

Original Research Article

Assessing the Performance Characteristics of the “CareStart™ Malaria HRP2 pf (CAT NO: G0141, ACCESSBIO)” Rapid Diagnostic Test for Asymptomatic Malaria in Mutengene-Cameroon

Abstract

Aim: The aim of this study was to determine the prevalence and density of malaria parasites in asymptomatic school children in Mutengene-Cameroon, and evaluate the performance characteristics of the ‘CareStart™ Malaria HRP2 pf (CAT NO: G0141, ACCESSBIO)’ rapid diagnostic test (RDT) using light microscopy as a gold standard.

Methodology: The study was a cross-sectional survey. The study was carried out in Mutengene, Fako Division, southwest Cameroon from February to March, 2013.

A total of 406 pupils aged 4-15 years were involved in the study. Demographic data was taken for each child and capillary blood collected by finger pricking. Thick and thin blood films were prepared for the assessment of parasite density and speciation respectively. A drop of blood was used on the RDT to determine the malaria status.

Results and Discussion

The mean age at 95% confidence interval (CI) was 8 ± 2 years (range =4-15 years) and the overall prevalence of malaria was 39.9% (CI = 35.3-44.7). The geometric mean parasite density (GMPD) was 2332.66 parasites/ μ L (range: 218-16000). More pupils were positive by microscopy (40.9%, CI =36.1-45.9) than by RDT (27.9%, CI = 23.7-32.7) and the difference was statistically significant ($\chi^2=16.1$, $P<0.0001$). The majority of the cases detected were those with high infection (≥ 5000 trophozoites/ μ L). Less than 50% of those with low (25.0%, CI=12.0-44.9), moderate (40.7%, CI=32.24-49.70) and high parasitaemia (75%, CI=5.00-89.82) were positive by RDT and the difference was statistically significant ($\chi^2= 10.09$, $P = 0.006$). The RDT showed a low sensitivity of 41.8% (CI=34.37-49.5%) and specificity of 82.3% (CI=76.8-86.7%) in diagnosing malaria. It was recommended that more research be done on this RDT to improve on its performance characteristics before it could be used in mass surveillance programmes for malaria management and control.

Keywords: Malaria, diagnosis, performance characteristics, ‘CareStart™ Malaria HRP2 pf (CAT NO: G0141, ACCESSBIO), Mutengene.

1.0 BACKGROUND

Malaria is a life-threatening disease and presents a diagnostic challenge to laboratories in most tropical countries where the diagnosis of malaria still relies predominantly on the clinical presentation and the century technique of microscopic examination of blood smears [1]. Several factors including endemic malaria, population movements, and travelers all contribute to presenting the laboratory with diagnostic problems for which it may have little expertise available.

Microscopy remains the gold standard for the detection of malaria parasites as it can provide information on both parasite species and density [2]. However, this procedure is labour-intensive, requiring substantial training and expertise. Sometimes, light microscopy is infrequently performed in endemic areas, resulting in misdiagnosis, misidentification of *Plasmodium* species, and therapeutic delays. There may be limited supply and maintenance of microscopes and reagents, delays in delivery of results, and inadequate quality control. All these diagnostic limitations affect the medical care provided, as malaria is a potentially fatal disease, but usually curable if diagnosed and treated on time [1]. Rapid diagnostic tests have thus been designed as alternatives to light microscopy [3].

Malaria diagnostic methods focus on the detection of malaria antigens such as the histidine rich protein 2 (HRP-2) for *P. falciparum* and the parasite specific lactate dehydrogenase (pLDH) or *Plasmodium* aldolase from the parasite glycolytic pathway found in all species [4], [5] and [6]. Some rapid diagnostic tests (RDTs) detect only HRP-2 of *P. falciparum* and cannot detect other species of *Plasmodium* infections. Although serological methods such as Enzyme-linked immunosorbent assay and molecular methods (polymerase chain reaction) are also available, for malaria diagnosis they are however cumbersome, expensive and beyond the reach of most malaria patients in endemic areas. Malaria especially that caused by *P. falciparum* can lead to severe disease and death [7] within a very short time, thereby necessitating rapid diagnosis followed by proper treatment. The malaria parasite that is endemic in Cameroon is *plasmodium falciparum* [8].

According to WHO [9] for results of a rapid diagnostic test to be acceptable, they should be as accurate as results derived from microscopy under routine field conditions. The sensitivity of the test should be at least 95% compared to microscopy and parasitaemia of 100 parasites/ul should be detectable [3]. Several studies have evaluated rapid diagnostic tests such as Wanji *et al.* [10] in Southwest Cameroon, Heutmekers *et al.* [11] in Belgium, Kosack *et al.* [12] in 'Medicine Sans Frontiers' programmes, Yan *et al.* [5] in China, Sani *et al.* [13], Ameh *et al.* [14] and Ojuronbo *et al.* [6] in Nigeria. However, an evaluation of all RDTs is important since a test may be valid but expensive or valid and reliable in the laboratory or hospital but with several inconveniences in the field [3]. The 'CareStart™ Malaria HRP2 pf (CAT NO: G0141, ACCESSBIO)' is a RDT that is currently used in health units in Fako Division, Southwest Cameroon. No study has been carried out on this RDT and no author has reported on its performance characteristics in Southwest Cameroon, an area endemic for *falciparum* malaria. It is therefore important

to evaluate the performance characteristics of this RDT using light microscopy as a gold standard. It is also necessary to determine the general prevalence of malaria in this area in order to know if the control programmes such as the early detection of parasites in symptomatic people and treatment with artemisinin combination therapy are yielding positive results [14] and [15].

2.0 MATERIALS AND METHODS

2.1 Study Sites

The study was carried out in Mutengene (Catholic School, C.S., Mutengene) in Fako Division, Southwest Cameroon. Mutengene (situated at 242 m above sea level, longitude 09° 18' 29" E and latitude 04° 05'57" N) is a road-junction town where major roads from Buea, Limbe, Tiko and Douala converge. This locality is found in the Mount Cameroon area. Weather records for the Mount Cameroon area from the Cameroon Development Corporation indicate a mean relative humidity of 80 %, an average rainfall of 4000 mm and a temperature range of 18°C - 29°C. There are two distinct seasons - a cold rainy season which spans from mid-March to November and a warm dry season with frequent light showers which runs from December to mid-March.

2.2 Study Population: The study included 406 pupils of both sexes aged 4 - 15 years. Pupils were enrolled into the study only if they were pupils in C.S. Mutengene, had received parental/guardian informed consent (came with signed consent forms) and succumbed to the blood collection procedure.

2.3 Study Design

The study was a cross-sectional survey where blood samples were collected from children in the months of February and March, 2013. A sensitization rally was organized with the teachers of the school to explain the purpose and benefits of the study (free malaria diagnosis for the children) before the sampling was done. Informed consent forms were sent to parents/guardians of all the pupils in the school (480) through the children stating the purpose of the study as well as the advantages and the amount of blood that had to be collected from each child. Only children who brought back signed informed consent forms were included in the study (406).

A simple semi-structured questionnaire was administered in English (and exceptionally in Pidgin English) to pupils to obtain data on each child's name, sex, age, and the socio-economic status of pupils were determined as indicated by Kimbi *et al.* [15] and Nyanga *et al.* [16]. The axillary temperature of each child was measured using a clinical thermometer. Fever was defined as a temperature $\geq 37.5^{\circ}\text{C}$. Blood was collected from each pupil by pricking the finger for the detection of malaria parasite by microscopy and RDT.

2.4 Collection, Preparation and Examination of Blood Samples

Demographic data such as the name, sex, age were obtained from the pupils. After cleaning the lobe of a finger with cotton wool soaked in methylated spirit, the finger was pricked using a sterile lancet. Blood was collected from the finger prick and used to prepare thick and thin blood films on labelled slides. Five (5) μL of blood was added to the sample well of the RDT device using a capillary supplied with the kit for this purpose and 1 - 2 drops of buffer were applied to the buffer well. The results were read after 20 minutes. The thick and thin blood films were air-dried and transported to the Malaria Laboratory of the University of Buea for further processing. Thin films were fixed with absolute methanol and both thick and thin films were later stained with 10 % Giemsa and examined for the presence of parasites and assessment of parasite density as stated by Cheesbrough [7].

Using a light microscope, asexual malaria parasites were counted against 200 leukocytes (or 500 leukocytes for low density infections) in thick films. These were used to compute the number of parasites per μL assuming a standard value for the leukocyte count to be 8,000WBC/ μL of blood. The slides were read by two independent microscopist and a slide was declared negative after viewing at least 100 high power microscopic fields without seeing any parasite. The microscopists were blinded to the results of the 'CareStart™ Malaria HRP2 pf (CAT NO: G0141, ACCESSBIO)'. Parasitaemia was classified as low (< 500 parasite/ μL of blood), moderate (501 - 5000 parasites/ μL of blood) and high (> 5000 parasites/ μL of blood [17].

2.5 Principle of the CareStart™ RDT and the test kit composition

The 'CareStart™ Malaria HRP2 pf (CAT NO: G0141, ACCESSBIO)' RDT is a chromatographic test for *in vitro* diagnosis that makes use of the HRP2 of *Plasmodium falciparum*. It has a long shelf-life of more than one year, with storage conditions ranging from 1°C to 40°C. Each kit is composed of 25 chromatographic test strips, a plastic container with diluents (5ml), a pack of 25 lancets and disposable alcohol swabs saturated with 70% isopropyl for disinfection. This test does not detect other malaria parasites like *P. ovale*, *P. malariae* and *P. vivax*.

2.6 Statistical Analysis

Data was entered into spread sheets using Microsoft excel and analyzed with the statistical package for social sciences (SPSS) version 17 (SPSS, Inc., Chicago, IL, USA). Means $\pm$ standard deviations (SD) were compared using Kruskal wallis test. The analysis of variance (ANOVA) was used where appropriate to compare means. Significant levels were measured at 95% confidence level with significant differences recorded at $p < 0.05$. Sensitivity and specificities were calculated using the formulae below:

Sensitivity = $\text{TP} / (\text{TP} + \text{FN})$.

134 Specificity = $TN / (TN + FP)$.

135 Positive predictive value = $TP / (TP + FP)$

136 Negative Predictive Value = $TN / (TN + FN)$ where TP = number of true positives, TN = number of true
 137 negatives, FP = number of false positives and FN = number of false negatives. The values obtained were
 138 expressed as percentages by multiplying by 100 [6].

139

140 3.0 RESULTS

141 3.1 Characteristics of the study population

142 A total of 406 pupils with a mean age of 8 ± 2 years (range =4-15 years) were evaluated for malaria
 143 prevalence by use of microscopy and 'CareStart™ Malaria HRP2 pf (CAT NO: G0141, ACCESSBIO)'
 144 RDT. The overall prevalence of malaria in the study population was 39.9% (CI = 35.3 – 44.7) (Table 1).
 145 Out of the 406 pupils examined by microscopy, 386 pupils were again examined using the CareStart™
 146 RDT and the prevalence among these pupils in relation to the two methods was 40.9% (n=158, CI =36.1-
 147 45.9) and 28%, (n=108, CI = 23.7-32.7) respectively.

148

149 Table 1: Characteristics of the study population

Factor	Category	Number examined	%
Sex	Male	214	52.7
	Female	192	47.3
Age (Years)	0-5	90	22.2
	6-10	246	60.6
	10-15	70	17.2
Socio-economic class	Poor	368	90.6
	Middle Class	8	2.0
	Rich	49	12.1
Total		406	100
Cartegory		Number positive	%
Prevalence of fever (for the 406 pupils)		110	27.1
Malaria parasite prevalence (overall for 406 pupils)		162	39.9

Malaria parasite prevalence for the 386 pupils by microscopy	158	40.9
Malaria parasite prevalence for the 386 pupils by RDT	108	28.0

150

151 3.2 Malaria prevalence and mean parasite densities by microscopy

152 Malaria prevalence was higher in males (42.1%, CI = 35.6-48.8 than in females (37.5%, n = 72, CI = 31-
153 44.5), but the difference was not statistically significant ($\chi^2 = 2.844$, $P = 0.426$).

154 Malaria prevalence was significantly highest among the middle class (50%, CI =21.5 - 78.5) when
155 compared with their poor (42.9%, CI = 37.9-48.04) and rich (0.0%) counterparts ($\chi^2 = 7.58$, $df = 2$, $P =$
156 0.023).

157 Among malaria positive pupils, the prevalence of fever was 39.1% (n = 36, CI = 29.79-49.35). However,
158 malaria prevalence was not significantly different between those who had fever and those who did not
159 have fever ($\chi^2 = 0.34$, $df = 1$, and $P = 0.056$) as shown in table 1.

160 The GMPD in the study population was 2332.66 parasites/ μ L (CI = 218-16000). It was higher in females
161 (2463.9 parasites/ μ L) than in males (2022.7 parasites/ μ L) and the difference between males and females
162 was statistically significant ($F = 0.96 = P = .003$), as shown in table 2. GMPDs were higher in pupils of the
163 age group 10 – 15 years (3172.50 parasites/ μ L) than in the other age groups and the difference was
164 statistically significant ($F = 5.28$, $df = 2$, $P = 0.006$) as shown in table 2. GMPD was higher in the middle
165 class (2327.3/ μ L) than in the poor and the difference was statistically significant ($F = 9.6$, $P = 0.001$)

166

167 Table 2: Malaria prevalence and GMPD as affected by sex, age and social class

Factor	Category	N	Malaria Prevalence % (n)	95% CI (%)	Test Statistics	GMPD/ μ L of blood)	Range	Test statistics
Sex	Males	214	42.1 (90)	35.6-48.8	$\chi^2 = 2.84$ $P = .42$	2022.8	218 -5333	$P =$ 0 .003 $F =$ 9.6
	Females	192	37.5 (72)	31-44.5		2463.7	615 -1600	

Age in years	0 - 5	90	26.7 (24)	18.6-36.6	$\chi^2 = 18$ P = 0.004.	2544.2	600 -5333	F = 5.28 P = 0.006
	6 - 10	246	45.5 (112)	39.4-51.8		1969.2	218 -5600	
	>10	70	37.1 (26)	26.8-48.9		3172.5	1600 -16000	
Social class	Poor	368	42.9 (158)	37.9-48.0	$\chi^2 = 7.585$ P = 0.023	2064.8	218 -16000	F= 9.6 P = 0.001
	Middle class	8	50.0 (4)	21.52-78.5		2327.3	615 -4800	
	Rich	49	00 (0)	00-00		00	00 - 00	
Total		406	40 (162)	35.3-44.7		2332.7	218 -16000	

N = number of pupils examined, n = number infected in the particular group

3.3 RDT status in relation to malaria prevalence by microscopy and mean parasite densities

More pupils were positive by use of microscopy (40.9%, CI =36.1-45.9) than by use of RDT (27.9%, CI = 23.7-32.7) and the difference in positivity was statistically significant ($\chi^2 = 16.1$, $P < 0.001$). Conversely, more pupils were negative by use of RDT than by use of microscopy (figure 1).

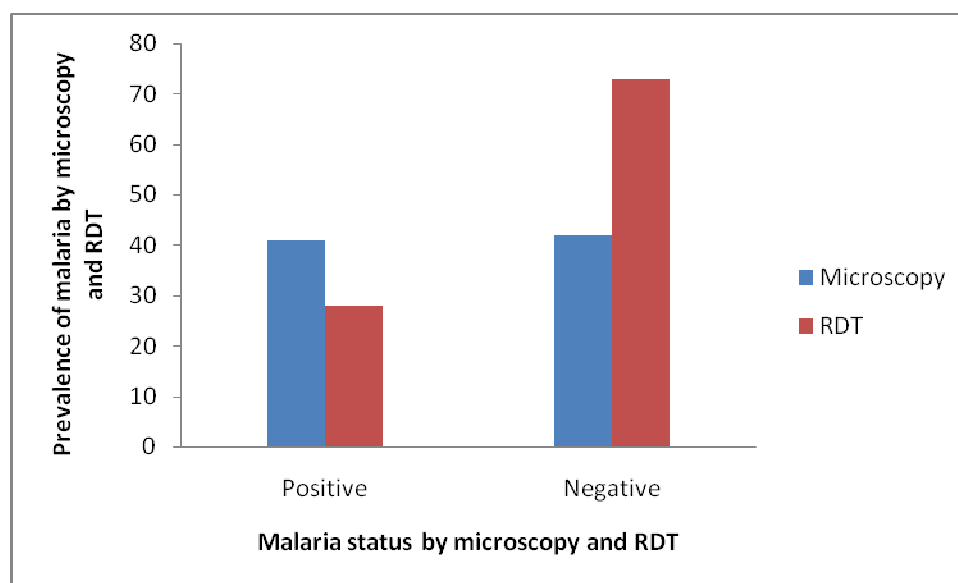


Figure 1: Prevalence of malaria by microscopy and RDT

The majority of malaria parasite infected cases detected by RDT were those with high infection (>5000T/ μ l). Less than 50% of those with low (25.0%, CI = 12.0-44.9) and moderate (40.7%, CI = 32.2-49.7) parasitaemia and 75% (CI = 5.0-89.8) of those with high parasitaemia were positive by use of RDT as shown in figure 2. The difference in malaria prevalence by use of the RDT in relation to parasite densities was statistically significant ($\chi^2 = 10.09$, df = 2, P = 0.006).

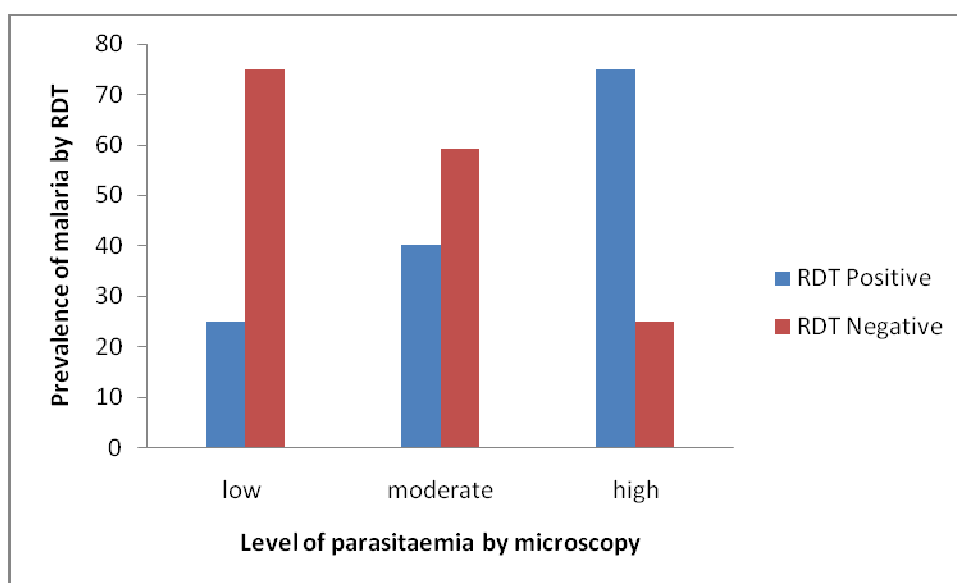


Figure 2: Malaria status by RDT in relation to mean parasite densities

3.4 False positive, false negative, true positive and true negative malaria cases

Positive malaria cases by use of microscopy and RDT (true positives) were 66 (41.8%, CI = 34.6 – 49.9, estimated population odds (EPO) = 0.73, CI= 0.53-0.99). Forty pupils (17.7%, CI= 13.28-23.2, EPO = 0.22, CI = 0.15-0.30) were positive by use of RDT, but negative by use of microscopy (false positive). Seventy pupils (22.3%, CI =18.0-27.2, EPO = 0.29, CI = 0.22 – 0.37) were positive when microscopy was used and negative when RDT was used (false negatives). The difference in malaria prevalence obtained by use of microscopy and RDT was statistically significant ($\chi^2 = 16.1$, P < 0.0001).

3.5 Sensitivity and specificity of the RDT

The sensitivity of the RDT was 41.8% (CI = 34.4 - 49.5) when compared with microscopy. The specificity of the RDT was found to be 82.3% (CI = 76.8 - 86.7). The positive and negative predictive values were 62.26% (CI = 52.8 - 70.9) and 66.9% (CI = 61.2 - 72.2) respectively. The RDT had a negative odds ratio of 0.7, positive odds ratio of 3.5 and diagnostic odds ratio of 2.58 as indicated in table 3.

Table 3: Performance characteristics of the RDT

Performance characteristic of RDT	Effective performance	95% Confidence interval
Sensitivity	41.8%	34.37% - 49.57%
Specificity	82.30%	76.8% - 86.7%
Negative likelihood ratio	0.707	0.61 – 0.82
Positive likelihood ratio	2.36	1.69 - 3.30
Diagnostic Odds Ratio	3.536	2.09 - 5.31
Positive predictive value	62.26	52.76 - 70.91%
Negative Predictive Value	66.9	61.18 - 72.17%

4.0 DISCUSSION

This study revealed that malaria is a major public health problem in Mutengene and there are still challenges in malaria diagnosis such as test duration (for microscopy) and test performance characteristics (for RDT). Malaria prevalence varied significantly by use of microscopy and 'CareStart™ Malaria HRP2 pf (CAT NO: G0141, ACCESSBIO)' RDT. The overall prevalence of malaria in this study was lower than that reported by some authors who worked in neighbouring areas in the Mount Cameroon area in recent years [18] and [19]. However, it was higher than the values reported by Kimbi *et al.* [8]. [15] in other neighboring villages in the Mount Cameroon area. The drop in malaria prevalence in the recent years is an indication that malaria control strategies in line with the Abuja Declaration of 2000 on Roll Back Malaria in the past few years are yielding positive results [8].

In line with Kimbi *et al.* [8], [15] and Sumbele *et al.* [20], more males were positive for malaria than females. However, in line with findings by Kimbi *et al.* [21], the geometric mean parasite densities were higher in females than in males.

Malaria prevalence was significantly higher in the middle class when compared with their poor and rich counterparts. This observation is contrary to the expectation and could likely be linked to the very large number of pupils in the poor class and the small numbers of pupils in the middle and rich classes. It is known that the rich are often more likely to afford appropriate control measures against malaria [22] and as such are less exposed to mosquito bites than the poor. Even when they get infected with the disease, they are better able to treat themselves than the poor.

The highest prevalence of malaria was found in the age group 6-10 years. Children in the age group 0-5 years constitute a high risk group and are better monitored by the parents especially the mothers. Their treatment at all health units is free of charge in Cameroon as decreed by the Head of State. Therefore, any case of malaria in such children can easily be treated regardless of the social status of the parents. On the other hand, children aged >10 years are generally more knowledgeable about malaria and can take personal protective measures than the younger ones. The drop in prevalence of malaria with increase in age could also be related to the acquisition of protective immunity due to repeated infections as children grow older in high transmission areas [15] and [21]. The level of acquired immunity also probably explains the highest value of GMPD observed in the youngest age group.

The majority of pupils examined in this study were asymptomatic and the prevalence of fever was low. More pupils were positive by use of microscopy than by use of RDT and the difference was significant. Although this RDT technique has the advantage of being cheap, faster, uses less blood, is less sophisticated, requires very little training and expertise, does not require reagent preparation, does not require electricity and can be used easily for field work, it however, performed far less than light microscopy and cannot differentiate malaria parasite species. According to Murray *et al.* [3], the ability of a test to distinguish between malaria species is important because infections due to some species such as *P. falciparum* warrant initial selection of additional drugs for treatment. The conditions of such patients can easily deteriorate within a short time and they therefore need special attention.

False positive and false negative results were recorded in this present study. With respect to the RDT used, the false positive results could be due to the presence of parasite antigens that could persist in the blood of the patient after chemotherapy [6]. False positive results resulting from misdiagnosis can unfortunately lead to wrong drug prescription especially when the clinical picture resembles that of malaria as is the case with acute respiratory infections, typhoid fever and meningitis. Consequently, patients could actually become chronically ill or even die from such diseases. Amexo *et al.* [23]. opined

that malaria over-diagnosis is still a major public health problem in Africa with studies suggesting between 50% and 99% of those prescribed antimalarial drugs are actually negative depending on the clinical setting. Wongsrichanalai *et al.* [24] believe that microscopy remains the gold standard for malaria diagnosis. However, if the microscopist is not well trained, there could be unacceptably high false-positive or high negative results.

False negative results could be due to very low parasitaemias so that the small quantity of blood used in the RDT test will not be enough to concentrate the parasites for them to be detected. Generally, a RDT can only be considered to be of good performance, if it is able to detect parasites at densities as low as 100parasites/ μ L of blood [3]. In this present study, false positive and false negative results were found at all the levels of parasitaemia indicating that the performance characteristics of the RDT did not strictly depend on the level of parasite density.

The sensitivity and specificity of the RDT used in the study falls short of the standards set by WHO. The standards for sensitivities and specificities of RDTs as set by WHO stipulates a minimal standard of 95% for *P. falciparum* sensitivity and specificity [3]. Other authors have worked on the performance characteristics of different CareStart RDTs produced by different manufacturers and making use of different antigens. Among these are Heutmekers *et al.* [12] in Belgium who worked on CareStart pLDH/pan-pLDH (G0121) and SDFK60 (HRP-2/pan HRP-2) in a reference setting. He reported sensitivity values > 90%. The difference in sensitivity observed in this present study and that of Heutmekers *et al.* [12] could have been as a result of the fact that the two RDTs detected different antigens. Again, Heutmekers *et al.* [12] worked in a non-endemic area and on stored blood samples for *P. falciparum*.

Performance characteristics of CareStart RDT (malaria pf rapid device, Biotec Laboratories, UK) were reported by Sani *et al.* [13] in Nigeria with a sensitivity of 90.2% and a Specificity of 95.4%. The high sensitivity and specificity recorded by Sani *et al.* [13] as compared to those in this study could have been as a result of the fact that they used patients presenting with signs of clinical malaria while in this present study we used pupils with asymptomatic malaria hence low parasitaemias. The postulations of Heutmekers *et al.* [11] that variations in RDT performance could be affected if they are produced by different manufacturers (with different product names) and targeting different antigens could explain the observed difference in performance characteristics of RDT in this study and that of Sani *et al.* [13].

Ashley *et al.* [25] reported sensitivities above 80% for three RDTs [CareStart[®] Malaria 3 line pLDH (Pan, Pf), OptiMAL-IT[®] pLDH (Pan, Pf) and CareStart[®] 2 line pLDH (Pan)]. Xiaodong *et al.* [26]. also reported a sensitivity and specificity of 89.68% and 98.26% respectively for CareStart[™] malaria HRP2/pLDH (Pf/pan). However, the RDTs they used could detect pLDH antigens from *P. falciparum* and other

Plasmodium species unlike the CareStart™ Malaria HRP2 pf (CAT NO: G0141, ACCESSBIO) that was used in this study. Ojuongbe *et al.* [6]. stated that several factors play a role during the manufacturing process of RDTs and environmental conditions may also affect performance of the RDT.

The positive and negative predictive values recorded in this study were higher than those recorded by Adugna *et al.* [27] in South central Ethiopia on CareStart™ malaria Pf/Pv combo test for *Plasmodium falciparum* and *Plasmodium vivax*. They reported seasonally varying RDT precision of as low as 14.3% positive predictive values, and 38.5% negative predictive values in health centre surveys and 40 - 63.6% positive predictive values in household surveys.

From this study, it was concluded that the sensitivity and specificity of the CareStart™ RDT are less than the standards stipulated by WHO. More research needs to be done on the RDT using clinically ill patients to determine if it is important to continue using it for malaria diagnosis in the study area or to improve on its performance characteristics before it could be considered a good diagnostic tool for mass surveillance programmes for malaria management and control in the study area.

5.0 CONFLICT OF INTEREST: none

6.0 ETHICAL CONSIDERATION: an ethical clearance was obtained from the South West Regional Delegation of Public Health. Administrative clearances were obtained from the Regional Delegation of Basic Education as well as from The Catholic Education Board.

7.0 INFORMED CONSENT: all pupils were issued consent forms to seek for their parents' approval. Pupils were accepted for screening when they brought back signed informed consent forms following the approval of their parents.

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