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Original Article

Evaluation of three rapigen biocredit point-of-care tests for malaria case management in Lagos, Nigeria

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Abstract

Introduction: Histidine-rich protein 2 (HRP2) antigen kits are widely used for malaria diagnosis in tropical regions due to their heat stability. The *Plasmodium* Lactate Dehydrogenase (pLDH) used in BIOCREDIT® malaria test kit is claimed to be heat-stable. This study aimed to evaluate the heat stability and field performance of three BIOCREDIT® kits.

Methodology: A cross-sectional community survey of malaria infection was carried out in Agbowa and Ikosi communities in Lagos State. Finger-prick blood was used to assess the performance of three BIOCREDIT® kits: Kit 1 (PfpLDH), Kit 2 (PfLDH/HRP2), and Kit 3 (PfLDH/PvLDH). Malaria microscopy and a reference mRDT (SD BIOLINE® *Pf*HRP2) were used as comparators. Heat stability testing was performed using *Plasmodium falciparum* panels after exposure to 35ºC and 45ºC for 60 days. A total of 834 participants were recruited.

Results: The sensitivity of BIOCREDIT® kits were: Kit 1 (PfLDH) 88.4%; Kit 2 PfHRP2 92% and PfLDH 86.6%; Kit 3 (PfLDH) 88.4%. The specificity of kits was: Kit 1 (Pf LDH) 91.7%; Kit 2 (PfHRP2) 82.3%) and (PfLDH) 92.1%; Kit 3 (PfLDH) 91.6%. The sensitivity and specificity of the reference kit were 92.9% and 85.9%. The three BIOCREDIT® Kits were stable. No potential case of *pfhrp2* gene deletion was observed.

Conclusions: The sensitivities of the three BIOCREDIT® malaria test kits were similar to the reference kit (SD BIOLINE mRDT). However, the specificities of PfLDH kits were significantly higher than the specificities of PfHRP2 kits. The BIOCREDIT® kits are suitable to be deployed in malaria-endemic tropical regions.

Key words: Cross-sectional studies; humans; microscopy; *Plasmodium falciparum*; histidine-rich protein; malaria.

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Introduction

Malaria remains a public health problem in Sub-Saharan Africa. It is estimated that there were 241 million malaria cases and 627,000 deaths worldwide in 2020, which represents around 14 million more cases and 69,000 more deaths than in 2019 [1]. Diagnosis is very important in case management of malaria. The two most important methods of diagnosing malaria are microscopy and the malaria rapid diagnosis tests (mRDT).

A major concern with the use of mRDT is the occurrence of false negative and positive results leading to misdiagnosis and eventual failure of patients to receive appropriate treatment for their health conditions [2–4]. One important factor contributing to false negative RDT test results is *pfhrp2* gene deletion which has been widely reported in many endemic countries [5–7]. However, a very low prevalence of *pfhrp2* and *pfhrp3* gene deletions in *P. falciparum* parasites has been reported in Nigeria [8]. Other factors that may lead to misdiagnosis are the RDT kit specificities, sensitivities, temperature tolerances, quality, and level of parasitemia [9–15]. PfHRP2-based mRDTs are recommended for routine diagnosis of malaria to guide the treatment of malaria patients in health facilities in Nigeria [16].

Histidine-rich protein 2 is a widely used target antigen for malaria diagnosis in tropical regions due to its heat stability of about 40 °C. However, this antigen can linger in the peripheral blood for up to 6 weeks, thereby giving false positive malaria test result [9,17,18]. Histidine-rich protein-2 (HRP2)-based test kits are specific for *Plasmodium falciparum* and

therefore cannot detect other malaria parasite species such as *P. malariae* and *P. ovale* present in our locality.

There are other mRDTs that are not species-specific (pan), which target enzymes produced by all plasmodia species such as Plasmodium lactate dehydrogenase (pLDH) and Aldolase [14,19]. pLDH are pan -specific enzymes that can detect all *Plasmodium falciparum* species. The isomers of pLDH that are specific for some *Plasmodium* species including, *P. falciparum* (PfLDH) and *P. vivax* (PvLDH) have been produced [20,21]. Isomers of pLDH specific to *P. malariae* (PmLDH) and *P. ovale* (PoLDH) are not commercially available for malaria test kit production. Generally, enzymes are denatured by heat, therefore pLDH and Aldolase-based kits are not recommended for use in tropical countries where ambient temperature can exceed 40 °C. Thus, heat stability is a requirement for malaria RDTs in areas where the room temperature exceeds 30 °C [22]. An ideal RDT should be able to tolerate temperatures of at least 40 °C with peaks of 50 °C [23]. RDTs may be exposed to high temperatures during transportation and storage. In Nigeria, room temperatures usually exceed 30 °C especially in the northern parts of the country. This underscores the need for heat stability testing of RDTs for use in Nigeria. In this study the heat stability and field performance of the RapiGEN BIOCREDIT Malaria Ag Pf (pLDH) and the RapiGEN BIOCREDIT Malaria Ag Pf (pLDH/HRPII) RDTs for detection of *P. falciparum* infections in comparison with reference RDT and Microscopy.

Methodology

Study Design and Sites

This was a cross-sectional community-based study, where participants were recruited through active case detection.

Agbowa-Ikosi community (6°39′ N, 3°43′ E) in Ikosi-Ejirin Local Council Development Area of Ikorodu Local Government Area in Lagos State. Agbowa-Ikosi is about 35 km from Lagos metropolis on the south bank of a creek that extends parallel to the sea from Lagos to Ikorodu with a mixture of indigenes and non-indigenes It has a population of about 107,283 people, their major occupations are farming, fishing, and pretty trading [24]. There are three public health facilities, one secondary (General Hospital Agbowa) and two primary health facilities (Agbowa-Ikosi Health Post (AIHP) and Agbowa Primary Healthcare Centre (APHC)). Malaria transmission is relatively high in these semi-urban areas compared to urban centres in Lagos. The selected health facilities have more than 500 outpatients per week. Participants were recruited at the peak of malaria transmission (August-October, 2022).

Before the commencement of the study, the investigators visited the site AIHP and APHC and explained the study to the Medical Officer with ethical approval and permission from the Lagos State Ministry of Health (LSMoH). Permission was granted to conduct the study and informed consent was obtained from each participant who participated in the study.

Study Duration

The study was conducted from August 2022 - February 2023, with sample collection as well as field and lab testing of investigational products. This also includes data analysis, report writing, and dissemination.

Investigational products

The three BIOCREDIT Malaria Antigen test kits evaluated in this study were produced by RapiGEN, INC. located at Dongan-gu, Anyang-si, Gyeonggi-do, Republic of Korea. The reference malaria rapid diagnostic test kit -SD BIOLINE Malaria Antigen *P.f* was manufactured by Standard Diagnostics Inc. in Boranhagai-ro, Giheung-gu, Yongin-si, Gyeonggi-do, Republic of Korea.

Ethical Approval

This study protocol was approved by the Institutional Review Board of the Nigerian Institute of Medical Research, Lagos (NIMR-IRB/22/030).

A total of 834 study participants were recruited for this study based on the reported hospital prevalence of 20.7% (Oladosu and Oyibo, 2013), The estimated sample size was 701 and a 10% non-response rate was added. The total number of participants recruited in this study meets the WHO recommended sample size of greater than 200 malaria-positive samples for field evaluation of malaria RDTs (World Health Organization, 2009).

Screening for malaria parasites in the community was carried out on all the recruited persons who gave consent to participate in the study regardless of the fever status. However, severely ill persons were referred to the clinics. The three kits that were assessed were BIOCREDIT Malaria Ag Pf (pfLDH), BIOCREDIT Malaria Ag Pf (pfLDH/HRPII) RDT, and BIOCREDIT Malaria Ag Pf/Pv (pfLDH/pvLDH). The reference test kit was SD BIOLINE Malaria Ag P.f HRP2 and the gold standard was microscopy in line with WHO guidelines of comparing new malaria diagnostic tools

to current gold standard for malaria diagnosis which is microscopy [25].

Laboratory procedures

Finger-prick blood samples were used for malaria diagnosis by RDT and Microscopy as the ideal blood sample because the density of developed trophozoites or schizonts is greater in blood taken from this capillary-rich area [26]. Biocredit and reference RDTs were performed by taking 5µL of the blood sample into the test well using an inverted cup transfer device for the Biocredit test kits 3 drops of buffer were added to the buffer well and timed for 25 minutes following the manufacturer's instruction. The reference RDT was timed for 15 minutes after adding 4 drops of buffer into the buffer well following the manufacturer's instruction. For the microscopy, thick and thin blood smears were made on frosted slides, and the thin smears were fixed with methanol, the blood films were stained with 3% Giemsa stain for 45 minutes in accordance with WHO stipulated malaria microscopy standards (https://www.who.int/publications/i/item/9241547820) . Each of the stained slides was read by two expert microscopists. Discordant readings were resolved before the final result was accepted.

All malaria-positive participants were treated following the standard of care for the treatment of uncomplicated malaria in Nigeria [16].

Malaria Parasite Panel Production

Malaria parasite panels of *Plasmodium falciparum* and non-*P. falciparum* (*P. ovale* and *P. berghei)* panels were prepared from malaria-positive samples. The panels had the following parasite densities: 200 parasites/µL of blood and 2000 parasites/ µL of blood which represent the low and high parasite densities that an optimal malaria RDT is expected to detect [27]. The panels were stored at -85 ºC for short-term and long-term duration in cryovials. Each cryovial contained 100 µL of parasitized blood.

Heat stability testing

Baseline testing of samples of the three products was conducted and documented before the products were stored at 35 ºC and 45 ºC for 60 days [28]. The testing of the kits with parasite-positive panels was repeated after exposure to heat for 60 days. The panel detection scores of the products at baseline and after 60 days were compared.

Test cassettes selected at random from 4 packs of each product (same lot) were tested. A total of 36 cassettes of each product were used for heat stability testing at baseline (12) and after exposure to 35 °C (12) and 45 °C (12) temperatures respectively.

Data management and statistical analysis

Data were analyzed using SPSS 25.0 for Windows (SPSS Inc. USA). Variables were summarized by descriptive statistics (frequency, percentage, mean, geometric mean, standard deviation). Proportions were compared by calculating chi-square, Fisher exact test or Mantel Haenszel tests depending on which is appropriate. Data not conforming to a normal distribution were compared by the Mann-Whitney U test, Kruskal-Wallis test or Wilcoxon ranked sum tests. A $p < 0.05$ was taken to indicate a significant difference. Estimates of 95% confidence interval (CI) for proportions were done using an online calculator on Vassarstat website for statistical computation (vassarstats.net/prop1.html). Panel detection score was used to compare RDT positivity before and after exposure to heat.

Results

Out of the 834 participants screened for malaria parasite, 306 (36.7%) were males and 528 (63.3%) were females and their age ranged from 1 year to 86 years with a mean age of 24.8 ± 19.8 years (Table 1). The temperature of the individuals ranged from 35.1- 39.7 °C. The hemoglobin (Hb) level of the children (315 children) ranged from 5.8 to 15.3 with a mean of 11.5 ±

Table 1. Baseline characteristics of the study participants.

Character	n	%
Sex		
Male	306	36.7
Female	528	63.3
Age (years)		
Mean ± SD	24.8 ± 19.8	
Range	1 – 86	
<5	81	9.7
5-10	192	23.0
11-15	138	16.5
16-25	74	8.9
>25	349	41.8
Weight (kg)		
Mean ± SD	48.3 ± 26.8	
Range	8.0 – 164.0	
Height (cm)		
Mean ± SD	127.3 ± 26.8	
Range	42.0 – 181.0	
Temperature (ºC)		
Mean ± SD	36.4 ± 0.3	
Range	35.1 - 39.7	
≥ 37.5	5	0.6
< 37.5	828	99.4
Hb (g/dL) (n = 315)		
Mean ± SD	11.5 ± 1.5	
Range	5.8 - 15.3	
Normal [≥ 11.0]	218	69.2
Anaemia [< 11.0]	97	30.8

Table 2. Malaria detection by different tests kits and microscopy.

Malaria Test	Positive cases	Percent (%)	95% CI
Kit 1 Pf pLDH	159	19.1	16.5 - 21.9
Kit 2 Pf pLDH	154	18.5	15.9 - 21.3
Kit 2 Pf HRP2	231	27.7	24.7 - 30.9
Kit 3 Pf pLDH	160	19.2	16.6 - 22.1
Kit 3 Pv pLDH	0	0	N/A
SD_HRP2	206	24.7	21.8 - 27.8
Microscopy	112	13.4	11.2 - 16.0
Species			
P falciparum	111	99.1	95.1 - 100.0
P. falciparum/P. malariae	1	0.9	0.0 - 4.9
Stage			
Gametocyte	1	0.9	0.0 - 4.9
Trophozoite	110	98.2	93.7 - 99.8
Trophozoite/Schizont/Gametocyte	1	0.9	0.0 - 4.9
Parasite density (parasite/µL)			
GMPD (range)	2049 (47-154,154)		
Low [1-1000]	42	37.8	28.8 - 47.5
Moderate [1001-10,000]	44	39.6	30.5 - 49.4
High [> 10,000]	25	22.5	15.1 - 31.4

*GMPD: Geometric Mean Parasite Density.

1.5. The normal and anemic Hb for the participants were 218 (69.2%) and 97 (30.8%) respectively for the 315 children (Table 1).

The malaria-positive rates based on the different diagnostic methods showed that the microscopy method had the least positivity rate of 13.4% (95% CI 11.2%-16.0%) whereas, the highest was kit 2 PfHRP2 27.7% (95% CI 24.7-30.9%). Positivity rate of kit 2 PfHRP2 27.7% (95% CI 24.7-30.9%) and SD Bioline HRP2 24.7 (95% CI 21.8-27.8%) were similar. The three Biocredit PfLDH kits had positivity rates that were significantly higher than microscopy but significantly lower than positivity rates of HRP2 test kits of Biocredit and SD Bioline (Table 2). Almost all the positive samples by microscopy had *P. falciparum* infection 111 (99.1%). Only 1 (0.9%) sample had mixed species of *P. falciparum* and *P. malariae*. The geometric mean parasite density (GMPD) was 2049 parasite/µL and ranged from 47-154,154 parasite/µL (Table 2).

The performance characteristics of RDT kits relative to malaria microscopy (gold standard) showed that the sensitivity and specificity of BIOCREDIT® kits were 88.4% (95% CI: 81.2-93.1%) and 91.7% (95% CI: 89.5-93.5%) respectively for kit 1 (PfLDH); 92% (95% CI: 85.4-95.7%) and 82.3% (95% CI: 79.3-84.9%) respectively for kit 2 (PfHRP2); 86.6% (95% CI: 79.1-91.7%) and 92.1% (95% CI: 89.9-93.9%) respectively for kit2 (PfLDH); 88.4% (95% CI: 81.2-93.1) and 91.6% (95% CI: 89.3-93.4) respectively for kit 3 (PfLDH). PvLDH in Kit 3 was not evaluated due to the absence of *P. vivax* in the study sites. SD Bioline HRP2 kit had sensitivity and specificity of 92.9% (95% CI: 86.5-96.3%) and 85.9% (95% CI: 83.1-88.2%) respectively (Table 3).

The positive predictive value (PPV) and negative predictive value (NPV) of the Pf pLDH were found to be 62.3% (95% CI = 54.5-69.4%) and 98.1% (95% CI = 96.7-98.9%) respectively, for Pf HRPII the PPV was 44.6% (95% CI = 38.3-51.0%) and NPV was 98.5%

Table 3. Performance Characteristics of Rapid Tests Evaluated.

Test kit	Microscopy		Total	Kappa	Sensitivity (%) [95% CI]	Specificity (%) [95% CI]	PPV (%) [95% CI]	NPV (%) [95% CI]
	Pos	Neg						
N	112 (13.4)	722 (86.6)	834					
Kit 1 [PfLDH]								
Pos	99 (88.4)	60 (8.3)	159	0.68	88.4 [81.2-93.1]	91.7 [89.5-93.5]	62.3 [54.5-69.4]	98.1 [96.7-98.9]
Neg	13 (11.6)	662 (91.7)	675					
Kit 2 [PfHRP2]								
Pos	103 (92.0)	128 (17.7)	231	0.512	92.0 [85.4-95.7]	82.3 [79.3-84.9]	44.6 [38.3-51.0]	98.5 [97.2-99.2]
Neg	9 (8.0)	594 (82.3)	603					
Kit 2 [PfLDH]								
Pos	97 (86.6)	57 (7.9)	154	0.679	86.6 [79.1-91.7]	92.1 [89.9-93.9]	63.0 [55.1-70.2]	97.8 [96.4-98.7]
Neg	15 (13.4)	665 (92.1)	680					
Kit 3 [PfLDH]								
Pos	99 (88.4)	61 (8.4)	160	0.677	88.4 [81.2-93.1]	91.6 [89.3-93.4]	61.9 [54.2-69.0]	98.1 [96.7-98.9]
Neg	13 (11.6)	661 (91.6)	674					
SD PfHRP2 (Comparator)								
Pos	104 (92.9)	102 (14.1)	206	0.581	92.9 [86.5-96.3]	85.9 [83.1-88.2]	50.5 [43.7-57.3	98.7 [97.5-99.4]
Neg	8 (7.1)	620 (85.9)	628					

Table 4. Comparison of result of HRP2 kits with pLDH kits.

HRP2 kits	N	Kit 1 (PfLDH)		Kit 2 (PfLDH)		Kit 3 (PfLDH)	
		Pos (%)	Neg (%)	Pos (%)	Neg (%)	Pos (%)	Neg (%)
Biocredit PfHRP2							
Pos	103	98 (99.0)	5 (38.5)	97 (100)	6 (40.0)	98 (99.0)	5 (38.5)
Neg	9	1 (1.0)	8 (61.5)	0 (0.0)	9 (60.0)	1 (1.0)	8 (61.5)
Total	112	99	13	97	15	99	13
Agreement		**94.6%**		**94.6%**		**94.6%**	
SD PfHRP2							
Pos	104	98 (99.0)	6 (46.2)	96 (99.0)	8 (53.3)	98 (99.0)	6 (46.2)
Neg	8	1 (1.0)	7 (53.8)	1 (1.0)	7 (46.7)	1 (1.0)	7 (53.3)
Total	112	99	13	97	15	99	13
Agreement		**93.8%**		**92.0%**		**93.8%**	

(95% CI = 97.2-99.2%), The PPV and NPV of the Pf pLDH were 63.0% (95% CI = 55.1-70.2%) and 97.8% (95% CI = 96.4-98.7%) respectively. The pLDH Pf also had PPV of 61.9% (95% CI = 54.2-69.0%) and NPV of 98.1% (95% CI = 96.7-98.9%), while SD BIOLINE HRPII RDT kits had PPV and NPV of 50.5% (95% CI = 43.7-57.3%) and 98.7% (95% CI = 97.5-99.4%) respectively (Table 3). There were no significant differences between the positive predictive values of the three Biocredit kits and the SD Bioline test kit, similar trend was observed in negative predictive values (Table 3).

The result of this study showed that out of the 112 participants that were positive by microscopy, 99 (88.4%), 97 (86.6%), 103 (92.0%), 99 (88.4%) and 104 (92.9%) participants were positive by Biocredit kit 1 PfLDH, Kit 2 PfLDH/HRP2, kit 3 PfLDH and SD Bioline HRP2 RDT kits respectively. Among the positive-microscopy cases, there was a case of discordance between the SD Bioline HRP2 Kit and BIOCREDIT® PfLDH kits. The SD Bioline HRP2 Kits were negative for malaria parasite and all three Biocredit® PfLDH kits were positive for that sample. The parasite density of the sample was 688 parasite/µL. Another case of discordance was observed between the SD Bioline HRP2 kit and the three Biocredit® PfLDH kits. The parasite density of the sample was 1000 parasite/µL. The agreement between the three Biocredit PfLDH kits and Biocredit HRP2 kits was the same (94.6%), however, the agreement between SD Bioline HRP2 kit and the three Biocredit PfLDH kits was similar: Kit 1 PfLDH 93.8%; Kit 2 PfLDH 92.0%; and Kit 3 PfLDH 93.8% (Table 4).

Comparison of positivity by parasite density using different malaria test kits showed that Biocredit kit 2 Pf HRP2 was the highest in detecting low parasite density (92.9%), followed by SD Bioline HRP2 (90.5%), while the kit that showed the least detection of low parasite density (83.3%) was kit 2 PfLDH. The kit that detected the highest parasite density was SD Bioline HRP2 (96.0%) while the Biocredit® kits were able to detect high density at the same rate (92.0%) (Figure 1).

The result of stability testing showed that all three Biocredit® Kits had panel detection scores of 12/12 (100%) at baseline and after 60 days of exposure to 35 °C and 45 °C (Table 5).

The three Biocredit PfLDH kits were not specific for *Plasmodium falciparum.* They were able to detect blood samples containing *P. ovale* and *P. berghei.* The fieldworkers reported that the three Biocredit® test kits

Figure 1. Positivity by parasite density using different malaria RDT kits.

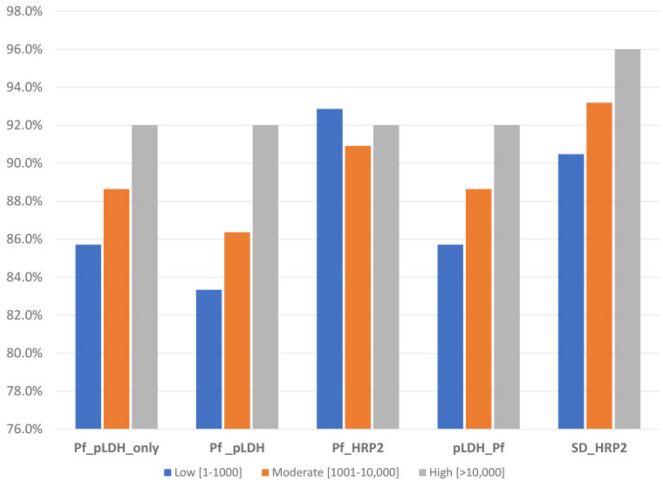

Table 5. Panel detection scores of the three BIOCREDIT® malaria RDT Kits before and after 60 days exposure to heat.

Product	Baseline (Day 0)	35 °C (Day 60)	45 °C (Day 60)
Kit 1 PfLDH	12/12 (100%)	12/12 (100%)	12/12 (100%)
Kit 2 PfLDH	12/12 (100%)	12/12 (100%)	12/12 (100%)
Kit 2 HRP2	12/12 (100%)	12/12 (100%)	12/12 (100%)
Kit 3 PfLDH	12/12 (100%)	12/12 (100%)	12/12 (100%)
Kit 3 PvLDH	ND	ND	ND

*ND: Not Done.

were easy to use in the field. The transfer device, an inverted cup, was also easy to use and the instruction leaflet was adequate. The package of the Biocredit kit has adequate content to conduct malaria tests.

Discussion

Many variable factors such as population climatic condition, local circulating parasite strains, parasite density, malaria prevalence rate, host/parasite genetic variation, quality of the RDT kits, user-friendliness of test methodology, kit format, local diagnostic practice and skills can influence the performance of RDT tests in different settings hence RDT test evaluations should be performed under the range of conditions in which they are to be used (World Health Organization, 2009). The heat stability and field performance capacities of three RapiGen BIOCREDIT® malaria Ag RDT kits: Pf (pLDH) only, Pf (pLDH/HRPII) and Pf/Pv (pLDH/pLDH) for accurate detection of malaria infection in our population was assessed using malaria microscopy thick/thin blood smear as a gold standard and SD BIOLINE® (PfHRPII) RDT test kit as a comparative standard.

The three RapiGen BIOCREDIT® malaria Ag RDT test kits have a valid ISO 13485:2003 certification and are undergoing WHO prequalification diagnostic performance assessment (https://www.who.int/teams/global-malaria-programme/case-management/diagnosis/rapid-diagnostic-tests/selection-and-procurement).

The Biocredit PfHRP2 and SD Bioline PfHRP2 had positive rates that were similar though the Biocredit PfHRP2 had a slightly higher positive rate. The similarity was expected because they both target the same antigen PfHRP2. Likewise, the positive rates of the three Biocredit PfLDH were similar. It is known that the HRP2 antigen persists in the blood after parasite clearance, unlike PfLDH which is a parasite enzyme that clears from the bloodstream when the parasite is no longer present [29,30]. This explains the significantly higher positivity rates of PfHRP2 kits compared to PfLDH kits. The positive rate of PfLDH was higher than microscopy in this study this does not agree with the study carried out in Ethiopia by Alemayahu *et al.* [31].

The sensitivities of the PfHRP2 kits (Biocredit and SD Bioline) reported from this study were similar to the PfHRP2 sensitivity reported from Papua Indonesia but higher than the report from Colombia [30, 32]. However, the PfHRP2 kits sensitivities were lower than the sensitivities reported from Nigeria and other African countries Ajumobi and associates from Nigeria and other recent studies from some countries in Africa [33–37].The sensitivities of the three Biocredit PfLDH kits were similar to the report by Hendriksen *et al.* [38].

The specificities of the Biocredit® PfLDH kits were significantly higher than both Biocredit® PfHRP2 and SD Bioline PfHRP2 kits**.** This study showed that the Biocredit® PfLDH could accurately detect the absence of malaria parasites among individuals. The specificities of Biocredit® PfLDH kits agree with the study carried out by Grandesso *et. al.* [34]. Lower specificities of PfLDH and PfHRP2 kits have been reported by other workers [37].

The PPV and NPV of the Biocredit PfLDH and PfHRP2 kits were similar to the SD Bioline PfHPR2 kit. Higher PPV and NPV have been reported by Alemayahu *et al.* and Hendriksen *et al.* [31,38]. The PPV and NPV of diagnostics kits are affected by malaria prevalence [39]. The relatively low PPV observed in this study may be due to the low prevalence of malaria in this study population.

The possibility of detecting HRP2 gene deletion was investigated by comparing PfHRP2 and PfLDH results for samples positive microscopy. It was however observed that none of the two discordant results met the criteria for suspecting gene deletion. The two potential cases were noted to be positive by either Biocredit PfHRP2 or SD Bioline PfHRP2. A very low prevalence of HRP2 gene deletion has been reported in Nigeria by other workers [8,40].

The stability testing of all three Biocredit® PfLDH and PfHRP2 kits at a baseline of 200 parasite/µL and 2000 parasite/µL panels were all positive with 100% panel detection score which shows that the RDT kits are heat stable even at high temperatures or climatic conditions that are obtainable in tropical African regions.

Conclusions

The sensitivities of the three RapiGen BIOCREDIT® mRDTs and SD BIOLINE Malaria Antigen (HRPII), which was the comparator, were similar. However, the specificities of BIOCREDIT kits detecting pLDH were significantly higher than both BIOCREDIT and SD BIOLINE kits detecting HRPII antigen. There was no case of suspected *hrp2* gene deletion observed among the blood samples that were positive by microscopy because all the malaria cases identified by PfLDH kits were positive by PfHRPII kits. The PfLDH test line of the three kits was not specific for *Plasmodium falciparum* as the PfLDH test line detected *P. ovale* and *P. berghei.* However, the PvLDH test line did not detect *P. falciparum, P. ovale* or *P.*

berghei. Malaria positivity was highest among children aged 11-15 years based on the results of all the malaria rapid test kits but this was not demonstrated by malaria microscopy. The fieldworkers testified and agreed that the three BIOCREDIT® mRDT test kits were very easy to use on the field. The three RapiGen BIOCREDIT® malaria RDT Kits were stable after 60 days of exposure to 35ºC and 45 ºC temperature. The three RapiGen BIOCREDIT® malaria RDT Kits are therefore suitable to be deployed in malaria-endemic tropical regions.

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