



Comparative Evaluation of Malaria Rapid Diagnostic Test Kits Commercially Available in Parts of South Eastern Nigeria

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Abstract

In 2010 world Health Organization (WHO) introduced Test Treat Track initiative for the management of malaria using antigen detecting rapid diagnostic test kits (RDTs) as a parasite based diagnosis to address the need for immediate diagnosis of malaria especially in remote limited areas. A diagnostic study was conducted in Imo state, Nigeria to evaluate the performance of four different RDTs in symptomatic patients. Patients were screened for malaria using blood samples collected from the at selected health from July 2013 to December 2013 facilities. Patients were tested with two Histidine rich protein RDTs and a two combination of Histidine rich protein 2 (HRP 2) antigens and Parasite lactose dehydrogenase enzyme (pLDH) RDTs. Microscopy was used as gold standard. Out of 100 participants enrolled and screened for malaria, 70 (70%) tested positive by Microscopy, 72 (72%), 72 (72%) tested positive by two of the HRP 2 RDTs respectively while 67 (67%), 67 (67%) tested positive with the HRP 2/pLDH RDTs HRP-2 RDTs are more sensitive than HRP 2/pLDH RDTs and could be a suitable alternative to microscopy to screen endemic malaria in rural Nigeria.

Keywords: Malaria; Test treat track initiative; Microscopy; RDT; Histidine rich protein; Sensitivity; Parasite lactose; Dehydrogenase enzyme

Introduction

Malaria remains a major public health problem to tropical and sub tropical regions of the world [1] In 2010, the World Health Organization (WHO) recommended parasitologic confirmation of suspected malaria cases before initiating anti-malarial treatment [2] and many malaria endemic countries have adopted this policy [3,4]. The scale-up of antigen-detecting malaria rapid diagnostic tests (RDTs) for *Plasmodium* species forms a vital part of the strategy to confirm malaria infection prior to treatment in resource-poor settings [5]. The immunochromatography antigen based malaria RDTs detect histidine rich protein -2 (HRP-2) antigen or Parasite lactosedehydrogenase (pLDH) enzyme. The HRP-2 antigen is specific for *P. falciparum* and Pan-pLDH detects all human infecting species [6,7]. Malaria confirmation can be achieved with RDTs in resource poor endemic settings where microscopy is not readily available. However, there are growing concerns about the accuracy of malaria RDTs results and their usefulness in providing informed decisions on malaria case management.

Previous studies have reported the performance of HRP 2 and pLDH based RDTs for detecting *Plasmodium falciparum* when compared to microscopy as the reference standard [8]. A systematic review of 48 studies describing malaria diagnostic performance indicated that although performance varied by species, parasite density and immunity, overall HRP2-detecting RDTs outperformed pLDH-based RDTs with high sensitivity and low specificity for diagnosing malaria in clinical cases in endemic areas [9]. However, HRP2-detecting RDTs are unsuitable for monitoring parasite clearance following anti-malarial treatment due to the persistence of the PfHRP2 antigen in the blood for up to four or five weeks following curative treatment of an infection [8-10].

The issue of persistent antigenaemia in endemic areas has been raised as a factor leading to reduced specificity of HRP2-detecting RDTs for diagnosing acute malaria and over-estimates of malaria prevalence in community surveys [11,12]. Studies have reported significant variations in RDT sensitivity and specificity [13-16] and particularly when RDTs are exposed to adverse conditions, such as higher temperature [17].

In Nigeria as in other endemic countries, the use of RDTs is strongly advocated. Following from this, several RDT kits have also flooded the markets. In an area where data and evidence to aid informed decision is lacking, it therefore becomes difficult to make recommendations on which types of RDTs to use for accurate detection and effective management of malaria.

This study aimed to comparatively evaluate 4 RDTs commercially available in South Eastern Nigeria using expert microscopy as standard.

Materials and Methods

Study setting

The study was conducted from July to December, 2013 in Aboh Mbaise, South Eastern Nigeria where malaria transmission is moderately high and seasonal. Prevalence of malaria in the general population (all age groups) in the rainy season (April-October) is 6.8 % with a predominance of *P. falciparum* followed by a cool, dry season from November to March [18].

A random sample of 100 participants were selected from 4 health facilities in Aboh Mbaise which include Aboh Mbaise general hospital, Umunneato Health Centre, Paulina memorial hospital and Ugochukwu clinic.

Participant enrolment and sample collection

A questionnaire consisting of socio-demographic information, age, sex, occupation, history of fever, drug use and axillary temperature were administered to the study participants and the caregivers of

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younger participants who could not answer for themselves. A finger-prick blood specimen was obtained from each study subject. The blood sample was collected in a transfer device (capillary tube) and placed in the appropriate well on the RDT where it was absorbed by the nitrocellulose paper. From the same finger prick an additional 2-3 drops of blood for a thick blood smear and 2-4 drops of blood for a thin blood smear were collected. Finger prick blood sample was taken to simultaneously test the four RDTs. Patients testing positive for malaria with any RDT were appropriately treated.

Microscopy

Thick and thin smears were made on clean grease free slide and stained with 5% Giemsa. The stained slides were taken to the Federal Medical Centre (FMC), Owerri, South Eastern Nigeria where Parasitological diagnosis was made independently by two microscopists, with discrepancies resolved by a third reader. A slide was declared negative if parasites were absent after examining 200 high power fields. Parasite density was quantified against 200 leukocytes on an assumed leukocyte count of 8000 per μL of blood.

Rapid diagnostic tests

The four RDTs included SD Bioline Malaria Ag Pf (Standard Diagnostics, Kyonggi, Korea), First Response Malaria Ag Pf[®] (Premier Medical Corporation Ltd, India), CareStart Malaria pLDH/HRP2 combo™ (AccessBio Inc., NJ, USA), AconMalaria pLDH/HRP2. The first two RDTs are histidine rich proteins, single and sensitive to only *Plasmodium falciparum* (Pf) species while the latter two are histidine rich proteins and Parasite lactose dehydrogenase enzyme (pLDH) combined RDTs that are sensitive to Pf and non falciparum species. The tests were performed and interpreted according to the RDTs manufacturer's instructions. Accordingly, two drops of buffer were used for SD Bioline[®] and First Response Malaria Ag Pf[®] and read within 15 min; for the other two RDTs, four drops of buffer were used and was read within 20 min. Malaria positivity was defined if any of the HRP-2 or pLDH bands were visible (plus the control band). If only HRP-2 band plus control band was visible it was considered as a *P. falciparum* infection and when both bands were positive simultaneously it was considered a *P. falciparum* infection or a mixed infection.

Quality control measures and RDT transport and storage

Room temperature and humidity in the RDT storage rooms in the health facilities were monitored and recorded using Tinytag™ Data Loggers with alarms set at 40°C. The recorded data was saved into the main database and verified fortnightly. The maximum humidity recorded was 80.1 rh % and temperature ranged between 25.1 and 27.5°C. Excessive exposure to direct sunlight was avoided during RDT transportation. Individual kits were opened only at the time of testing and were checked for the presence of the desiccant. A single batch of each RDT with same LOT number was purchased from the local market.

Statistical analysis

Data were double entered using SPSS and Epi version 6.04. The results of the four RDTs were compared against expert microscopy as the reference test. Qualitative data were represented in tables, bar charts and 2x2 contingency which was used to compare variable. Mean difference between the four kits and Microscopy was analysed using t-test.

Ethical clearance

The study was approved by the Federal University of technology

Owerri (FUTO) Nigeria Research and Ethics Review Committee. Verbal agreements to use patients' samples for this analysis were acquired at the district health management level and permission was granted to conduct this study in the selected health facilities. Adequate verbal information was provided for the participants, letting them know about the need and essence of their blood samples, and also the nature of the research. Informed oral consent was also obtained from the study participants.

Results

A total of 100 study participants were enrolled from July to December, 2013. About 61 (61%) of them were males while 39 (39%) were females. A greater percentage 37 (37%) belonged to the age group 0-11 years (Table 1).

Seventy (70%) of the subjects tested malaria positive for stained film microscopy while the RDT kits- SD-Bioline, First Response, CareStart Pf/pan and Acon Pf/pan showed positive results of 72 (72%), 72 (72%), 67 (67%) and 67 (67%) respectively. The Performance characteristics of the RDT kits, SD Bioline, First Response, CareStart Pf/pan and Acon Pf/pan kits were sensitivity- 98.6%, 98.6%, 92.9% and 92.9%; specificity- 90.0%, 90.0%, 93.3% and 93.3%; test accuracy 97%, 97%, 92% and 92%; positive predictive value- 95.8%, 95.8% , 97.0% and 97.0%; Negative predictive value- 96.4%, 96.4%, 84.4% and 84.4% (Table 2).

Table 3 depicts the distribution of patients screened for Malaria using HRP-2 test kits. In this study, microscopy was used as the 'Gold Standard'. A total of 100 people were screened among which 70% tested positive. These 100 people included 61 males and 39 females of whom 51(83.6) and 19 (48.7%) respectively were positive. The study participants were categorized among the following age groups, 0-11 years (37), 12-25 years (11), 26-49 years (29) and ≥ 50 years (23). The results of the age groups respectively showed the following positive results: 30 (81.1%), 5 (45.5%), 21 (72.4%), 14 (60.9%).

Seventy two (72) out of the 100 participants screened who represents 72% tested positive in the diagnosis with SD Bioline Pf test kit. About

Characteristics (n=100)	Frequency	Percentage (%)
Sex		
Male	61	61
Female	39	39
Age		
0-11	37	37
12-25	11	11
26-49	29	29
≥ 50	23	23
Occupation		
Students	31	31
Housewife	14	14
Farming	20	20
Trading	11	11
Self-employment	16	16
Civil Servants	8	8
Marital Status		
Married	71	71
Single	26	26
Widowed	3	3

Table 1: Socio-demographic characteristics of study subjects.

RDTs	Sensitivity (%)	Specificity (%)	Test Accuracy (%)	Positive Predictive Value (%)	Negative Predictive Value (%)	False Positive Rate (%)	False Negative Rate (%)
SD BIOLINE	98.6	90	96	95.8	96.4	3	1
First Response	98.6	90	96	95.8	96.4	3	1
Care Start Pf/Pan	92.9	93.3	93	97	84.4	2	5

Table 2: Performance of the rapid diagnostic tests relative to those of microscopy.

Criteria	Total Tested	No. Positive	%Positive
Microscopy			
Total	100	70	70
Male	61	51	83.6
Female	39	19	48.7
0-11	37	30	81.5
12to25	11	5	45.5
26-49	29	21	72.4
≥ 50	23	14	60.9
SD Bioline			
Total	100	72	72
Male	61	49	80.3
Female	39	23	60
0-11	37	30	81.1
12-25	11	7	63.6
26-49	29	20	69
≥50	23	15	65.2
First Response			
Total	100	72	72
Male	61	49	80.3
Female	39	23	60
0-11	37	30	81.1
12to25	11	7	63.6
26-49	29	20	69
≥ 50	23	15	65.2

Table 3: Distribution of study participants screened for malaria with hrp-2 test kits.

49 (80.3%) and 23 (60.0%) of males and females tested positive from total 61 and 31 respectively. The infection rates among four age groups as shown by this diagnostic tool as included in the table shows that the highest infected age group is 0-11 years 30 (81.1%), followed by 26-49 years which represent 20 (69%), while the 12-25years 7 (63.6%) had the least infected age group. The results recorded for First Response Pf was the same as that of SD Bioline Pf.

The distribution of participants screened for malaria with HRP-2/pLDH RDT kits is depicted in Table 4. The result by the test devices showed that out of 100 participants screened, 67 (67%) tested positive for malaria parasite. 46 (75.4%) out of 61 males and 21 (53.8%) out of 39 females were positive. Age group distribution shows that the highest infected age group was 0-11years 28 (75.7%) and the least infected was 26-49 representing 20(69%). The results of AconPf/pLDH was also the same with CareStartPf/pLDH

Table 5 shows the comparison of the four RDTs with results of microscopy. The data were represented in a 2x2 contingency table. For SD BiolinePfRDT, results were positive in 72 (72%) cases and negative in 28 (28%) out of 100 patients. Among the 70 positive cases diagnosed by expert microscopy, SD BiolinePfRDTs result was true positive (by both microscopy and PfHRP-2 RDTs) in 69 cases. SD Bioline failed to detect 1 cases positive by microscopy yielding one false negative results,

Criteria	Total Tested	No. Positive	%Positive
Microscopy			
Total	100	70	70
Male	61	51	83.6
Female	39	19	48.7
0-11	37	30	81.5
12-25	11	5	45.5
26-49	29	21	72.4
≥50	23	14	60.9
CareStart Pf/pLDH			
Total	100	67	67
Male	61	46	75.4
Female	39	21	53.8
0-11	37	28	75.7
12-25	11	5	45.5
26-49	29	20	69
≥50	23	14	60.9
Acon Pf/pLDH			
Total	100	67	67
Male	61	46	75.4
Female	39	21	53.8
0-11	37	28	75.7
12-25	11	5	45.5
26-49	29	20	69
≥50	23	14	60.9

Table 4: Distribution of study participants screened for malaria with hrp-2/pldh test kits.

also among the 30 negative cases detected by expert microscopy, 3 cases were SD BiolinePf positive result, yielding 3 false positive results and 27cases which were both SD Bioline and microscopy negative.

For First Response RDT, results were positive in 72 (72%) cases and negative in 28 (28%) out of 100 patients. Among the 70 positive cases diagnosed by expert microscopy, First Response RDTs result was true positive (by both microscopy and Pf HRP-2 RDTs) in 69 cases. First Response failed to detect 1 case positive by microscopy yielding one false negative results, also among the 30 negative cases detected by expert microscopy, 3 cases were First Response positive result, yielding 3 false positive results and 27cases which were both First Response and

	Microscopy			P.value
	Positive Frequency (%)	Negative Frequency (%)	Total Frequency (%)	
RDTs				
SD Bioline Pf				
Positive	69 (98.6%)	3(10)	72 (72)	
Negative	1 (1.4%)	27 (90)	28 (28)	
	70 (100)	30 (100)	100 (100)	0
First Response				
Positive	69 (98.6%)	3(10)	72 (72)	
Negative	1 (1.4%)	27 (90)	28 (28)	
	70 (100)	30 (100)	100 (100)	0
Carestart Pf/pLDH				
Positive	65 (92.9)	2(2)	67 (67)	
Negative	5 (7.1)	28 (28)	33 (33)	
	70 (100)	30 (100)	100 (100)	0
Acon Pf/pLDH				
Positive	65 (92.9)	2(2)	67 (67)	
Negative	5 (7.1)	28 (28)	33 (33)	
	70 (100)	30 (100)	100 (100)	0

Table 5: Comparison of the four rdts with microscopy.

microscopy negative.

For Care Start *Pf*/Pan RDT test kit using 2x2 contingency table, *Plasmodium falciparum* (line 1) is in agreement (true positive) in 65 cases and disagreement (False negative) in 5 cases out of 70 positive diagnoses by expert microscopy. Out of 30 expert microscopy negative cases, 28 were true negative(negative for both Care Start *Pf*/Pan RDT and expert microscopy) and 2 cases was positive by Care Start *Pf*/Pan RDT but negative by expert microscopy thus making it false positive.

For Acon*Pf*/Pan RDT test kit using 2x2 contingency table, *Plasmodium falciparum* (line 1) is in agreement (true positive) in 65 cases and disagreement (False negative) in 5 cases out of 70 positive diagnoses by expert microscopy. Out of 30 expert microscopy negative cases, 28 were true negative(negative for both Acon*Pf*/Pan RDT and expert microscopy) and 2 cases was positive by Acon*Pf*/Pan RDT but negative by expert microscopy thus making it false positive.

Table 6 indicates the variation of sensitivity of the Four RDTs Kits by Parasite density with Patients tested with concordant results. The *Pf* density was divided into six ranges as follows: ≤ 100 parasites/μl, 101-500 parasites/μl, 501-1000 parasites/μl, 1001-5000 parasites/μl, 5001-10000 parasites/μl and ≥10000 parasites/μl. The results of the *Pf* density groups respectively showed the following positive results for SD Bioline, First Response, CareStart*Pf*/Pan and Acon*Pf*/Pan and Microscopy 0(0), 0(0), 0(0), 0(0), and 0(0), at the second range 7 (10.1%), 7 (10.1%), 7 (10.8%) 7 (10.8%) and 7 (7%), at the third range 23 (33.3%), 23 (33.3%), 21 (32.3%) 21 (32.3%) and 23 (23%) at the fourth range 19 (27.5%), 19 (27.5%), 19 (29.2%) 19 (29.2%) and 19 (27.1%) at the fifth range 8(11.6%), 8 (11.6%), 7 (10.7%), 7 (10.7%) and 8(11.4%) and lastly 12 (17.4%), 12 (17.4%), 10 (15.4), 10 (15.4) and 11 (16.9%). The 501-1000 parasites/μl of SD Bioline showed the highest rate of 33.3%.

Discussion

Parasite-based routine malaria diagnosis is focused on detection of asexual parasite stage in the stained blood smears using microscopy or detection of parasite antigen using RDTs. The present study has compared the performance of four RDTs using two histidine rich protein and a combination of histidine rich protein and parasite lactose dehydrogenase enzyme (pLDH) RDTs. The Study results revealed that the four RDTs showed an appreciable effectiveness though the HRP-2 RDTs showed a higher ability to detect *P. falciparum* in malaria endemic areas.

Peripheral blood film microscopy and Rapid diagnostic test showed *Plasmodium falciparum* was the only species found in positive slides. The sensitivity of HRP-2/pLDH is consistent with similar studies in Madagascar [19]. The Underlying reason is that *Pf* based RDTs which detect HRP-2 is a very stable antigen [20] and has a superior heat stability than pLDH which degrades easily [21] although such was not demonstrated in the present study. The performance of the SD Bioline HRP-2 and First Response malaria RDT observed in the present study was agrees with previous studies with other HRP-2 based commercial RDTs such as Paracheck*Pf*TM (Orchid Biomedical system) [22] Binax Now MalariaTM (Binax, Inc, Inverness Medical Professional Diagnostic, USA) [19]. The sensitivity of pLDH RDTs was consistent with previous studies done with *Pf*/Pan based commercial RDTs such as SD Bioline*Pf*/Pan (Standard Diagnostics, Inc, Korea [23].

The sensitivity of HRP-2 RDTs meets the WHO recommendation of ≥ 95% and is consistent with previous reports with using *Pf* only RDTs in eastern Tanzania [14] and in Uganda [24]. However, the results of HRP-2 kits are at variance with other studies which showed lower sensitivities. Studies conducted in Enugu Nigeria [25] and in Ethiopia

Density of Pf	SD Bioline (%)	First Response	CareStart Pf/Pan	Acon Pf/Pan	Microscopy	Sensitivity			
						SD Bioline	First Response	CareStart	Acon
≤ 100	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	Not Applicable			
101-500	7 (10.1)	7 (10.1)	7(10.8)	7(10.8)	7(7)	98.6	98.6	92.2	92.2
501-1000	23 (33.3)	23 (33.3)	21 (32.3)	21 (32.3)	23 (23)	98.6	98.6	92.2	92.2
1001-5000	19 (27.5)	19 (27.5)	19 (29.2)	19 (29.2)	19 (27.1)	98.6	98.6	92.2	92.2
5001-10000	8 (11.6)	8 (11.6)	7 (10.7)	7 (10.7)	8 (11.4)	98.6	98.6	92.2	92.2
>10000	12 (17.4)	12 (17.4)	11 (16.9)	11 (16.9)	13 (18.6)	98.6	98.6	92.2	92.2
	69(100)	69(100)	65(100)	65(100)	70(100)	98.60%	98.60%	92.20%	92.20%

Table 6: Variation of sensitivity of four rapid diagnostic test kits by parasite density (number of patients tested with concordant results).

found a sensitivity of 69.7% for Global device RDT [26] and 42.3% for a Pf RDT [27] and 47.5% for Panscreen, an HRP-2 and pLDH-2 based- RDT respectively [28]. These have been ascribed to decreasing sensitivity with reduction in parasite density and thus patients with low density malaria parasites are missed, these could also be caused by failure of the parasite to express the antigen due to deletion of the gene Pf HRP-2 [29,30]. The sensitivity of HRP-2/pLDH RDTs was low compared to that of HRP-2. These could be explained based on several factors such as decreased pLDH activity with antimalarial therapy that may have resulted in remnant non-viable and non pLDH producing strains [31], however parasites may be seen on blood smears [13]. Host metabolic and immune factors could also reduce target antigens or interfere with their binding to detecting antibodies [32]. Overall Specificity of HRP-2 RDTs, was lower than that of teh HRP-2/pLDH RDTs. This is also in consonance with previous [33,34]. The reason behind the low specificity of HRP-2 RDTs may be due to a high false positives of *Plasmodium falciparum* in the suspected cases. This might be due to any previous history of infection with *Plasmodium falciparum*.

Variation in parasite density had no effect on the sensitivity of the RDTs as this was constant. This is contrary to findings in non-immune visitors to Asia, sub-saharan Africa and south America, where 11.7% (48 of the 409 positive thick smear) had parasite density of <100/μl [32]. A plausible explanation for this could be the fact that the parasite density was consistently above 100 parasites/μl, which is a possibility in a typical endemic setting.

Negative Predictive value was higher for HRP-2 RDTs than HRP-2/pLDH. Similarly, high NPV for HRP-2 RDT test has been reported in a study in Bangladesh [35]. The NPV was reported low for HRP/pLDH in another study conducted in 2003 [36], but in another study conducted in 2007, it was significantly higher [26]. In the case of HRP-2 test the high NPV thus permitted us to assertively diagnose negative test patients as non-malarial patients [37]. Hence, the possibility of missing a positive case is less by HRP-2 than HRP/pLDH RDTs.

The RDTs however detected malaria in some patients, who were tested negative by microscopy, these were denoted as false positive. False positives have been reported in other investigations [36,38], but they appear to play a minor role in the usefulness of malaria test for clinical settings. False positive reactions may occur in individuals who have been recently treated for malaria as reported by [39,40], other causes of false positive results are persistent viable asexual stage parasitaemia, below the detection limit of microscopy and persistence of antigens due to sequestration. This sequestration of malaria parasite at the time of blood collection is a factor that is quite interesting. There is evidence of parasitaemia clinically, the RDT kits also test positive

whereas microscopy couldn't detect the parasite at any stage [41]. It was further discovered by thorough microscopic examination that malaria pigment were seen in the peripheral blood leucocytes of these patients. This finding added weight to the usefulness of the RDTs, its advantages over microscope when the parasites are sequestered in the cerebral and placental malaria [41].

The detection of the two RDTs as negative with HRP-2 and HRP-2/pLDH with the later having a higher negative value whereas the corresponding microscopy was positive was encountered. This test, that is false negatives have also been encountered in other studies as [42-44]. This could be as a result of level of parasitaemia, deletion of HRP-2 genes and the activity of the immune system of individual, which combats the antigens of the parasite in the peripheral blood, this makes the antigens to be absent, while present and seen in a stained film at microscopic examination. Although, there was a slight difference in sensitivity and specificity of HRP-2 and HRP-2/pLDH RDTs, there was no significant mean difference paired for both and the microscopy standard which means there performances are relatively the same. Therefore any of the four brands would probably be suitable as a potential alternative to field microscopy or for use in the clinics where microscopy is not available in malaria endemic areas.

Conclusion

The diagnostic accuracy to detect malaria among febrile patients was slightly better with HRP-2 RDTs but overall differences between the four RDTs was not very significant and performance was comparable to field microscopy.

This study has found HRP-2 RDT to be a reliable diagnostic tool in malaria endemic areas. Heat Stability remains a major concern for the pLDH tests. Stability testing should form part of quality assurance and quality control of malaria diagnostic tests.

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