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Comparison of clinical, microscopic, and rapid diagnostic test methods in the diagnosis of *Plasmodium falciparum* malaria in four districts in the Eastern Region, Ghana

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Abstract

Background: The WHO recommends all suspected malaria cases to be confirmed by laboratory diagnosis (microscopy or rapid diagnostic test (RDT)), wherever possible, before the commencement of antimalarial treatment. In deprived settings, getting laboratory confirmation can be challenging and treatment may be based on a presumptive clinical diagnosis of malaria. However, the accuracy of clinical diagnosis is variable, and a universal predictive clinical algorithm is nonexistent.

Objective: This study aimed to compare the use of clinical, microscopic and RDT methods in the diagnosis of *Plasmodium falciparum* malaria in four districts in the Eastern Region of Ghana.

Methods: Patients initially seen and clinically diagnosed with malaria by a physician and referred for laboratory confirmation (microscopy and RDT) were recruited. Each patient provided a blood sample for malaria parasite detection. Microscopy was considered the diagnostic "gold standard". The performance analysis included sensitivity, specificity, receiver operating characteristics (ROC), kappa and Youden index.

Results: In all 500 patients were recruited [males (33.20%, n = 166); mean age = 29.60 ± 20.30 years). Seventeen different symptoms were reported — fever (84.40%, n = 422), headache (64.20%, n = 321) and chills (54.40%, n = 272) were the highest. Hyperpyrexia (62.80%, n = 341) and splenomegaly (38.80%, n = 194) were the commonest of the 6 vital signs recorded. Only *Plasmodium falciparum* parasites were identified by both microscopy and RDT. Prevalence values of 96.80% (n = 484), 90.80% (n = 454), and 43.00% (n = 215) were obtained for microscopy, RDT and clinical diagnosis, respectively ($p < 0.05$). Mean parasite density by microscopy was 16229.41 ± 10533.62 parasites/mL. Using microscopy as the gold standard, RDT reported a higher sensitivity (91.32%) compared to clinical diagnosis (43.60%), but a lower specificity (25.00% against 80.00%). Both RDT and clinical diagnosis had low negative predictive values, 8.18% and 4.20% respectively, against microscopy. There was poor consensus (kappa < 0.20) between all three diagnostic approaches.

Conclusion: All clinically diagnosed malaria cases should be confirmed with a laboratory test, preferably microscopy before antimalarial treatment starts.

Keywords: Malaria, microscopy, rapid diagnostic test, clinical diagnosis, Ghana

INTRODUCTION

Malaria is hyperendemic in Ghana. According to the WHO [1], of the 10 highest-burden countries in Africa, Ghana and Nigeria reported the highest absolute increases in cases of malaria in 2018 compared with 2017. In Ghana, malaria is one of the main causes of adult morbidity and the leading cause of workday loss to illness. It also accounts for 44% of outpatient attendance, 13% of all hospital deaths, and 22% of mortality among children less than five years of age [2]. Malaria also affects economic development in Ghana as it puts a heavy burden on health and productivity. According to a study in 2016 by Nonvignon et al. [3], businesses in Ghana in 2014 lost approximately 6.6 million United States dollars due to malaria infections. About 90% of the financial loss was attributed to direct costs (e.g. direct treatment cost of reported illness among staff and their dependents). Similarly, businesses in Ghana lost a total of 3913 productive days from 2012 to 2014 as a result of malaria infections. Malaria is preventable and treatable. However,

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for the treatment of malaria to be effective, an accurate diagnostic method is required [4]. Misdiagnosis of malaria when it occurs may end up in overdiagnosis, over-prescription with an increase in the cost of anti-malaria drugs, underdiagnosis, and inappropriate treatment of non-malaria febrile patients [5]. Misdiagnosis can also end up in the death of the patient [5]. Though there is a clear need for improvement, malaria diagnosis is the most neglected area of malaria research [6].

Improved diagnosis of malaria has enormous benefits. In 2006, Rafael et al. [7] estimated that over 100,000 deaths and about 400 million unnecessary treatments would be averted with a diagnostic test requiring minimal infrastructure and having 95% sensitivity and 95% specificity. The two main approaches to malaria diagnosis are the presumptive clinical diagnosis and test-based approaches. Clinical diagnosis of malaria is based on patients' signs and symptoms or physical findings during examination [8, 9] and malaria is the most common clinical diagnosis for febrile patients in Sub-Saharan Africa [10]. Though clinical diagnosis is imprecise, it is still the basis for therapeutic care for febrile patients in malaria-endemic areas, where laboratory support is often out of reach. Unfortunately, malaria symptoms overlap with those of other tropical diseases like typhoid fever, respiratory tract infections and viral infections and these impair the specificity of presumptive diagnosis thereby encouraging indiscriminate use of antimalarials in endemic areas. The accuracy of clinical diagnosis also varies with the level of endemicity, malaria season, and age group [11]. Recently, it was demonstrated that machine learning techniques can be successfully applied to predict malaria using patient information [12]. In addition, eight significant features, seven significant symptoms and patients' history of malaria as a non-symptom-related factor, have been shown to contribute most to malaria diagnosis in Indonesia [13].

In Ghana, malaria diagnosis has largely been presumptive over the years [14] though there has been a progressive shift to laboratory confirmation using microscopy or the rapid diagnostic tests (RDT) as the basis for treatment. Since 2010, the national malaria control programme of Ghana has promoted the 'T3' policy — for testing, treating, and tracking malaria infections — as recommended by WHO for the management of uncomplicated malaria cases. The percentage of suspected malaria cases to be tested by RDT in public health facilities is projected to reach 85% by 2022 [15]. However, in deprived settings, getting laboratory confirmation can be challenging. Malaria is also presumptively diagnosed by mothers as they depend mainly on the presence or history of fever for diagnosis. Data from the 2019 Ghana malaria indicator survey shows that only 34% of children < 5 years with fever had blood taken for malaria test [16]. There is a need to continually assess the diagnostic efficiency of various malaria diagnostic methods in health facilities. This study aimed to compare the use of clinical diagnosis, microscopic and RDT in the diagnosis of *P. falciparum* malaria in four districts in the Eastern Region of Ghana.

MATERIALS AND METHODS

Study design and sites

A cross-sectional study design was employed. Samples were collected from seven health centres in four districts in the Eastern Region. The district and health facilities were: Akuapen North District (Tetteh Quarshie Memorial Hospital); Lower Manya Krobo District (Atua District Hospital, Somanya Health Centre, and St Martin De Pores Hospital); Atiwa East District (Enyiresi Government Hospital); and New Juaben District (Koforidua Regional Hospital and Koforidua Polyclinic). The Eastern Region lies between latitudes 6º and 7º North and between longitudes 1º30' West and 0º30' East. The Region has a land area of 19,323 square kilometres (which constitutes 8.1% of the total land area of Ghana) [17]. Koforidua is the administrative capital. The 2017 projected population for the Eastern Region based on the 2010 population census figure of 2,633,154 and an annual growth rate of 2.50% was 2,952,399. The population is 49% male and 51% female with an urban-rural split of 43.33% to 56.62% respectively. About 41.30% of the population is < 5 years. Agriculture is the main economic activity in the region and employs about 53% of the population, 10.70% of the population is in industry, and about 22% is in the services sub-sector.

Sample size and patient population

The 'StatCalc' function of Epi Info™ software, version 7.2.4.0 (Centre for Disease Control, USA) was used to calculate the sample size. Using a population size of 2,952,399 (https://ghanadistricts.com/), an expected frequency of 50%, an acceptable margin of error of 5%, a design effect of 1 and a cluster effect of 4, we calculated a sample size of 384 with a cluster size of 96. Therefore, at least 96 patients were needed from each site for the study. Blood samples were only collected from a patient presenting with symptoms consistent with uncomplicated malaria who came to the selected health facilities and had been referred by a physician to the outpatient laboratory for malaria confirmation with microscopy and RDT in line with national malaria treatment guidelines. The study excluded patients diagnosed with serious malaria or other conditions requiring immediate diagnosis or care, and pregnant women. We also excluded hospitalized patients, patients reporting for medical examinations, and those with unrelated causes of malaria.

Data collection

All participants had the study explained to them following which written informed consent was obtained. For children aged 12 – 17 years, child assent was obtained while parental consent was obtained from parents and guardians of all children. Demographic and clinical details of patients were gathered at the time of registration. A finger-prick blood sample was collected from each patient for thick and thin blood smears and RDT. Physicians at the outpatient departments of the health centres performed a clinical diagnosis based on fever (temperature > 37.5 ºC) with or without a history of fever. Other symptoms and vital signs were also considered for clinical diagnosis.

Rapid diagnostic tests

Each RDT device was labelled with the corresponding identification code for each study participant. Approximately 2 mL of whole blood was taken from consented patients into EDTA tubes by standard technique. For each sample, malaria testing was done with the SD BIOLINE Malaria Ag Pf (PfHRP2) RDT (SD Bioline™, USA). A 5 µL loop was used to sample blood from an EDTA tube into an RDT cassette sample well after which four drops of assay diluent were dispensed into the assay diluent well. The result was read at 15 to 30 minutes. The results were interpreted following the instructions provided by the manufacturers. A negative result was recorded when one band appeared in the control area. The presence of two bands — one band in the control area and the other band in the test area — was noted as positive for *P. falciparum.*

Microscopy

Thick and thin blood smears were prepared on the same slide for each blood sample after the analysis of RDT. Two drops of blood were placed on a clean labelled glass slide about 1 cm distance apart. For the thick blood film smear, the blood spot was stirred in a circular motion with the corner edge of another slide. The thin blood film was prepared by placing the smooth edge of the spreader slide on the drop of blood at an angle of 45° and quickly smeared forward on the slide surface. The blood smears were allowed to air dry, and the thin film was fixed with methanol. The slides were then stained with 10% Giemsa for 15 minutes after which the stain was washed off and air-dried. Slides were examined using a light microscope with 100 x oil immersion and 100 fields were scanned before a particular smear was declared negative. Parasitaemia was calculated by counting the number of parasites observed per 200 leukocytes and assuming a total of 8,000 leukocytes per microlitre.

Statistical analysis

Data collected was cleaned up and analysed using the IBM-Statistical Package for Social Sciences (IBM-SPSS) version 26 and MedCalc Statistical Software for Windows Version 19.5.3 (MedCalc Software Bvba, Austria) at https://www.medcalc.be/. Continuous variables were summarized into means and standard deviations (SD), and categorical variables reported as frequencies and percentages were used to evaluate the descriptive statistics. Using light microscopy of Giemsa-stained thick blood film as the gold standard, the sensitivity and specificity of each of the other diagnostic methods were calculated. This offered 100% hypothetical sensitivity, precision, and positive and negative predictive values for the microscopy [18]. Formulas were then used to calculate the sensitivity, specificity, and predictive values [19]. Sensitivity was defined as the probability that a truly infected individual will test positive and specificity as the probability that a truly uninfected individual will test negative. The weighted kappa statistic was calculated using the interrater agreement analysis to determine how the results provided by RDT and by clinical diagnosis agreed with microscopy in the diagnosis of malaria. The quality of combined test methods with microscopy and clinical diagnosis as a medical diagnostic tool was assessed using the Youden J statistic index on a scale of 0 – 1 [18,19].

RESULTS

Characteristics of patients

Table 1 shows the characteristics of all 500 patients involved in the study. Females (66.80%, n = 334) made up the majority of patients. The ages ranged from 1 year to 89 years with a mean of 29.60 ± 20.30 years. The age group < 18 had the highest number of patients (37.60%, n = 188) while patients aged 51 – 60 years (6.60%, n = 33) had the least number of patients. The majority (77.00%, n = 385) of the patients had no prior anti-malaria treatment.

Table 1: Characteristics of 500 patients involved in the study

Characteristics (n = 500)	Number	Percentage
Site		
Akuapem North	199	39.80
Atiwa East	82	16.40
Lower Manya Krobo	94	18.80
New Juaben Municipal	125	25.00
Gender		
Male	166	33.20
Female	334	66.80
Age (years)		
< 18	188	37.60
19 – 30	99	19.80
31 – 40	77	15.40
41 – 50	50	10.00
51 – 60	33	6.60
> 60	53	10.60
Prior anti-malaria treatment		
Anti-malaria drug	46	9.20
Herbal treatment	69	13.80
None	385	77.00

Clinical features of patients

Based on physicians' records at the district hospitals, 17 different symptoms were reported (Figure 1a) by patients. The most prevalent symptoms reported were fever (84.40%, n = 422), headache (64.20%, n = 321) and chills (54.40%, n = 272). All three symptoms were reported to be present or absent simultaneously in 43.00% (n = 215) and 6.20% (n = 31) of the patients, respectively (Figure 1b). The least reported symptoms were diarrhoea (0.8%, n = 4), joint pains (0.6%, n = 3) and night sweat (0.4%, n = 2). Figure 1c also shows the vital signs recorded by the physicians. Hyperpyrexia (62.80%, n = 341) and splenomegaly (38.80%, n = 194) were the highest vital signs recorded. Both were present or absent simultaneously in time in 38.60% (n = 193) and 37.00% (n = 185) of the patients, respectively (Figure 1d). Axillary temperatures recorded ranged between 36.93 °C and 39.92 °C (mean = 38.53 °C).

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Malaria prevalence versus diagnostic techniques

The only malaria parasite identified by both microscopy and RDT was *P. falciparum*. Prevalence values of 96.80% (n = 484/500), 90.80% (n = 454/500), and 43.00% (n = 215/500) were obtained for microscopy, RDT, and clinical diagnosis, respectively. The mean parasite density by microscopy was 16229.41 ± 10533.62 parasites/µL.

Diagnostic performance of tests methods

The RDT reported a sensitivity of 91.32% and a specificity of 25.00% using microscopy as the gold standard, while clinical diagnosis reported a sensitivity of 43.60% and a specificity of 80.00% (Table 2). Both RDT and clinical diagnosis were found to have low negative predictive values of 8.18% and 4.20%, respectively, against microscopy. The correlation of RDT and clinical diagnosis to parasite density observed by microscopy is shown in Table 3. Out of 496 positive patients, 96.77% (n = 480) had parasite count >1000 per mL by microscopy and 91.13% (n = 452) and 87.30% (n = 213) were positive for RDT and clinical diagnosis, respectively. Table 4 shows the level of agreement estimated between the different diagnostic methods for malaria. The weighted kappa showed poor consensus (< 0.20) between all three diagnostic approaches. The Youden index revealed a higher diagnostic ability when microscopy and clinical diagnosis are combined compared to any other combinations.

DISCUSSION

In the WHO African Region, malaria kills one child every 2 minutes and over a quarter of all young child deaths in Africa occur due to malaria [1] and thus the importance of a timely and accurate diagnosis of the disease cannot be overemphasized. This study compared the test performance of three commonly used diagnostic methods, microscopy, clinical diagnosis by a physician and RDT. The study showed an indication of wide differences or substantial nonoverlap between the test methods in the positive detection of malaria among the study population.

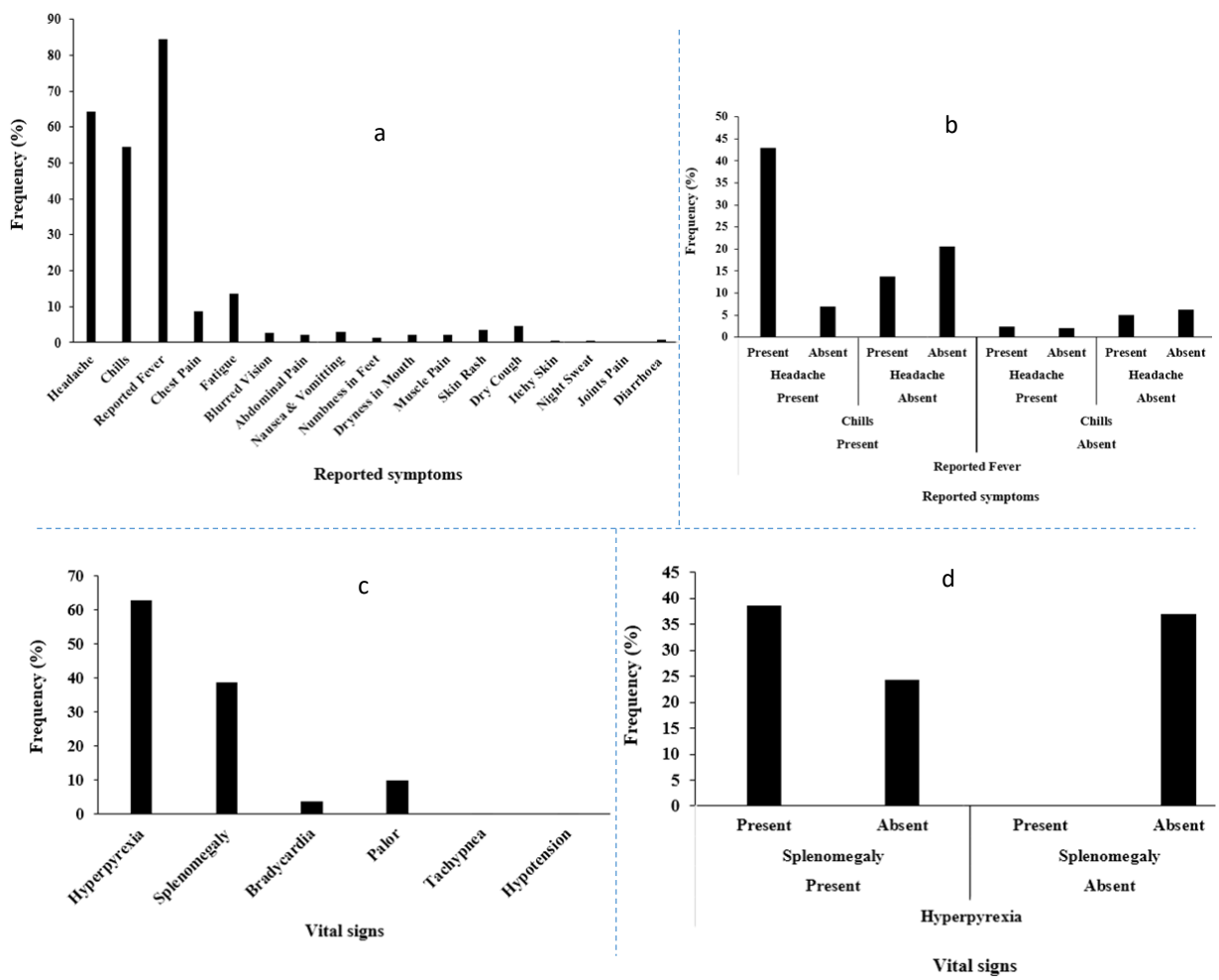

Figure 1: (a) Symptoms reported by the patients; (b) Aggregate of the three most important symptoms; (c) Vital signs recorded by physicians; (d) Aggregate of the two highest vital signs

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Prevalence values of 96.80%, 90.80%, and 43.00% were obtained for microscopy, RDT and clinical diagnosis, respectively ($p < 0.05$). Presumptive diagnosis or clinical diagnosis of malaria based on non-specific signs and symptoms like headaches, fever, weakness, dizziness, pruritus etc. [18], is commonly used because it is cheap and allows for prompt treatment of the patient [9]. Populaces of endemic areas are often familiar with this combination of symptoms, and frequently self-diagnose malaria based on symptoms alone. For example, though respondents in an irrigated farming community in Ghana displayed high knowledge of some common clinical malaria symptoms, only 3% out of 337 survey respondents indicated they sought immediate treatment at a health facility whenever they suspected malaria. According to the majority (about 97%) of the survey respondents, it was only if they felt the suspected malaria illness was severe and/or other treatment options had failed that they sought treatment at a health facility [20]. A recent study involving day and night market traders in 10 selected markets within the Greater Accra Region reported similar findings [21]. Malaria diagnosis that is free of charge in the public sector is not yet a policy adopted in Ghana [22] and this could be a reason for the poor attitudes and practices observed.

Table 2: Sensitivity and specificity of clinical diagnosis and RDT diagnosis of malaria using microscopy as the gold standard

Parameter	Sensitivity	Specificity	PPV	NPV
Microscopy	100.00	100.00	100.00	100.00
RDT	91.32	25.00	97.52	8.18
Clinical diagnosis	43.60	80.00	98.60	4.20

All figures are in percentages; PPV, positive predictive value; NPV, negative predictive value; RDT, rapid diagnostic tests

Table 3. Correlation of RDT and clinical diagnosis to parasite density *observed* by microscopy

Parameter		Parasitic count range (count/μL)		
		1 – 100	101 – 1000	>1000
Number observed		0	4	496
MPC /μL (range)		0	897.50 (650 – 1000)	16353.00 (1150 –68674)
Microscopy	*P.falcip*+	0	4 (100.00%)	480(967.74%)
RDT	*P.falcip*+	0	2 (50.00%)	452 (91.10%)
	Negative	0	2 (50.00%)	44 (8.90%)
Clinical diagnosis	Positive	0	2 (100.00%)	213 (87.30%)
	Negative	0	0	31 (12.70%)

MPC, mean parasite count; RDT, rapid diagnostic test; *P. falcip, Plasmodium falciparum*; +, positive diagnostic tests

Table 4. Level of agreement between different diagnostic methods for malaria

Diagnostic methods	*p* value	kappa statistic	Youden
Microscopy and RDT	0.026	0.09	0.16
Microscopy and clinical diagnosis	0.031	0.02	0.24
Clinical diagnosis and RDT	0.052	-.05	0.15

Kappa < 0.20, poor; 0.41 – 0.60, moderate; 0.61 – 0.80, good; 0.81 – 1, very good. *p* value is significant at < 0.05

In this study, 17 different symptoms were reported by the patients as recorded by the physicians. The most prevalent symptoms reported were fever (84.40%), headache (64.20%), and chills (54.40%). Forty-three per cent (n = 215) of the patients reported all three symptoms present simultaneously. According to the patients, these had persisted for over a week. The many patients (62.80%) reporting fever agreed with the vital sign hyperpyrexia (mean axillary temperatures = 38.50ºC) recorded by the physicians. However, these symptoms generally overlap with those of other tropical diseases like typhoid fever, respiratory tract infections and viral infections. Thus, it was surprising clinical diagnosis showed generally high specificity in comparison with microscopy (80.0%) which was the gold standard. This value is different to the 25.8% finding of the study in Ho [23] though a recent study by Wogu and Nduka [24] in Nigeria indicated a specificity of 95.93%. The high specificity of this study implies that clinical diagnosis with microscopy may diagnose patients having malaria as being truly infected. This will likely avoid the situation where all fevers are presumptively treated as malaria, thus masking underlying potentially fatal conditions [25].

According to the 2020 world malaria report, either there was no available data reported to WHO or a policy was adopted but not implemented in 2019 in Ghana on RDT use at the community level [22]. Using microscopy as the gold standard, histidine-rich protein 2 (PfHRP2) RDT has shown different levels of accuracy for malaria infection in field studies [26, 27]. In this study, the sensitivity and specificity of RDT when compared with microscopy were 91.20% and 25.00%, respectively. These agree with some earlier studies [28, 29]. This sensitivity, however, was below WHO recommended minimal standard of 95% sensitivity for *P. falciparum* densities of 100/μL and a specificity of 95% for an acceptable RDT for malaria [30]. Several factors in the manufacturing process and environmental conditions may affect RDT performance, and these include suboptimal sensitivity at low parasite densities, storage temperature and humidity, and the inability to accurately identify parasites to the species level or quantify infection density [31]. Compared with microscopy, RDT demonstrated a low

NPV (8.180) but high PPV (97.40%). Thus, using RDT implies a test result may indicate a false negative result by showing that an infection is not present when it is. The persistence of PfHRP2 protein in circulation after parasite clearance contributes to a lower specificity level of RDT [28, 29]. False-negative results using RDT have been reported because of the deletion of PfHRP2 antigens or genetic polymorphisms in the PfHRP2 gene in certain *P. falciparum* parasites [32-34]. Also, in this study, 44 (8.80%) patients confirmed RDT negative and recorded parasitic densities of ≥ 1000 parasites/μL. Studies have reported patients with high levels of parasitemia that give false negative RDT results [35, 36]. There was a poor level of agreement between both RDT (kappa: 0.087) and microscopy. Moderate agreements have been reported between RDT and field microscopy in Cameroon [37] and Ghana [23]. Based on the results of this study, RDT must be used alongside microscopy in the study area because RDT detection of *P. falciparum* has lower sensitivity than other methods [38]. There was also a poor level of agreement between clinical diagnosis (0.02) and microscopy. Clinical diagnosis alone is thus likely to result in malaria misdiagnosis.

Conclusion
Clinical diagnosis may not be reliable as a stand-alone malaria diagnostic technique. Malaria control campaigns must encourage the timely report and confirmation of all suspected clinical malaria cases with a laboratory test, most preferably, microscopy.

DECLARATIONS

Ethical considerations
Ethical approval was obtained from the Ethics and Protocol Review Committee of the College of Health Sciences (CHS-Et/M.7-4.15/2018-2019), University of Ghana. Approval was sought from all health facilities.

Consent to publish
All authors agreed to the content of the final paper.

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Competing Interests
No potential conflict of interest was reported by the authors.

Author contributions
CAB, AG conceived and designed the study. ARL contributed to data collection and analysis. All authors contributed to data interpretation. ARL drafted the article. CAB, AG provided critiques and revisions to the drafted article. All authors contributed to the final manuscript. All authors read and approved the final manuscript.

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Availability of data
Data is available upon request to the corresponding author.

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