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Assessing the Diagnosis of Malaria by Comparing Microscopy, Rapid Diagnostic Tests, and Polymerase Chain Reaction among Suspected Malaria Cases in Upper Denkyira East Municipality, Central Region, Ghana

Prince Yaw Boakye[1,2*], Godfred Yaw Boanyah[3], Gabriel Ofosu Agyapong[2], Daniel Azumah Nayembil[2], Christopher Bijabdo Jato[2], Irene Adjoa Anderson[2], Patrick Kojo Ahiabor[2], Isaac Owusu-Frimpong[4], Prince Osei Akumiah[2]

[1]Department of Clinical Microbiology, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana
[2]Department of Physician Assistantship, Presbyterian University, Agogo, Ghana
[3]International Center of Insect Physiology and Ecology, Nairobi, Kenya
[4]Department of Molecular Microbiology and Immunology, Bloomberg School of Public Health, Johns Hopkins University, Baltimore, Maryland, USA
Email: *pyboakye@presbyuniversity.edu.gh

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Abstract

Background: Malaria has been one of the major causes of death in Ghana, especially in children. Appropriate diagnosis is important in the treatment of malaria. Microscopy is the gold standard for malaria diagnosis; however, most rural communities have resorted to the use of Rapid Diagnostic Tests (RDTs) due to the lack of electricity and ease of use. It is therefore important to assess the effectiveness of RDTs in giving accurate results. The study participants were aged 1 - 90 years. **Methodology/Principal Findings:** This study is a hospital-based cross-sectional study conducted at Dunkwa-on-Offin Municipal Hospital, St. Mark Hospital, and Kyekyewere Health Center. It involved patients with suspected malaria cases who consented to be part of the study. Blood samples were collected from participants for microscopy, RDTs, and Polymerase Chain Reaction (PCR) testing. A total of 310 patients were involved in the study. 67.1% and 32.9% were females and males, respectively. The prevalence of malaria using microscopy, Care Start RDT, Aria Rapid RDT, Acro Biotech RDT, and PCR was 31.3%, 34.5%, 34.5%, 34.5%, and 44.8%, respectively. The prevalence was highest in Kyekyewere (50%), followed by St. Mark Hospital (35%), and lowest in Dunkwa-on-Offin Municipal Hospital (31.3%).

Mixed infections were higher than single infections among those who tested positive using all the diagnostic methods. Using PCR as a reference, the sensitivity and specificity of microscopy were 69.57% and 99.42%, respectively. The sensitivity of CareStart, Aria Rapid test, and Acro Biotech RDTs was 79.10%, and their specificities were all 99.43%. **Conclusion:** The research revealed that PCR had the highest sensitivity compared to RDTs and microscopy. Nevertheless, it cannot be used in routine diagnosis in developing countries and endemic areas due to the high cost involved. The study also established that microscopy should still be the gold standard for diagnosing malaria infection. In remote areas without electricity or microscopy expertise, RDTs can be utilised for early diagnosis to avoid complications associated with late detection.

Keywords

Microscopy, Diagnosis, Rapid Diagnostic Test (RDTs), Polymerase Chain Reaction (PCR)

1. Introduction

Malaria has been the leading cause of child mortality in sub-Saharan Africa [1]. Malaria is a global burden, and according to the WHO 2023 malaria report, an estimated number of 608,000 deaths and 249 million cases of malaria infection were recorded globally in 2022. The percentage of total malaria deaths in children aged under 5 years decreased from 87% in 2000 to 76% in 2015. Since then, there has been no change [2]. An estimated 90% of malaria deaths occur in sub-Saharan Africa, and children less than 5 years old account for the majority of these deaths (78%) [3]. In Ghana, about 20,000 children die from malaria yearly [3]. Children less than five years old account for 25% of these deaths [3]. Malaria is highly associated with poverty, and most malaria-endemic countries, such as Ghana, are developing countries [4].

Diagnosis and treatment of malaria infection are concerns in the country. The WHO recommends the use of microscopy as a gold standard for diagnosing malaria infection; however, most rural communities employ the use of Rapid Diagnostic Tests (RDTs) in diagnosing malaria. This is due to the lack of electricity and qualified personnel to operate the microscope. Again, WHO recommends diagnosis of all patients suspected of malaria before treatment is administered, and RDTs have the potential to improve the quality of managing malaria infection, especially in remote areas [5].

Rapid diagnostic test is a technique that identifies malaria antigen in a small quantity of blood, usually about 5 - 15 μL, by immunochromatographic assay with monoclonal antibodies directed towards the target parasite antigen and fixed on a test strip [6]. RDTs have the ability to detect histidine-rich protein 2 (HRP 2), aldolase, or *Plasmodium* lactose dehydrogenase (pLDH). The histidine-rich pro-

tein 2 (HRP 2) is exclusively for the detection *of Plasmodium falciparum* (*Pf*RDTs). The aldolase or the *Plasmodium* lactose dehydrogenase (*pLDH*) detects the other *Plasmodium* species (Pf/Pan RDTs).

In about 5 - 20 minutes, the result is obtained. RDT tests are easy to perform and simple to interpret. They do not require much capital, nor do they need electricity. The RDTs used by most government facilities in Ghana can detect only *P. falciparum* (CareStart RDT). Nevertheless, there are some that detect both *P. falciparum* and other species of *Plasmodium* parasite [7]. Commercial test kits are made with different mixtures of target antigens to fit the epidemiology of malaria in the locality. The malaria antigen which is mainly targeted is Histidine-Rich Protein 2 (HRP-2), which is specific for *P. falciparum*. Some commercial test kits have both genus-specific aldolase enzyme and HRP-2 assays; this makes it possible to distinguish *P. falciparum* and non-*P. falciparum* malaria [7]. Although the efficacy of RDTs has been determined by many specialists by comparison with microscopy as the gold standard [5], it is relevant to compare them with a molecular technique (PCR). The limitations of both RDTs and microscopy can reduce the reliability of results, and therefore a more sensitive and specific method is needed to assess the accuracy of these diagnostic methods. PCR has been shown to detect the presence of the malaria parasite below the detection limit of RDTs and microscopy [8].

A recent study in Ethiopia showed that *Plasmodium falciparum* parasites that do not have the histidine-rich protein 2 (pfhrp2) and 3 (pfhrp3) genes may avoid detection by these RDTs. However, it is uncertain whether these gene deletions provide enough of an advantage to cause a rapid increase in the parasite population [9]. Hence, there is a need to periodically assess the efficacy of RDTs that have been approved by the Ministry of Health and are in use at various rural health facilities.

This study aimed to evaluate the diagnostic ability of three RDTs—Care Start RDT, Aria Rapid RDT, and Acro Biotech RDT used in rural health facilities in Ghana by comparing them with microscopy and PCR. The results of this research will help improve diagnosis and eventually the treatment of malaria infection in rural communities. It would also provide data to further improve the quality of RDTs.

2. Materials and Methods

2.1. Ethics Statement

The Committee on Human Research, Publication and Ethics of the School of Medical Sciences, Kwame Nkrumah University of Science and Technology granted approval for the study (CHRPE/AP/580/19).

2.2. Study Design and Location

The study was cross-sectional. Participants were selected from patients who were

suspected of having malaria using the inclusion and exclusion criteria of the study. Dunkwa-on-Offin Municipal Hospital (200 patients), Kyerewere Health Center (50 patients), and St. Mark Hospital (60 patients) were selected from the municipality. These study sites were chosen from among three hospitals, four health centers, three clinics, and nineteen CHPS Compounds. Patients who were included in the study were from the surrounding villages. The study sites were chosen because most people from the rural communities attend these hospitals.

2.3. Inclusion and Exclusion Criteria

Patients who showed symptoms of malaria and were asked by a physician to perform a malaria test were included in the study. Those who read and understood the participant information leaflet and gave their consent were included in the study. Guardians of children who were below the age of 18 gave consent for their wards. The research protocol was explained to patients who could not read before they were included in the study. Patients who were not asked to perform a malaria test were excluded from the study, and patients who did not consent to the study were excluded from the study. A total of 310 participants were included in the research.

2.4. Sample Size

The sample size was calculated using the sample size formula

$$n = z^2 \cdot P(1-P)/M^2,$$

where: n = sample size, Z = 1.96 standard score at 95% confidence interval, M = margin of error of 5.0%, and P = estimated prevalence of malaria (28%) [10] Therefore, $n = 1.96^2 \times 0.28(1 - 0.28)/0.05^2 = 310$. A total of 310 participants were selected for the research. The sample size was divided among the study sites based on the size of the facility and the frequency with which patients from villages attend them. The information concerning the attendance of patients was obtained from the Records Department of the hospitals. (**Figure 1**)

2.5. Samples Collection

Ethylenediaminetetraacetic acid (EDTA) tube was labeled with the participant's initials, date, and time of collection. The site of blood collection was cleaned using 70% alcohol. 2 mL of venous blood was collected with a syringe and needle and then transferred into the labeled EDTA tube. The blood sample in the tube was well mixed. Microscopy and Rapid Diagnostic Test were done immediately after blood collection [11].

Frosted-end slides were cleaned and labeled with numbers and dates. Two thick and two thin films were prepared immediately after the samples were collected. For the thin film, 2 µL of blood was pipetted and placed on the slide. A clean spreader slide was held at a 45˚ angle, toward the blood on the specimen slide. The spreader was pushed forward smoothly and rapidly.

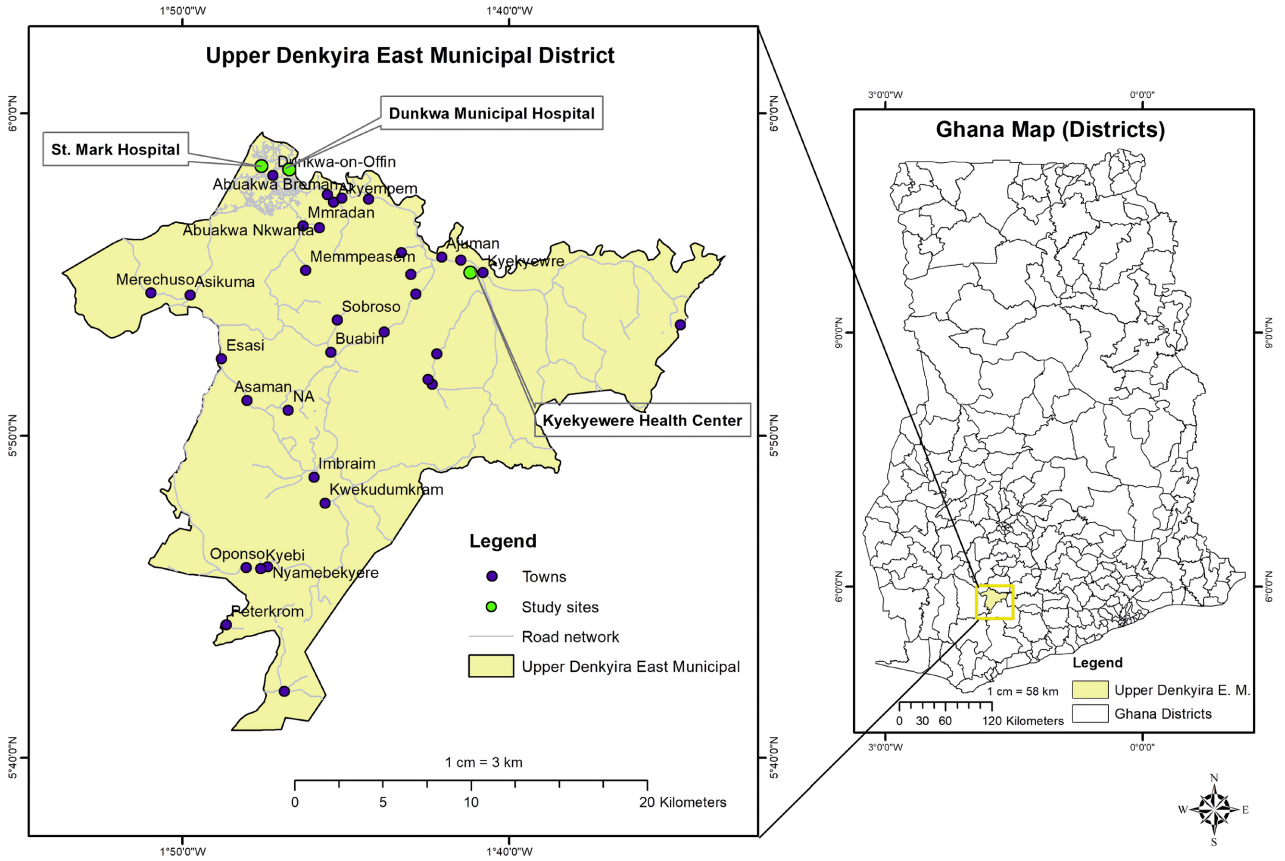

Figure 1. The map of Ghana showing the location of the study in the Upper Denkyira East Municipality. The map was developed by one of the authors with ESRI ArcMap 10.8 using data from the Ghana Open Data Initiative and the Open Street Map Foundation.

To prepare the thick film, 6 µL of blood was pipetted and placed on a specimen slide. The blood was spread using the corner of a different clean slide. The spread was made into a circle with a diameter of 1 - 2 cm. The smear was made such that it was easy to read through. Both the thin and thick films were allowed to dry properly. The thin film was fixed in absolute methanol before staining [11].

2.6. Preparation and Staining of Blood Smears

Firstly, 9 mL of buffered water with a pH of 7.2 was poured into a beaker. Giemsa stock solution was filtered through Whatman filter paper into a 25 mL container. Using a clean pipette, 1 mL of the filtered Giemsa solution was added to the buffered water. The freshly prepared Giemsa solution was used within a maximum of 15 minutes.

The slide was placed in the working Giemsa stain for about 45 - 60 minutes. The thin film was rinsed by dipping it in the Giemsa buffer about 3 - 4 times, while the thick smear was left in the buffer for about 5 minutes. The stained slides were dried upright in a rack.

2.7. Examination of Thick Films

The thick film was used to detect malaria parasites and mixed infections. The pre-

pared slide (smear) was first screened with a low magnification (×10) to detect parasites and then switched to the 100× oil immersion objective lens. Slides were declared negative after observing over 100 fields, with each containing about 200 white blood cells (WBCs).

2.8. Examination of Thin Films

The thin film was used for the species identification of the parasites. The prepared thin film was observed under the microscope using low magnification (×10 objective lens) to further detect parasites. The slide was examined thoroughly using the 100× oil immersion objective lens to identify species. *Plasmodium* species were distinguished based on the morphological characteristics of a blood smear. These include: the size of infected red blood cells and the nature of trophozoites observed in the blood film [11]. Two independent certified malaria microscopists read both the thick and thin smears, and the results were compared to ensure validation. The first microscopist examined the blood smear (thick and thin films) and reported the presence or absence of malaria parasites, species identification, and parasite density. The second microscopist independently re-examined the same slide without knowing the results of the first reader and provided his own diagnosis. All discrepancies between the results of the two independent certified microscopists were settled by a third, more experienced microscopist (tie-breaker).

2.9. Determination of Parasite Density

The parasite density determination was done in the thick smear. Two tally counters were used to count the number of parasites against the WBCs. The count was done until at least 200 WBCs were counted. The number of parasites/μL of blood was determined by finding the number of parasites in relation to the standard number of 8000 WBCs/μL.

$$\text{Number of Parasites per μL of blood} = \frac{\text{Number of parasites counted}}{\text{Number of WBCs counted}} \times 8000 \text{ (WBCs/μL)}$$

8000 WBCs/μL is considered as the standard number of white blood cells per μL of blood [11].

2.10. Rapid Diagnostic Test (RDT)

Malaria tests were done using three different RDTs. They were CareStart Malaria Pf (HRP2) Ag RT, Aria Malaria Pf/Pan Ag Rapid Test, and Acro Biotech Malaria Pf/Pan Ag Rapid Test.

2.11. Procedure for CareStart Malaria *Pf* (HRP2) Ag RT

The cassette was labeled with the patient's initials. 5 μl of blood was pipetted from the sample already collected into an EDTA tube. The 5 μl of blood collected was transferred into the "S" well. Two drops of the buffer solution were added to the "A" well. The result was read after 20 minutes. The test was declared invalid when

a line did not appear at the "C" portion. Such results were repeated. A result was declared negative when a line appeared at the "C" portion only. A result was declared positive for *P. falciparum* (*Pf*) when two lines appeared, one at the "C" portion and one at the "T" portion. The sensitivity and specificity of the CareStart test Kit were 98% and 97.5%, respectively, from the manufacturer.

2.12. Procedure for the Aria Malaria *Pf*/Pan Ag Rapid Test and the Acro Biotech Malaria *Pf*/Pan Ag Rapid Test

The cassette was labeled using the participant's initials. 5 µl of blood was pipetted from the EDTA tube. Two drops of blood lysis buffer were added to the centre of the buffer well (B well). The result was read within 20 - 30 minutes. The result was declared as negative when only the "C" line was present. The result was declared *Pf* positive or reactive when only the *Pf* line developed in addition to the control "C" line. This indicated the presence of the *Plasmodium falciparum* histidine-rich protein-II (pHRP-II) antigen [11].

The result was read as Pf negative but positive for any of the other three *Plasmodium* species (*Plasmodium ovale*, *Plasmodium malariae*, *Plasmodium vivax*) when only the Pan line developed together with the control line ("C"). This showed the presence of *Plasmodium* lactate dehydrogenase (pLDH) antigen. When both the Pf and the Pan lines together with the control line ("C") developed, the result was read as Pf positive and any of the other three *Plasmodium* species also positive. This showed the presence of both pLDH and pHRP-II antigens. The result was read as invalid when no control (C) line developed.

The sensitivity and specificity of the Aria Rapid test kit were 100% and 100% for the detection of Pf and 95% and 100% for the detection of Pan, respectively. The sensitivity of the Acro Biotech test kit was 96.5% and 90.3% for detecting Pf and Pan, respectively. The specificity for detecting both Pf and Pan was 99.4%. The above sensitivity and specificity of the Aria Rapid Test and Acro Biotech RDTs were reported as given by the manufacturer.

2.13. Molecular Analysis of Blood Samples

2.13.1. Extraction of *Plasmodium* DNA

The samples were packed out of the storage freezer (−20˚C) and allowed to thaw. DNA isolation was performed using the Quick DNA kits (Zymo Research, USA; https://www.zymoresearch.com) with the manufacturer's protocol. The procedure used was as follows: Blood samples were mixed thoroughly by pipetting up and down, and 100 µL aliquots were transferred into a labelled sterile 1.5 ml microcentrifuge tube. After which, 1 mL of TE (1 × Tris-EDTA buffer) was added, vortexed at 3000 rpm for 30 seconds, and then incubated at 4˚C for 10 minutes. After the incubation, samples were centrifuged at 12,000 ×g for 5 minutes, and the supernatant was discarded. The sediment was then suspended in a mixture containing 300 µL Genomic Lysis Buffer and 5 µL Proteinase K and mixed completely by vortexing for 15 - 30 seconds. At this stage, the whole mixture was incubated at 56˚C overnight.

After the overnight incubation, the mixture was vortexed again for 15 - 30 seconds and incubated at 80˚C for 10 minutes for improved lysis and inactivation of the proteinase K enzyme. The mixture was then vortexed and transferred into a labeled Zymo-Spin™ IIC column in a microcentrifuge tube, after which centrifugation was performed at 12,000 ×g for one minute. The flow-through was discarded along with the microcentrifuge tube. At this point, the Zymo-Spin™ IIC column was placed in a new microcentrifuge tube to begin the washing process. An aliquot of 200 µl of DNA Pre-Wash Buffer and 500 µl of g-DNA Wash Buffer were used independently for the first and second washing of DNA, respectively, with the previous centrifugation conditions.

To remove traces of washing solutions, centrifugation was repeated to dry the spin column. The dried spin column was transferred into a clean labeled micro centrifuge tube for the elution of DNA bound to the silica membrane. Finally, 70 µl DNA Elution Buffer was added to the spin column, incubated for 15 minutes at room temperature, and then centrifuged at 3000 rpm for 30 seconds to collect the DNA. The eluted DNA was stored immediately at −20˚C until ready for the Nested PCR test [11].

2.13.2. The ssrRNA Gene Amplification from the *Plasmodium* DNA

The amplification of the ssRNA gene of the *Plasmodium sp.* involved nested-PCR as described by Snounou and Singh (2002) [12]. The pair of genus-specific oligonucleotide primers, rPLU1 and rPLU5, targets a 1.6 - 1.7 kb (kilobase) fragment of the gene for the first run of PCR (Nest 1; **Figure 2**). Subsequently, to further confirm the amplification of the Nest 1 PCR product, a Nest 2 PCR was run with a pair of genus-specific primers (RPLU 3 and 4) to amplify 235 bp (base pair) of the Nest 1 PCR product. In addition, the speciation of the *Plasmodium* involved the use of species-specific primers; *P. falciparum* (RFAL 1 and 2), *P. malariae* (RMAL and 2), *P. ovale* (OVA1 and RPLU2) and *P. vivax* (VIV1 and 2) to amplify 206 bp, 145 bp, 226 bp and 120 bp of the gene, respectively, of the Nest 1 PCR product (Nest 2; **Figure 2**).

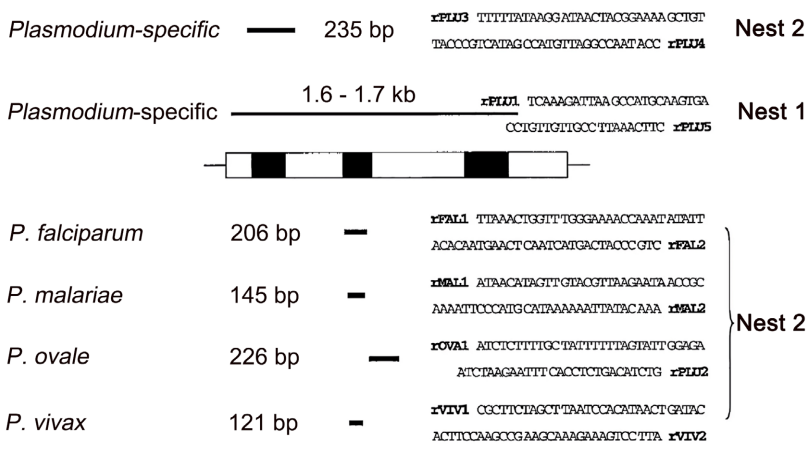

Figure 2. Schematic diagram showing the Nest 1 and Nest 2 *Plasmodium* primers, their sequences, and corresponding fragment sizes. Source: [12].

3. Data Analysis

Statistical Package for Social Scientists (SPSS version 23) was used for the analysis. Descriptive statistics such as frequency and percentage were used. The chi-square test of association was further used to test the association between the area where suspected malaria cases were sampled and the result obtained from the test, as well as the type of species detected. In all the analyses, a p-value of 0.05 was considered significant.

4. Results

Although microscopy is regarded as the gold standard for diagnosing malaria, PCR was taken as a reference for measuring the sensitivity and specificity of RDT and microscopy.

The study comprised 102 (32.9%) male and 208 (67.1%) female participants. The age group with the highest number of participants was 5 - 14 years, representing 24.5%, followed by <5 years (19%). The age group 55 to 64 years had the lowest number of participants, representing 5.2%. Table 1 below shows the demographic characteristics of participants in the study area.

Table 1. Demographic characteristics of study participants.

Characteristics	Frequency	Percentage (%)
Sex		
Male	102	32.9
Female	208	67.1
Total	310	100
Age Groups (years)		
<5	59	19
5 - 14	76	24.5
15 - 24	58	18.7
25 - 34	36	11.6
35 - 44	17	5.5
45 - 54	23	7.4
55 - 64	16	5.2
≥65	25	8.1
Total	310	100

4.1. Distribution of *Plasmodium* Parasites at Study Sites

In terms of *Plasmodium spp* parasitemia among the samples collected from the study centers, Kyekyewere Health Center recorded the highest, followed by Dunkwa Municipal Hospital, and St. Mark Hospital recorded the least. Figure 3 below shows the distribution of *Plasmodium* parasites in the study area.

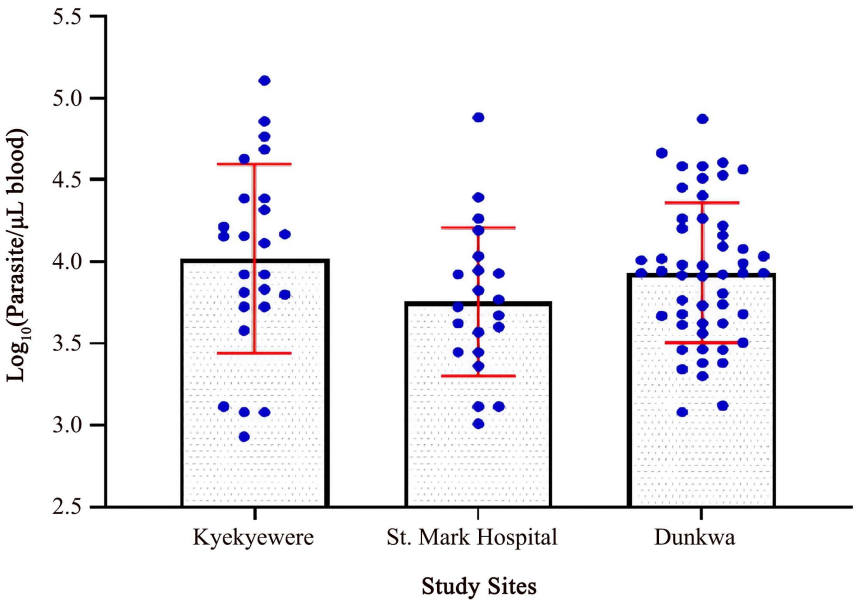

Figure 3. Distribution of parasitemia by study site.

4.2. The Performance of RDTs against Various *Plasmodium* Parasite Densities

It was observed that the ability of the RDTs to detect positive results increases as the parasite density (*Plasmodium* parasitemia) increases. Ninety-seven positive results by microscopy were compared to the CareStart, Aria Rapid Test, and Acro Biotech RDTs. At a parasite density of ≤1000 parasites/µL, microscopy detected one positive, while the RDTs did not detect any positives. At a parasite density of 1001 - 5000 parasites/µL, CareStart, Aria Rapid Test, and Acro Biotech RDTs all recorded 24 (77.40%) positives respectively out of the 31 positives for microscopy. At 5001 - 10,000 parasites/µL, out of the 27 positives for microscopy, CareStart, Aria Rapid Test, and Acro Biotech RDTs all recorded 24 (88.90%) positives. Out of 38 positives detected by microscopy, at a parasite density > 10,000, all the RDTs detected 37 (97.37%) positives. Table 2 below shows parasite density versus positive malaria cases identified by microscopy and RDT.

Table 2. Parasite density versus malaria positivity identified by microscopy and RDT.

Parasite Density (parasites/µL)	Microscopy	Rapid Diagnostic Tests		
		CareStart	Aria Rapid Test	Acro Biotech
≤1000	1	0 (0)	0 (0)	0 (0)
1001 - 5000	31	24/31 (77.4 %)	24/31 (77.4%)	24/31 (77.4%)
5001 - 10000	27	24/27 (88.9%)	24/27 (88.9%)	24/27 (88.90%)
>10000	38	37/38 (97.4%)	37/38 (97.4%)	37/38 (97.4%)

RDT positive with microscopy negative was considered a false positive and not included as a malaria-positive case for Table 2 only. The various RDTs were compared with microscopy at various parasite densities.

4.3. Nested PCR Products

The figures below, thus, Figures 4-8, show Nested PCR products under UV light. The DNA bands of various sizes (bp) clearly show the presence of various *Plasmodium* species. P1, P2, P3, P4, P5, P6, P7, P8, P9, P8, P9, P10 and P11 show some of the patients' samples used for the Nested PCR. PC and NC show positive and negative control, respectively. Figures 4-8 were taken by our team during the research.

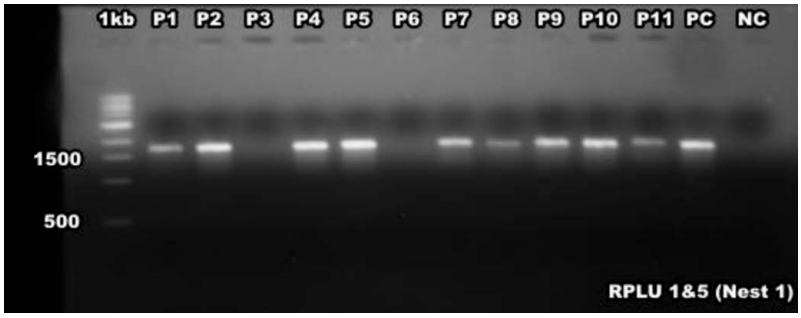

Figure 4. Gel electrophoresis image of *Plasmodium* PCR product (Nest 1).

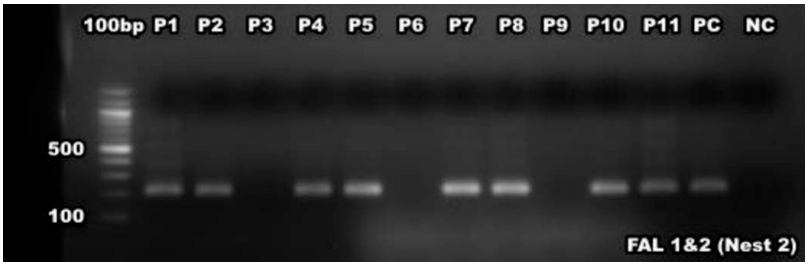

Figure 5. Gel electrophoresis image of *Plasmodium falciparum* Nest 2 PCR product.

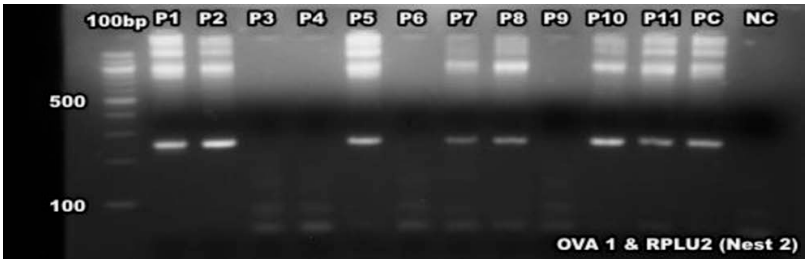

Figure 6. Gel electrophoresis image of *Plasmodium ovale* Nest 2 PCR product.

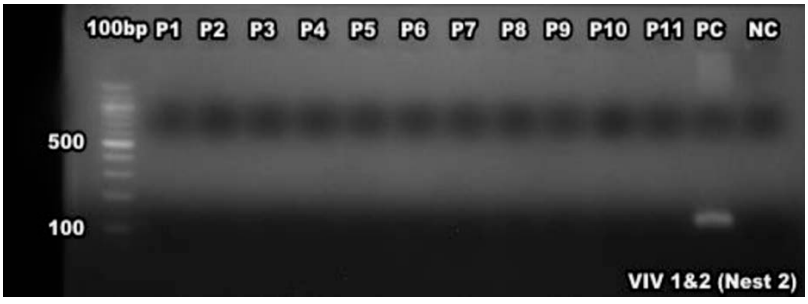

Figure 7. Gel electrophoresis image of *Plasmodium vivax* Nest 2 PCR product.

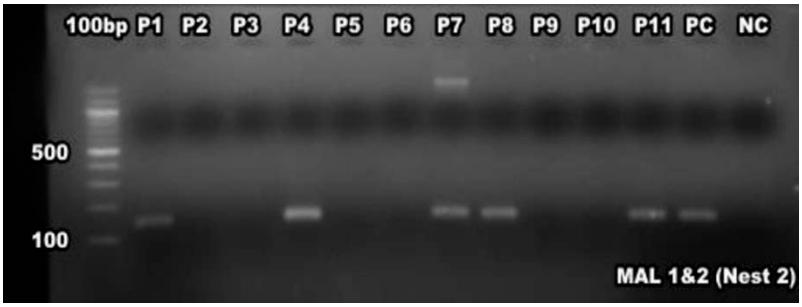

Figure 8. Gel electrophoresis image of *Plasmodium malariae*.

4.4. *Plasmodium* Species Detected by Nested PCR, RDT, and Microscopy

Speciation was done for all 310 samples using Nested PCR, RDT, and Microscopy. Mixed species infections were recorded by all diagnostic techniques. **Figure 9** below shows *Plasmodium* species detected by Nested PCR, RDT, and Microscopy.

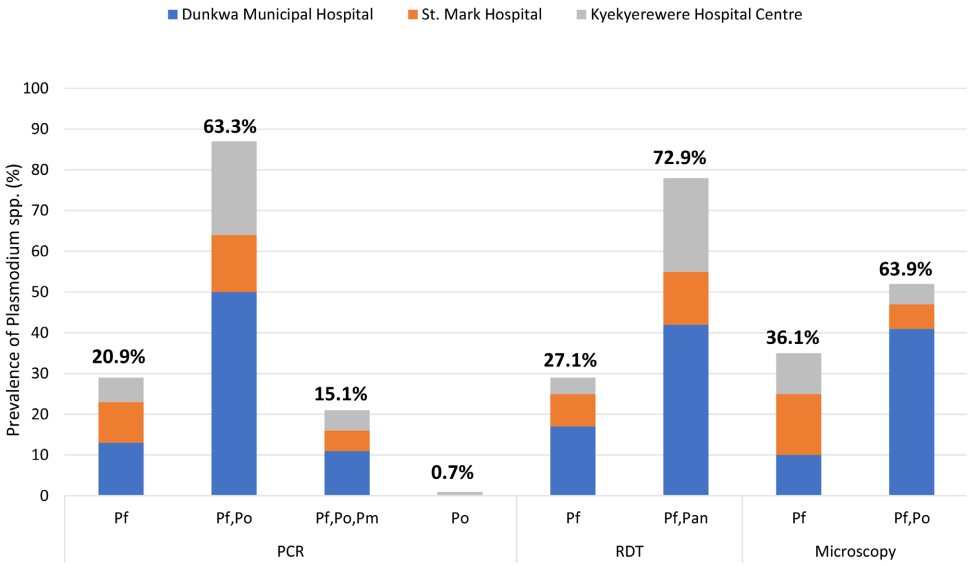

Figure 9. Distribution of *Plasmodium* species detected by PCR, RDT, and Microscopy.

4.5. Comparison of CareStart, Aria Rapid Test, Acro Biotech RDTs, and Microscopy to PCR

When PCR was used as the "Standard Reference of comparison", the sensitivity of CareStart, Aria Rapid Test, and Acro Biotech RDTs was 79.10%. Their corresponding specificities were all 99.43%. When the ROC analysis was performed, it was observed that CareStart, Aria Rapid Test, and Acro Biotech RDTs all had an AUC (Area under the ROC curve) value of 0.94. The kappa value of 0.57 obtained for all RDTs showed a modest agreement between PCR and the RDTs. A chi-square Test of Association indicated a significant difference between the various RDTs and PCR ($p = 0.001$). Microscopy had a sensitivity and specificity of 69.57% and 99.42%, respectively. **Table 3** shows the diagnostic evaluation of RDTs and Microscopy using PCR as reference.

Table 3. Evaluating the performance of CareStart, aria rapid test, acro biotech RDTs, and microscopy with PCR as a reference.

Performance Indicators	Rapid Diagnostic Test			Microscopy
	Aria	Acro	CareStart	
Sensitivity (%) (CI_{95})	79.10 (75.00 - 83.00)	79.10 (75.00 - 83.00)	79.10 (75.00 - 83.00)	69.57 (65.8 - 72.0)
Specificity (%) (CI_{95})	99.43 (96.60 - 100.00)	99.43 (96.60 - 100.00)	99.43 (96.60 - 100.00)	99.42 (96.60 - 100.00)
Positive Predictive Value (%) (CI_{95})	99.07 (95.20 - 100.00)	99.07 (95.20 - 100.00)	99.07 (95.20 - 100.0)	98.96 (96.6 - 99.80)
Negative Predictive Value (%) (CI_{95})	86.21 (76.80 - 93.00)	86.21 (76.80 - 93.00)	86.21 (76.8 - 93.00)	80.82 (74.20 - 87.40)
Accuracy (%) (CI_{95})	95.74 (91.60 - 97.90)	95.74 (91.60 - 97.90)	95.74 (91.60 - 97.90)	84.21 (79.80 - 88.00)
AUC (CI_{95})	0.94 (0.91 - 0.96)	0.94 (0.91 - 0.96)	0.94 (0.91 - 0.96)	0.80 (0.78 - 0.86)
Kappa Value (κ), SE p-value	0.57 <0.001	0.57 <0.001	0.57 <0.001	0.45 <0.001

4.6. Comparing the Distribution of Malaria and the Diagnostic Efficiency of the Three Diagnostic Methods

Out of the 310 cases, 141 patients tested positive by microscopy or PCR. The percentages of positives for PCR, microscopy, and RDT were 98.6%, 68.9%, and 75.9%, respectively. Table 4 shows the comparison of the distribution of malaria-positive cases by diagnostic techniques.

Table 4. Distribution of Malaria and the diagnostic efficiency of the three diagnostic methods (N = 310).

Number of cases with the following test results							
Variable	RDT+ microscopy+ PCR+	RDT+ microscopy− PCR+	RDT+ microscopy+ PCR−	RDT− microscopy+ PCR+	RDT− Microscopy+ PCR−	RDT− Microscopy− PCR+	RTD+ Microscopy− PCR−
Positive Cases	84	22	1	11	1	22	0
Diagnostic efficiency of three diagnostic methods							
Type of Diagnostic Method	RDT		Microscopy		PCR		
Diagnostic efficiency % (no. of positives/total positives)	75.9% (107/141)		68.9% (97/141)		98.6% (139/141)		

5. Discussion

The research aimed at evaluating the performance of RDTs, microscopy, and PCR

in the detection of *Plasmodium* parasites in blood. PCR was used as a reference to compare with microscopy and RDTs.

The percentage of males and females obtained is similar to a work conducted in Cameroon on malaria diagnostic methods [8]. Their study recorded 67.1% females and 32.9% males. However, the male-to-female ratio differed from a study conducted in Nigeria which recorded 52.7% males and 47.3% females [13].

From the positive cases of malaria obtained by microscopy, females and males recorded 56.7% and 43.3%, respectively. Females further recorded 54% for patients who had mixed parasite infections compared with 46% of male patients. The higher prevalence of malaria in females was also reported in Tanzania [14]. In addition, the number of females recruited in the study was larger than that of males, hence accounting for the difference seen.

The higher prevalence of malaria in Kyekyewere is partly due to illegal mining activities in the area where pits are left uncovered after mining and are filled with water, which serves as a breeding site for mosquitoes. Moreover, Kyekyewere is a bushy area; hence, these bushes serve as breeding sites for mosquitoes, leading to the increased incidence of malaria. On the other hand, Dunkwa-on-Offin Municipal and St. Mark Hospital are located in the district capital, which is a semi-urban community. A similar difference in prevalence among study sites was recorded in southern Ethiopia [15]. The study sites were mining and farming communities; according to the Presidential Malaria Initiative, the prevalence of malaria in rural communities was 28% [10]. The prevalence in this study was 31.3%. Presumptive treatment is very common among dwellers in rural areas. Most of them buy drugs from pharmacies at the onset of any symptom, and they may end up taking the wrong drug while the parasites still persist in their blood. A study conducted at Kintampo North and South recorded a prevalence of 58% for malaria, which is higher than the prevalence obtained in this study [16].

It was noted in this study that the ability of the RDTs to give accurate results increased as parasite density increased. With a parasite density of >10,000 parasites/μL, the RDTs detected 97.4% of the positive results obtained by microscopy. At a parasite density of 5001 to 10,000 parasites/μL, the ability of the RDTs to detect positive results decreased to 88.9%. At a parasite density of 1001 to 5000 parasites/μL, 77.4% of the positives detected by microscopy were recorded by RDT. Lastly, at a density ≤ 1000, the RDTs could not detect one positive sample detected by microscopy. Similar results were reported in Nigeria [17]. It is likely that the RDT may give false negative results for patients with low parasite density. In rural areas where only RDT is used for diagnosis, it is likely that some true malaria cases may be missed.

Using PCR as a reference, this study presented sensitivities of 79.10% and 69.57% for RDTs and microscopy, respectively. Their respective specificities were 99.43% and 99.42%. The result differs from studies conducted in Cameroon [8] as the sensitivity and specificity recorded were 95.3% and 94.3%, respectively. The reduced sensitivity shown in this study is higher than the 78.4% recorded in Zaira,

Nigeria [18]. Another study conducted in Northern Nigeria reported a low sensitivity of 40.3% and specificity of 89.6% [19]. The low sensitivity and specificity recorded by the RDTs may be due to extreme environmental conditions such as high temperature and humidity during storage in these regions [20]. The manufacturing process can also affect the quality of the RDTs. Again, low parasite density can affect the sensitivity of RDTs; as the parasite density reduces, the sensitivity of the RDT consequently reduces [8]. Furthermore, conditions such as mutation or deletion of the HRP-2/HRP-3 gene have a probability of reducing the sensitivity and specificity of RDTs. In such instances, the parasite no longer produces the antigen or produces a mutant (different) antigen which cannot be recognized by antibodies embedded on the RDT strip [21]. However, the study did not conduct a test to detect the gene deletion or mutation of pfhrp2/3. Future research can focus on detecting deleted/mutated pfhrp2/3 in the study area.

AUC (Area under the ROC curve) values of 0.94 and 0.80 were recorded for the RDTs and microscopy, respectively. This showed that RDT and microscopy were excellent and very good in detecting positive and negative samples. Kappa values of 0.57 and 0.45 were recorded for RDTs and microscopy, respectively. The kappa values obtained by the RDTs and microscopy showed a moderate agreement with microscopy.

A study conducted by Opoku *et al.*, 2023 and Ahmad *et al.*, 2021 recorded similar sensitivities to those obtained by this research [22] [23]. Opoku *et al.*, 2023 recorded sensitivities of 55.7% and 39.3% for RDTs and microscopy, respectively. Ahmad *et al.*, 2021 recorded sensitivities of 82% and 84.2% for RDTs and microscopy, respectively. The reduced sensitivities of 79.10% and 69.57% for RDTs and microscopy, respectively, may partly be due to the fact that PCR has a higher detection limit of between 0.7 and 0.2 parasites/μL [24]. Another reason that might have accounted for the higher number of positives by PCR may be the persistence of parasite antigen (HRP-II) in blood circulation even after treatment of malaria [25].

Similar studies were conducted by Quakyi *et al.* (2018) and Acquah *et al.* (2021) [26] [27] in Ghana. They recruited children less than 5 years old who presented to health facilities with a history of fever in the past 72 hours and afebrile volunteers aged 3 and above, including pregnant women, respectively. This research, on the other hand, involved people who presented visible symptoms of malaria such as fever. Again, their research focused primarily on *Plasmodium falciparum*. However, our study included other *Plasmodium* species such as *Plasmodium ovale*, *Plasmodium vivax* and *Plasmodium malariae*. It was necessary to look out for the presence of other species in the study population because the RDTs used at most Government facilities in Ghana are the CareStart HPR2(Pf). This research found that *Plasmodium ovale*, *Plasmodium malariae* together with *Plasmodium falciparum* were among the positive cases obtained. Most of the positive cases were coinfections of *Plasmodium falciparum* and *Plasmodium ovale* using all three methods of diagnosis. Therefore, using CareStart HPR2 (Pf), which detects only *Plas-*

modium falciparum, is not adequate for complete diagnosis. *Plasmodium ovale* infection requires additional treatment with primaquine to prevent relapses of parasites from hypnozoites. In order to guarantee early and comprehensive diagnosis and treatment of malaria in rural populations, the research recommends that health policy makers adopt the use of RDTs that can identify other *Plasmodium* species in addition to *P. falciparum*. Although PCR has higher sensitivity than microscopy and RDTs, it is not suitable for routine diagnosis of malaria infection, especially in malaria-endemic countries such as Ghana. This is because parasite antigens may still remain in the blood after clearing of the parasites from a person's blood [10].

This will give false positive results, hence a wrong diagnosis. Again, the cost involved in the PCR test is high and requires a well-trained technician to perform the test. This will make it difficult for developing countries to use it as a routine diagnostic tool. However, it is relevant in confirming cases of malaria, speciation, and detecting mixed species infections.

141 out of 310 respondents were positive for PCR or microscopy. The percentage of positives for RDT, microscopy, and PCR for the 141 samples was 75.9%, 68.9%, and 98.6%, respectively. 53.3%, 47.5%, and 87.4% were obtained for RDT, microscopy, and PCR from a study conducted by Chourasia *et al.* (2017) [28].

6. Conclusions

PCR has a higher sensitivity than microscopy and RDTs. However, it is not suitable for routine diagnosis in developing countries such as Ghana due to the high cost involved, inadequately trained personnel, and lack of electricity in some rural communities.

Microscopy remains the gold standard for diagnosing malaria. RDTs can be used as an emergency tool, especially in remote rural communities, for early diagnosis to prevent complications associated with late diagnosis. RDTs used in health facilities should be able to detect other *Plasmodium* species apart from *Plasmodium falciparum*.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] D'Acremont, V., Lengeler, C., Mshinda, H., Mtasiwa, D., Tanner, M. and Genton, B. (2009) Time to Move from Presumptive Malaria Treatment to Laboratory-Confirmed Diagnosis and Treatment in African Children with Fever. *PLOS Medicine*, **6**, e252. https://doi.org/10.1371/journal.pmed.0050252

[2] WHO (2023) World Malaria Report 2023. https://www.who.int/teams/global-malaria-programme/reports/world-malaria-report-2023#:~:text=Latest%20malaria%20report%20spotlights%20the,the%20malaria%2Dcarrying%20Anopheles%20mosquito

[3] Yankson, R., Anto, E.A. and Chipeta, M.G. (2019) Geostatistical Analysis and Map-

ping of Malaria Risk in Children under 5 Using Point-Referenced Prevalence Data in Ghana. *Malaria Journal*, **18**, Article No. 67. https://doi.org/10.1186/s12936-019-2709-y

[4] Teklehaimanot, A. and Mejia, P. (2008) Malaria and Poverty. *Annals of the New York Academy of Sciences*, **1136**, 32-37. https://doi.org/10.1196/annals.1425.037

[5] Guthmann, J.P., Ruiz, A., Priotto, G., Kiguli, J., Bonte, L. and Legros, D. (2002) Validity, Reliability and Ease of Use in the Field of Five Rapid Tests for the Diagnosis of *Plasmodium falciparum* Malaria in Uganda. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **96**, 254-257. https://doi.org/10.1016/s0035-9203(02)90091-x

[6] Tangpukdee, N., Duangdee, C., Wilairatana, P. and Krudsood, S. (2009) Malaria Diagnosis: A Brief Review. *The Korean Journal of Parasitology*, **47**, 93-102. https://doi.org/10.3347/kjp.2009.47.2.93

[7] Bell, D., Wongsrichanalai, C. and Barnwell, J.W. (2006) Ensuring Quality and Access for Malaria Diagnosis: How Can It Be Achieved? *Nature Reviews Microbiology*, **4**, 682-695. https://doi.org/10.1038/nrmicro1474

[8] Moyeh, M.N., Ali, I.M., Njimoh, D.L., Nji, A.M., Netongo, P.M., Evehe, M.S., *et al.* (2019) Comparison of the Accuracy of Four Malaria Diagnostic Methods in a High Transmission Setting in Coastal Cameroon. *Journal of Parasitology Research*, **2019**, 1-8. https://doi.org/10.1155/2019/1417967

[9] Feleke, S.M., Reichert, E.N., Mohammed, H., Brhane, B.G., Mekete, K., Mamo, H., *et al.* (2021) *Plasmodium falciparum* Is Evolving to Escape Malaria Rapid Diagnostic Tests in Ethiopia. *Nature Microbiology*, **6**, 1289-1299. https://doi.org/10.1038/s41564-021-00962-4

[10] Ghana Malaria Operational Plan FY18 (2018) African Malaria Task. Presidential Malaria Initiative.

[11] Centers for Disease Control and Prevention (2023) Malaria. https://www.cdc.gov/dpdx/malaria/index.html

[12] Snounou, G. and Singh, B. (2002) Nested PCR Analysis of *Plasmodium* Parasites. In: Doolan, D.L., *Malaria Methods and Protocols*, Humana Press, 189-204. https://doi.org/10.1385/1-59259-271-6:189

[13] Bashir, M., Sunday, E., Mohammed, B., Ali, I., Isa, H., Sambo, K.H., *et al.* (2019) Evaluation of the Efficacy of Rapid Diagnostic Tests Compared to Microscopy in the Diagnosis of Malaria Infection. *International Journal of Mosquito Research*, **6**, 37-41.

[14] Kinimi, E., Patrick, B.N. and Misinzo, G. (2018) Serological Evidence of Chikungunya and Malaria Co-Infection among Febrile Patients Seeking Health Care in Karagwe District, Tanzania. *Tanzania Journal of Health Research*, **20**, 1-8. https://doi.org/10.4314/thrb.v20i4.1

[15] Shamebo, T. and Petros, B. (2019) Trend Analysis of Malaria Prevalence in Halaba Special District, Southern Ethiopia. *BMC Research Notes*, **12**, Article No. 190. https://doi.org/10.1186/s13104-019-4215-2

[16] Owusu-Agyei, S., Asante, K.P., Adjuik, M., Adjei, G., Awini, E., Adams, M., *et al.* (2009) Epidemiology of Malaria in the Forest-Savanna Transitional Zone of Ghana. *Malaria Journal*, **8**, Article No. 220. https://doi.org/10.1186/1475-2875-8-220

[17] Obeagu, E.I., Uo, C. and Is, E. (2018) Malaria Rapid Diagnostic Test (RDTs). *Annals of Clinical and Laboratory Research*, **6**, 1-3.

[18] Sheyin, Z. and Bigwan, I.E. (2013) Comparison of CARE START HRP2 Rapid Malaria Test with Light Microscopy for Guiding Patient's Treatment of Fever in Nigerian

Endemic Areas. *The Journal of Medical Sciences*, **4**, 353-356.

[19] Abdulkadir, I., Rufai, H., Ochapa, S., Malam, M., Garba, B., Oloko, A.G., *et al.* (2015) Malaria Rapid Diagnostic Test in Children: The Zamfara, Nigeria Experience. *Nigerian Medical Journal*, **56**, Article 278. https://doi.org/10.4103/0300-1652.169744

[20] Chiodini, P.L., Bowers, K., Jorgensen, P., Barnwell, J.W., Grady, K.K., Luchavez, J., *et al.* (2007) The Heat Stability of *Plasmodium* Lactate Dehydrogenase-Based and Histidine-Rich Protein 2-Based Malaria Rapid Diagnostic Tests. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **101**, 331-337. https://doi.org/10.1016/j.trstmh.2006.09.007

[21] Kumar, N., Pande, V., Bhatt, R.M., Shah, N.K., Mishra, N., Srivastava, B., *et al.* (2013) Genetic Deletion of HRP2 and HRP3 in Indian *Plasmodium falciparum* Population and False Negative Malaria Rapid Diagnostic Test. *Acta Tropica*, **125**, 119-121. https://doi.org/10.1016/j.actatropica.2012.09.015

[22] Opoku Afriyie, S., Addison, T.K., Gebre, Y., Mutala, A., Antwi, K.B., Abbas, D.A., *et al.* (2023) Accuracy of Diagnosis among Clinical Malaria Patients: Comparing Microscopy, RDT and a Highly Sensitive Quantitative PCR Looking at the Implications for Submicroscopic Infections. *Malaria Journal*, **22**, Article No. 76. https://doi.org/10.1186/s12936-023-04506-5

[23] Ahmad, A., Soni, P., Kumar, L., Singh, M.P., Verma, A.K., Sharma, A., *et al.* (2021) Comparison of Polymerase Chain Reaction, Microscopy, and Rapid Diagnostic Test in Malaria Detection in a High Burden State (Odisha) of India. *Pathogens and Global Health*, **115**, 267-272. https://doi.org/10.1080/20477724.2021.1893484

[24] Bell, A.S. and Ranford-Cartwright, L.C. (2002) Real-Time Quantitative PCR in Parasitology. *Trends in Parasitology*, **18**, 338-342. https://doi.org/10.1016/s1471-4922(02)02331-0

[25] Moody, A. (2002) Rapid Diagnostic Tests for Malaria Parasites. *Clinical Microbiology Reviews*, **15**, 66-78. https://doi.org/10.1128/cmr.15.1.66-78.2002

[26] Quakyi, I.A., Adjei, G.O., Sullivan, D.J., Laar, A., Stephens, J.K., Owusu, R., *et al.* (2018) Diagnostic Capacity, and Predictive Values of Rapid Diagnostic Tests for Accurate Diagnosis of *Plasmodium falciparum* in Febrile Children in Asante-Akim, Ghana. *Malaria Journal*, **17**, Article No. 468. https://doi.org/10.1186/s12936-018-2613-x

[27] Acquah, F.K., Donu, D., Obboh, E.K., Bredu, D., Mawuli, B., Amponsah, J.A., *et al.* (2021) Diagnostic Performance of an Ultrasensitive HRP2-Based Malaria Rapid Diagnostic Test Kit Used in Surveys of Afebrile People Living in Southern Ghana. *Malaria Journal*, **20**, Article No. 125. https://doi.org/10.1186/s12936-021-03665-7

[28] Chourasia, M.K., Raghavendra, K., Bhatt, R.M., Swain, D.K., Meshram, H.M., Meshram, J.K., *et al.* (2017) Additional Burden of Asymptomatic and Sub-Patent Malaria Infections during Low Transmission Season in Forested Tribal Villages in Chhattisgarh, India. *Malaria Journal*, **16**, Article No. 320. https://doi.org/10.1186/s12936-017-1968-8