Journal of Bacteriology and Parasitology

Kimbi et al., J Bacteriol Parasitol 2012, 3:7
DOI: 10.4172/2155-9597.1000153

Research Article Open Access

# Asymptomatic Malaria in School Children and Evaluation of the Performance Characteristics of the Partec Cyscope® in the Mount Cameroon Region

**Helen Kuokuo Kimbi[1]*, Hilda Uforka Ajeagah[1], Frederick Chi Keka[1], Emmaculate Lum[1], Hervé Nyabeyeu Nyabeyeu[2], Calvin Fotsing Tonga[1], Asaah Humphrey Gah[2] and Leopold Gustave Lehman[2]**

[1]*Department of Zoology and Animal Physiology, Faculty of Science, University of Buea, P.O. Box 63, Buea, SWR, Cameroon*
[2]*Department of Animal Biology, Faculty of Science, University of Douala, P.O. Box 2701, Douala, Cameroon*

## Abstract

The key to the effective management of malaria is prompt and accurate diagnosis followed by effective treatment. The aim of this study was to determine asymptomatic malaria parasite prevalence and density and evaluate the performance characteristics of the Partec CyScope® (fluorescent microscope) in school children in the Mount Cameroon region using light microscopy as a gold standard. A total of 541 pupils aged 4 – 16 years were recruited into the study. After recording demographic data on each child, capillary blood was collected for the preparation of thin and thick blood films for the assessment of parasite density and speciation respectively. Five µl of blood was placed on the dye-labeled portion of the slide, cover-slipped, incubated for 1 minute and observed under the CyScope® for parasites. Performance characteristics of CyScope® were calculated. The overall prevalence of malaria was 64.0% and 58.4% for light microscopy and Partec CyScope® respectively. The overall geometric mean parasite density (GMPD) was 2255.22 (range 320-35040). The sensitivity of the test was 91.3% while the specificity was 86.7%. The Partec CyScope® showed a relatively high sensitivity and specificity in diagnosing malaria in school children and could therefore be used in mass surveillance programmes for the management and control of malaria.

**Keywords:** Evaluating; Performance characteristics; Partec CyScope®; Asymptomatic malaria; Diagnosis; Cameroon

## Introduction

Malaria is responsible for high rates of morbidity and mortality in sub-Saharan Africa leading to over one million deaths annually [1]. It is the most common single diagnosis made in most African countries and is responsible for 40% hospital attendance in Southwest Cameroon [2]. One of the contributing factors to these high rates of morbidity and mortality is delayed or inaccurate results as malaria presents a diagnostic challenge to laboratories in endemic countries. The diagnosis of malaria in many areas still relies predominantly on its clinical presentation which has limited specificity [3] hence many cases go undiagnosed and sometimes untreated. The key to effective management of malaria is prompt and accurate diagnosis. The WHO [4] recommends that malaria case management where possible should be based on parasitological diagnosis, except when considering young children in endemic areas where lack of resources or urgency of response temporarily limits its application. This is important in order to avoid the unnecessary use of anti-malarial drugs especially the recently introduced artemisinin-based combination therapies (ACTs) as this could lead to the development of drug resistance. Therefore, the priority of the Roll Back Malaria Program for endemic countries needs to be a balance between the unnecessary use of ACTs and case management [5,6].

Examination of blood smears using light microscopy remains the gold standard for malaria diagnosis, but it is labour intensive, requires skilled microscopists and generally there is limited supply and maintenance of microscopes and reagents thus leading to delays in delivery of results. New Rapid Diagnostic Techniques (RDTs) have been developed and evaluated in recent years to overcome the limitations of light microscopy. However, the rapid introduction, withdrawal and modification of commercially available RDTs, variable quality control in manufacturing, and potential decrements in test performance related to the stability of stored test kits have rendered these reviews obsolete [7].

The Partec CyScope® (fluorescent microscope) has recently been introduced into the market as an alternative for the rapid diagnosis of malaria (a special RDT meant to overcome the deficiencies of test kits). The method has been used in malaria endemic countries such as Ghana [8,9] and Sudan [5,10]. No study has been done using this method in the Mount Cameroon region to evaluate the performance characteristics of the technique. This needs to be done particularly as high rates of asymptomatic malaria have been reported in this area [11-13]. Thus, the aim of this study was to determine asymptomatic malaria prevalence and density and evaluate the performance characteristics of the Partec CyScope® in school children in the Mount Cameroon region using light microscopy as a gold standard.

## Materials and Methods

### Study site

The study was carried out in the Mount Cameroon region in two primary schools (Government Practicing School Molyko, and Government School Bomaka). Malaria is endemic in the Mount

***Corresponding author:** Helen K Kimbi, Department of Zoology and Animal Physiology, Faculty of Science, University of Buea, P.O. Box 63, Buea, SWR, Cameroon, Tel: +237 7783 66 03/9403 53 55; Fax: +237 3332 22 72; E-mail: hkimbi@yahoo.co.uk

**Received** September 10, 2012; **Accepted** September 26, 2012; **Published** September 30, 2012

**Citation:** Kimbi HK, Ajeagah HU, Keka FC, Lum E, Nyabeyeu HN, et al. (2012) Asymptomatic Malaria in School Children and Evaluation of the Performance Characteristics of the Partec Cyscope® in the Mount Cameroon Region. J Bacteriol Parasitol 3:153. doi:10.4172/2155-9597.1000153

Cameroon region and *P. falciparum* is the most prevalent species in the area. Weather records 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-27°C. There are two distinct seasons, a cold rainy season that extends from mid-March to October and a warm dry season that lasts from November to mid-March.

### Study population

The study population consisted of primary school children aged 4 to 16 years and of both sexes. Contact visits were made to the schools and the benefits of the study and procedures were explained to the Head teacher/teachers (that all children positive for malaria will be treated). Informed consent forms were given to the pupils to take home to their parents/legal guardians to ask for their consent to participate in the study. Those included in the study were the children whose parents/legal guardians consented by signing the consent forms. A total of 1000 consent forms were given out to children in the two schools and 541 children brought back signed (approved) forms and all of them were included in the study. The study was carried out between the months of March and June, 2010, a period that coincides with the high transmission season for malaria in the area.

### Ethical considerations

Ethical and administrative clearances for this study were obtained from the Ethics Committee of the Regional Delegations of Public Health and Basic Education respectively, South West Region, Cameroon. All the children found to be positive for malaria were treated with malartin (artesunate) and fansidar.

### Collection of blood samples

Demographic data such as the name, sex, age and auxiliary temperature of each child were recorded before blood collection. Blood was collected from a finger prick and used to prepare thick and thin blood films for the assessment of parasite density and speciation respectively. Five (5) µl of blood was collected using a micropipette for the preparation of slides to be observed under the Partec CyScope®. Heparinised capillary tubes were filled with blood for the determination of Packed Cell Volume (PCV).

### Staining and microscopic examination of blood films by light microscopy

Thin and thick blood films were stained with 10 % Giemsa and examined under the oil immersion (x100) objective of a UNICO® light microscope. Thick films were considered positive when asexual forms (trophozoites and schizonts) and or gametocytes were present in the blood film. Slides were declared negative after observing at least 100 high power fields without detecting any parasites. Parasites were counted against 200 leucocytes and expressed as parasites per microliter (µl) of blood, assuming a white blood cell count of 8000/µl of blood [14]. Thin blood films were used for *Plasmodium* speciation with the aid of identification charts of Cheesbrough [15].

### Malaria diagnosis using the Partec CyScope® rapid malaria test

The tests were performed according to the manufacturer's (PARTEC GmbH, Münster, Germany) instructions. Briefly, the slide used to observe malaria parasites under the Partec CyScope® is supplied with the dried reagent (4'-6 Di Amidino -2-Phenyl Indole, DAPI) which is a DNA-staining fluorochrome. The capillary blood pipetted from the finger prick was placed on the dye-labeled portion of the slide. The slide was tilted gently in all directions so that the blood could spread over the reagent. A cover slip was then placed over the blood sample. After incubating (that is, allowing the stain to penetrate the blood cells) at room temperature for 1 minute, the slide was then observed at 100x objective under UV light of the Partec CyScope® for malaria parasites. The presence of distinct bright, shiny, tiny dots observed under the UV light indicated the presence of malaria parasites besides nuclei of white blood cells. In order to prevent the slides from drying out, they were kept in a wet chamber. Positive and negative controls from known samples were observed on each batch of ready-to-use slides.

### Data analysis

After all the tests were performed the values for sensitivity, specificity, predictive values and accuracy were calculated using light microscopy as a gold standard. The variables measured were the number of true positives (TP), number of true negatives (TN), number of false positives (FP) and number of false negatives (FN).

Sensitivity was calculated as TP/(TP+FN), Specificity was calculated as TN/(TN+FP), Positive predictive value (PPV) was calculated as PPV=TP/TP+FP, Negative predictive value (NPV) was calculated as NPV=TN/TN+ FN, Accuracy=(TP+TN)/number of all tests and the overall measure of reliability=(TP x TN)– (FP x FN)/(TP+ FN) (TN+FP) [16].

## Results

### Demographic characteristics of the study population

A total of 541 school children (300 females and 241 males) were recruited into the study. The mean age of the study population was 9.22 ± 2.13 (range: 5 – 16) years. The mean temperature at enrolment was 36.68 ± 2.28°C (range: 35.2 – 39.4). The mean PCV value was 36.54 ± 4.02 (range 19 - 50) as shown on Table 1.

### Malaria parasite prevalence in the study population

The overall malaria parasite prevalence was 64.0% (346) and 58.4% (313) by light microscopy and Partec CyScope® respectively. The GMPD was 2255.22 parasites/µl (range 320 -35040) for light microscopy (Table 1). Two *Plasmodium* species were identified, *P. falciparum* and *P. malariae*. *P. malariae* was found only in cases of mixed infections. A total of 317 out of 346 pupils positive for malaria parasites by light microscopy were found to be infected with *P. falciparum* alone giving a percentage of 91.6% while 29 cases were mixed infections of *P. falciparum* and *P. malariae* (8.4%).

### Comparing the results of Partec CyScope® using light microscopy as a gold standard

From a total of 541 pupils included in the study, 316 pupils were

| Characteristic | | Value (%) |
|---|---|---|
| Sex | Male | 241 (44.5%) |
| | Female | 300 (55%) |
| Mean Age (years ± SD) | | 9.22 ± 2.134 (range: 5–16) |
| Mean axillary temperature (°C ± SD) | | 36.68 ± 2.28 (range: 35.2 – 39.4) |
| Overall malaria parasite prevalence using light microscope | | 346 (64%) |
| Overall malaria parasite prevalence using PartecCyScope® | | 316 (58.4%) |
| Overall geometric mean parasite density | | 2255.22 (range 320 - 35040) |
| Overall mean packed cell volume | | 36.54 ± 4.02 (range 19 - 50) |

**Table 1:** Baseline Characteristics of the Study Population.

positive for both light microscopy and the Partec CyScope® (TP), 30 pupils were positive with the Partec CyScope® but negative with light microscopy (FP), 30 pupils were negative with the Partec CyScope® but positive with light microscopy (FN) and 195 were true negatives for both the Partec CyScope® and light microscopy (TN) as shown in Table 2.

### Performance characteristics of the Partec CyScope®

The general performance characteristics of the CyScope® were also evaluated. The sensitivity of the test was 91.3% with a confidence interval (CI) of 88.9 – 93.3, specificity was 86.7% with a confidence interval value of 82.9 - 89.7. The positive predictive value, negative predictive value and reliability (j-index) were 91.3 (CI=88.9 – 93.3), 86.7 (CI=82.9 - 89.7) and 78 (CI=0.72 – 0.83) respectively.

## Discussion

Malaria is endemic in the Mount Cameroon area and *P. falciparum* is the predominant species accounting for over 90% of all malaria cases [12]. Falciparum malaria is known to be the most fatal and can lead to dire consequences within a short period of time especially in high risk individuals such as children below five years, pregnant women and non-immune individuals. Thus, falciparum malaria is an important cause of severe malaria and therefore needs proper management. The primary tool for the effective management of this disease remains the early and accurate diagnosis as well as treatment of clinical cases [6-8]. Definitive diagnosis leads to accurate epidemiological assessment of the infection in a particular setting.

The malaria parasite prevalence values for light microscopy and Partec CyScope® reported in this study are in conformity with previous studies carried out in the Mount Cameroon region where some authors reported prevalence values of up 60.0% and above [12,13]. The study was carried out between the months of March and June, 2010 (rainy season) and this has been reported to be the peak period of malaria transmission in the study area [11]. This high malaria parasite prevalence underscores the fact that, malaria is still a heavy burden in this area. Therefore, appropriate control measures such as long lasting bed nets and proper environmental management need to be intensified.

The study results showed comparable performance characteristics of the Partec CyScope® using light microscopy as a gold standard. This high value for the light microscopy could be due to the expertise of the highly trained microscopists that observed the slides as they were able to detect parasites at even very low parasitaemia. Hassan et al. [10] recorded similar results for light microscopy in pregnant women in Sudan and this was attributed to well-trained microscopists. Light microscopy has historically been the mainstay of laboratory diagnosis of malaria in endemic countries and continues to be the gold standard despite some drawbacks. It is time consuming, labour intensive and cannot be performed where there is no electricity. The Giemsa stain needs to be freshly prepared with a neutral buffer and to be filtered before use. This cumbersome process is rarely followed in laboratories of rural areas and frequently produces poor quality of malaria diagnostics. As a result of these limitations, alternative techniques for the rapid diagnosis of malaria that are easier and quick to perform as well as cheaper, such as the Partec CyScope® fluorescent microscope, have been developed and the performance characteristics need to be evaluated [8].

| Results of PartecCyScope® | Results of light microscopy (gold standard) | | Total |
|---|---|---|---|
| | Positive | Negative | |
| Positive | 316 (TP) | 30 (FP) | All with positive test TP+FP= 346 |
| Negative | 30 (FN) | 195 (TN) | All with negative test FN+TN =225 |
| Total | All with disease (346) | All without disease (255) | Everyone=TP+FP+FN+TN (541) |

**Table 2:** Results of the PartecCyScope® test using light microscopy as gold standard.

The false positive results for the Giemsa stain could be due to artifacts while those for the Partec CyScope® could be attributed to the presence of fragmented nuclei from damaged reticulocytes or white blood cells [8] and this often happens on warmer days when CyScope® slides are left for a few hours [6]. It could also be due to the presence of bacterial or other microbial DNA which are able to fluoresce and are misinterpreted as plasmodial DNA [6,8]. In another study in Uganda, Sousa-Figueiredo et al. [6] suggested that false positive results by the Partec CyScope® method could be linked to the presence of schistosomes (in the blood stream) which usually regurgitate their blood meals sloughing their intestinal cells that could likely contain broken down nuclei (that can fluoresce and be detected by the Partec CyScope®) into the host blood stream. In addition, field studies, unlike carefully controlled laboratory, hospital or clinically based studies are sometimes vulnerable to different elements and unidentified DNA containing bodies or dust particles which could also cause problems at certain times [6].

The accepted level of sensitivity for a rapid diagnostic test in diagnosing malaria is a sensitivity of 95%. The sensitivity value recorded in the present study is in line with several other studies which also recorded sensitivity values close to 95%; in Uganda [6], Sudan [5,10], in Ghana [9] and Cameroon [17].

When compared to other commercially available rapid tests (such as ParaSight F, which detects only histidine rich protein-2 and the Hexagon Malaria Combi™ rapid test), the Partec CyScope® is highly commendable because it can detect parasites at very low concentrations with good sensitivities and specificities at different parasite densities, thereby giving it a greater potential for use as an epidemiological tool for the control of malaria. This test also performed better than the OptiMAL™ rapid malaria test, which failed to detect asymptomatic malaria in an area of Thailand endemic for *P. falciparum* and *P. vivax* [18]. However, a major limitation of this rapid test is its inability to differentiate between *Plasmodium* species and to quantify the parasite density [6]. Despite the limitations of the light microscope with Giemsa-stained thick and thin blood films, it can however be used for both qualitative and quantitative purposes and is the only cheap method that can be used for species identification. However, it is more difficult to use in rural areas of malaria endemicity where there is no electricity [8].

The Partec CyScope® method has several advantages over light microscopy. Only a small quantity of blood (5 µl) is needed when compared to 10 µl for light microscopy, making the Partec CyScope® method ideal for child patients [8]. It is less sophisticated and faster to use than light microscopy as it requires very little training and expertise. Reagent preparation is not necessary since reagents are already dried on the slides, and this may likely prolong the shelf life of the test reagents. The Partec CyScope® is battery operated, making it ideal for field work (as was the case in this study) and areas where there is no electricity [8].

The high parasite prevalence of *P. falciparum* observed in this study is in conformity with earlier reports from Africa in general and some

parts of Cameroon in particular which described *P. falciparum* as the most common cause of malaria infection. All cases of malaria in the study were caused by *P. falciparum* either as single or mixed infections with *P. malariae*. This can be very dangerous since *P. falciparum* is the most pathogenic of all *Plasmodium* species. This implies that the Partec CyScope® can be comfortably used in this area without much problem since species identification is not much of a problem as *P. falciparum* is responsible for more than 90% of all infections.

### Acknowledgements

We are grateful to all the parents/guardians for their consent and the children who participated in this study. Kakwa Biofarm, Limbe, Cameroon supplied the drugs.

### References

1. http://www.who.int/malaria/world_malaria_report_2010/malaria2010_summary_keypoints_en.pdf
2. Ikome LE, Ndamukong KJ, Kimbi H (2002) Prevalence and case-control study of cerebral malaria in Limbe of the South-West Cameroon. Afr J Health Sci 9: 61-67.
3. Singh N, Mishra AK, Shukla MM, Chand SK, Bharti PK (2005) Diagnostic and prognostic utility of an inexpensive rapid on site malaria diagnostic test (ParaHIT f) among ethnic tribal population in areas of high, low and no transmission in central India. BMC Infect Dis 5: 50.
4. http://whqlibdoc.who.int/publications/2008/9789241596305_eng.pdf
5. Hassan Sel-D, Okoued SI, Mudathir MA, Malik EM (2010) Testing the sensitivity and specificity of the fluorescence microscope (Cyscope) for malaria diagnosis. Malar J 9: 88.
6. Sousa-Figueiredo JC, Oguttu D, Adriko M, Besigye F, Nankasi A, et al. (2010) Investigating portable fluorescent microscopy (CyScope) as an alternative rapid diagnostic test for malaria in children and women of child-bearing age. Malar J 9: 245.
7. Murray CK, Gasser RA Jr, Magill AJ, Miller RS (2008) Update on rapid diagnostic testing for malaria. Clin Microbiol Rev 21: 97-110.
8. Nkrumah B, Agyekum A, Acquah SE, May J, Tannich E, et al. (2010) Comparison of the novel Partec rapid malaria test to the conventional Giemsa stain and the gold standard real-time PCR. J Clin Microbiol 48: 2925-2928.
9. Nkrumah B, Acquah SE, Ibrahim L, May J, Brattig N, et al. (2011) Comparative evaluation of two rapid field tests for malaria diagnosis: Partec Rapid Malaria Test® and Binax Now® Malaria Rapid Diagnostic Test. BMC Infect Dis 11: 143.
10. Hassan Sel-D, Haggaz AE, Mohammed-Elhassan EB, Malik EM, Adam I (2011) Fluorescence microscope (Cyscope) for malaria diagnosis in pregnant women in Medani Hospital, Sudan. Diagn Pathol 6: 88.
11. Kimbi HK, Tetteh KK, Polley SD, Conway DJ (2004) Cross-sectional study of specific antibodies to a polymorphic *Plasmodium* falciparum antigen and of parasite antigen genotypes in school children on the slope of Mount Cameroon. Trans R Soc Trop Med Hyg 98: 284-289.
12. Kimbi HK, Awah NW, Ndamukong KJ, Mbuh JV (2005) Malaria infection and its consequences in school children. East Afr Med J 82: 92-97.
13. Nkuo Akenji TK, Ntonifor NN, Ching JK, Kimbi HK, Ndamukong KN, et al. (2005) Evaluating a malaria intervention strategy using knowledge, practices and coverage surveys in rural Bolifamba, southwest Cameroon. Trans R Soc Trop Med Hyg 99: 325-332.
14. Moody A (2002) Rapid diagnostic tests for malaria parasites. Clin Microbiol Rev 15: 66-78.
15. Cheesbrough M (2005) District Laboratory Practice in Tropical Countries. Part I. Second edition. Cambridge University Press. Low Price Edition.
16. Jaeschke R, Guyatt G, Sackett D (1994) Users' Guides to the Medical Literature: III. How to use an article about a diagnostic test: B. What are the results and will they help me in caring for my patients? JAMA 271: 703-707.
17. Wanji S, Kimbi HK, Eyong JE, Tendongfor N, Ndamukong JL (2008) Performance and usefulness of the Hexagon rapid diagnostic test in children with asymptomatic malaria living in the Mount Cameroon region. Malar J 7: 89.
18. Coleman RE, Maneechai N, Ponlawat A, Kumpitak C, Rachapaew N, et al. (2002) Short report: Failure of the OptiMAL rapid malaria test as a tool for the detection of asymptomatic malaria in an area of Thailand endemic for *Plasmodium* falciparum and P. vivax. Am J Trop Med Hyg 67: 563-565.