

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.

Experimental design: The study was a cross-sectional survey.

Place and Duration of Study: The study was carried out in Mutengene, Fako Division, southwest Cameroon from February to March, 2013.

Methodology: 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 ($\chi^2=16.02$, $P<0.0001$). 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.

31 **Keywords:** Malaria, diagnosis, performance characteristics, 'CareStart™ Malaria HRP2 pf (CAT NO:
32 G0141, ACCESSBIO), Mutengene.

33 Introduction

34 Malaria is a life-threatening disease and presents a diagnostic challenge to laboratories in most tropical
35 countries where the diagnosis of malaria still relies predominantly on the clinical presentation and the
36 century technique of microscopic examination of blood smears (Neeru *et al.*, 2005). Several factors
37 including endemic malaria, population movements, and travelers all contribute to presenting the
38 laboratory with diagnostic problems for which it may have little expertise available.

39 Microscopy remains the gold standard for the detection of malaria parasites as it can provide information
40 on both parasite speciation and density (Moody, 2002). However, this procedure is labour-intensive,
41 requiring substantial training and expertise. Sometimes, light microscopy is infrequently performed in
42 endemic areas, resulting in misdiagnosis, misidentification of *Plasmodium* species, and therapeutic
43 delays. There may be limited supply and maintenance of microscopes and reagents, delays in delivery of
44 results, and inadequate quality control. All these diagnostic limitations affect the medical care provided,
45 as malaria is a potentially fatal disease, but usually curable if diagnosed and treated on time (Neeru *et al.*,
46 2005). Rapid diagnostic tests have thus been designed as alternatives to light microscopy (Murray and
47 Bennet, 2009).

48 Malaria diagnostic methods focus on the detection of malaria antigens such as the histidine rich protein 2
49 (HRP-2) for *P. falciparum* and the parasite specific lactate dehydrogenase (pLDH) or *Plasmodium*
50 aldolase from the parasite glycolytic pathway found in all species (Abanyie 2011;Yan et 2013;
51 **Ojurongbe, 2013.**). Some rapid diagnostic tests (RDTs) detect only HRP-2 of *P. falciparum* and cannot
52 detect other species of *Plasmodium* infections. Although serological methods such as Enzyme-linked
53 immunosorbent assay and molecular methods (polymerase chain reaction) are also available, for malaria
54 diagnosis they are however cumbersome, expensive and beyond the reach of most malaria patients in
55 endemic areas. Malaria especially that caused by *P. falciparum* can lead to dire consequences within a
56 very short time, thereby necessitating rapid diagnosis followed by proper treatment.

57 According to WHO (2000), for results of a rapid diagnostic test to be acceptable, they should be as
58 accurate as results derived from microscopy under routine field conditions. The sensitivity of the test
59 should be at least 95% compared to microscopy and parasitaemia of 100 parasites/ul should be
60 detectable (Murray *et al.*, 2008). Several studies have evaluated rapid diagnostic tests such as Wanji *et*
61 *al.* (2008) in Southwest Cameroon, Heutmekers *et al.* (2012) in Belgium, Kosack *et al.* (2013) in
62 'Medicine Sans Frontiers' programmes, Juan *et al.* (2013) in China, Sani *et al.* (2013) and Ojurongbo *et*
63 *al.* (2013) in Nigeria. However, an evaluation of all RDTs is important since a test may be valid but
64 expensive or valid and reliable in the laboratory or hospital but with several inconveniences in the field

(Neeru *et al.*, 2005). 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.

Materials and Methods

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.

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.

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 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.

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.* (2013a). 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.

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 (2010).

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 (Allen *et al.*, 1992).

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*.

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

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

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

133 Negative Predictive Value = $TN / (TN + FN)$ where TP = number of true positives, TN = number of true
134 negatives, FP = number of false positives and FN = number of false negatives. The values obtained were
135 expressed as percentages by multiplying by 100 (Ojurongbe *et al.*, 2013).

136 Ethical Considerations

137 An ethical clearance was obtained from the South West Regional Delegation of Public Health.
138 Administrative clearances were obtained from the Regional Delegation of Basic Education as well as from
139 the Catholic Education Board. All pupils were issued informed assent forms to seek for their parents'
140 approval. Pupils were accepted for screening when they brought back signed assent forms following the
141 approval of their parents. They also gave their verbal assent for blood collection and had the right to
142 refuse blood collection.

143

144 Results

145 Characteristics of the study population

146 A total of 406 pupils with a mean age of 8 ± 2 years (range =4-15 years) were evaluated for malaria
147 prevalence by use of microscopy and 'CareStart™ Malaria HRP2 pf (CAT NO: G0141, ACCESSBIO)'
148 RDT. The overall prevalence of malaria in the study population was 39.9% (CI = 35.3 – 44.7) (Table 1).
149 Out of the 406 pupils examined by microscopy, 386 pupils were again examined using the CareStart™
150 RDT and the prevalence among these pupils in relation to the two methods was 40.9% (n=158, CI =36.1-
151 45.9) and 28%, (n=108, CI = 23.7-32.7) respectively.

152

153 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	Poor	368	90.6

class	Middle Class	8	2.0
	Rich	49	12.1
Total		406	100
Prevalence of fever		406	27.0
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

154

155 **Malaria prevalence and mean parasite densities by microscopy**

156 Malaria prevalence was higher in males (42.1%, CI = 35.6-48.8 than in females (37.5%, n = 72, CI = 31-

157 44.5), but the difference was not statistically significant ($\chi^2 = 2.844$, $P = 0.426$).

158 Malaria prevalence was significantly highest among the middle class (50%, CI =21.5 - 78.5) when

159 compared with their poor (42.9%, CI = 37.9-48.04) and rich (0.0%) counterparts ($\chi^2 = 7.58$, $df = 2$, $P =$

160 0.023).

161 Among malaria positive pupils, the prevalence of fever was 39.1% (n = 36, CI = 29.79-49.35). However,

162 malaria prevalence was not significantly different between those who had fever and those who did not

163 have fever ($\chi^2 = 0.34$, $df = 1$, and $P = 0.056$) as shown in table 1.

164 The GMPD in the study population was 2332.66 parasites/ μ L (CI = 218-16000). It was higher in females

165 (2463.9 parasites/ μ L) than in males (2022.7 parasites/ μ L) and the difference between males and females

166 was statistically significant ($F = 0.96 = P = .003$,) as shown in table 2. GMPDs were higher in pupils of the

167 age group 10 – 15 years (3172.50 parasites/ μ L) than in the other age groups and the difference was

168 statistically significant ($F = 5.28$, $df = 2$, $P = 0.006$) as shown in table 2. GMPD was higher in the middle

169 class (2327.3/ μ L) than in the poor and the difference was statistically significant ($F = 9.6$, $P = 0.001$)

170

171 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	$\chi^2 = 2.84$	2022.8	218 -5333	$P =$

	Females	192	37.5 (72)	31-44.5	P = .42	2463.7	615 -1600	0 .003 F = 9.6
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	

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

173

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

175 More pupils were positive by use of microscopy (40.9%, CI =36.1-45.9) than by use of RDT (27.9%, CI =
176 23.7-32.7) and the difference in positivity was statistically significant ($\chi^2 = 26.9$, P < 0.001). Conversely,
177 more pupils were negative by use of RDT than by use of microscopy (figure 1).

178

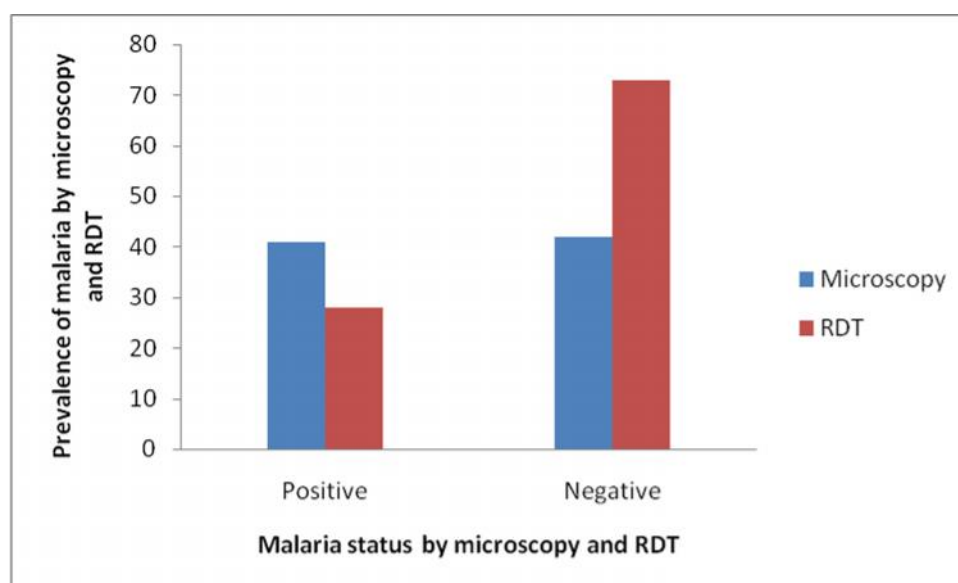


Figure 1: Prevalence of malaria by microscopy and RDT

The majority of malaria parasite infected cases detected by RDT were those with high infection (>5000T/μl). 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).

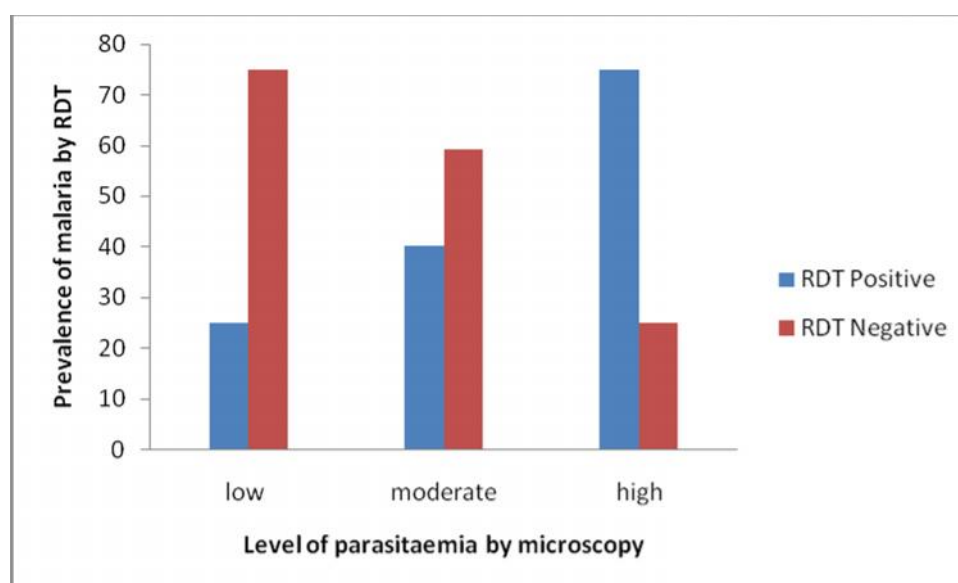


Figure 2: Malaria status by RDT in relation to mean parasite densities

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 ($\chi^2 = 16.1$, $P < 0.0001$) as shown in table 3.

Table 3: Malaria prevalence by microscopy and RDT

Malaria parasite status			95% CI in % for estimated population proportion	Test statistics
Microscopy	RDT			
	Positive % (N)	Negative % (N)		
Positive	42.1 (67) True	58.2 (92) False Negative	34.7 - 49.9	$\chi^2 = 16.1$ df = 1,

	positive	by RDT		P < 0.001
Negative	18.1 (41) False positive by RDT	82.3 (186) True negative	13.6 - 23.6	
Total	27.3 (108)	72.4 (278)	23.4 - 32.3	

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 4.

Table 4: 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%

210

211 DISCUSSION

212 This study revealed that malaria is a major public health problem in Mutengene and there are still
213 challenges in malaria diagnosis such as test duration (for microscopy) and test performance
214 characteristics (for RDT). Malaria prevalence varied significantly by use of microscopy and 'CareStart™
215 Malaria HRP2 pf (CAT NO: G0141, ACCESSBIO)' RDT. The overall prevalence of malaria in this study
216 was found to be 39.9%. The prevalence value was lower than that reported by some authors who worked
217 in neighbouring areas in the Mount Cameroon area in recent years (Nkuo-Akenji *et al.*, 2008, Achidi *et al.*,
218 2008). However, it was higher than the values reported by Kimbi *et al.* (2012, 2013) in other neighboring
219 villages in the Mount Cameroon area. The drop in malaria prevalence in the recent years is an indication
220 that malaria control strategies in line with the Abuja Declaration of 2000 on Roll Back Malaria in the past
221 few years are yielding positive results (Kimbi *et al.*, 2012).

222 In line with Kimbi *et al.* (2012), Kimbi *et al.* (2013), and Sumbele *et al.*, 2010 more males were positive
223 for malaria than females. However, in line with findings by Kimbi *et al.* (2013b), the geometric mean
224 parasite densities were higher in females than in males.

225 Malaria prevalence was significantly higher in the middle class when compared with their poor and rich
226 counterparts. This observation is contrary to the expectation and could likely be linked to the very large
227 number of pupils in the poor class (368) and the small numbers of pupils in the middle (8) and rich
228 classes (49). It is known that the rich are often more likely to afford appropriate control measures against
229 malaria (Legesse *et al.* 2007) and as such are less exposed to mosquito bites than the poor. Even when
230 they get infected with the disease, they are better able to treat themselves than the poor.

231 The highest prevalence of malaria was found in the age group 6-10 years. Children in the age group 0-5
232 years constitute a high risk group and are better monitored by the parents especially the mothers. Their
233 treatment at all health units is free of charge in Cameroon as decreed by the Head of State. Therefore,
234 any case of malaria in such children can easily be treated regardless of the social status of the parents.
235 On the other hand, children aged >10 years are generally more knowledgeable about malaria and can
236 take personal protective measures than the younger ones. The drop in prevalence of malaria with
237 increase in age could also be related to the acquisition of protective immunity due to repeated infections
238 as children grow older in high transmission areas (Kimbi *et al.*, 2013). The level of acquired immunity also
239 probably explains the highest value of GMPD observed in the youngest age group.

240 The majority of pupils examined in this study were asymptomatic and the prevalence of fever was low
241 (27.0%, CI = 20.1 -35.0). More pupils were positive by use of microscopy than by use of RDT and the
242 difference was significant (P <0.001). 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.* (2008), 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 presence of parasite antigens that could persist in the blood of the patient after chemotherapy (Ojurongbe *et al.*, 2013). 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.* (2004) 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.* (2007) 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 (Murray *et al.*, 2008). 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 (41.77% and 82.3% respectively) 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 (Murray *et al.*, 2008). 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.* (2012) 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.* (2012) could have been as a result of the fact that the two

RDTs detected different antigens. Again, Heutmekers *et al.* (2012) 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.* (2013) in Nigeria with a sensitivity of 90.2% and a Specificity of 95.4%. The high sensitivity and specificity recorded by Sani *et al.* (2013) 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.* (2012) 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.* (2013).

Elizabeth *et al.* (2009) 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.* (2013) 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.* (2013) 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 (62.26% and 66.9% respectively) were higher than those recorded by Adugna *et al.* (2013) 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.

CONFLICT OF INTEREST: none

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.

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.

References

Achidi EA, Apinjo TO, Mbunwe E, Besingi R, Yafi C, Wenjighe A, Ajua A and Anchang JK. Febrile status, malarial parasitaemia and gastro-intestinal helminthiases in schoolchildren resident at different altitudes, in south-western Cameroon. *Annals of Tropical Medicine & Parasitology*. 2008;102(2):103–118.

Amexo M, Tolhurst R, Barnish G, and Bates I. "Malaria misdiagnosis: effects on the poor and vulnerable," *The Lancet*. 2004;364 (9448):1896–1898.

Cheesbrough M. District laboratory practice in tropical countries part1 and 2. Cambridge low price editions. Cambridge University press. 2010. 454pp

Heutmekers M, Philippe G, Lieselotte C, Emmanuel B, Marjan VE, Jessica M and Jan J. Evaluation of the malaria rapid diagnostic test SDFK90: detection of both PfHRP2 and Pf-pLDH. *Malaria Journal* 2012;11:359 <http://www.malariajournal.com/content/11/1/359>

Kimbi HK, Sumbele IUN, Nweboh M, Anchang-Kimbi JK, Lum E, Nana Y, Ndip LM, Njom H and Lehman LG Malaria and haematologic parameters of pupils at different altitudes along the slope of Mount Cameroon: a cross-sectional study *Malaria Journal* 2013a, 12:193

Kimbi HK, Keka FC, Nyabeyeu HN, Ajeagah HU, Tonga CF, et al. An Update of Asymptomatic *Falciparum* Malaria in School Children in Muea, Southwest Cameroon. *J Bacteriol Parasitol* 2012;3:154. doi:10.4172/2155- 9597.1000154

Kimbi HK, Nana Y, Sumbele IN, Anchang-Kimbi JK, Lum E, Tonga C, Nweboh M and Lehman L. Environmental Factors and Preventive Methods against Malaria Parasite Prevalence in Rural Bomaka and Urban Molyko, Southwest Cameroon. *J Bacteriol Parasitol*. 2013 b:4:162. <http://dx.doi.org/10.4172/2155-9597.1000162>

- 338
- 339 Kosack C, Naing WT, Pirious E, Shanks L. Routine parallel diagnosis of malaria using microscopy and
340 the malaria rapid diagnostic test SD 05FK60: The experience of Médecins Sans Frontières in
341 Myanmar. *Malaria Journal*. 2013;12:167. <http://www.malariajournal.com/content/12/1/167>
- 342 Legesse Y, Tegegn A, Belachew T, Tushune K: Knowledge, attitude and practice about malaria
343 transmission and its preventive measures among households in urban areas of Assosa zone,
344 Western Ethiopia. *Ethiop J Health Dev* 2007, 21: 2
- 345
- 346 Moody A. "Rapid diagnostic tests for malaria parasites," *Clinical Microbiology Reviews*. 2002;15 (1):66–
347 78,
- 348 Murray CK, Gasser RA, Magill AJ and Miller RS. Update on Rapid Diagnostic Testing for Malaria. *Clinical*
349 *microbiology reviews*. 2008;21 (1): 97–110.
- 350 Murray CK, Gasser R A, Alan J, Magill J and Miller RS. Update on Rapid Diagnostic Testing for Malaria.
351 *Infectious Disease Service, Brooke Army Medical Center, Fort Sam Houston, Texas1; General*
352 *Internal Medicine Service, Clinical Microbiology Reviews*. 2008;21:97–110. No. 10893-
353 8512/08/\$08.00_0 doi:10.1128/CMR.00035-07.
- 354 Nkuo- Akenji T K., Sumbele I, Mankah EN, Njunda AL, Samje M and Kamga L. The burden of malaria
355 and malnutrition among children. *African Journal for Food, Agriculture, Nutrition and*
356 *Development*. 2008: 8(3):252-264.
- 357 Ojurongbe O, Olunike OA, Sunday ST, Oyeboade A, Terry A, Olugbenga AO, Taiwo A O, Oloyede SB,
358 and Oluwaseyi AA. Assessment of Clinical Diagnosis, Microscopy, Rapid Diagnostic Tests, and
359 Polymerase Chain Reaction in the Diagnosis of *Plasmodium falciparum* in Nigeria. *Malaria*
360 *Research and Treatment*. 2013:2013, Article ID 308069, 5 pages,
361 <http://dx.doi.org/10.1155/2013/308069>
- 362 Sani UM, Jiya NM, Ahmed H. Evaluation of a malaria rapid diagnostic test among febrile children in
363 Sokoto, Nigeria. *International journal of medicine and Biomedical Sciences*. 2013: 3(5):434-440.
- 364 Tjitra E, Suprianto S, Dyer M, Currie JB, and Anstey NM. Field Evaluation of the ICT Malaria P.f/P.v
365 Immunochromatographic Evaluation for Detection of *Plasmodium falciparum* and *P. vivax* in
366 Patients with a Presumptive Clinical Diagnosis of Malaria in Eastern Indonesia. *Journal of Clinical*
367 *Microbiology*. 1999;37 (8): 2412-2417.

- 368 Wanji S, Kimbi HK, Eyong JE, Tendongfor N and Ndamukong JL. Performance and usefulness of the
369 Hexagon Rapid Diagnostic Test in Children with Asymptomatic malaria living in the Mount
370 Cameroon Region. *Malaria Journal*. 2008; 8(7): 643-649.
- 371 WHO. Severe and complicated malaria. *Transactions of the Royal Society of Tropical Medicine and*
372 *Hygiene*. 2000; 94 (1): 1–90.
- 373 Wongsrichanalai C, Barcus MJ, Muth S, Sutamihardja A, and Wernsdorfer WH. “A review of malaria
374 diagnostic tools: microscopy and rapid diagnostic test (RDT),” *The American Journal of Tropical*
375 *Medicine and Hygiene* 2007; 77(no. 6, supplement):119–127.
- 376 World Malaria Report (2005, 2007, 2009, 2010 and 2011.
377 <http://www.nobelprize.org/educational/physics/microscopes/fluorescence/index.html>
- 378 Juan Y, Nana L, Xu Z, Peipei L, Zhenjun Z, Lili W, Siying L, Xiaomei L, Ying W, Shuying L, Zhaoqing Y,
379 Bin Z, Guofa Z, Guiyun Y, Liwang C, Yaming C and Qi F. Performance of two rapid diagnostic
380 tests for malaria diagnosis at the China-Myanmar border area. *Malaria Journal*. 2013;12:73.
- 381 Ashley Elisabeth, Touabi Malek, Ahrer Margareta, Hutagalung Robert, Htun Khayae, Lwin Min Myo,
382 Koscalova Alena, Comte Eric, Hamade Prudence, Anne-Laure, Luchavez Page Jennifer, Proux
383 Stephane, Nosten Francois, Guerin Philippe. Evaluation of 3 rapid diagnostic tests (CareStart[®]
384 Malaria 3 line pLDH (Pan, Pf), OptiMAL-IT[®] pLDH (Pan, Pf) and CareStart[®] 2 line pLDH (Pan)
385 for the diagnosis of malaria in Myanmar. *Malaria Journal*. 2009;8:241
- 386 Xiaodong ST, Ambo E, Chun W, Zhibin C, Yan D, Jian W, Jiazhi W, Xiaonong Z. Diagnostic performance
387 of CareStart[™] malaria HRP2/pLDH (Pf/pan) combo test versus standard microscopy on
388 falciparum and vivax malaria between China-Myanmar endemic borders. *Malaria Journal*. 2013
389 Jan 7;12:6. doi: 10.1186/1475-2875-12-6.
- 390 Adugna Woyessa, Wakgari Deressa, Ahmed Ali, and Bernt Lindtjörn Evaluation of CareStart[™] malaria
391 Pf/Pv combo test for *Plasmodium falciparum* and *Plasmodium vivax* malaria diagnosis in Butajira
392 area, south-central Ethiopia. *Malaria Journal* 2013, 12:218 doi:10.1186/1475-2875-12-218
- 393 Abanyie AF. Arguin PM., and Gutman J. “State of malariadiagnostic testing at clinical laboratories in the
394 United States, 2010: a nationwide survey,”*Malaria Journal*, vol. 10, no. 1, article 340, 2011.
395 Sumbele IUN, Nkuo-Akenji T, Samje M, Ndzeize T, Ngwa EM and Titanji VPK. Haematological
396 changes and recovery associated with treated and untreated *Plasmodium falciparum* infection in
397 children in the Mount Cameroon Region. *Journal of Clinical Medicine and Research*. 2010;2(9):
398 143-151.