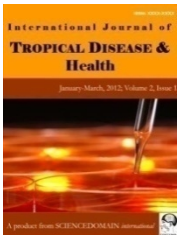

International Journal of TROPICAL DISEASE & Health
4(9): 1011-1023, 2014

SCIENCEDOMAIN *international*
www.sciencedomain.org

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

Judith Lum Ndamukong-Nyanga[1,2*], Helen K. Kimbi[2], Irene Ule Ngole Sumbele[2], Lum Emmaculate[1,2], Malaika Nain Nweboh[2], Yannick Nana[2], Sunjo Cyrilla Bertek[3] and Kenneth J. N. Ndamukong[1]

[1]Department of Biological Sciences, Higher Teachers Training College, University of Yaoundé I, Yaoundé, Cameroon.
[2]Department of Zoology and Animal Physiology, Faculty of Science, University of Buea, P.O.Box 63, Buea, SWR, Cameroon.
[3]Saint Francis Higher Institute of Medical and Biomedical Sciences (Aka Biaka), P.O.Box 77, Buea, SWR, Cameroon.

Authors' contributions

This work was carried out in collaboration between all authors. Author JLNN designed the study, wrote the protocol, carried out field and laboratory work and wrote the first draft of the manuscript. Author HKK designed the study, wrote the protocol, carried out field work, read and corrected the manuscript. Author IUNS performed the statistical analysis, read and corrected the manuscript. Authors LE, MNN, YN, SCB and KJNN participated in the data collection and literature searches. All authors read and approved the final manuscript.

Original Research Article

Received 9th April 2014
Accepted 30th June 2014
Published 1st August 2014

ABSTRACT

Aim: The aim of this study was to determine the prevalence and density of malaria parasites in asymptomatic school children in Mutengene and evaluate the performance

**Corresponding author: Email: ndamju@yahoo.com;*

characteristics of the 'CareStart™ Malaria HRP2 pf (CAT NO: G0141, ACCESSBIO)' rapid diagnostic test (RDT) using light microscopy as a gold standard.
Study Design: The study was a cross-sectional survey.
Place and Duration of Study: The study was carried out in Mutengene, from February to March, 2013.
Methodology: A total of 406 pupils were studied. Demographic data was taken for each child and capillary blood was collected. Blood films were prepared for the assessment of parasite density and speciation. A drop of blood was used on the RDT to determine the malaria status.
Results: 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% (162) by microscopy. The geometric mean parasite density (GMPD) was 2332.7 parasites/μL (range: 218 - 16000). Only 386 pupils were examined by both methods. 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 (χ^2 = 16.1, P <0.0001). The majority of those detected had high infection (≥ 5000 parasite/μL). 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 significant (χ^2 = 10.09, P = 0.006). The RDT showed a low sensitivity of 48.5% (CI = 40.3 – 56.9%) and specificity of 84.0% (CI = 80.0- 88.2%).
Conclusion: More research needs to be done on the RDT to improve on its performance characteristics before it could be used in mass surveillance programmes.

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

1. INTRODUCTION

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 travellers all contribute to presenting the laboratory with diagnostic problems for which it may have little expertise available [2].

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 a 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 [4,5,6]. 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 *P. falciparum* and it is responsible for more than 90% of all malaria cases in the country [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/μL 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 Ojurongbo 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^{TM} 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 [14,15].

2. 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 09º 18' 29" E and latitude 04º 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 18ºC - 29ºC. 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 480 pupils in the school 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 the 406 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. [15] and Nyanga et al. [16]. The axillary temperature of each child was measured using a clinical thermometer. Fever was defined as a temperature ≥37.5ºC. Blood was collected from each pupil by pricking the finger for the detection of malaria parasites by microscopy and RDT.

2.4 Collection, Preparation and Examination of Blood Samples

Demographic data such as the name, sex and 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 *P. falciparum* 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 microscopists 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^{TM} 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]. Speciation was done using the diagnostic aids of Cheesbrough [7] and only pure *P. falciparum* cases were used in comparing the two diagnostic methods.

2.5 Principle of the CareStart^{TM} RDT and the test kit composition

The 'CareStart^{TM} Malaria HRP2 pf (CAT NO: G0141, ACCESSBIO)' RDT is a chromatographic test for *in vitro* diagnosis that makes use of the HRP2 of *P. falciparum*. It has a long shelf-life of more than one year, with storage conditions ranging from 1ºC to 40ºC. 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 Case Definitions

According to Cheesbrough [7], true positives (TP) are positive RDT cases which are also positive by microscopy and are used to determine the sensitivity of the test. False positives (FP) are positive cases by RDT (but which are negative by microscopy) due to persistence of parasite antigens following treatment or the presence of other substances. True negatives (TN) are cases that are negative by both methods [6]. False negatives (FN) are those cases that are found to be negative by RDT but positive by microscopy. FN gives the difference needed to make a sensitivity of 100% e.g. 95% sensitivity implies 5% false negatives [7].

2.7 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 ± standard deviations (SD) were compared using t-test and analysis of variance (ANOVA) where appropriate. The malaria parasite density was log transformed before analysis. Proportions were compared using chi-square (χ^2). Significant levels were measured at 95% confidence intervals and values were considered significant at $P < 0.05$. Sensitivity and specificities were calculated using the formulae below:

Sensitivity = TP/ (TP+FN).
Specificity = TN/ (TN+FP).
Positive predictive value = TP/ (TP+FP)
Negative predictive value = TN/ (TN+FN). The values obtained were expressed as percentages by multiplying by 100 [6].

3. RESULTS

3.1 Characteristics of the Study Population

A total of 406 pupils with a mean age of 8±2 years (range = 4 - 15 years) were evaluated for malaria prevalence by use of microscopy. The overall prevalence of malaria in the study population was 39.9% (CI = 35.3 – 44.7) as shown in Table 1. *P. falciparum* constituted 97.5% of the infections while the rest were mixed infections *of P. falciparum* and *P. malariae.* Out of the 406 pupils examined by microscopy only 386 were examined using the CareStart™ Malaria HRP2 pf (CAT NO: G0141, ACCESSBIO) RDT. Twenty of the pupils did not succumb to blood collection for the RDT. The prevalence of malaria by RDT was 28% (n = 108, CI = 23.7 - 32.7).

3.2 Malaria Prevalence and GMPD by Microscopy

Malaria prevalence and GMPD were similar with respect to sex (Table 2).The GMPD in the study population was 2332.7 parasites /µL. It was higher in females (2463.9 parasites /µL) than males (2022.7 parasites /µL) and the difference was not statistically significant. GMPD was highest in pupils of the age group 10 – 15 years (3172.50 parasites/µL) than in the other age groups and the difference was statistically significant (F = 5.28, P = 0.006).

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
Category		**Number positive**	**%**
Prevalence of fever (for the 406 pupils)		110	27.1
Malaria parasite prevalence (overall for 406 pupils) by microscopy		162	39.9
Malaria parasite prevalence by RDT		108	28

Table 2. Malaria prevalence and GMPD as affected by sex and age

Factor	Category	N	Malaria prevalence % (n)	GMPD/µL of blood	Range
Sex	Males	214	42.1 (90)	2022.8	218 - 333
	Females	192	37.5 (72)	2463.7	615 -1600
Test	$\chi^2 = 2.84$			t = -1.72	
P- value	P = 0 .42			P = 0.08	
Age in years	0 - 5	90	26.7 (24)	2544.2	600 - 5333
	6 - 10	246	45.5 (112)	1969.2	218 - 5600
	11 - 15	70	37.1 (26)	3172.5	1600 -16000
Total		406	40 (162)	2332.7	218 -16000
Test	$\chi^2 = 18$			ANOVA = 5.28	
P- value	P = 0.004			P = 0.006	

With reference to social class, none of the pupils in the rich class was infected. Malaria prevalence was significantly higher ($\chi^2 = 7.58$, P = 0.023) among the middle class (50%, CI = 21.5 - 78.5) when compared with their poor counterparts (42.9%, CI = 37.9 - 48.04). The GMPD was higher in the middle class (2327.3/µL) than in the poor (2068.8/µL) and the difference was statistically significant (ANOVA = 9.6, P = 0.001) as shown in Fig. 1.

3.3 RDT Status in Relation to Malaria Prevalence by Microscopy and Mean Parasite Densities

Out of the 386 pupils examined by both microscopy and RDT, 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 < 0.001) as shown in Fig. 2.

The majority of malaria parasite infected cases detected by RDT were those with high infection (≥5000 parasites/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 RDT as shown in Fig. 3. The difference in malaria

prevalence by RDT in relation to parasite densities was statistically significant (χ^2= 10.09, P = 0.006).

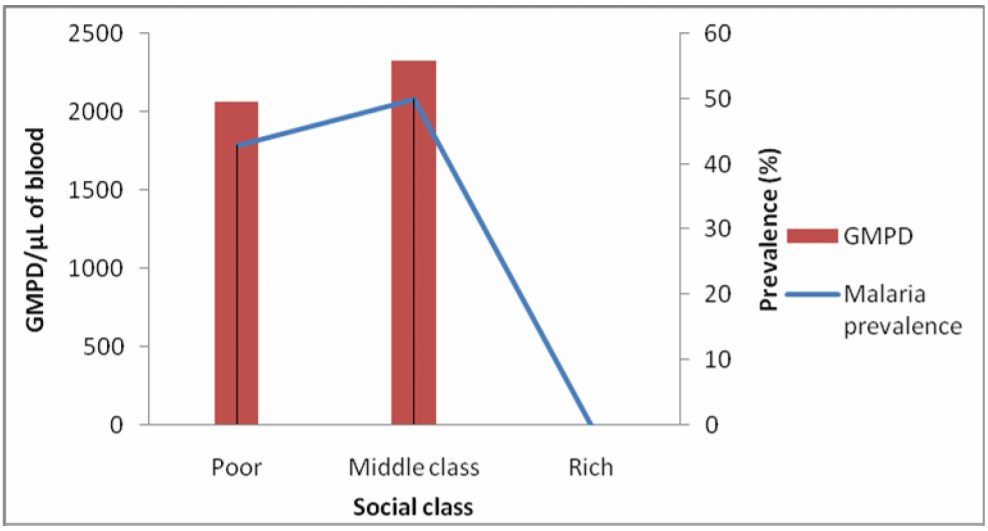

Fig. 1. GMPD and prevalence of malaria parasites in relation to socio-economic status of pupils

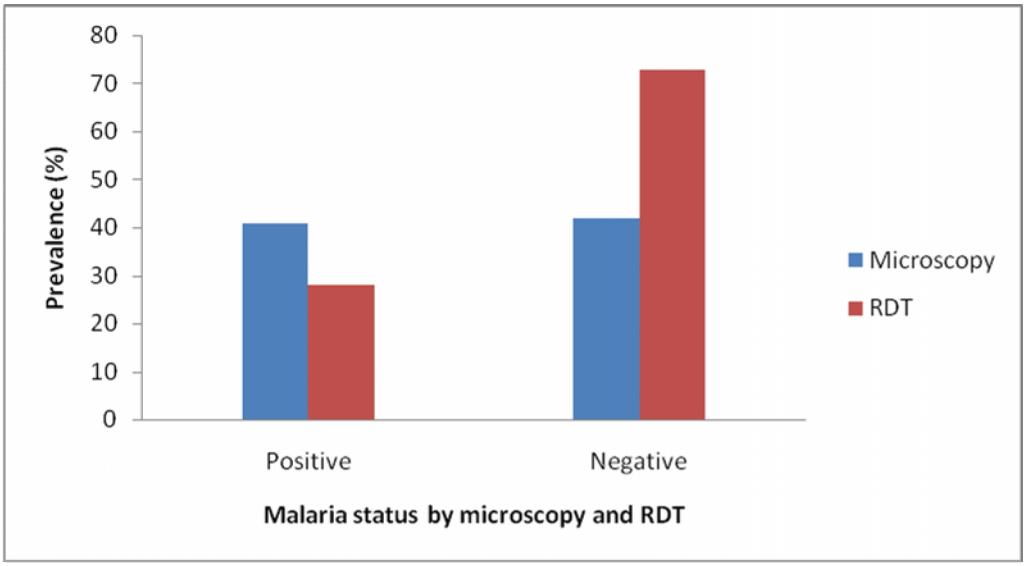

Fig. 2. Overall prevalence of malaria by microscopy and RDT

3.4 False Positive, False Negative, True Positive and True Negative Malaria Cases

Of the 158 pure *P. falciparum* cases diagnosed by microscopy, only 66 [41.8%, CI = 34.6 – 49.9; estimated population odds (EPO) = 0.73, CI= 0.53 - 0.99] were TP. The number of FP cases in the study population was 40 (17.7%, CI= 13.28 - 23.2; EPO = 0.22, CI = 0.15 -

0.30) while the number of FN was 70 (22.3%, CI =18.0 - 27.2, EPO = 0.29, CI = 0.22 – 0.37) and the difference was statistically significant (χ^2 = 16.1, P < 0.0001).

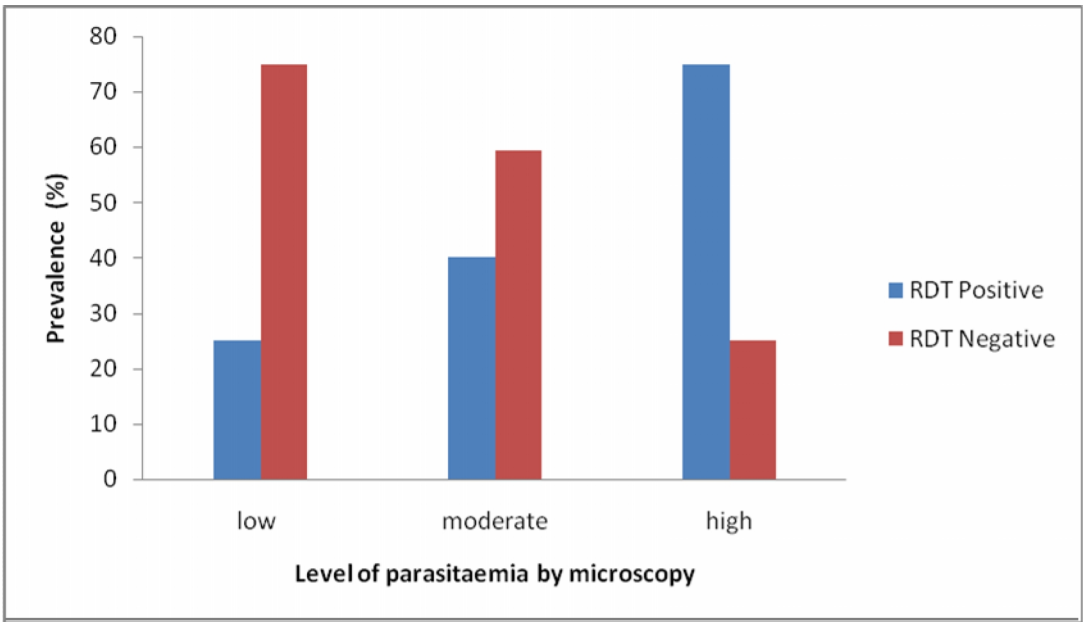

Fig. 3. Malaria status by RDT in relation to mean parasite densities

3.5 Sensitivity and Specificity of the RDT

The sensitivity of the RDT was 48.5% (CI = 40.3 – 56.9) when compared with microscopy. The specificity of the RDT was 84.0% (CI = 79.0 – 88.0). The positive and negative predictive values were 62.3% (CI = 52.8 - 70.9) and 75.0% (CI = 69.6 – 79.7) respectively as indicated in Table 3.

Table 3. Performance characteristics of the RDT

Performance characteristic of RDT	Effective performance	95% Confidence interval
Sensitivity	48.5%	40.3 - 56.9%
Specificity	84%	79.0 – 88.0%
Negative likelihood ratio	3.0	2.2 – 4.2
Positive likelihood ratio	0.6	0.5 – 0.7
Diagnostic odds ratio	5.0	3.1 – 8.0
Positive predictive value	62.3%	52.8 - 70.9%
Negative predictive value	75.0%	69.6 – 79.7%

4. 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^{TM} 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 [18,19]. However, it was higher than the values reported by Kimbi et al. [8,15] in other neighbouring villages in the Mount Cameroon area. The drop in malaria prevalence in 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 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 [15,21]. The low 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 cases 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/μL 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] when compared with those in this study could be due to the fact that patients presenting with signs of clinical malaria were used while in this study asymptomatic pupils were studied. 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 may 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 [CareStartÔ Malaria 3 line pLDH (Pan, Pf), OptiMAL-IT® pLDH (Pan, Pf) and CareStartÔ 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 Woyessa et al. [27] in South Central Ethiopia on CareStart™ malaria Pf/Pv combo test for *P. falciparum* and *P. vivax.* They reported seasonally varying RDT precision 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.

5. CONCLUSION

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.

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/guardians.

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.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Tjitra E, Suprianto S, Dyer M, Currie JB, Anstey NM. Field evaluation of the ICT malaria P.f/P.v Immunochromatographic evaluation for detection of *Plasmodium falciparum and P. vivax* in patients with a presumptive clinical diagnosis of malaria in Eastern Indonesia. J Clin Microbiol. 1999;37(8):2412-2417.
2. Moody A. Rapid diagnostic tests for malaria parasites, Clin Microbiol Rev. 2002;15 (1):66–78.
3. Murray CK, Gasser RA, Alan J, Magill J, Miller RS. Update on rapid diagnostic testing for malaria. ClinMicrobiol Rev. 2008;21:97-110.
4. Abanyie AF. Arguin PM., Gutman J. State of malaria diagnostic testing at clinical laboratories in the United States, 2010: a nationwide survey. Malar J. 2011;10:340.
5. Juan Y, Nana L, Xu Z, Peipei L, Zhenjun Z, Lili W, et al. Performance of two rapid diagnostic tests for malaria diagnosis at the China-Myanmar border area. Malar J. 2013;12:73.
6. Ojurongbe O, Olunike OA, Sunday ST, Oyebode A, Terry A, Olugbenga AO, et al. Assessment of clinical diagnosis, microscopy, rapid diagnostic tests, and polymerase chain reaction inthe diagnosis of *Plasmodium falciparum* in Nigeria. Malaria Res Treat. 2013;2013:308069.
7. Cheesbrough M. District laboratory practice in tropical countries part 1 and 2. Cambridge low price editions. Cambridge University press; 2010.
8. 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.
9. WHO. Severe and complicated malaria. Trans R Soc Trop Med Hyg. 2000;94 (1):1-90.

10. Wanji S, Kimbi HK, Eyong JE, Tendongfor N, Ndamukong JL. Performance and usefulness of the Hexagon rapid diagnostic testin children with asymptomatic malaria living in the Mount Cameroon Region. Malar J. 2008;8(7):643-649.
11. Heutmekers M, Philippe G, Lieselotte C, Emmanuel B, Marjan VE, Jessica M, Jan J. Evaluation of the malaria rapid diagnostic testSDFK90: detection of both PfHRP2 and Pf-pLDH. Malar J. 2012;11:359.
12. Kosack C, Naing WT, Pirious E, Shanks L. Routine parallel diagnosis of malaria using microscopy and the malaria rapid diagnostic test SD 05FK60: The experience of Médecins Sans Frontières in Myanmar. Malar J. 2013;12:167.
13. Sani UM, Jiya NM, Ahmed H. Evaluation of a malaria rapid diagnostic test amongfebrile children in Sokoto, Nigeria. Int J med Biomed Sci. 2013;3(5):434-440.
14. Ameh J**,** Ahmad RM, Ekeh N, Linga P, Mangoro Z, Imam SU, Akeredolu P Hudu S. Laboratory diagnosis of malaria: comparing Giemsa stained thick blood films with rapid diagnostic test (RDT) in an endemic setting in North West Nigeria. J Med Lab Diagn. 2012;3(2):10-15
15. Kimbi HK, Sumbele IUN, Nweboh M, Anchang-Kimbi JK, Lum E, Nana Y, et al. Malaria and haematologic parameters of pupils at different altitudes along the slope of Mount Cameroon: a cross-sectional study. Malar J. 2013;12:193.
16. Nyanga JLN, Kimbi HK, Sumbele IUN, Lum E, Nweboh MN, et al. Socio-demographic and environmental factors influencing asymptomatic malaria and anaemia incidence among school children in Fako Division, South West Cameroon. BJMMR. 2014;4(20):3814-3827.
17. Allen SJ, Bennet S, Ridley EM, Rowe PA, Jakobsen PH, O'Donnel A, Greenwood BM. Morbidity from malaria and immune responses to define*Plasmodium falciparum* antigens in children with sickle cell trait in the Gambia. Trans R Soc Trop Med Hyg.1992;86:494-498.
18. Nkuo- Akenji TK, Sumbele I, Mankah EN, Njunda AL, Samje M, Kamga L. The burden of malaria and malnutrition among children. AJFAND. 2008;8(3):252-264.
19. Achidi EA, Apinjo TO, Mbunwe E, Besingi R, Yafi C, Wenjighe A, Ajua A, Anchang JK. Febrile status, malarial parasitaemia and gastro-intestinal helminthiases in school children resident at different altitudes, in South–Western Cameroon. Ann Trop Med Parasitol. 2008;102(2):103-118.
20. Sumbele IUN, Nkuo-Akenji T, Samje M, Ndzeize T, Ngwa EM, Titanji VPK. Haematological changes and recovery associated with treated and untreated *Plasmodium falciparum* infection in children in the Mount Cameroon Region. J Clin Med Res. 2010;2(9):143-151.
21. Kimbi HK, Nana Y, Sumbele IN, Anchang-Kimbi JK, Lum E, Tonga C, et al. Environmental factors and preventive methods against malaria parasite prevalence in rural Bomaka and urban Molyko, Southwest Cameroon. J Bacteriol Parasitol. 2013;4:162.
22. Legesse Y, Tegegn A, Belachew T, Tushune K. Knowledge, attitude and practice about malaria transmission and its preventive measures among households in urban areas of Assosa zone, Western Ethiopia. Ethiopian J Health Dev. 2007;21:157.
23. Amexo M, Tolhurst R, Barnish G, Bates I. Malaria misdiagnosis: effects on the poor and vulnerable. Lancet. 2004;364(9448):1896–1898.
24. Wongsrichanalai C, Barcus MJ, Muth S, Sutamihardja A, Wernsdorfer WH. A review of malaria diagnostic tools: microscopy and rapid diagnostic test (RDT). Am J Trop Med Hyg. 2007;77(6 suppl):119-127.

25. Ashley E, Touabi M, Ahrer M, Hutagalung R, Htun K, Lwin MM, et al. Evaluation of 3 rapid diagnostic tests (CareStartÔ malaria 3 line pLDH (Pan, Pf), OptiMAL-IT® pLDH (Pan, Pf) and CareStartÔ 2 line pLDH (Pan) for the diagnosis of malaria in Myanmar. Malar J. 2009;8:241.
26. Xiaodong ST, Tambo E, Chun W, Zhibin C, Yan D, Jian W, et al. Diagnostic performance of CareStart™ malaria HRP2/pLDH (Pf/pan) combo test versus standard microscopy on falciparum and vivax malaria between China-Myanmar endemic borders. Malar J. 2013;7:126.
27. Woyessa A, Deressa W, Ali A, Lindtjørn B. Evaluation of CareStart™ malaria Pf/Pv combo test for *Plasmodium falciparum* and *Plasmodium vivax* malaria diagnosis in Butajira area, south-central Ethiopia. Malar J. 2013;12:218.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=597&id=19&aid=5610