amounts of blood samples[4, 5]. Results of these tests usually show up as a colored test line and may be obtained within 5 to 20 min. Several formats of malarial RDTs exist. The major variation between the formats is in the type of antigen the kits are able to detect. These antigens which are specific for the detection of unique *Plasmodium* sp or *Plasmodium* spp in general include; histidine-rich protein 2 (HRP-2) for *P. falciparum*, aldolase for *Plasmodium* spp in general and plasmodial lactate dehydrogenase (pLDH) either for *P. vivax* (Pv-pLDH), *P. falciparum* (Pf-pLDH) or *Plasmodium* sp in general (pan-pLDH).

At present, assays that detect HRP-2 are more common, contributing to greater than 90% of RDTs in use[6]. These assays are particularly useful in Sub-Saharan Africa where *P. falciparum* is the main causative agent of malaria. HRP-2 was the first antigen used in the commercial preparation of malaria RDTs[7]. This protein has several characteristics, which make it a good target. HRP-2 is a water-soluble protein distinct to *P. falciparum*. This protein occurs in the cytoplasm of the parasite as well as on membranes of infected erythrocytes[8] and increases in concentration as parasite development takes place. It is easily diffusible into plasma and may be detected when low levels of parasites are present.

Since the introduction and commercial availability of malaria RDTs, these tests have gained widespread use worldwide and in Africa. In 2012, World Health Organization (WHO) reported an increase in the use of RDTs in Africa, and pegged it as the main

contributing factor to the increase in confirmatory diagnosis in the region[9]. The advantages of RDT over the standard blood film microscopy include a lack of requirement for trained personnel, electricity and rapidity. These make this technique useful even in interior places. In recent years, the use of RDTs has equally gained increasing widespread application in Nigeria. A 2016 study[10] carried out on formal private health facilities, found that RDTs were employed in the confirmation of about 50% of presumptive diagnosis by plasmodial detection. Another study which compared two different healthcare facilities, reported a higher use of RDTs (85.2%) in public health facilities, than in private (32.9%) [11]. This increase in use of RDTs has translated to an increase in manufacture and supply of malarial RDT products. A 2013 review reported more than 200 of these RDT products at that time[12]. A current WHO list of recommended RDT products published online (http://www.who.int/malaria/publications/atoz/rdt-selection-criteria.pdf) contains just over 60 products, with inclusion criteria based on the product meeting a minimum set of criteria. WHO recommended RDT standards includes a sensitivity of 95% for the detection of 100/µl of *P. falciparum* and a 95% specificity[13]. These values serve as the benchmark for determining the diagnostic accuracy of an RDT. This diagnostic accuracy has however been found to vary based on geographical region[14] making an assessment of RDT performance by region essential. By 2008 when two major reviews on malarial RDTs had been published[4, 14], only one Nigerian study was cited. A more recent Cochrane Review[15] mentioned only two Nigerian papers published in 2001 and 2007 respectively[16, 17]. This similar trend was also reported in a 2013 review[12]. There is therefore a need to identify what has been done so far in Nigeria, analyze how the results of these studies fit in with data worldwide.

The Nigerian Story

A survey of the literature on RDT use in Nigeria was therefore carried out. This was aimed at assessing the efficacy of the use of RDTs as a diagnostic tool for the detection of malaria infection in Nigeria and exploring possible factors that could impact on its capabilities. The literature survey noted reports carried out on different segments of the population, but mostly those presenting with febrile symptoms resembling malaria. A number of Nigerian studies[18, 19, 20, 21, 22, 23, 24] reported only on the use of RDTs as a diagnostic tool, making an assessment of the accuracy of the test impossible. Hence they were not included for further analysis. Other studies however (Table 1), carried

out diagnosis using both RDT and microscopy. Hence the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of the RDT kits could be assessed and therefore, the diagnostic accuracy. Fourteen of these studies described data exclusively related to children, one on women, while the rest described data from mixed age groups, or just adults. Eleven different brands of RDTs were used in these studies: SD-Bioline, Carestart, FirstResponse, Paracheck-Pf, Biotec, Acon Malariatest, AcumenPF, Diaspot, Global, Parasight-F, ICT. These brands varied both in manufacturers and antigen detected, with test kits either detecting HRP-2 and/or *Pf*-*p*LDH and/or pan-*p*LDH. These studies reported sensitivity values ranging from 8.3% to 100% (mean: 76.7%), specificity values ranging from 40.7% to 100% (mean: 91.2%), PPV ranging from 50% to 100% (mean: 90.2%) and NPV ranging from 34.29% to 100% (mean: 81.8%).

Table 1: Summary of studies reporting on malaria diagnosis by RDT and microscopy

S/No	Study (Ref)	Study Pop	Test Kit	Antigen	Sensitivity (%)	Specificity (%)	NPV (%)	PPV (%)	REF
1.	Nwuba *et al.,* 2001	Children	ICT Pf	HRP-2	93.1	95.8			[16]
2.	Happi *et al.,* 2004	Children	Parasight-F	HRP-2	80	-	-	-	[25]
3.	Oguonu and Okafor 2007	Children	Paracheck Pf	HRP-2	42.31	93.65	-	-	[17]
4.	Adesanmi *et al.,* 2011	Children	Paracheck-pf	HRP-2	82	91.5	91.5	82	[26]
5.	Houmsou *et al.,* 2011	Mixed	NI	HRP-2	89.5	100	93.9	100	[27]
6.	Rabiu *et al.,* 2011	Children	Paracheck-Pf®	HRP-2	86.2	81.98	-	-	[28]
7.	Aladenika *et al.,* 2012	Children			Ch/Ad	Ch/Ad	Ch/Ad	Ch/Ad	[29]
		Adult	Biotech		96:30	99:99	96:59	99:97	
			Acon		96:46	98:98	97:65	98:96	
			Acumen		99:78	97:97	99:82	97:96	
			Diaspot		98:75	97:96	99:79	97:95	
			Global		96:73	98:97	97:78	98:96	
8.	Ameh *et al.,* 2012	Mixed	SD-Bioline	*Pf*-*p*LDH and/or pan-*p*LDH	75.2	80.4	57.5	90.2	[30]
9.	Falade *et al.,* 2013	Adults	Paracheck-Pf®	HRP-2	55.4	90.3	-	-	[31]
10.	Sani *et al.,* 2013	Children	Biotec	HRP-2	96.9	-	-	-	[21]
11.	Sheyin and Bigwan 2013	NI	Carestart™	HRP-2	78.4	97.6	80.1	97.3	[32]
12.	Bagbi *et al.,* 2014	Children	Paracheck-pf	HRP-2	96.7	88.5	-	-	[33]
13.	Elendu and Oyibo 2014	Mixed	SD-Bioline	HRP-2	96.7	98.7	-	-	[34]
14.	Abdulkadir *et al.,* 2015	Children	Carestart™	HRP-2	40.3	89.6	56.5	81.8	[35]
15.	Ajumobi *et al.,* 2015	Children	SD-Bioline	HRP-2	100	98.5	100	88.6	[36]
16.	Dougnon *et al.,* 2015	Mixed	SD-Bioline	*Pf*-*p*LDH and/or pan-*p*LDH	45.45	100	86.95	100	[37]

S/No	Study (Ref)	Study Pop	Test Kit	Antigen	Sensitivity (%)	Specificity (%)	NPV (%)	PPV (%)	REF
17.	Elechi *et al.,* 2015	Children	Acon	HRP-2	8.3	100	74	100	[38]
18.	Idowu *et al.,* 2015	Mixed	SD-Bioline	HRP-2	71.8	87.1	-	-	[39]
19.	Mohammed *et al.,* 2015*	Women	Not stated		88.8	100	83	100	[40]
20.	Ayogu *et al.,* 2016	Mixed	First Response®	HRP-2	82.17	100	34.3	100	[41]
21.	Dozie and Chukwocha 2016	Mixed	SD-Bioline	HRP-2	98.6	90	96.4	95.8	[42]
			First Response®	HRP-2	98.6	90	96.4	95.8	
			Carestart™	pLDH+	92.9	93.3	84.4	97	
			Malariatest (Acon)	pLDH+	92.9	93.3	84.4	97	
22.	Falade *et al.,* 2016	Children	SD-Bioline	HRP-2	94.3	41.6	86.1	65.6	[3]
					92.8	40.7	82.7	65.0	
23.	Garba *et al.,* 2016	Children	Carestart™	HRP-2	9.09	92.06	53.7	50	[43]
24.	Ogunniyi *et al.,* 2016	Mixed	Carestart™	HRP-2	76	96	93.6	84.7	[44]
25.	Okangba *et al.,* 2016	Mixed	SD-Bioline	HRP-2	83.3.	94.7	96.9	73.5	[45]
26.	Oyeyemi *et al.,* 2016	Mixed	First Response®	HRP-2	Inf: 44.3	97.5	72.8	92.1	[46]
					NPW: 78.3	77.5	70.5	83.9	
					PW: 83.3	83.9	72.4	78.4	

NI: Not Indicated; Inf: Infant; NPW: Non-pregnant women; PW: Pregnant women
*Only study not carried out on febrile patients

The actual occurrence distribution of these values differed; 26.8% of cases reported sensitivity values above 95%, 53.8% for specificity, 59.4% for PPV and 28.1% for NPV (Table 2). Considering that generally, low values for the different parameters (<60%), were only reported in a few cases (21.9% and 5.1% for sensitivity and specificity respectively), the sensitivity values in this study were similar to previous review reports on values ranging from 65% to 100%, and 83.6% to 100%[4, 14] and the more current Cochrane review[15], which reported a range of 42% to 100% with most values clustering between 80% and 100%. These sensitivity values are also quite similar to other reports from Africa and non-African malaria endemic regions, such as India, and other parts of the world. A 2008 comprehensive study reporting on RDT diagnosis in Kenya and Uganda over a 4-year period[47] reported sensitivity values of 90% and 91%. Higher sensitivity values of 97% were however noted for febrile patients as opposed to the 89% noted in non-febrile patients. Also Hopkins *et al.,* 2008 carrying out a study in Uganda, noted a sensitivity of 97%[48]. This study involved the evaluation of 1000 patients at 7 different sites around the country (N = 7000). Most other studies generally reported high (>90%) sensitivity values[49, 50, 51, 52]. A variation from this was a 2014 report from Central African Republic who reported sensitivity values ranging from 85.4% to 88.2% and associated this with low parasitaemia[53].

Table 2: Distribution of values reported by the different studies

	% Distribution			
Range	Sensitivity	Specificity	PPV	NPV
> 95%	26.8	53.8	59.4	28.1
80% - 95%	31.7	38.5	25	34.4
70% - 79%	19.5	2.6	6.3	6.3
60% - 69%	0	0	6.3	3.1
50% - 59%	2.4	0	3.1	12.5
< 50%	19.5	5.1	0	3.1

Considering the sheer number of variables involved, generalized analysis and extrapolations could not be drawn from these data. A few studies highlight why comparisons even within a region may be difficult. First is the comparative 2012 study by Aladenika and colleagues, which reported on diagnosis efficiency of different RDT brands in both children and adults[29]. This study consistently reported lower sensitivity values for all five RDT brands when testing adult populations. This however differed from the 2016 report by Oyeyemi and colleagues who used a single RDT brand against differing populations and noted a much lower sensitivity value in children than adults[46]. Another possible factor affecting sensitivity data is the parasite density. While most Nigerian studies provided no data for this, reduced parasite density is generally associated with decreased sensitivities. Previous studies have reported a decrease in sensitivity values at parasite densities <500/μl[4, 14]. Okangba and colleagues noted this when they observed that for five microscopy positive but RDT negative cases, the parasite density was below 200/μl[45]. A similar trend was observed by Sani and colleagues who clearly noted an increase in sensitivities from 0% to 100% as parasite density per μl increased from <200 to >12800 (21). All of these, point at a need for standardization in research design to enable a country wide comparison which would help inform policies. The difference in sensitivity based on parasite density can be quite significant. A report on an RDT trial noted a 53.9% sensitivity of parasite densities per μl between 0 and 100 as opposed to a 92.6% sensitivity at parasite densities per μl between 500 and 1000 and a 99.7% density at parasite density > 5000 per μl[4].

One key data often lacking in the studies was whether patients were excluded from the study based on antimalarial use in the preceding two weeks. Abdulkadir and colleagues who recorded a low sensitivity of 40.3% reported that 79% of their study population (febrile under-fives) had received inappropriate antimalarial therapy[35].

Specificity values in this study were on average higher than sensitivity values. These values ranged from 40.7% to 100%, with a mean of 91.13%. 71.8% of the cases reporting on both sensitivity and specificity had a higher specificity value. Previous reviews had expressed concerns about the potential of HRP2-based RDTs to produce false positive results in patients recently treated for malaria, thereby limiting specificity. This potential was described as being linked with the ability of HRP2 to persist in the bloodstream for up to 28 days, even after the clearance of the parasite[6, 54]. HRP2-based RDTs have therefore previously been reported to generally have a lower specificity but higher sensitivity than pLDH based RDTs[48, 49, 52]. A 2011 review[15], reported that for every 100 cases of malaria, HRP2 based tests detected 2 more cases than the pLDH tests, but also gave 4 false positives per every 100 non-cases. Numerous other studies gave similar reports of lower specificity values than sensitivity[55, 56, 57]. One study assessing the relationship between specificity and duration from last malaria episode reported a specificity of 11% in people who had malaria in the previous 2 weeks, and 89% in those who had malaria in the in the previous 10 weeks[52]. This relationship between last malaria episode and low specificity (hence false positives), has been noted to pose an issue particularly in areas with moderate to high transmission of malaria[58], thereby compromising cost effectiveness and user perception of RDTs, and potentially contributing to drug misuse and hence resistance.

Exceptions to this have however been noted[59, 60, 61], with some studies linking the exceptions to hypoendemic regions with low transmission of malaria [47, 55]. Singh *et al.,* 2005 reported higher specificity values as opposed to sensitivity (70% vs 93%) in low transmission areas. One of these studies also study reported higher specificity values in older aged patients, afebrile patients and towards the end of transmission season[47]. Similar findings of higher specificity values were noted among Nigerian studies. It would therefore be essential to explore if indeed the generalized association of HRP2 with lower specificities still stand or if this needs to be revised based on more current data.

CONCLUSION

This study presents a summary of work done so far on the use of RDTs in malaria diagnosis in Nigeria. This practice is on the increase, with majority of articles on the subject matter published in the past two years. Despite the wide range of RDTs available commercially, over 50% of studies made use of WHO recommended products. Generally, findings from these studies have reported acceptable levels of sensitivity, though the specificity values differed from what seems to be the accepted dogma of lower specificity values of HRP2-based RDTs. Majority of these studies were independent localized studies however. There is therefore a need for a country wide collaborative multisite study to be carried out, taking in all the associated variables which could impact on both sensitivity and specificity values, and employing a standardized study protocol. This would provide a more robust set of data to properly understand the pros and cons of the use of RDTs in malaria diagnosis and inform on future policies in this fight to end the scourge of malaria in Nigeria.

REFERENCES

1. WHO: World Malaria Report 2013. World Health Organization; Geneva. 2013.

2. Ojurongbe O, Adegbosin OO, Taiwo SS, Alli OA, Olowe OA, Ojurongbe TA, Bolaji OS, Adeyeba OA. Assessment of clinical diagnosis, microscopy, rapid diagnostic tests, and polymerase chain reaction in the diagnosis of *Plasmodium falciparum* in Nigeria. *Malaria Research and Treatment*. 2013; Article ID: 308069.

3. Falade CO, Ajayi IO, Nsungwa-Sabiiti J, Siribié M, Diarra A, Sermé L, Afonne C, Yusuf OB, Gansane Z, Jegede AS, Singlovic J. Malaria rapid diagnostic tests and malaria microscopy for guiding malaria treatment of uncomplicated fevers in Nigeria and prereferral cases in 3 African countries. *Clinical Infectious Diseases*. 2016; 63(Suppl 5): S290 – S297.

4. Murray CK, Gasser RA, Magill AJ, Miller RS. Update on rapid diagnostic testing for malaria. *Clinical Microbiology Reviews*. 2008; 21(1): 97 – 110.

5. Chilton D, Malik AN, Armstrong M, Kettelhut M, Parker-Williams J, Chiodini PL. Use of rapid diagnostic tests for diagnosis of malaria in the UK. *Journal of Clinical Pathology*. 2006; 59(8): 862 – 866.

6. Mouatcho JC, Goldring JD. Malaria rapid diagnostic tests: challenges and prospects. *Journal of Medical Microbiology*. 2013; 62(10): 1491 – 1505.

7. Shiff CJ, Premji Z, Minjas JN. The rapid manual ParaSight®-F test. A new diagnostic tool for *Plasmodium falciparum* infection. Transactions of the Royal Society of *Tropical Medicine and Hygiene*. 1993; 87(6): 646 – 648.

8. Howard RJ, Uni S, Aikawa M, Aley SB, Leech JH, Lew AM, Wellems TE, Rener J, Taylor DW. Secretion of a malarial histidine-rich protein (Pf HRP II) from *Plasmodium falciparum*-infected erythrocytes. *The Journal of Cell Biology*. 1986; 103(4): 1269 – 1277.

9. WHO: World Malaria Report 2012. World Health Organization; Geneva. 2012.

10. Mokuolu OA, Ntadom GN, Ajumobi OO, Alero RA, Wammanda RD, Adedoyin OT, Okafor HU, Alabi AD, Odey FA, Agomo CO, Edozieh KU. Status of the use and compliance with malaria rapid diagnostic tests in formal private health facilities in Nigeria. *Malaria Journal*. 2016; 15(1): 4.

11. Bamiselu OF, Ajayi I, Fawole O, Dairo D, Ajumobi O, Oladimeji A, Steven Y. Adherence to malaria diagnosis and treatment guidelines among healthcare workers in Ogun State, Nigeria. *BMC Public Health*. 2016; 16(1): 828.

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

13. WHO. Towards quality testing of malaria rapid diagnostic tests: evidence and methods. WHO, Western Pacific Region, Manila, Philippines. 2006.

14. Wongsrichanalai C, Barcus MJ, Muth S, Sutamihardja A, Wernsdorfer WH. A review of malaria diagnostic tools: microscopy and rapid diagnostic test (RDT). *The American Journal of Tropical Medicine and Hygiene*. 2007; 77(6 Suppl): 119 – 27.

15. Abba K, Deeks JJ, Olliaro PL, Naing CM, Jackson SM, Takwoingi Y, Donegan S, Garner P. Rapid diagnostic tests for diagnosing uncomplicated *P. falciparum* malaria in endemic countries. *Cochrane Database Systematic Review.* 2011; (7): CD008122.

16. Nwuba RI, Anumudu CI, Omosun YO, Sodeinde O, Nwagwu M. Evaluation of a rapid immunochromatographic card test for *Plasmodium falciparum* in Ibadan, Nigeria. *African Journal of Medicine and Medical Sciences*. 2001; (1-2): 123 – 124.

17. Oguonu T, Okafor HU. Comparison of clinical, microscopic and rapid diagnostic test methods in the diagnosis of *Plasmodium falciparum* malaria in Enugu, Nigeria. *Nigerian Postgraduate Medical Journal*. 2007; 14(4): 285 – 289.

18. Ayorinde AF, Oyeyiga AM, Nosegbe NO, Folarin OA. A survey of malaria and some arboviral infections among suspected febrile patients visiting a health centre in Simawa, Ogun State, Nigeria. *Journal of Infection and Public Health*. 2016; 9(1): 52 – 59.

19. Kolawole OM, Mokuolu OA, Olukosi YA, Oloyede TO. Comparative prevalence of *Plasmodium falciparum* malaria in patients attending Okelele Health Centre, Okelele, Ilorin, Nigeria. *Indian Journal Health Science Biomedical Research*. 2017; 10: 57 – 62.

20. Oladokun RE, Ige OK, Ogunbosi B, Brown B. Challenges of malaria diagnosis in paediatric patients at a Nigerian hospital. *International Journal of Tropical Disease & Health*. 2015; 5(4): 269 – 275.

21. Sani UM, Jiya NM, Ahmed H. Evaluation of a malaria rapid diagnostic test among febrile children in Sokoto, Nigeria. *International Journal of Medicine and Medical Science*. 2013; 3(5): 433 – 440.

22. Nwaneri DU, Adeleye OA, Ande AB. Asymptomatic malaria parasitaemia using rapid diagnostic test in unbooked pregnant women in rural Ondo-south district, Nigeria. *Journal of Preventive Medicine and Hygiene*. 2013; 54(1): 49 – 52.

23. Gobir Z, Tukur Z. Prevalence of malaria parasitemia using rapid diagnostic test among apparently healthy children in Kano, Nigeria. *Journal of Medicine in the Tropics*. 2014; 16(1): 1 – 4.

24. Uzochukwu BS, Obikeze EN, Onwujekwe OE, Onoka CA, Griffiths UK. Cost-effectiveness analysis of rapid diagnostic test, microscopy and syndromic approach in the diagnosis of malaria in Nigeria: implications for scaling-up deployment of ACT. *Malaria Journal*. 2009; 8(1): 265.

25. Happi CT, Gbotosho GO, Sowunmi A, Falade CO, Akinboye DO, Oladepo O, Oduola AM. Malaria diagnosis: false negative parasight-F tests in falciparum malaria

patients in Nigeria. *African Journal of Medicine and Medical Sciences*. 2004; 33(1): 15 – 18.

26. Adesanmi T A, Okafor H U, Okoro A B, Mafe A G. Diagnosis of malaria parasitemia in children using a rapid diagnostic test. *Nigerian Journal of Clinical Practice*. 2011; 14: 195 – 200.

27. Houmsou RS, Amuta EU, Sar TT, Adagba AH. Malarial infection among patients attending a Nigerian semi-urban based hospital and performance of HRP-2 pf Rapid diagnostic Test (RDT) in screening clinical cases of *Plasmodium falciparum* malaria. *Translational Biomedicine*. 2011; 2(1): 5.

28. Rabiu OR, Kosoko AM, Falade CO, Ademowo OG. Evaluation of the performances of two rapid diagnostic tests (Cyscope® mini and Paracheck-Pf®) in the diagnosis of malaria among febrile children in southwest Nigeria. *Medical Principles and Practice*. 2012; 22(3): 255 – 259.

29. Aladenika S, Tatfeng M, Nwobu G, Osakue E, Ehiaghe A, Ehiaghe JI, Okwu MU, Ikusemoro AI. Sensitivity and specificity of five malaria rapid diagnostic kits used in south-south region of Nigeria. *Open Journal of Clinical Diagnostics*. 2012; 2: 70 – 75

30. Ameh J, Ahmad RM, Ekeh N, Linga P, Mangoro Z, Imam AU, Akeredolu P, Hudu S. Laboratory diagnosis of malaria: Comparing giemsa stained thick blood films with rapid diagnostic test (RDT) in an endemic setting in North-west Nigeria. *Journal of Medical Laboratory and Diagnosis*. 2012; 3(2): 10 – 15.

31. Falade CO, Adesina-Adewole B, Dada-Adegbola HO, Ajayi IO, Akinyemi JO, Ademowo OG, Adewole IF, Kanki P. Evaluation of Paracheck-Pf(TM) rapid malaria diagnostic test for the diagnosis of malaria among HIV-positive patients in Ibadan, south-western Nigeria. *Pathogens and Global Health*. 2013; 107(2): 69 – 77.

32. Sheyin Z, Bigwan IE. Comparison of CareStart HRP2 Rapid Malaria Test with Light Microscopy for Guiding Patient's Treatment of Fever in Nigeria Endemic Areas. *Journal of Medicine and Medical Sciences*. 2013; 4: 353 – 356.

33. Bagbi BM, Obieche A, Enato EFO. Prevalence of Malaria Among Symptomatic Children Presumptively Treated with Anti-Malarial Medications in Edo State Nigeria. *African Journal of Pharmaceutical Research & Development*. 2014; 6(1): 17 – 23.

34. Elendu JA, Oyibo W. Performance of Hrp2 Based Malaria Rapid Diagnostic Test (Sd-Bioline) in Whole Blood and Saliva in Patient's in Lagos State. *International Journal of Innovation and Applied Studies*. 2014; 9(2): 642 – 647.

35. Abdulkadir I, Rufai HA, Ochapa SO, Malam MS, Garba BI, Oloko AG, George II. Malaria rapid diagnostic test in children: The Zamfara, Nigeria experience. *Nigerian Medical Journal*. 2015; 56(4): 278 – 282.

36. Ajumobi O, Sabitu K, Nguku P, Kwaga J, Ntadom G, Gitta S, Elizeus R, Oyibo W, Nsubuga P, Maire M, Poggensee G. Performance of an HRP-2 rapid diagnostic test in

Nigerian children less than 5 years of age. *The American Journal of Tropical Medicine and Hygiene*. 2015; 92(4): 828 – 833.

37. Dougnon TV, Bankole HS, Hounmanou YM, Echebiri S, Atchade P, Mohammed J. Comparative study of malaria prevalence among travellers in Nigeria (West Africa) using slide microscopy and a rapid diagnosis test. *Journal of Parasitology Research*. 2015; Article ID: 108707.

38. Elechi HA, Rabasa AI, Bashir MF, Gofama MM, Ibrahim HA, Askira UM. Uncomplicated malaria in children: The place of rapid diagnostic test. *Nigerian Medical Journal*. 2015; 56(2): 85 – 90.

39. Idowu AO, Odeyemi MO, Babalola SA. Assessment of Accuracy and Effectiveness of Rapid Diagnostic Test for Malaria Diagnosis at Primary Health Centres in Abeokuta Nigeria. *International Journal of Tropical Disease & Health*. 2015; 8(3): 90 – 97.

40. Mohammed Y, Dabo NT, Kawo AH, Yakubu AI. Comparison of Microscopic Examination and Rapid Diagnostic Tests Used To Diagnose Malaria among Pregnant Women in Kano, North –Western, Nigeria. *International Journal of Science and Technology*. 2015; 5(2): 47 – 52.

41. Ayogu EE, Ukwe CV, Nna EO. Assessing the reliability of microscopy and rapid diagnostic tests in malaria diagnosis in areas with varying parasite density among older children and adult patients in Nigeria. *Journal of Postgraduate Medicine*. 2016; 62(3): 150 – 156.

42. Dozie UW, Chukwuocha UM. Comparative Evaluation of Malaria Rapid Diagnostic Test Kits Commercially Available in Parts of South Eastern Nigeria. *Journal of Tropical Diseases & Public Health*. 2016; 4:201.

43. Garba BI, Muhammad AS, Musa A, Edem B, Yusuf I, Bello NK, Adeniji AO, Kolawole T. Diagnosis of malaria: A comparison between microscopy and rapid diagnostic test among under-five children at Gusau, Nigeria. *Sub-Saharan African Journal of Medicine*. 2016; 3: 96 – 101.

44. Ogunniyi A, Dairo MD, Dada-Adegbola H, Ajayi IO, Olayinka A, Oyibo WA, Fawole OI, Ajumobi O. Cost-Effectiveness and Validity Assessment of Cyscope Microscope, Quantitative Buffy Coat Microscope, and Rapid Diagnostic Kit for Malaria Diagnosis among Clinic Attendees in Ibadan, Nigeria. *Malaria Research and Treatment*. 2016; Article ID: 5242498.

45. Okangba C, Elikwu CJ, Shobowale EO, Shonekan O, Nwadike V, Tayo B, Omeonu AC, Faluyi B, Meremikwu C, Falade O, Osoba D. Histidine rich protein 2 performance in determining the prevalence of malaria among patients presenting with clinical symptoms of malaria. *Scientific Journal of Pure and Applied Sciences*. 2016; 5(1): 339 – 350.

46. Oyeyemi OT, Sode OJ, Adebayo OD, Mensah-Agyei GO. Reliability of rapid diagnostic tests in diagnosing pregnancy and infant-associated malaria in Nigeria. *Journal of Infection and Public Health*. 2016; 9(4): 471 – 477.

47. Abeku TA, Kristan M, Jones C, Beard J, Mueller DH, Okia M, Rapuoda B, Greenwood B, Cox J. Determinants of the accuracy of rapid diagnostic tests in malaria case management: evidence from low and moderate transmission settings in the East African highlands. *Malaria Journal*. 2008; 7(1): 202.

48. Hopkins H, Bebell L, Kambale W, Dokomajilar C, Rosenthal PJ, Dorsey G. Rapid diagnostic tests for malaria at sites of varying transmission intensity in Uganda. *Journal of Infectious Diseases*. 2008; 197(4): 510 – 518.

49. Hopkins H, Kambale W, Kamya MR, Staedke SG, Dorsey G, Rosenthal PJ. Comparison of HRP2-and pLDH-based rapid diagnostic tests for malaria with longitudinal follow-up in Kampala, Uganda. *The American Journal of Tropical Medicine and Hygiene*. 2007; 76(6): 1092 – 1097.

50. Gabrielli S, Bellina L, Milardi GL, Katende BK, Totino V, Fullin V, Cancrini G. Malaria in children of Tshimbulu (Western Kasai, Democratic Republic of the Congo): epidemiological data and accuracy of diagnostic assays applied in a limited resource setting. *Malaria Journal*. 2016; 15(1): 81.

51. Alareqi LM, Mahdy MA, Lau YL, Fong MY, Abdul-Ghani R, Ali AA, Cheong FW, Tawfek R, Mahmud R. Field evaluation of a Pf HRP-2/pLDH rapid diagnostic test and light microscopy for diagnosis and screening of falciparum malaria during the peak seasonal transmission in an endemic area in Yemen. *Malaria Journal*. 2016; 15(1): 49.

52. Mbabazi P, Hopkins H, Osilo E, Kalungu M, Byakika-Kibwika P, Kamya MR. Accuracy of two malaria rapid diagnostic tests (RDTs) for initial diagnosis and treatment monitoring in a high transmission setting in Uganda. *The American Journal of Tropical Medicine and Hygiene*. 2015; 92(3): 530 – 536.

53. Djallé D, Gody JC, Moyen JM, Tekpa G, Ipero J, Madji N, Breurec S, Manirakiza A. Performance of Paracheck™-Pf, SD Bioline malaria Ag-Pf and SD Bioline malaria Ag-Pf/pan for diagnosis of falciparum malaria in the Central African Republic. *BMC Infectious Diseases*. 2014; 14(1): 109.

54. Kakkilaya BS. Rapid diagnosis of malaria. *Laboratory Medicine*. 2003; 8(34): 602 – 608.

55. Singh N, Mishra AK, Shukla MM, Chand SK, Bharti PK. Diagnostic and prognostic utility of an inexpensive rapid on site malaria diagnostic test (ParaHIT f) among ethnic tribal population in areas of high, low and no transmission in central India. *BMC Infectious Diseases*. 2005; 5(1): 50.

56. Bharti PK, Silawat N, Singh PP, Singh MP, Shukla M, Chand G, Dash AP, Singh N. The usefulness of a new rapid diagnostic test, the First Response® Malaria Combo (pLDH/HRP2) card test, for malaria diagnosis in the forested belt of central India. *Malaria Journal*. 2008; 7(1): 126.

57. Singh N, Shukla MM, Shukla MK, Mehra RK, Sharma S, Bharti PK, Singh MP, Singh A, Gunasekar A. Field and laboratory comparative evaluation of rapid malaria diagnostic tests versus traditional and molecular techniques in India. *Malaria Journal*. 2010; 9(1): 191.

58. Aydin-Schmidt B, Mubi M, Morris U, Petzold M, Ngasala BE, Premji Z, Björkman A, Mårtensson A. Usefulness of Plasmodium *falciparum*-specific rapid diagnostic tests for assessment of parasite clearance and detection of recurrent infections after artemisinin-based combination therapy. *Malaria journal*. 2013; 12(1): 349.

59. 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. *Malaria Journal*. 2013; 12(1): 218.

60. Sahu SS, Gunasekaran K, Jambulingam P. Field performance of malaria rapid diagnostic test for the detection of Plasmodium falciparum infection in Odisha State, India. *The Indian Journal of Medical Research*. 2015; 142(Suppl 1): S52 – S58.

61. Fransisca L, Kusnanto JH, Satoto TB, Sebayang B, Supriyanto, Andriyan E, Bangs MJ. 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. *Malaria Journal*. 2015; 14(1): 103.