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Comparison of the Partec CyScope® Rapid Diagnostic Test with Light Microscopy for Malaria Diagnosis in Rural Tole, Southwest Cameroon

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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 SCB, KL, KNL, MKG, NYAL, NNH, ANPB, MSM, CT, KJNN and LGL participated in the data collection and literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Introduction/Aim: Malaria is a major public health problem and can lead to fatal consequences within few days if not diagnosed and promptly treated. The aim of this study was to determine the malaria parasite prevalence and assess the performance characteristics of the Partec CyScope® rapid diagnostic test (RDT) in Tole.

Experimental Design, Place and Duration of Study: The study was a cross-sectional survey, carried out in Tole, Southwest Cameroon in July 2014.

Methodology: A total of 231 children were studied. Information on demographic data, temperature and malaria risk factors was recorded. Capillary blood was collected by finger pricking. Thick and thin blood films were prepared for malaria parasite detection and speciation. Ten µL of blood was added unto the DAPI coated slides and read under the Partec CyScope®. Haemoglobin values were determined.

Results and Conclusion: The overall prevalences of malaria parasites, fever and anaemia were 66.2%, 35.9% and 86.6% respectively. Although not statistically significant, malaria parasite prevalence was highest in children aged 1 – 5 years, higher in females, those that had stagnant water and bushes around their homes as well as those who did not use insecticide-treated bed nets and insecticide residual spraying when compared with their respective counterparts. Overall geometric mean parasite density (GMPD) was 3691 (range = 100 - 48000) parasites/µL of blood). GMPD was significantly higher (*P* = 0.03) in febrile than afebrile children. Prevalence of anaemia was significantly higher (P = 0.01) in malaria positive (68.5%) than negative (45.2%) children. More cases of infections were detected by light microscopy than by Partec CyScope®. The sensitivities and specificities of Partec CyScope® were 87.6% (CI = 81.4-91.1%) and 94.9% (CI = 87.5-98.0%) respectively while the positive and negative predictive values were 97.1% and 79.6% respectively. Partec CyScope® can therefore be used for mass malaria surveillance.

Keywords: Malaria diagnosis; partec CyScope® rapid diagnostic test; performance characteristics; Cameroon.

1. INTRODUCTION

Malaria is a parasitic disease that is endemic in sub-Saharan Africa including Cameroon where the disease is prevalent throughout the year [1]. If it is not diagnosed and treated on time, the disease can lead to fatal consequences within a few days. As such rapid and efficient diagnosis needs to be made [2]. Sometimes malaria is diagnosed based on the patient's symptoms and physical findings such as enlarged spleen at examination especially in children in resource-poor countries [3]. These clinical assessments are often not specific and can affect the medical care provided if the wrong diagnosis is made [4]. Thus, early clinical findings need to be confirmed by a laboratory test. Slide microscopy is a key element in parasitological diagnosis of malaria, both for clinical care and quality control of malaria [5]. Light microscopy (LM) therefore remains a gold standard for malaria diagnosis despite some disadvantages such as inability to detect parasites at low parasitaemia, is time consuming, requires electricity, and elaborate training to produce results [6]. Rapid diagnostic tests (RDTs) which counter these disadvantages have thus been developed and they focus on the detection of malaria antigens or antibodies [7,8], or malaria DNA or entire parasites in red blood cells [9]. Such tests provide results in 2 to 15 minutes. Two RDTs are most widely used in Cameroon - the 'CareStart™ Malaria HRP2 pf [10], and the Partec CyScope® [11].

The Partec CyScope® falls within a new generation of fluorescent microscopes and uses a fluorescent stain called 4'-6 Di Amidino -2-Phenyl Indole (DAPI) that stains cellular DNA [12]. It is based on the principle that some molecules can absorb light rays of certain wavelengths and then re-emit light waves whose wavelength falls within the visible region of the electromagnetic spectrum [9]. The use of RDT does not completely eliminate the need for malaria light microscopy because there are risks of obtaining false-negative RDT results [13]. Kimbi et al. [11] reported a sensitivity of >80% for Partec CyScope® in a semi urban area in the Mount Cameroon region, Muea. As time goes on improvements in RDTs could lead to improved performance, necessitating an assessment of performance characteristics for each RDT over time and in different settings. On the other hand, if the performance characteristics of RDTs are

not checked frequently, dropping standards in production may set in leading to reduced performance characteristics. There is therefore the need to assess the performance characteristics of the Partec CyScope® diagnostic technique especially in a rural setting like Tole.

Tole is a village in the Mount Cameroon area which never had a health centre before June 2014, although it hosts the labourers of the Tole Tea Plantation, a major tea plantation in Southwest Cameroon. Although some work has been reported on malaria in several areas in the Mount Cameroon area [14-17] none has specifically focused on Tole village. There is therefore the need to assess malaria prevalence in this area in relation to various predisposing factors so that effective control measures can be put in place to help curb the burden of malaria. Thus, the objectives of this study were to determine the malaria parasite prevalence and density in relation to various predisposing factors and investigate the performance characteristics of the Partec Cyscope® diagnostic test.

2. MATERIALS AND METHODS

2.1 Study Site

The study was carried out in Tole village, Southwest Cameroon in July, 2014. Tole (situated at 578m above sea level, longitude 09º 18' 23" E and latitude 04º 05'54'' N) is found in the Mount Cameroon area. The inhabitants are mainly tea plantation workers or subsistence farmers. Most of them are poor and live in houses made up of plank with holes and crevices in the walls. The houses are concentrated around the same area and are very close to each other. Minor roads separate the settlement into streets. 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 Participants

Following consultations with the Chief of Tole and his village councillors, community health agents were sent out for a door to door education. A date for data collection was communicated and the venue was the newly created Health Centre in Tole. On the day of sample collection, another team went out into the community to remind parents/guardians about the screening exercise. The sample population was made up of children of both sexes aged 1 - 15 years who came to the Health Centre on the appointed date, lived in Tole, had received parental/guardian informed consent/came with signed assent forms and succumbed to the blood collection procedure. The younger children were brought to the health Centre by their parents/guardians.

2.3 Study Design/Period

The study was a cross-sectional survey where blood samples were collected from children in the month of July 2014. The Chief of the village was visited a week prior to the date of data collection. The purpose and benefits of the study (free malaria diagnosis and treatment for the children) were discussed with the chief, notables in the village, community health agents and village 'town-crier' before the sampling was done. Informed consent forms explaining the purpose and benefits of the study as well as the amount of blood that had to be collected from each child were sent to parents/guardians through the community health agents. Only children who brought back signed informed consent/assent forms on the appointed day of the screening, accompanied by parents or guardians, were attended to at the village Health Centre and were thus included in the study.

A simple semi-structured questionnaire was administered in English (and exceptionally in Pidgin English) to children or parents/guardians to obtain data on each child's name, sex and age while the socio-economic status of pupils were determined as indicated by Kimbi 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 child by pricking the finger for the detection of malaria parasite by microscopy and Partec CyScope®. Haemoglobin measurements were done by use of a haemoglobinometer (URIT 12 digital haemoglobinometer).

2.4 Collection, Preparation and Examination of Blood Samples for Light Microscopy

Blood was collected from the finger prick and used to prepare thick and thin blood films on

labelled slides for the assessment of parasite density and speciation respectively as stated by Cheesbrough [18]. Using a light microscope *Plasmodium falciparum* parasites were counted against 200 leukocytes in thick films. These were used to compute the number of parasites per µL assuming a standard value for the leukocyte count to be 8,000 WBC/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 Partec CyScope®. Parasitaemia was classified as low (<500 parasite/µL of blood), moderate (501-5000 parasites/µL of blood) and high (>5000 parasites/µL of blood) according to Allen et al. [19]. All patients diagnosed with malaria were treated with Artesunate–Amodiaquine combination therapy in consultation with the health authorities of the village health centre.

2.5 Preparation and Examination of Blood Samples by Partec CyScope®

The supplied microscope slides were pre-coated with the fluorochrome 4'-6 Di Amidino-2-Phenyl Indole (DAPI) as indicated by the manufacturer's instructions. Ten (10) µL of blood was then added unto the DAPI coat and covered using a cover slide. After incubating for ten minutes, the slides were mounted on the Partec CyScope® and read as either positive or negative as indicated by the manufacturer.

2.6 Statistical Analysis

Data was entered into spread sheets using Microsoft excel and analysed with the statistical package for social sciences (SPSS) version 17 (SPSS, Inc., Chicago, IL, USA). The analysis of variance (ANOVA) was used where appropriate to compare means and chi-square (χ^2) was used to compare proportions. Significant levels were measured at 95% confidence level (CI) with significant differences recorded 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) where TP = number of true positives, TN = number of true negatives, FP = number of false positives and FN = number of false negatives. The values obtained were expressed as percentages by multiplying by 100 [8,10].

According to Cheesbrough [18] true positives are positive RDT cases which are also positive by microscopy and are used to determine the sensitivity of the test. False positives 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 are cases that are negative by both methods (microscopy and RDT). False negatives 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.

3. RESULTS

3.1 Characteristics of the Study Population

A total of 231 children with a mean age of 6 ± 4 years (range = 1-15 years) were evaluated for malaria parasite prevalence by use of light microscopy and Partec CyScope®. More females (125) than males (106) were involved in the study. The majority of the children were of the age group 0-5 years as shown in Table 1. All the children who participated in this study were in the poor socio-economic class.

3.2 Malaria Prevalence by Light Microscopy in Relation to Sex, Age, Fever and Anaemia Status

The overall prevalence of malaria parasites, fever and anaemia in the study population was 66.2 % (CI = 59.9-72.0%), 35.9% (CI = 30.0 – 42.3%) and 86.6%, (CI = 81.6 - 90.4%) respectively. Malaria parasite prevalence was similar (χ^2 = 77.8, *P* = .45) in females (67.2%, CI = 59.4-75.5) and males (65.1%, CI = 55.6-73.5). Although not significantly different (χ^2 = 3.1, *P* = .38), malaria was most prevalent among children of the age group 0 – 5 years (72.2%) than in the age groups 6 – 10 years (61.9%) and 11-15 years (59.0%). The prevalence of malaria parasite was higher in children without fever (70.3%) than in those with fever (59.1%) although the difference was not statistically significant (χ^2 = 5.2, *P* = .07). Malaria parasite prevalence was significantly higher (χ^2 = 6.5, *P* = .01) in the children who were anaemic (68.5%) than those none anaemic (45.2%) as shown in Fig. 1.

3.3 Geometric Mean Parasite Densities (GMPDs) by Light Microscopy in Relation to Sex, Age, Fever and Anaemic Status

The geometric mean parasite density (GMPD) in the study population was 3691 (range = 100-48000) parasites/μL of blood. Although not statistically significant, the GMPDs/μL of blood was higher in females (4235) than males (3027), in pupils of the age group 0 – 5 years (5768) than in the other age groups and non-anaemic children (4116) than those who were anaemic (3648). The GMPDs were higher in children who had fever (4111 parasites/μL) than among those who were afebrile (2968 parasites/μL) and the difference was statistically significant (t = 1.02, *P* = .03) as shown in Table 2.

3.4 Malaria Prevalence by Light Microscopy in Relation to Predisposing Factors

Although not statistically significant, malaria parasite was more prevalent among those who had stagnant water (66.0%, N = 64) and bushes around their houses (71.9%, N = 69), did not use bed nets (66.7%, N = 108) or insecticide residual spraying (IRS) (79.4%, N = 177) than their respective counterparts as shown in Fig. 2.

Table 1. Baseline characteristics of the study population

Factor	Category	Number examined	Population proportion	95% CI
Sex	Males	106	45.9	39.6 –52.3
	Females	125	54.1	47.7 – 60.4
Age group (Years)	0 – 5	108	46.8	40.4 – 53.2
	6 – 10	84	36.4	30.4 – 42.7
	10 – 15	39	16.9	12.6 – 22.3
Total		231	100	

Table 2. Malaria GMPD as affected by sex, age, anaemia and fever

Factor	Category	GMPD/μL of blood)	Range	Test statistics
Sex	Males	3691	100 - 48000	t = 6.97
	Females	4235	100 - 48000	*P* = .5
Age group in years	0 – 5	3618	100 - 24000	F = 0.72
	6 – 10	4458	180 - 11600	*P* = .75
	>10	2271	200 - 10640	
Anaemia	Yes	3646	100 - 48000	t = 1.04
	No	4116	240 - 24000	*P* = .31
Fever	Yes	4110	100 - 48000	t = 1.02
	No	2968	100 - 24000	*P* = .03
Total		3690.9	100 - 4800	

N = Number of pupils examined; n = Number infected in the particular group

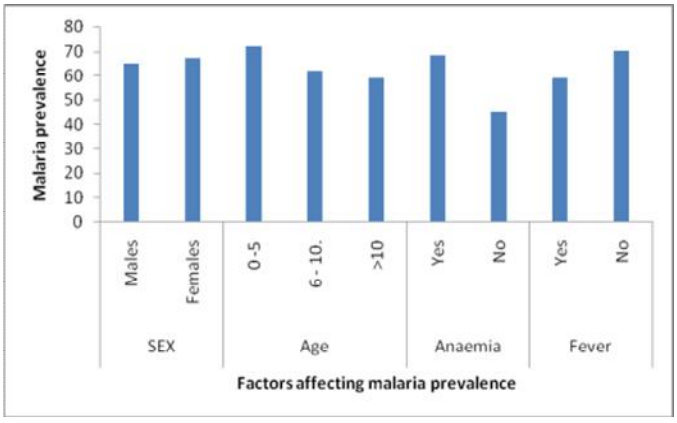

Fig. 1. Malaria prevalence by light microscopy in relation to sex, age, fever and anaemic status

3.5 Partec CyScope® Malaria Status in Relation to Malaria Prevalence and Mean Parasite Densities by Microscopy

More children were positive for malaria parasites by use of light microscopy (66.23%, CI = 59.9 - 72.0%) than by use of Partec CyScope® (59.7%, CI = 53.1-65.9%). The difference in positivity between the two methods was however not statistically significant (χ^2 = 0.627, *P* = .43). The two methods showed the same pattern with the highest cases of low parasite density infection being detected than those of moderate and high infection as shown in Fig. 3. Although not statistically significant (χ^2 = 4.3, *P* = .23), more cases of low, moderate and high infections were detected by light microscopy than were positive by use of Partec Cyscope® as shown in Fig. 3.

3.6 False Positive, False Negative, True Positive and True Negative Malaria Cases by Use of Partec CyScope®

Participants who were malaria parasite positive by use of both light microscopy and Partec CyScope® (true positives) were 134 [58.0%, CI = 51.6 – 64.2%; estimated population odds (EPO) = 1.4, CI = 1.1–1.8]. Four children (1.7%, CI = 0.7-4.4%; EPO = 0.02, CI = 0.01-0.4) were positive by use of Partec microscopy, but negative by use of light microscopy (FP). Nineteen children (8.2%, CI = 5.3 – 12.5%; EPO = 0.09, CI = 0.06–0.14%) were positive when light microscopy was used and negative when Partec CyScope® was used (FN). Seventy four children were negative by use of both Partec CyScope® and light microscopy (TN) with an estimated population proportion of 32.0% (CI = 26.3-38.2%; EPO = 47.1, CI = 35.8 – 62.0) as shown in Table 3.

3.7 Performance Characteristics of Partec CyScope®

The sensitivity of the Partec CyScope® was 87.6% (CI= 81.4-91.9%) when compared with light microscopy while the specificity was 94.9% (CI = 87.5-98.0%). The positive and negative likelihood ratios were 17.1 (CI = 6.6 – 44.4) and 0.1 (CI = 0.1 – 0.2) respectively. The positive and negative predictive values were 97.1% and 79.6% respectively (Table 4).

4. DISCUSSION

This study revealed that malaria is still a major public health problem in Tole village despite intervention strategies that have been put in place in Cameroon over the years such as the free distribution of bed nets and sensitization on radio and television. The overall malaria prevalence was 66.2% and this value is higher than the value reported by Kimbi et al. [16] in other villages in the Mount Cameroon area.

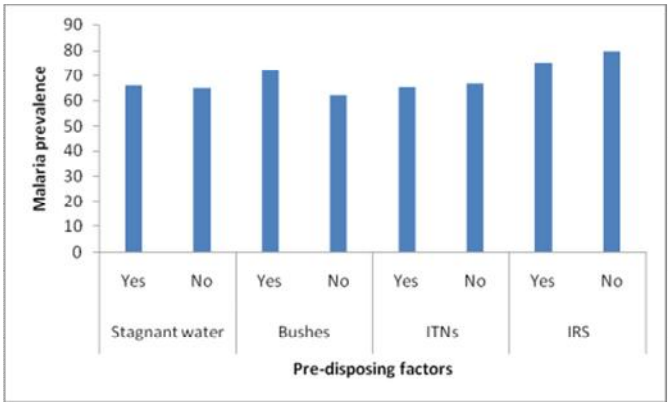

Fig. 2. Malaria prevalence by light microscopy in relation to pre-disposing factors

Table 3. False positive, false negative, true positive and true negative malaria cases by use of Patec CyScope®

Test (Partec CyScope®)	Reference or standard (light microscopy)		
	Positive	Negative	Total
Positive	134 (TP)	4 (FP)	138
Negative	19 (FN)	74 (TN)	93
Total	153	78	231

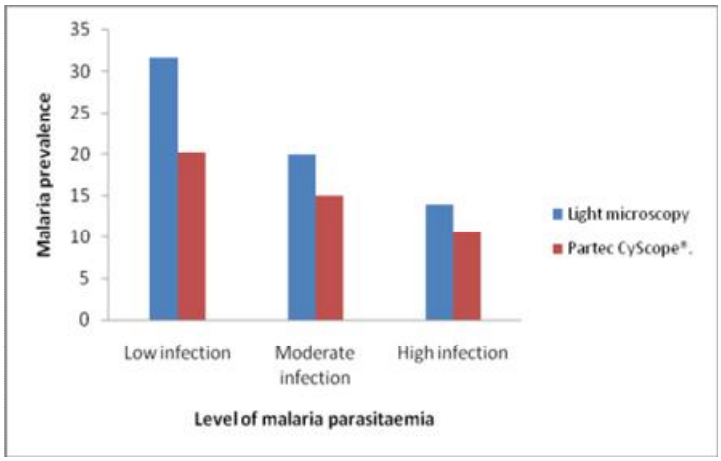

Fig. 3. Malaria status by Partec CyScope® in relation to mean parasite densities by light microscopy

Table 4. Characteristics of the Partec CyScope®

Characteristic	Effective performance	95% Confidence interval
Sensitivity	87.6%	81.4 - 91.9%
Specificity	94.9%	87.5 - 98.0%
Positive likelihood ratio	17.1	6.6 – 44.4
Negative likelihood ratio	0.1	0.1 – 0.2
Positive predictive value	91.1%	92.8 - 98.9%
Negative predictive value	79.6%	70.3- 86.5%

The higher prevalence could be highlighting some of the challenges faced by inhabitants of the village such as: lack of health centres for consultation and treatment, lack of RDT kits or techniques, unavailability of drugs, poverty leading to inability to pay for screening and treatment, or negligence on the part of the villagers (parents). It is worth noting that the first health centre in this village only became operational in June, 2014, a few days before the start of this study. Before the establishment of the Health Centre, patients had to travel long distances to neighbouring villages to seek for medical attention. This lack of medical attention might have probably led to self-medication and chronic malaria cases in the community that could have served as reservoirs for infection and consequently higher transmission rates. All the participants included in this study belonged to the poor socio-economic class and were therefore less likely to afford appropriate control measures against malaria [20]. However, the overall prevalence of malaria (by light microscopy) was lower than that reported by Nkuo-Akenji et al. [21] in Muea, a locality in the Mount Cameroon area (96.2%). The authors however attributed the high malaria prevalence to lack of education on proper treatment and control.

The highest prevalence of malaria was found in the age group 0 - 5 years. Children in this age group constitute a high risk group and need better monitoring by the parents especially the mothers. Although their treatment in all health units is free of charge in Cameroon as decreed by the Head of State [22], the absence of a health centre in the community until a few days to the start of this study probably deprived the inhabitants of Tole the right to free screening and treatment for this age group. Most of the parents are also farmers with no formal education hence they probably have limited knowledge on malaria control. This may explain the presence of many uncleared bushes around the houses that could have served as breeding sites for mosquitoes. On the other hand, older children are generally more knowledgeable about malaria and are better able to take personal protective measures than the younger ones. These measures include the active killing of mosquitoes and wearing of protective clothing leading to a reduction in man-vector contact and subsequently the number of infective bites. 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 malaria endemic areas [16]. The low level of acquired immunity also probably explains the highest value of GMPD observed in the youngest age group.

The prevalence of fever (35.9%) among the study participants indicated that both clinical and asymptomatic malaria parasitaemia were present. It is known that frequent exposure to malaria parasites leads to low grade asymptomatic parasite load. This asymptomatic malaria parasitaemia becomes symptomatic when immunity drops as a result of stress, poor nutrition or other diseases [23].

The higher prevalence of malaria in children who had anaemia probably indicate that malaria is a contributing factor to anaemia in the study population. Anaemia is the most frequent malaria complication among infants and young children living in malaria endemic areas with high levels of transmission [24]. In severe anaemia apparent loss of erythrocytes exceeds the proportion of erythrocytes infected by parasites [25]. Takem et al. [26] reported malaria parasitaemia to be responsible for 44% of anaemia in those with malaria infection. This agrees with findings of Sumbele et al. [27]. Anaemia among the malaria negative children might have been due to malnutrition and other parasitic infections like intestinal helminths. It has been reported that high levels of poverty in most African countries and reliance on subsistence farming by the majority of the population increases vulnerability to disease prevalence and that anaemia is more common among the poor than the rich [28].

Malaria was more prevalent among children who did not use bed nets, insecticide sprays in the houses or had bushes and stagnant water around their houses indicating that the application of vector control measures has a vital role in the control of malaria as they prevent vector-man contact and consequently transmission and decreased disease burden [29]. Poor vector control leads to increased mosquito bites and an increased burden of the disease. According to WHO [30], each vector species inter-relates with its environment to be able to survive and in doing so transmits disease (bionomics). The presence of stagnant water bodies around human habitation serves as breeding and survival sites for mosquito larvae while the bushes serve as resting sites for mosquitoes.

The low and ineffective utilization of the mosquito bednets might have exacerbated the impact of the house type (most of the houses in Tole are wooden houses with cracks and crevices on the walls). As such, tactile stimuli emanating from the host skin could easily be perceived outdoors and would have attracted mosquitoes to feed on the hosts.

More children were positive by use of light microscopy than by use of the Partec CyScope® although the difference was not significant. This RDT technique has the advantage of being faster, uses less blood, is less sophisticated, requires very little training and expertise, does not require electricity and can be easily used for field work by a mobile team of researchers. However, this RDT technique cannot differentiate malaria parasite species. The ability of a test to distinguish between malaria species is important [2]. This is due to the fact that some antimalarial drugs are made to specifically target particular *Plasmodium* species and when antimalarials are used that do not target the species of parasite in the patient, the situation can become fatal within a few days. Therefore, light microscopy remains supreme in this regard as speciation can be done using the technique. However, it has its own disadvantages which include the fact that it needs elaborate training, is time-consuming and needs electricity [2,6].

False positive and false negative results were recorded by the use of Partec CyScope® in mild, moderate and heavy infections. According to Nkrumah et al. [9], false positive results for Partec CyScope® could be due to the presence of artifacts such as non-specific aggregated DAPI, immature erythrocytes or bacterial cells which might have been misinterpreted as plasmodial DNA. Amexo et al. [31] indicated that at least 50% of those prescribed antimalarials could actually be negative for malaria. False positive results pose a problem because they lead to wrong drug prescriptions. This constitutes a problem to patients because antimalarial drugs have serious side-effects such as nausea and body weakness [31]. Drugs in the body also over-work the liver and kidney since detoxication has to be done for clearance and excretion [32] and so undue drug consumption should be avoided whenever necessary.

False negative results could be due to the small quantity of blood used in the Partec CyScope® test (10 µL) which may not be enough to concentrate the parasites for them to be detected. It is also worth noting that in very low parasitaemic cases, infections could be missed. Murray et al. [2] indicated that a RDT should be able to detect parasites at densities as low as 100 parasites/µL of blood for it to be considered to be of good performance.

WHO stipulates a minimal standard of 95% for *P. falciparum* sensitivity and specificity [2]. The sensitivity and specificity of Partec CyScope® used in the study falls slightly below the standards set by WHO but are however both greater than 80% and similar to those of Hassan et al. [33] and Kimbi et al. [11]. Nkrumah et al. [9] in Ghana recorded higher performance characteristics with sensitivities of 100% and specificities of 97.4% for Partec CyScope®. Sousa-Figueiredo et al. [12] reported specificity of <40% and sensitivity of 86.7% lower than that reported in this present study in children for Partec CyScope®in Uganda.

5. CONCLUSION

From this study, it was concluded that the sensitivity and specificity of Partec CyScope® was close to the standards stipulated by WHO. However, considering its advantages it could be a good diagnostic tool for mass surveillance programmes or for routine malaria diagnosis in the especially in remote areas that lack electricity.

CONSENT

All children were issued consent/assent forms to seek for their parents' approval. Children were accepted for screening when they brought back signed informed consent Forms following the approval of their parents (for older children) or when the parents/guardians accompanied them to the health centre with signed consent/assent forms (for the younger children).

ETHICAL APPROVAL

An ethical clearance was obtained from the South West Regional Delegation of Public Health. Administrative clearances were obtained from the chief of Tole village. All children were issued informed consent forms to seek for their parents' approval. Children were only included in the study when they brought back signed informed consent forms following the approval of their parents and accepted the sample collection procedure.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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