Performance and Comparison of two Malaria Rapid Diagnostic Tests among Symptomatic Patients in Lagos, Southwest Nigeria

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ABSTRACT

Malaria remains a public health problem in Sub Saharan Africa. Microscopic identification of Plasmodium spp. is the gold standard for malaria diagnosis. However, malaria rapid diagnostic test kits are also available for prompt diagnosis, effective treatment and are important in reducing morbidity and mortality associated with malaria especially among high-risk groups. The use of malaria rapid diagnostic tests (RDTs) has improved *Plasmodium falciparum* diagnosis, especially in settings where quality microscopy is not available. Field evaluation of RDTs would assist in confirming the potency, performance of the RDTs and in ruling out or confirming malaria parasite. In this study, the performance of two RDTs were assessed for malaria diagnosis, using thick and thin blood smears as the gold standard. A total of 1,271 patients suspected to have malaria who gave consent were enrolled in the study within six months at St Matthew''s Primary Health Center Amukoko in Ajeromi Ifelodun LGA of Lagos state. The study population age range between 2-67years. This is a cross-sectional study involving patients with history of malaria symptoms. The SD Bioline and First Response HRP2 based MRDTs were evaluated in this study. Of the patients 1,271 screened for malaria, 185 were slide positive for malaria parasites. Blood smears and HRP2 First response RDT showed malaria prevalence rate of 14.6%, while the HRP2 SD Bioline RDT showed prevalence rate of 15.5%. The SD Bioline HRP2 RDTs showed a sensitivity of 61.1%, specificity of 92.3%, PPV of 57.4% and NPV of 93.3%, while the First response RDT showed a sensitivity of 58.9%, specificity of 93.1%, PPV of 59.6% and NPV of 92.9%. In this study, the sensitivity of the RDT increased with parasite density (>1000p/µl showed sensitivity >90%). All the malaria rapid diagnostic test kits performed relatively well and can be used in emergencies. Concerning cost-effectiveness, using the malaria RDTs at the hospital is cheaper than light microscopy for diagnosing malaria. However, for malaria diagnosis, malaria RDT kits cannot be relied upon alone; hence, microscopic confirmation is always required.

INTRODUCTION

In malaria-endemic settings, prompt and efficient diagnosis is required for effective disease management (Oyeyemi *et al*., 2015). The World Health Organization (WHO) recommends that all febrile patients in

malaria-endemic settings are appropriately diagnosed before commencing any treatment. This recommendation enhances anti-malarial drugs' effective and efficient use and prevents unnecessary treatments that could contribute to drug resistance (Abba *et al*., 2011). Malaria is a major public health concern in Nigeria, with an estimated 68 million cases and 194,000 deaths due to the disease in 2021 contract malaria yearly, with most deaths among children under 5 years (WHO, 2020). At the same time, *Plasmodium falciparum* malaria remains one of the most significant diseases in Nigeria and sub-Saharan Africa, recording a high burden on health services (Ansah *et al.,* 2010). Generally, examining malaria blood film for *Plasmodium spp*. identification using a light microscope remains the gold standard for confirming malaria in suspected patients (Mukry *et al*., 2017; Berzosa, *et al.,* 2020). However, in remote rural settings where skilled personnel or microscopes are unavailable, Rapid Diagnostic Test (RDT) kits are recommended to screen for malaria before commencing anti-malarial treatment (Ajakaye *et al*., 2020; Odeniyi *et al.,* 2020; Okangba *et al*., 2020). Hence, in Nigeria, several malaria RDT kits are in the market (Ilesanmi *et al*., 2017; Oluwaremilekun, *et al*., 2020; Okangba *et al*., 2022) and the main difference between these malaria RDT kits is the detected antigen. RDTs do not require much infrastructure or technical expertise and as such can be done easily in remote rural settings, hence discouraging the presumptive treatment of malaria in the absence of microscopy. Malaria RDTs exist in different formats. The major difference between them is in the antigen type detected by the kit. The histidine-rich protein 2 (HRP-2) is specific for the detection of P. falciparum, aldolase for detection of 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) (Kennedy and Otokunefor 2017). HRP-2 can detect the presence of the Plasmodium protein whether dead or alive and so tends to be very sensitive, but less specific because of it results in false positive occasionally (FMOH 2015). At present, more than 90% of RDT in use are the most common assays that detect HRP-2 These assays are valuable in Sub Saharan Africa where the main causative agent of malaria is *P. falciparum* (Mouatcho and Goldring 2013). Some RDT kits show different performances in different geographical settings (Ugah *et al.,* 2017). There is, therefore, a need to continually evaluate the performance of malaria RDT kits used in primary healthcare in different geographical settings. This study evaluated two routinely used malaria RDT kits at St. Matthew's Primary Health Centre Amukoko, Lagos. Amukoko in Ajeromi Ifelodun Local Government Area (LGA) of Lagos State using blood film for P. falciparum parasites identification as the gold standard.

MATERIALS AND METHODS

Study Design and Study Site

The study is a cross-sectional study and it was conducted from April to October 2017 among persons suspected of malaria during their visit to St. Matthew's Primary Health Centre Amukoko, Lagos. Amukoko in Ajeromi Ifelodun Local Government Area (LGA) of Lagos State. This hospital serves the indigenes of the Amukoko District in the Southwest Region. Inclusion criteria: Patient with history of malaria symptoms (axillary temperature $\geq 37.5^{\circ}C$), No intake of anti-malaria in the two weeks preceding presentation at clinic, presence of *P. falciparum* parasite and gave consent. Exclusion criteria: Patient with no history of malaria symptoms, refuse to give consent, recent intake of antimalarials (in the last 2 weeks) and Patient with clinical anaemia

Study Population

All persons suspected of malaria visiting the St. Matthew's Primary Health Centre Amukoko, Lagos. Amukoko in Ajeromi Ifelodun Local Government Area (LGA) of Lagos State.. The study was conducted at St. Matthew's Primary Health Centre Amukoko, Lagos. Amukoko in Ajeromi Ifelodun Local Government Area (LGA) of Lagos State. The study area was selected because it is densely populated (estimated population of 684, 105) and is near to the Atlantic Ocean and Lagos Lagoon. The outpatient

department of the Primary Healthcare Centre (PHC) handles an average of 150 suspected malaria cases weekly. Children and adults, age between 2- 67years who presented in the health facility with documented fever (≥37.5^{O}C) or symptoms of fever and other related symptoms and who gave consent were recruited into the study.

Sample Size Determination.

The sample size was calculated using a prevalence of malaria reported in Nigeria, 14.7% (Aina *et al*., 2013), The Assumptions were: Degree of precision is put at 0.05 and the population of people with malaria more than 10,000

$$\mathbf{n} = z^2pq/d^2$$

prevalence of malaria in Lagos is 14.7% (Aina *et al*., 2013);

Minimum sample size of 342

Participants Enrollment

A case report form (CRF) was obtained for each patient presenting with fever after taking verbal consent. The CRF contained information on previous antimalarial used, age, malaria symptoms and age. Three millilitres of venous blood was collected by the laboratory personnel into ethylene diaminetetra acetic acid(EDTA) bottles from the patient for microscopy and rapid diagnostic test. Patients blood were screened with two different Rapid Diagnostic Tests kits. The RDTs were performed and the results were interpreted in the laboratory.

Preparation of blood films for parasite diagnosis /quantification

A. Thin and thick smear preparation

Thin and thick blood films, using a template diameter of 15mm for thick were made on the same frosted end, grease free glass slide, with date, patient's study 1D number. Also duplicate slides were prepared for each patient, which were stained, dried and boxed. The thin film was fixed in absolute methanol for 1-2 seconds and air dried, while the thick malaria blood film (MBF) were not fixed, this allow the red blood cells (RBCs) to lyse, making the malaria parasites to be left intact and concentrated.

B. Staining Procedure

The MBFs were stained after 48 hours with 3% Giemsa stain solution at pH 7.2. Thereafter the stained slides were rinsed with buffer water of pH 7.0 – 7.2 after 45minutes.

C. Examination of blood films for Parasite Diagnosis

The stained slides were read by first and second microscopist to determine the parasite density; number of malaria parasites (parasite count) and leukocytes were counted per high power field. The absolute parasite density was calculated using the formula below:

$$\frac{\text{No of parasites counted}}{\text{No of WBC counted}} \times \underline{\text{Total WBC count}} = \text{No of parasite/µl blood}$$

A malaria blood film (MBF) was considered positive when a reddish chromatin dot with a purple or blue cytoplasm of the malaria parasite are seen together. A MBF was declared negative after viewing 500 high

power fields, using x100 oil immersion objective lens and no asexual and sexual stages of the parasite were seen.

Performance of the Rapid Diagnostic Test

The test kit contains a membrane strip which is pre-coated with monoclonal antibody with two separate lines, one serves as the control line while the other is for *Plasmodium falciparum* HRP2. 5ml of blood sample were collected with the kits calibrated dropper and transferred into the sample well of the RDTs This was followed by adding four drops of buffer for SD-bioline and three drop for First response into the buffer well., the test kits have one control line to indicate the validity of the test procedure and its working condition .Control and test lines appeared within 15minutes in a reading window the test was done following the manufacturer's instructions.

Interpretation of RDT test Results

The principle of reading the colour intensity of the test line is graded as follows: 0= No test line (Negative), 1+ (Positive with very faint test line), 2+ (Positive equal to test line), 3+ (Strong positive, greater than test line). The presence of two bands indicate a positive result for *Plasmodium falciparum*, one band for control line and the other is for the test line, while the presence of only one band in the control area indicates a negative result.

ETHICAL CONSIDERATION

Ethical approval for this study was obtained from the Research, Grants and Experimentation Committee of the College of Medicine of the University of Lagos, Nigeria (CM/COM/08/XXIV).. Participants gave informed consent and assent from children with corresponding consent from their Parents/Guardian after explaining the purpose of the research before they were enrolled. Patients that declined to participate were not denied access to the available health care service. The entire study was conducted in line with good clinical laboratory practice.

Data Analysis: The data generated from the study were analyzed using EPIINFO 3:4:3 version statistical software (CDC, Atlanta, USA). Tests for associations and differences were done by chi square analysis, Fisher Exact test and analysis of variance where appropriate. The performance characteristics of the MRDTs was measured using the following performance characteristics: sensitivity, specificity, negative predictive value, positive predictive value, negative likelihood ratio and positive likelihood ratio, recover operator characteristics and area under the curve. Test of statistical significance was set at P value less than 0.05 at 95% confidence interval.

RESULTS

Demographic and Patients Information

A total of 1271 malaria suspected patients participated in the study at St Matthew Primary Health Centre. Their baseline characteristics are summarized in table 1. The study participants had a mean age of 26.3 ± 16.7years and mean temperature of $36.9\pm0.8^{\circ}$C. The age range of 25-34 had the highest population of 308 (24.3%) followed by 15-24 age range 275(21.70%) and least was <5 age group 156(12.3%) . The study population were more of female 767(60.3%), than male 505(39.7%). Patients with temperature of <37.4°C (afebrile) were more 1025(81.3%) than those with ≥37.5 temperature (febrile patients) 246(18.7%). The major drug taken by participant at home before coming to the health care centre was Agbo 305(23.9%) followed by ACT 208(16.3%) and PCM 165(16.1%). The least drug used at home was Antibiotics 54(5.3%). Most of the study participants 808 (63.5%) had fever, which is the clinical symptom with the

highest frequency, this is followed by yellow urine 594(46.6), headache 441(33.6%) and chills 420(33.0%) (table 2a). Diarrheal and itching exhibited the lowest clinical symptoms frequency in the study population (table 2a). In table 2b, among the afebrile patients, those with fever exhibited the highest frequency of 60.6% and 77.1% among the febrile patients while diarrheal was the lowest (1.2%) for afebrile and itching (0%) for febrile patients

Prevalence of malaria and Performance characteristics among study Participants

In this study the prevalence of malaria using blood specimen with microscopy method was 14.6%, while SD Bioline HRP2 RDT was 15.5% and First Response RDT was 14.6%. The study showed that participants >5 years had the highest documented fever 61(39.1%), while participants >44 years recorded the lowest among the study population 20(10%) (figure 1). There was a significant difference (c^2 = 76.5, df = 5; P= < 0.001) between age groups and documented fever among the study population. The older age group of >44 years had the lowest body temperature (<37.5^{O}C), while for body temperature >=37.5^{O}C had the highest frequency among study population of children < 5 years (figure 1). The comparative performance of the two different RDTs with microscopy method in (table 3) indicated that microscopy method had the prevalence rate of 14.6%, while, SD Bioline and First Response had a prevalence rate of 15.5% and 14.6% respectively. The sensitivity and specificity of the RDTs used for this study varied. SD Bioline RDT showed the highest sensitivity of 61.1% while, FR was 58.9% and their specificities were 92.3% and 93.1% on blood specimen respectively. (Table 4). There was a relationship between age and malaria prevalence among the study group. The age group >5 had malaria prevalence of 13(8.4%), while age group >5-14 years exhibited the highest prevalence of malaria 46 (26.6%), and age group 35-44years had the lowest prevalence of 14(9%) among the study population (table 5). Malaria infection was significantly associated with age (c^2 = 41.85, df = 5; P<0.001). There was no significant difference between malaria prevalence and the participants sex (c^2 = 1.53, P< 0.216). Although, there was relationship between body temperature group and malaria prevalence (c^2 = 22.97; P< 0.001). The participants having >=37.5^{O}C 58(24.6%) exhibited high malaria prevalence than, participants having <37.5^{O}C 126(12.3%). The effect of parasite density on the malaria RDT sensitivity is seen in table 6. The higher the parasitaemia level, the higher the sensitivity of the test.

Body weakness had a significant association with age (c^2 = 14.49; P< 0.001). Age group <5 years had the least frequency of body weakness 156(26%), while age >44years showed the highest frequency of 200 (56%). There was relationship between age and fever (c^2 = 32.9; P< 0.001). The frequency of fever among the study participants reduced as the age increases. There was also significant association with joint ache (c^2 = 84.81; P< 0.001). The least frequency was seen among participants of age <5 years and the highest value was seen in age group >44 years (table 7).

Table 1: Baseline characteristics of the study participants

Description	n (%)	Afebrile (n=1,023)	Febrile (n=246)	Total (n=1249)
No of Participants	1271			
Sex				
Female	767(60.30)	620(81.8)	138(18.2)	758
Male	505(39.70)	402(80.4)	98(19.6)	500

Age (years) Mean±SD	26.3±16.7			
<5	156(12.30)	95(60.9)	61(39.1)	156
5-14	173(13.60)	122(70.9)	50(29.1)	172
15-24	275(21.70)	226(83.7)	44(16.3)	270
25-34	308(24.30)	265(87.7)	37(12.3)	302
35-44	157(12.40)	134(85.9)	22(14.1)	156
>44	200(15.80)	180(90.0)	20(10.0)	200
Temperature(^{O}C)	Chi square =76.5	df = 5; P <0.001		
Mean±SD	36.9±0.8	36.1±0.6	36.9±1.1	
Home treatment drugs ACTs	208(16.3)	128(60.1)	80 (39.9)	
Agbo	305(23.9)	200(67.2)	105(32.8)	
Paracetamol	165(16.1)	112(74.0)	53(24.0)	
SP	24 (1.9)	16(66.7)	8 (33.3)	
Chloroquinine	133(10.5)	97(76.9)	36(23.1)	
Antibiotics	54 (5.3)	44(80.5)	10(19.5)	

Table 2a: Frequency of Clinical symptoms among Study Participants

Symptoms	Frequency	Percent	95% Conf Limits	
Fever	808	63.5%	60.8%	66.1%
Yellow urine	594	46.6%	43.9%	49.4%
Joint ache	549	43.1%	40.4%	45.9%
Headache	441	34.6%	32.0%	37.3%
Chills	420	33.0%	30.4%	35.7%
Body weakness	320	25.1%	22.8%	27.6%
Loss of Appetite	221	17.4%	15.3%	19.6%
Stomach ache	194	15.2%	13.3%	17.3%
Cough	191	15.0%	13.1%	17.2%
Vomiting	146	11.5%	9.8%	13.4%
Dizziness	107	8.4%	7.0%	10.1%
Isomnia	49	3.8%	2.9%	5.1%

Nausea	43	3.4%	2.5%	4.6%
Chest pain	28	2.2%	1.5%	3.2%
Diarrhea	16	1.3%	0.7%	2.1%
Itching	16	1.3%	0.7%	2.1%

Table 2b: Frequency of Clinical symptoms among Afebrile and Febrile Study Participants

| Symptoms	Frequency for Afebrile Patients (%)	95% Conf. Limits (Afebrile)	Frequency for Febrile Patients (%)	95% Conf. Limits (Febrile)
Body weakness	257(25.1)	22.5 – 27.9	58(24.6)	19.2 -30.6
Cough	148(14.5)	12.4 -16.9	48(18.2)	13.5 – 23.7
Chill	323(31.6)	28.8 -34.5	91(38.6)	32.3 – 45.1
Chest pain	26 (2.5)	1.7 – 3.7	1 (0.4)	0.0 – 2.3
Diarrhea	12(1.2)	0.6 -2.1	4(1.7)	0.5 -4.3
Dizziness	95(9.3)	7.6 -11.3	10(4.2)	2.1 -7.7
Fever	620(60.6)	57.5 – 63.6	182(77.1)	71.2 – 82.3
Headache	355(34.7)	31.8 – 37.7	78(33.1)	27.1 – 39.4
Isomnia	40(3.9)	2.8 – 5.3	8(3.4)	1.5 -6.6
Itching	16 (1.6)	0.9 – 2.6	0(0.0)	0.0 -1.6
Joint ache	456(44.5)	41.5 – 47.6	85(36.0)	29.9 – 42.5
Loss of Appetite	182 (17.8)	15.5 -20.3	34(14.4)	10.2 – 19.5
Nausea	37(3.6)	2.6 -5.0	3 (1.3)	0.0 – 1.6
Stomach ache	170 (16.6)	14.4 – 19.1	22(9.3)	5.9 -13.8
Vomitting	103(10.1)	8.3 -12.1	43 (18.2)	13.5 – 23.7
Yellow urine	472(46.1)	43.0 – 49.2	116(49.2)	42.6 – 55.7
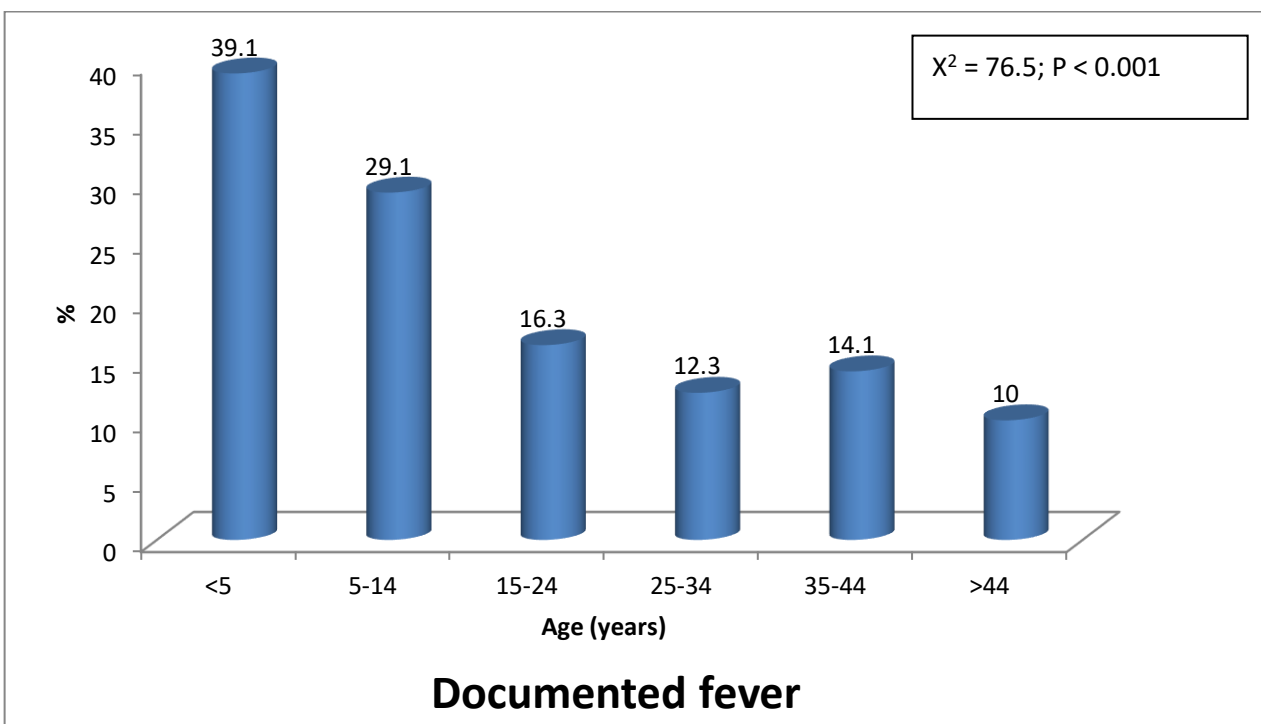
Figure 1: Comparison of documented fever (Body temperature ≥37.5°C) versus age of study participants

Table 3: Prevalence of malaria using different malaria RDTs and Microscopy Method

Specimen	Diagnostic method	Total	Positive for malaria	Prevalence
Blood	Microscopy	1271	185	14.6
	Hs_SD	1269	197	15.5
	Hs_FR	1251	183	14.6

Hs_SD=HRP2 single SD Bioline, Hs_FR =HRP2 single First response Hs

Table 4: Performance characteristics of RDTs using Blood specimen

RDTs	Sensitivity	Performance characteristic				
		Specificity	PPV	NPV	LR(+)	LR(-)
Hs_SD	61.1	92.3	57.4	93.3	7.9	0.4
Hs_FR	58.9	93.1	59.6	92.9	8.5	0.4

Hs_SD=HRP2 single SD Bioline, Hs_FR =HRP2 single First response RDT kit, PPV=Positive Predictive Value, NPV=Negative Predictive Value, PLR=Positive Likelihood Ratio, NLR= Negative Likelihood Ratio

Table 5: Comparison of malaria prevalence by age, sex and body temperature

	Microscopy		TOTAL	Chi square	P
	NEG	POS			
Age (n=1265)					
<5	141 (91.6)	13 (8.4)	154		
5-14	127 (73.4)	46 (26.6)	173		
15-24	218 (79.6)	56 (20.4)	274	41.85	<0.001
25-34	271 (88.0)	37(12.0)	308		
35-44	142 (91.0)	14 (9.0)	156		
>44	181 (90.5)	19 (9.5)	200		
SEX (n=1268)					
Female	661 (86.4)	104 (13.6)	765	1.53	0.216
Male	422 (83.9)	81 (16.1)	503		
Temp (n=1257)					
<37.5(Afebrile)	895(87.7)	126 (12.3)	1021	22.97	<0.001
>=37.5 (febrile)	178 (75.4)	58(24.6)	236		

Table 6: Effect of parasite density on malaria RDT sensitivity

Parasite density group	n	Sensitivity of RDTs at different parasite densities	
		Hs_SD_bld	**Hs FR_bld**
1-200	61	26.2	21.3
201-500	26	61.5	61.5

501-1000	19	73.7	73.7
>1000	79	84.8	83.5

Hs_SD_bld=HRP2 single sd bioline blood, Hs_FR_bld =HRP2

Table 7: Presenting signs and symptoms in relation to age of the study participants

agegp	Presenting signs and symptoms															
	TOTAL	BWK	CGH	CHL	CHP	DIA	DZN	FEV	HAC	ISM	ITCH	JAC	LAP	NAU	SAC	VOM	YELURI
<5	156	26	35	48	2	2	6	121	45	3	1	31	31	3	13	30	62
Row %	100	16.7	22.6	30.8	1.3	1.3	3.8	77.6	28.8	1.9	0.6	19.9	19.9	1.9	8.3	19.2	39.7
5-14	172	35	22	58	3	1	13	129	56	3	5	55	33	8	18	33	84
Row %	100	20.3	12.9	33.7	1.7	0.6	7.5	75	32.7	1.7	2.9	32	19.2	4.6	10.5	19.2	48.8
15-24	275	69	32	83	8	7	25	170	88	7	2	107	37	5	57	20	125
Row %	100	25.1	11.6	30.2	2.9	2.5	9.1	61.8	32	2.5	0.7	38.9	13.5	1.8	20.7	7.3	45.5
25-34	307	95	44	102	7	4	30	179	108	12	5	143	62	13	49	35	141
Row %	100	30.9	14.3	33.2	2.3	1.3	9.7	58.3	35.1	3.9	1.6	46.4	20.2	4.2	15.9	11.4	45.8
35-44	157	38	20	51	2	1	11	92	55	11	2	83	29	4	28	13	77
Row %	100	24.2	12.7	32.5	1.3	0.6	7	58.6	35	7	1.3	52.9	18.5	2.5	17.8	8.3	49
>44	200	56	38	76	6	1	22	113	85	13	1	126	27	10	29	12	102
Row %	100	28	19.1	38	3	0.5	11	56.5	42.5	6.5	0.5	63	13.5	5	14.5	6	51
TOTAL	1267	319	191	418	28	16	107	804	437	49	16	545	219	43	194	143	591
Row %	100	25.2	15.1	33	2.2	1.3	8.4	63.5	34.5	3.9	1.3	43	17.3	3.4	15.3	11.3	46.6
chi square		14.4731	13.2993	3.6672			7.3967	32.8742	8.928			84.8121	7.9645	6.47	16.2	32	5.4543
P		0.013	0.021	0.598			0.193	<0.001	0.112			<0.001	0.158	0.26	.006	<0.001	0.363

BWK=Body weakness; CGH=Cough; CHL=Chill; CHP=Chest pain; DIA= Diarrhea; DZN=Dizziness; FEV=Fever; HAC= Headache; ISM=Isomnia; ITCH=Itching; JAC=Joint pain; LAP=Loss of appetite; NAU=Nausea; SAC= Stomach ache; VOM=Vomitting; YELURI= Yellow urine

DISCUSSION

Malaria remains a major public concern, with over 90% of global cases occurring in Africa. Prompt and accurate diagnosis of malaria is the fundamental first step to effectively identify, treat and interrupt disease transmission (Lander *et al.*, 2016). An excellent modelling analysis predicts that > 100,000 malaria-associated deaths would be averted by a 95% sensitive and 95% specific diagnostic tool that require minimal infrastructure. (Rafael *et al.,* 2006). Among children, the prevalence and mortalities associated with malaria are higher than among adults, and in 2019, about 67% of children died of malaria worldwide (Prah *et al.*, 2021; Mohammed *et al.,* 2022). Studies have shown that many anti-malarial drugs have been misused on patients with non-malarial diseases due to a lack of prompt and accurate laboratory diagnosis (Bell *et al.,* 2006). Hence, in malaria-endemic settings, RDT kits are essential in remote areas where microscopes or skilled personnel are unavailable to ensure the effective use of anti-malarial drugs. It has been reported that temperatures in the tropics can denature antibodies in the membrane or damage the nitrocellulose membrane forming the strip of the RDT kits, thereby changing its flow characteristics or causing the antibody to detach

from the membrane (Chiodini *et al*., 2007). Hence, evaluating the performance of these RDT kits in different geographical settings is imperative. In this current study, the sensitivities of the RDT kits was 61.1 % for the First Response Malaria AgPf kit to 58.9% for the SD Bioline Ag Pf kit. In other parts of the world, sensitivities of RDT kits ranging from 96.0% to 97.6% have been reported (Msellem *et al.,* 2009; Stauffer *et al.,* 2009; Niscarti *et al.,* 2009; Ajumobi *et al.,* 2015; Domfeh *et al.,* 2023), which implies that the evaluated RDT kits have lower sensitivities. The specificities of RDT kits in this current study was 93.1% for the First Response Malaria Ag Pf kit to 92.3% for the SD Bioline Malaria Ag Pf kit, and specificities of RDT kits within the ranges of 87%–100% have also been reported in previous studies (Buchachart *et al*., 2004; Msellem *et al.,* 2009; Dougnon *et al.,* 2015). Considering the WHO standard of choosing malaria RDT kits, based on 95% sensitivity (Ajakaye *et al*., 2020), the SD Bioline Ag Pf kit is the preferred choice. However, all the malaria RDT kits evaluated recorded specificities lower than the WHO recommendation of 97% specificity (Ajakaye *et al*., 2020), . Hence, microscopic confirmation of malaria is always required after using RDT kits in primary healthcare settings. The sensitivities observed in this study is similar to results obtained by several field studies (Houze *et al*., 2009, Buchachart, 2010; Ameh *et al*., 2012; Oyeniran *et al*., 2014; Ilesanmi *et al*., 2017; Oluwaremilekun *et al.,* 2020) which reported sensitivity ranging from 50.3% -74.3%, but different from the results obtained from several other studies (Bharti *et al*., 2008; Khainar, 2009; Okonko*, et al*., 2009; Ansah, 2010, Chinkhumba *et al.,* 2010; Kalu *et al*., 2012; Sani *et al*., 2014; Adekunle *et al*., 2014; Oboh, *et al*., 2021), which reported a variable sensitivity of 84.2-100%, but considerably favourable with specificity of 82.8%-98%. Variations in test sensitivity between these studies may be due to differences in the types of RDTs formats used or due to variations in epidemiologic characteristics of the study population, level of parasitaemia, test methodology and skill of microscopists (Oboh *et al*., 2021).

The sensitivity and specificity value recorded in this study is lower than the WHO recommendation of 95% sensitivity and 97% specificity for malaria RDTs. This is a major issue to be considered when using RDTs in diagnosis of malaria in primary health care or for malaria control programmes (WHO, 2019). Several factors have been reported to be responsible for low performance of RDTs in field conditions which includes effect of environmental conditions such as high temperatures during transportation and storage, quality issues, disease related factors e.g. parasite species and density etc., and also host factors such as treatment history (Emmanuel *et al*. 2018). The Federal Ministry of Health, National Malaria and Vector Control Division, Nigeria has recommended that RDTs cannot be used to replace microscopy as the only means of diagnosis for malaria (FMOH, 2015). This is because there are variations in the performances of RDTs. The effect of high temperature and humidity which is characteristic of tropical regions on RDTs is a serious limitation. As such, microscopy remains the gold standard for malaria diagnosis and should be used for diagnosis of malaria where possible especially in severe illness where malaria is suspected.

CONCLUSION

In conclusion, RDTs are very useful in areas where microscopic diagnosis of malaria is not feasible however they should not be used to replace microscopy. The performance of the RDTs evaluated in this study had a very low sensitivities when compared with other studies. This is linked to the low parasitaemia seen in the microscopy results. There is need for further investigation on the RDTs used in this study to determine the reason for the low sensitivity recorded

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COMPETING INTERESTS

The authors declare no competing interests

REFERENCES

1. Abba, K., Deeks, J.J., Olliaro, P. et al. (2011) "Rapid diagnostic tests for diagnosing uncomplicated P. falciparum malaria in endemic countries," Cochrane Database of Systematic Reviews, vol. 7, pp. 7–19.
2. Adekunle NO, Sam-Wobo SO, Dedeke GA, Ojo DA, Abimbola WA, Adeleke MA, Surakat OA (2014) Evaluation of rapid methods in malaria diagnosis from persons attending primary health facil ities, Ogun State, Nigeria. *Niger J Parasitol* 35(1–2):19–25
3. Ameh J, Ahmad RM, Ekeh N, Linga P, Mangoro Z, Imam AU, Akeredolu P, Hudu S (2012) Laboratory diagnosis of malaria: comparing giemsa stained thick blood films with rapid diagnos tic test (RDT) in an endemic setting in North-west Nigeria. *J Med Lab Diagn* 3(2):10–15
4. Ajakaye, O., Ibukunoluwa, A.R. (2020)."Performance evaluation of a popular malaria RDT in Nigeria compared with microscopy," *Journal of Parasitic Diseases*, vol. 44, no. 1, pp. 122–125
5. Ajumobi, O., Sabitu, K., Nguku, P. et al. (2015). "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,* vol. 92, no. 4, pp. 828–833.
6. Ansah, E.K., Narh-Bana, S. Epokor, M (2010). "Rapid testing for malaria in settings where microscopy is available and peripheral clinics where only presumptive treatment is available: a randomised controlled trial in Ghana," *British Medical Journal*, vol. 340, no. mar051, p. c930.
7. Aina, O.O., Agomo, C.O., Olukosi, Y.A., Okoh, H.I., Iwalokun, B.A., Egbuna. K.N., Orok, A.B., Ajibaye, O., Enya, V.N.V., Akindele, S.K., Akinyele, M.O., and Agomo, P.U. (2013). Malariometric Survey of Ibeshe Community in Ikorodu, Lagos State: Dry Season. *Malaria Research and Treatment*, 1-9.
8. Berzosa P, de Lucio A, Romay-Barja M, Herrador Z, González V, García L, et al. (2018). Comparison of three diagnostic methods (microscopy, RDT, and PCR) for the detection of malaria parasites in representative samples from Equatorial Guinea. Malar J. 17:333.
9. Bharti, P.K., Chandel, H.S., Ahmad, A., Krishna, S., Udhayakumar, V. and Singh, N. (2016). Prevalence of pfhrp2 and/or pfhrp3 gene deletion in *Plasmodium falciparum* population in eight highly endemic states in India. *PLoS One.* 11(8)
10. Buchachart, P.K., Silawat, N., and Singh, P.P. (2010). The usefulness of a new rapid diagnostic test, the First Response® MalariaM Combo (pLDH/HRP2) card test, for malaria diagnosis in the forested belt of central India. *Malar J.* 7:126.
11. Chiodini, P.L., Bowers, K., Jorgensen, P., Barnwell, J.W., Grady, K.K., Luchavez, J., Moody, A.H., Ceniza, A., Bell, D. (2007). The stability of Plasmodium lactate dehydrogenase-based and histidine-rich protein II based malaria rapid diagnostic tests. *Transaction of the Royal Society of Tropical Medicine and hygiene*. 91:479-483
12. Chinkhumba, J., Skarbinski, J., Chilima, B., Campbell, C., Ewing, V., San Joaquin, M., Sande, J., Ali, D., and Mathanga (2010). Comparative field performance and adherence to test results of four malaria rapid diagnostic tests among febrile patients more than five years of age in Blantyre, Malawi. *Malaria Journal,* 9: 209-214
13. Domfeh, S.A., Boateng Y. Darkwa, Y., Raymond K. G., Adu-Asamoah, E., Obirikorang, C.(2023.) Evaluation of Four Malaria Rapid Diagnostic Test Kits Used at the Enyiresi Government Hospital in the Eastern Region of Ghana Journal of Parasitology Research Volume Article ID 4226020, 6 pages https://doi.org/10.1155/2023/4226020
14. Dougnon, T., Bankole, H. S., Hounmanou,Y. M. G., Echebiri, S., Atchade, P., Mohammed, J. (2015).

"Comparative study of malaria prevalence among travellers in Nigeria (West Africa) using slide microscopy and a rapid diagnosis test," *Journal of Parasitology Research*, vol. , p. 4, .

15. Emmanuel, I.O., Chijioke, U.O., Ekelozie, I.S., (2018). Malaria rapid dagnostic test (RDTs). Ann Clin Lab Res 6(4):275. https://doi.org/10.21767/2386-5180.100275

16. Federal Ministry of Health (2015) National guidelines for diagnosis and treatment of malaria—3rd edition. Publications of the Federal Ministry of Health, Nigeria, The Federal Ministry of Health, National Malaria and Vector Control Division. Available at Publications nmcp.gov.nh. accessed

17. Houzé, S., Boly, M.D., Le Bras, J., Deloron, P., Faucher, J.F.(2009). *Pf*HRP2 and *Pf*LDH antigen detection for monitoring the efficacy of artemisinin-based combination therapy   (ACT) in the treatment of uncomplicated *falciparum* malaria. *Malar J* 8:211

18. Ilesanmi, R.I., Olalubi, O.A., Ilesanmi, A.O., Effedua, H., Amoo, A.O., (2017). Comparative assessment of some rapid diagnostic test (RDT) kits for malaria diagnosis in Ibadan, Nigeria. *MWJ* 8:17

19. Kalu, K.M., Obasi, N.A., Nduka, F.O., Otuchristian, G. (2012). A comparative study of the prevalence of malaria in Aba and Umuahia urban areas in Abia State, Nigeria. *Res J Parasitol* 7:17–24

20. Kennedy, T. and Otokunefor, K (2017). "Diagnostic efficiency of rapid diagnosis test kits in malaria diagnosis- the Nigerian story," Gazette of Medicine, vol. 5, no. 2, pp. 1–15.

21. Mohammed, K., Salifu, M.G., Batung, E. et al. (2022) "Spatial analysis of climatic factors and plasmodium falciparum malaria prevalence among children in Ghana," Spatial and Spatio-temporal Epidemiology, vol. 43, p. 100537.

22. Landier, J., Parker, D. M., Thu, A.M., Carrara, V.I,, Lwin, K. M, Bonnington CA, et al. (2016). The role of early detection and treatment in malaria elimination. *Malar J*. 15:363.

23. Moody, A.(2002) Rapid diagnostic tests for malaria parasites Clinical Microbiology Revolution 15:66-78.

24. Mouatcho J. C, Goldring J. D (2013). Malaria rapid diagnostic tests: challenges and prospects. *J Med Microbiol* 62(10):1491–1505

25. Mukry, S.N., Saud, M., Sufaida, G., Shaikh, K., Naz, A., Shamsi,T.S. (2017). "Laboratory diagnosis of malaria: comparison of manual and automated diagnostic tests," *Canadian Journal of Infectious Diseases & Medical Microbiology*, vol. pp. 1–7.

26. Msellem, M.I., Mårtensson, A., Rotllant, G., Bhattarai, A., Strömberg, J., Kahigwa, E., et al (2009). Influence of rapid malaria diagnostic tests on treatment and health outcome in fever patients, Zanzibar — a crossover validation study. PLoS Med;6(4).

27. Oboh, A.M., Orieroa, E.C., Ndiayeb, T., Badianeb, A.S., Ndiayeb, D., Amambua-Ngwa, A. (2021). Comparative analysis of four malaria diagnostic tools and implications for malaria treatment in Southwestern Nigeria *International Journal of Infectious Diseases* 108; 377–381

28. Odeniyi, O . M. , Agomo C. O. , Okangba, C. C., Olaniyan, K. , Oyibo, W. A. Comparison of Performance Characteristics of Two Malaria Rapid Diagnostic Tests in Detecting malaria infection among febrile patients in urban Area of Lagos, Nigeria *Ife Journal of Science* vol. 22, no. 2 (2020)

29. Okangba, C. C, Oyibo W.A., Thompson, E. T., Ejime E., Ajani, T.A., Elikwu, C.J., Okangba, K. K (2020). The Relevance of Positive Control Wells in Monitoring the Performance of Malaria Rapid Diagnostic Test in the Field for Effective Case Management of Malaria *International Journal of Research and Scientific Innovation.| Volume VII, Issue X, | ISSN 2321–2705

30. Okangba, C. C., Funwei, R. I., Elikwu, C. J., Nwadike, V. U., Okangba, K. K., Osinowo, A. O., Tayo, B., Taiwo, A. A. and Ogunsola, A. O. (2022). Comparative Evaluation of BinaxNOW™ Malaria Rapid Diagnostic Test, Microscopy and Polymerase Chain Reaction for Plasmodium falciparum Detection among Individuals Suspected with Malaria in Lagos, Southwest Nigeria. Nigerian Journal of Parasitology 43(2) 324-334

31. Okonko, I.O., Soleye, F.A., Amusan, T.A., Ogun, A.A., Udeze, A.O., Nkang, A.O., Ejembi, J., Faleye, T.O.C (2009). Prevalence of malaria plasmodium in abeokuta Nigeria. Malays J Microbiol 5(2):113–118

32. Oyeniran OA, Ajayi OO, Afolabi AY, Oladipo EK, Adepeju AA (2014) Comparison of rapid

diagnostic tests and microscopy for malaria. Afr J Clin Exper Microbiol 15(3):158–174. https://doi.org/10.4314/ajcam.v15i3.7

33. Oyeyemi, O.T., Ogunlade, A.F., Oyewole, A. (2015). "Comparative assement of microscopy and rapid diagnostic test (RDT) as malaria diagnostic tools," Research Journal of Parasitology, vol. 10, no. 3, pp. 120–126.

34. Prah, J.K., Amoah, S., Yartey, A.N., Ampofo-Asiama, A and Ameyaw, A.E. (2021) "Assessment of malaria diagnostic methods and treatments at a Ghanaian health facility," Pan African Medical Journal, vol. 39, no. 1, p. 251.

35. Rafael, A.M.E., Taylor, T., Magill, A., Lim, Y., Girosi, F., Allan, R. (2006). Reducing the burden of childhood malaria in Africa : the role of improved diagnostics. *Nature*. 444(Suppl 1):39–48.

36. Sani, U.M., Jiya, N.M., and Ahmed, H. (2013). Evaluation of malaria rapid diagnostic test among febrile children in Sokoto, Nigeria. *Int. J. Medi Sci., ISSN* 2167, 3(5), 434-440.

37. Stauffer, W.M., Cartwright, C.P., Olsonetal, D.A. (2009) "Diagnostic performance of rapid diagnostic tests versus blood smears for malaria in US clinical practice," Clinical Infectious Diseases, vol. 49, no. 6, pp. 908–913.

38. Ugah, U.I., Alo, M.N., Owolabi, J.O et al (2017)."Evaluation of the utility value of three diagnostic methods in the detection of malaria parasites in endemic area," Malaria Journal, vol. 16, no. 1, pp.1–8

39. WHO (2019). The use of malaria rapid diagnostic tests. Second edition. WHO Library cataloguing in Publication Data Geneva.

40. WHO (2020). Guideline for the treatment of malaria, second edition, (http:/whqlibdoc.who.int/publications/2020/9789241547925.eng.pdf)

41. WHO (2011) Universal access to malaria diagnostic testing, An operational manual. https://www.who.int/malaria/publications/ atoz/9789241502092/en/. Accessed 12 Sept, 2018

42. WHO (2015) Guidelines for the treatment of malaria. 3rd edition. https://www.who.int/malaria/publications/atoz/9789241549127/ en/. Accessed 12 Sept, 2018