

## Research Article

# Comparative Diagnostic Performance of Microscopy, SD-Bioline Rapid Diagnostic Test, and Polymerase Chain Reaction in the Detection of Malaria Infection among Pregnant Women at Delivery in Kumba Health District Area in the Southwest Region of Cameroon

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**Introduction.** Malaria during pregnancy is a major cause of morbidity and mortality in sub-Saharan Africa. Microscopy and rapid diagnostic test (RDT) recommended by the World Health Organization for clinical diagnosis have poor sensitivity to detect individuals with very low levels of parasitemia. Previous studies have shown that malaria in pregnancy is associated with mastitis and excessive uterine blood loss during delivery. However, information evaluating the performance of these tools in detecting malaria in pregnancy at the national level is limited. This study therefore evaluates the performance of microscopy, RDT, and nested polymerase chain reaction (nPCR) in the detection of pregnancy-associated malaria at delivery. **Methods.** A total of 227 participants constituting of 201 pregnant women without and 26 with HIV were recruited from five health facilities within the Kumba health district area. Mother venous and cord blood were collected at delivery to test for malaria using the thick-film microscopy, SD-bioline RDT, and 18SrRNA-nested PCR. **Results.** The percentage of malaria-positive cases detected by thick-film microscopy (TFM), RDT, and PCR in pregnant women with and without HIV was 7.69%, 53.85%, and 50% and 3.48%, 23.38%, and 49.25%, respectively. *Plasmodium falciparum* was detected in 1.99% cord blood samples of women without HIV by PCR. The positivity rate in at least two of the test methods (composite positive) was 42.31% for women with and 19.90% for women without HIV. The sensitivity of TFM and RDT when using PCR as a reference was 7.21% and 49.00%, respectively, in all samples. The specificity was 99.14% and 90.55% with kappa values of 0.064 and 0.461, respectively. When using the composite test as a reference, the sensitivity of TFM, RDT, and nPCR was 15.69%, 94.12%, and 100%, respectively. Specificity was 99.43%, 93.18%, and 65.34% with kappa values of 0.213, 0.821, and 0.458, respectively. **Conclusion.** This study shows that PCR is more sensitive in the detection of malaria parasite followed by SD-bioline RDT kit. However, in resource-limited settings where access to molecular diagnosis of malaria is a problem, RDT should be considered as the first option to microscopy in the diagnosis of malaria.

## 1. Introduction

Accurate and early diagnosis of malaria infection remains the key to malaria elimination in malaria-endemic areas as early and effective treatment relies on this aspect. Even though the World Health Organization (WHO) recommends the use of microscopy and rapid diagnostic test for malaria detection in clinical settings, these tools are faced with several limitations rendering them not efficient enough in the detection of malaria-infected individuals [1]. The sensitivity of microscopy relies on the expertise of the technician as studies have shown that an experienced microscopist can only detect a minimum parasitemia range of between 40 and 100 parasites/ $\mu\text{L}$  of blood [2]. The rapid diagnostic test (RDT) on its own detects histidine rich protein II (*pfhrp2*) produced by *P. falciparum* and lactate dehydrogenase produced by the other species of *Plasmodium* [2, 3]. RDT most often will miss-diagnose individuals infected with mutant strains of *Plasmodium* having *pfhrp2* gene deleted [4], and at the same time, the presence of *pfhrp2* in the blood does not necessarily signify the presence of an active infection as studies have shown that this protein can still be detected in blood weeks after parasite clearance [4].

Malaria during pregnancy is a major cause of morbidity and mortality in sub-Saharan Africa, particularly among HIV-positive pregnant women [5]. Malaria infection in sub-Saharan Africa is mostly caused by four *Plasmodium* species: *Plasmodium falciparum*, *Plasmodium malariae*, *Plasmodium ovale*, and *Plasmodium vivax* with the most virulent being *Plasmodium falciparum* [6]. *P. vivax* infection is mostly common among individuals with the Duffy-blood group [7]. However, recently studies have shown traces of *P. vivax* infection in sub-Saharan Africa including Cameroon [8]. Previous studies have also reported severe cases of malaria infection associated with *P. vivax* infection [9].

In a country such as Cameroon where between 2015 and 2019, there were 1,310,000 pregnancies per year and 201,000 resulted in abortions [8]; malaria remains a major public health threat, with the entire country at a risk of transmission [10]. Despite significant progress in recent years, the disease remains prevalent, with a high number of suspected cases in health care facilities ranging between 3.3 and 3.7 million per year [11]. This situation turns out to be amplified among HIV-infected pregnant women as studies have shown increased malaria prevalence which may lead to spontaneous abortion, placenta malaria infection, anemia, and even transplacental infection from mother to fetus [12]. The national estimate of HIV prevalence among pregnant women is estimated at 5% [13].

In malaria-endemic areas where immunity to malaria is high, most of the individuals infected with malaria are asymptomatic carriers [14]. Since the recommendation of sulfadoxine-pyrimethamine and cotrimoxazole to pregnant women as prophylaxis against malaria [13], the request for malaria diagnosis among pregnant women during antenatal care (ANC) visit is mostly based on the manifestation of clinical signs and symptoms of malaria. Therefore, most

asymptomatic and submicroscopic malaria-infected individuals remain undiagnosed at each ANC visit [4, 13]. This is risky in the fight against malaria infection because these individuals may serve as potential sources for malaria transmission [2].

However, asymptomatic and submicroscopic malaria-infected individuals can only be detected through consistent routine screening at each ANC visit by highly sensitive molecular assays, such as nested polymerase chain reaction (nPCR) and loop-mediated isothermal amplification (LAMP) [1, 15]. The limit of detection for nPCR and LAMP is expected to be 0.1–10 parasites/ $\mu\text{L}$  of blood, and it can even be lower with quantitative real-time polymerase chain reaction (qRT-PCR) [16].

Nevertheless, to achieve the zero-malaria elimination goal [17], effective tools for malaria diagnosis are needed as clinical diagnosis of this infection remains the key, before making an informed choice on antimalarial medication. It is therefore critical to assess current malaria diagnostic tools at the national level, particularly in resource-limited settings, to determine which tool is more suitable for the detection of malaria infection among pregnant women, as accurate diagnosis of this infection must be followed by immediate treatment, as studies have shown that malaria, particularly in pregnancy, is associated with postpartum complications such as mastitis [18], and excessive uterine blood loss [19]. This will guide policy on the clinical management of pregnant women and strategy toward the elimination of malaria in pregnancy.

**1.1. Objectives.** The current study screened pregnant women at delivery in Kumba health district area in the Southwest region of Cameroon to evaluate the diagnostic performances of TFM, SD-bioline RDT, and nested PCR techniques in the detection of malaria parasites during pregnancy.

## 2. Methods

**2.1. Study Area and Period.** The study was conducted within the Kumba health district area, Meme Division of the South West region of Cameroon. The study started on the 15<sup>th</sup> of March 2022 and ended on the 30<sup>th</sup> September 2022. Participants were enrolled from five different Health institutions: Regional Hospital Annex Kumba, District Hospital Kumba-town, Presbyterian Hospital Kumba, Catholic Hospital Fiango, and the Kossala Integrated Health Center.

An estimated 400,000 people live in Kumba (4°38'N 9°26'E), with the majority of them being young adults [20]. Kumba is a cosmopolitan town located in a dense, evergreen tropical rain forest (Figure 1 [20]). The climate is equatorial, with two distinct seasons: rainy and dry seasons. From March to November, the rainy season lasts eight months, while the dry season lasts from November to March. In August, there is a two-week break in rainfall, which is known as the August break. Kumba has an average annual



FIGURE 1: The map of Kumba showing the different major quarters of the town [20].

temperature of 31°C and an annual precipitation total of 3512 mm [20]. Currently, Kumba is one of the areas affected by the anglophone crisis, with the majority of its inhabitants internally displaced and living in bushes, while others have migrated to neighboring towns as well as neighboring countries such as Nigeria [21, 22].

The result of a previous study showed a prevalence of 90.3% malaria infection among feverish patients in the Kumba health district [23], while among HIV patients, the prevalence is around 27.2% [24]. However, the prevalence of malaria among pregnant women reported by Asoba and collaborators is 43.2%, 25.2%, and 6.8% in the first, second, and third trimester of pregnancy, respectively [25]. The national HIV prevalence among pregnant women ranges between 4 and 5.55% [26, 27].

**2.2. Study Design and Population.** This was a hospital-based cross-sectional study. The study enrolled pregnant women with and without HIV infection who were in their third trimester (26–32 weeks) and willingly consented to participate. During delivery, the mother venous blood and cord blood were collected and questionnaires were administered and filled.

**2.3. Ethical Consideration.** Ethical clearance for this study was obtained from the Institute for Advanced Medical Research and Training (IAMRAT), College of Medicine, University of Ibadan, Nigeria (ID NHREC/05/01/2008a). However, ethical clearance from Cameroon was obtained from the Institutional Review Board of the Faculty of Health Sciences at the University of Buea (ID 2022/1655-02/UB/SG/IRB/FHS). Administrative clearance was obtained from the Ministry of Public Health, Regional Delegation of Public Health for the South West Region (ID R11/MINSANTE/SWR/RDPH/PS/276/281). Further authorization was

obtained from the Meme Health District service (ID 12/022/MINSANTE/RDPHSW/KHD/DMO/048). Directors of the health facilities involved also provided an authorization letter for the study to take place in their various facilities. Participants who wished to participate in the study had to first sign an informed consent form. For participants who were unable to read or write, the information was read out and explained to them, and their thumbprints were taken. The study considered pregnant women with and without HIV who were in the third trimester of pregnancy and were fully aware of their viral load counts.

**2.4. Sample Size Determination.** The estimated sample size for the study was calculated using the Cochrane formula [28], with a previous prevalence of 81% [5]. The critical value and standard value for the corresponding level of confidence (Z) was 1.96, and the margin of error (e) was 0.05. After substituting the variables, a minimum sample size of 236 was required. However, 227 participants constituting of 201 and 26 HIV negative and HIV positive pregnant women were recruited.

## 2.5. Exclusion and Inclusion Criteria

**2.5.1. Inclusion Criteria.** The study enrolled both HIV positive and HIV negative pregnant women who were within the third trimester of pregnancy (26–32 weeks) and were committed to delivering in their health facility of enrolment. It also took into consideration those who were aware of their HIV status as well as their viral load.

**2.5.2. Exclusion Criteria.** Pregnant women who were in the early stage of pregnancy (<26 weeks) and those with multiple pregnancy were excluded from the study.

**2.6. Sampling Method.** A probability proportionate to the size sampling method was used to determine the number of participants to be recruited in each of the selected health facilities for the study (Table 1). A randomized sampling method was used each day to recruit participants as they came for their ANC in the selected health facilities.

**2.7. Sociodemographic and Clinical Baseline of Study Participants.** A well-structured questionnaire was used to collect demographic information such as age, gravidity, occupation, location, educational level, marital status, and HIV status. The result of the rapid diagnostic test and microscopy for each participant was made available to them. This was to ensure that those positive for malaria should be placed immediately on treatment.

### 3. Laboratory Procedures

**3.1. Sample Collection, Processing, and Storage.** During labor, 3 mL of mother venous blood was collected using a 5 mL syringe and placed in an ethylene diaminetetraacetic acid (EDTA) tube.

Immediately after delivery, the delivered placenta with the clamped umbilical cord was collected and the cord was cleaned and disinfected using povidone-iodine (betadine). Using a 5 mL syringe, 3 mL of cord blood was aspirated from the umbilical cord and placed in a sodium heparinized tube. This process was done immediately after the placenta was delivered when it was still warm to prevent clotting. The sample in the tube was then rotated gently to ensure it was properly mixed with the anticoagulant. The collected samples were transported to the lab for processing.

In the laboratory, the maternal venous blood was used to prepare a thick film. A drop of the blood was placed on a clean slide and rocked properly to lyse and expose the infected red blood cells. The slide was then allowed to air dry and packaged to prevent contamination in preparation for further analysis.

The remaining whole blood sample for maternal venous blood and cord blood was then centrifuged to separate the plasma from the concentrated red cells (pellet). The plasma and pellet were then aliquoted into separate and well-labeled Eppendorf tubes. The samples were then stored in a  $-20^{\circ}\text{C}$  refrigerator in preparation for further analysis. After the field work, the samples were transported using a sample transportation box to the immunology and molecular biology laboratory of the Biotechnology Center of the University of Yaoundé I for further analyses. Upon arrival, 50  $\mu\text{L}$  of the pellet for both the maternal venous sample and the cord blood sample was blotted on a 185 mm Whatman filter paper and allowed to air dry. The samples were then packaged and stored in separate, clean, and well-labeled medicine zip-lock bags for downstream assays.

**3.2. Malaria Parasite Detection Using Thick Film Microscopy (TFM).** A drop of the mother venous blood as well as the cord blood sample was placed on a well-cleaned slide and rocked properly to ensure lysis of the red blood cells (RBCs). The thick film was then allowed to air dry and further stained for

15 minutes with 10% Giemsa. This was rinsed with clean water, and the slides were allowed to air dry. The slides were examined under the 100x oil emission objective, and parasitemia for those positive was calculated using the following formula [29] (see Figure 2(a)):

$$\frac{\text{number of parasites counted} \times 8000}{\text{number of white blood cells}} = \frac{\text{parasites}}{\mu\text{L}} \text{ of blood.} \quad (1)$$

**3.3. Detection of Malaria Infection Using the Rapid Diagnostic Test (RDT).** Venous blood from mothers was used to test for the presence of histidine rich protein II (HRP 2) and lactate dehydrogenase (LDH) using the SD-bioline RDT kit (SD Standard Diagnostics, Inc) following the manufacturer instructions (Figure 2(b)).

**3.4. Extraction of Plasmodium DNA for Molecular Diagnosis.** The DNA for the *Plasmodium* parasites was extracted using the hot Chelex method as described by Plowe et al. [29, 30]. A total of about 50  $\mu\text{L}$  of whole blood pallet sample was blotted on a 185 mm Whatman filter paper and allowed to air dry. This was later cut and placed in a 1.5 mL Eppendorf tube. A total of 1 mL 0.5% saponin was later added to the tube, inverted several times, and allowed to incubate overnight (18–24 hrs) at  $+4^{\circ}\text{C}$ . The supernatant was discarded in 10% bleach, and 1% phosphate buffer saline (PBS) was added to each Eppendorf tube. The tubes were capped and inverted several times and allowed to incubate at  $+4^{\circ}\text{C}$  for 20 minutes. The supernatant was removed, and the filter paper was removed and placed in a clean labeled Eppendorf tube. A total of 150  $\mu\text{L}$  of nuclease-free water was added which was followed by the addition of 50  $\mu\text{L}$  of 20% heated Chelex. The tube was then vortexed vigorously for 30 seconds and allowed to stand in a water bath for 10 minutes at a temperature of  $100^{\circ}\text{C}$ . The supernatant was later transferred into a clean sterile tube and centrifuged at 14000 rotations per minute (rpm) for 2 minutes. The supernatant was finally pipetted into a fourth final tube and stored at  $-80^{\circ}\text{C}$  for further downstream analyses.

**3.5. Nested Polymerase Chain Reaction for Plasmodium species Identification.** Nested PCR was done using the 18S rRNA primer which targets the multicopy 18S rRNA plasmodial gene. Using a modified protocol from Snounou et al. [30, 31], the protocol for the PCR reaction was developed. For each sample, 14  $\mu\text{L}$  of PCR reaction was prepared consisting of 7  $\mu\text{L}$  of 2x OneTaq® quick-load master mix (New England Biolabs, Inc), 3  $\mu\text{L}$  of nuclease-free water, 0.5  $\mu\text{L}$  each of 10  $\mu\text{M}$  forward and reverse primers, and 3  $\mu\text{L}$  of template DNA from the extract. For the nest-1 reaction of the PCR, templated DNA extract plus forward and reverse primers were used (rPLU5: 5'-CCTGTTGTTGCCTTAAACTTC-3' and rPLU6: 5'-TTA AAATTGTTGCAGTTAAAACG-3'). For the nest-2 reaction of the PCR, the amplicons from nest-1 reaction were used as template DNA together with species-specific forward and reverse primers for the different *Plasmodium* species (Table 2).

TABLE 1: The number of participants enrolled from each of the different health facilities for the study.

Sample	Regional hospital annex Kumba, n (%)			District hospital Kumba-town, n (%)			Presbyterian hospital Kumba, n (%)			Kossala integrated health center Kumba, n (%)			Catholic hospital Kumba, n (%)		
	2021 stat <sup>a</sup> n (%)	p value	PSS <sup>b</sup> n (%)	2021 stat n (%)	p value	PSS n (%)	2021 stat n (%)	p value	PSS n (%)	2021 stat n (%)	p value	PSS n (%)	2021 stat n (%)	p value	PSS n (%)
Pregnant women with HIV, (n = 26)	10/718 (1.4)	0.014	11/26 (42.3)	10/280 (3.6)	0.035	5/26 (19.2)	20/800 (2.5)	0.025	5/26 (19.2)	13/765 (6.3)	0.063	0 (0)	17/685 (2.5)	0.025	5/26 (19.2)
Pregnant women without HIV (n = 206)	708/718 (98.6)	0.986	22/206 (10.6)	270/280 (96.4)	0.964	45/206 (21.8)	780/800 (97.5)	0.975	13/206 (6.31)	752/765 (98.3)	0.983	36/206 (17.4)	668/685 (97.5)	0.975	90/206 (43.6)

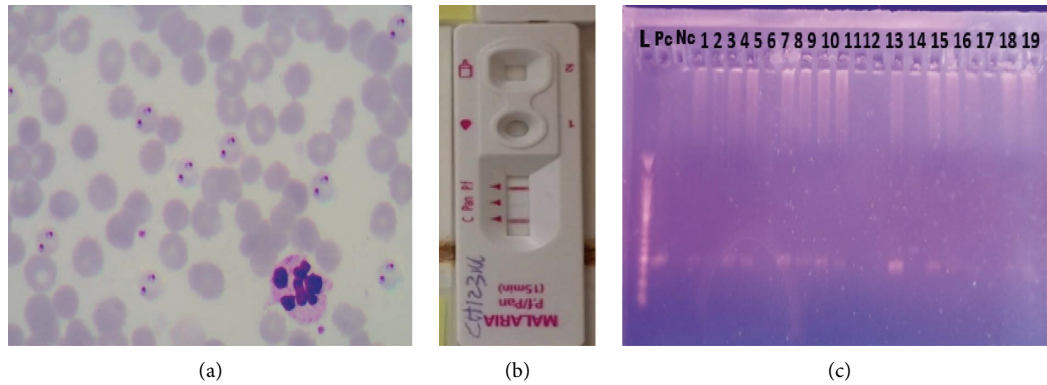


FIGURE 2: Representative photograph of the three different test methods used in the study. (a) The presence of malaria parasites in a slide smear, (b) malaria-positive RDT kit, and (c) bands of samples positive for malaria using the nested PCR assay.

TABLE 2: Sequences of primers for *Plasmodium* species identification.

	Sequence (5'-3')
<i>Plasmodium falciparum</i>	rFAL1: 5'-TTAAACTGGTTTGGGAAAACCAAATATATT-3' rFAL2: 5'-ACACAATGAACCTCAATCATGACTACCCGTC-3'
<i>Plasmodium malariae</i>	rMAL1: 5'-ATAACATAGTTGTACGTTAAGAATAACCGC-3' rMAL2: 5'-AAAATTCCCATGCATAAAAAATTATACAAA-3'
<i>Plasmodium ovale</i>	rOVAL1: 5'-ATCTCTTTTGCTATTTTGTAGTAATTGGAGA-3' rOVAL2: 5'-GGAAAAGGACACATTAATTGTATCCTAGTG-3'
<i>Plasmodium vivax</i>	rVIV1: 5'-CGCTTCTAGCTTAATCCACATAACTGATAC-3' rVIV2: 5'-ACTTCCAAGCCGAAGCAAAGAAAGTCCCTT-3'

Purified MRA 736G genomic DNA for *Plasmodium falciparum* while known positive samples for *Plasmodium malariae* and *Plasmodium ovale* were used as positive control. Nuclease-free water was used as negative control. Reaction conditions used for amplification are as follows: For nest-1: initial denaturation: 95°C for 5 minutes in 1 cycle, denaturation: 94°C for 1 minute, annealing: 58°C for 1 minute, final extension: 72°C for 2 minutes in 25 cycles, and final extension: 72°C for 2 minutes in 1 cycle. For the nest-2, the reaction conditions remain the same as in nest-1. The nest-2 products were electrophoresed on ethidium bromide-stained 1.5% agarose gels and visualized on the Gel Doc™ XR + System (Bio-Rad, USA). Samples were qualitatively identified as negative or positive (Figure 2(c)) if 205 bp bands were observed for *P. falciparum*, 144 bp bands for *P. malariae*, 800 bp band for *P. ovale*, and 120 bp for *P. vivax*. Figure 3 shows a summary flow chart of the study.

**3.6. Definition of Terminologies.** Individuals considered positive for malaria were those either positive by thick-film microscopy (TFM), RDT, and PCR. Individuals with body temperature  $\leq 37.5^\circ\text{C}$  were considered asymptomatic, while those with temperature  $> 37.5^\circ\text{C}$  were considered symptomatic. Those positive for malaria by either RDT or PCR but negative by microscopy were considered as submicroscopic infected individuals. Composite-positive individuals were those positive for at least two of the diagnostic test methods used for sample analysis (see Figure 3).

### 3.7. Data Quality Assurance

**3.7.1. Data Quality Management.** All laboratory materials such as rapid test kits, slides, thermometers, collection tubes, and sample transporting systems were checked by experienced laboratory professionals to ensure that they are not expired as well as performing well. Samples collected were also checked for hemolysis and labeling. Those hemolyzed were sorted and discarded immediately. Sample collection was done by staff members at the maternity who were trained before the study on cord blood and venous blood collection procedures. The rapid test kit was examined for its expiration date and inbuilt control appearances. Tests that were indeterminate were rerun to confirm their RDT results.

**3.7.2. Data Processing and Analysis.** Data were coded, entered, and cleaned in Microsoft Excel spreadsheet version 16. Statistical analysis of the data was performed using Stata version 17 software [32] and GraphPad version 8.0.1 software [33]. The Fisher exact test was used to ascertain the difference in the proportion between HIV positive and HIV negative groups. Descriptive statistic was used to represent the number of cases of malaria detected by the different test methods. Descriptive GraphPad prism chart was used to express the relationship between SP dosages and parasitemia levels. Mann-Whitney U and Kruskal-Wallis tests were used to test for the relationship between doses of SP taken and parasitemia levels. *p* values less than 0.05 were considered to be statistically significant. A diagnostic

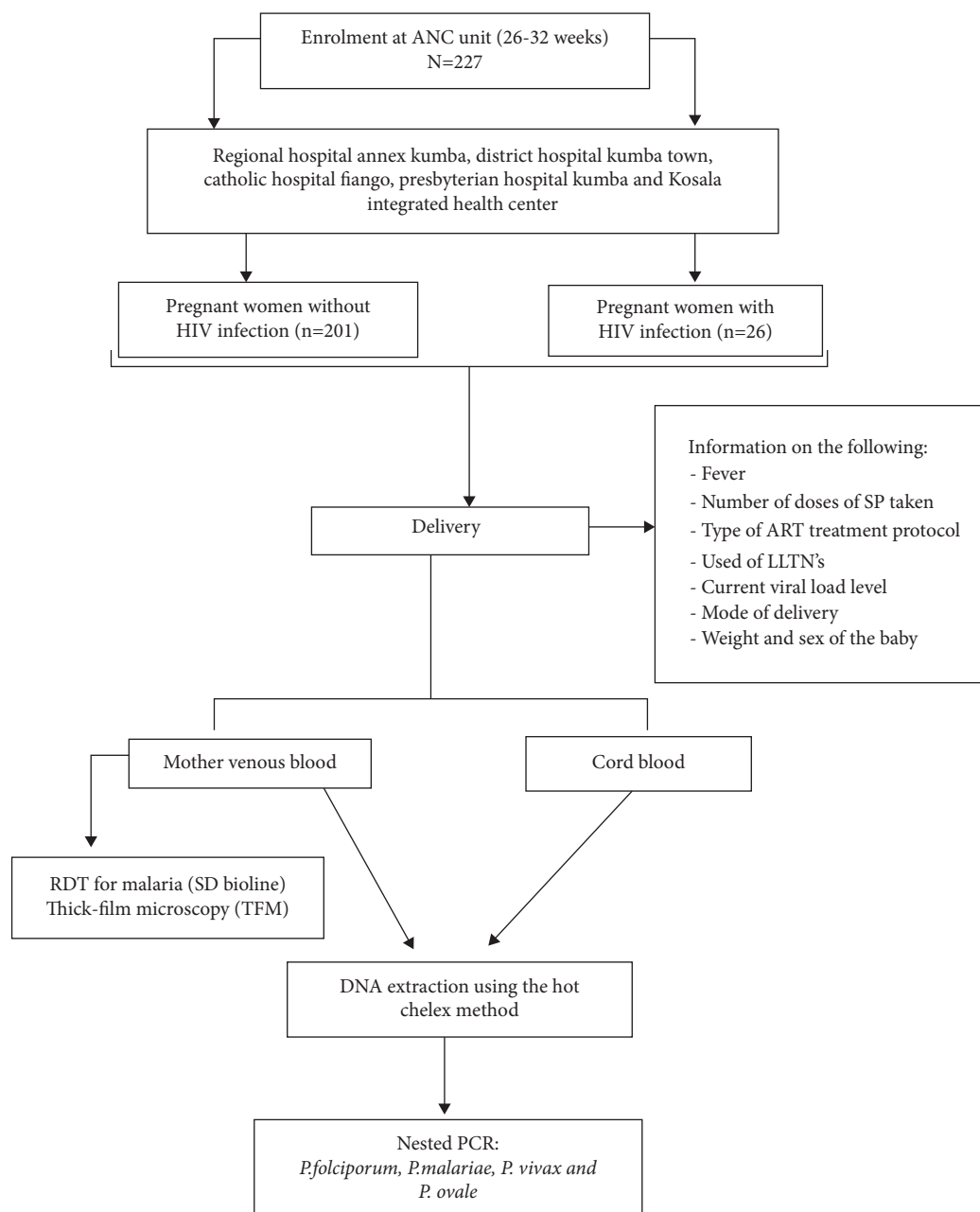


FIGURE 3: Summary flow chart for the recruitment of participants for the study of malaria among pregnant women with and without HIV infection in Kumba.

performance test using nPCR and composite test (individuals positive with at least two of the three test methods) as reference standard was determined using the MedCalc statistical software version 20.218.

## 4. Results

**4.1. Sociodemographic Characteristics of the Study Population.** Table 3 shows the age range of the HIV-positive and the HIV-negative population with the mean age being  $30.57 \pm 4.86$  (range: 20–41 years) and  $26.13 \pm 5.53$  (range: 15–42 years), respectively. A greater proportion of those enrolled for both the HIV-negative and HIV-positive group

were multigravidas 133/66.2% and 24/92.3%, respectively. More than half of the HIV-negative (139/69.2%) and HIV-positive (21/80.7%) population were married. Greater number of the HIV-negative (87/43.2%) and the HIV-positive (12/46.2%) population had at least secondary school education. Occupation was civil service, student, business, and farming. Majority of the participants were into business (94/46.7% and 13/50.00%, respectively) for both the HIV-negative and HIV-positive groups with a disproportionate share of both the HIV-negative (153/76.1%) and HIV-positive (24/92.3%) participants testifying of sleeping under insecticide-treated bed net. Also, 172/201 (75.77%) of the HIV-negative women had a normal vagina delivery

TABLE 3: Baseline characteristics of the HIV-positive and HIV-negative study population.

Variables	HIV-negative (201)		HIV-positive (26)		<i>p</i> value
	Frequency	(%)	Frequency	(%)	
Age group					
15–19	26	12.9	0	0.00	<b>0.001</b>
20–24	55	27.4	2	7.69	
25–30	79	39.3	11	42.3	
≥31	41	20.4	13	50.0	
Gravidity					
Primigravida	68	33.8	2	7.69	<b>0.006</b>
Multigravida	133	66.2	24	92.3	
Marital status					
Single	57	28.4	5	7.69	0.524
Married	139	69.2	21	80.7	
Divorced	5	2.5	0	0.00	
Educational level					
Primary	56	27.9	9	34.6	0.580
Secondary	87	43.2	12	46.2	
Tertiary	58	28.9	5	19.2	
Occupation					
Civil service	29	14.4	5	19.2	<b>0.021</b>
Student	38	18.9	0	0.00	
Business	94	46.7	13	50.0	
Farming	40	19.9	8	30.7	
Insecticide treated bed net usage					
No	48	23.9	2	7.69	0.077
Yes	153	76.1	24	92.3	
Mode of delivery					
CS	29	12.78	5	19.23	0.558
ND	172	75.77	21	80.76	
Sex of baby					
Male	99	49.25	13	50.00	0.943
Female	102	50.74	13	50.00	

(ND), while among their HIV counterparts recorded 21/26 (80.76%) with greater proportion of the women without HIV 102/201 (50.74%) giving birth to female children compared to the other group where the proportions were the same. There was a significant difference between age group, gravidity, and occupation of both groups of the study population.

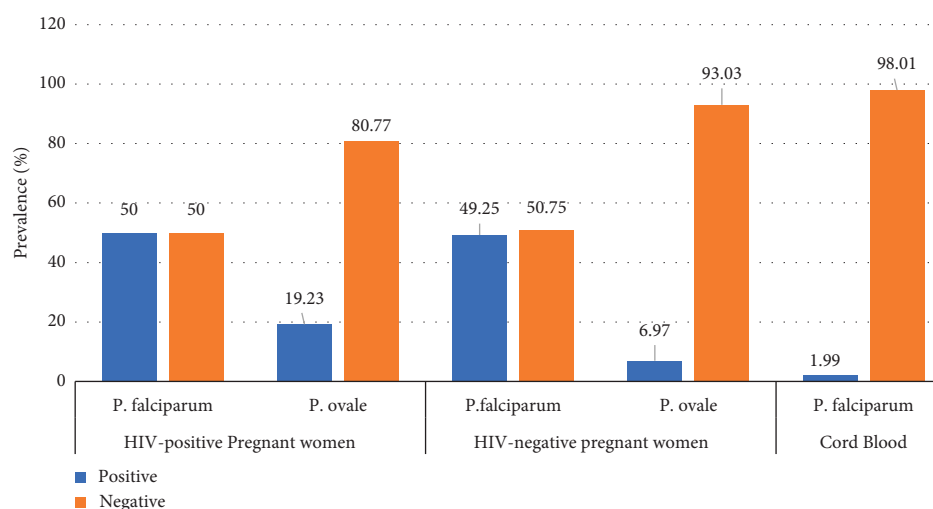
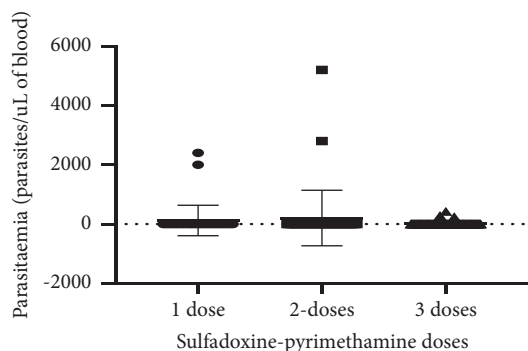
**4.2. The Percentage of Diagnosed Malaria Cases among the HIV-Positive and HIV-Negative Pregnant Women at Delivery.** The highest percentage of (99/49.25%) malaria infection among the HIV-positive group of pregnant women was detected by RDT; while among the HIV-negative group, the highest percentage of (14/53.85%) was detected by PCR. Among the HIV-negative group, those positive for malaria in at least two of the three diagnostic test methods (composite test) used in the study were just 40 (19.9%), while among the HIV positive group, 11 (42.31%) were positive (Table 4). PCR detected 4 (1.99%) cord blood infections (transplacental malaria) among the unexposed HIV cord blood. There was a significant difference in the results of RDT and composite test result of both women with and without HIV infection with a *p* value of 0.002 and 0.022, respectively.

**4.3. *Plasmodium* species Responsible for Maternal and Transplacental Malaria Infection among Pregnant Women as Determined by Nested Polymerase Chain Reaction.** *Plasmodium falciparum* and *Plasmodium ovale* were the two species of parasites responsible for malaria infection among the study population (Figure 4). The HIV-positive group recorded 13/26 (50.00%) *P. falciparum* cases, while the HIV-negative group recorded 99/201 (49.25%) cases. The percentage of *P. ovale* detected among the HIV-positive and HIV-negative groups was 5/26 (19.23%) and 14/201 (6.97%), respectively. *P. falciparum* was the only species responsible for cord blood infection with a percentage of 4/201 (1.99%).

**4.4. Relationship between Intermittent Preventive Treatments (IPTp-SP) on *Plasmodium* species Distribution and Malaria Parasitemia Level.** Out of the 201 HIV negative participants, a decreased level of parasitemia was observed among those who took ≥3 doses of SP. Those who took 2-doses had the highest level of parasitemia compared to those who took just a single dose of SP (Figure 5). The mean parasitemia level for both groups of study population was  $29.23 \pm 107.0$  (range: 280–480 parasite/μL of blood) and  $66.36 \pm 469.3$  (range: 240–5200 parasite/μL of blood) among the HIV-positive and HIV-negative groups, respectively.

TABLE 4: The percentage of diagnosed malaria cases among the HIV-negative and HIV-positive population using the different diagnostic methods.

	Mothers' peripheral blood				Cord blood
	TFM (%)	RDT (%)	PCR (%)	* Composite test (%)	PCR (%)
HIV-negative ( <i>n</i> = 201)	7 (3.48)	47 (23.38)	99 (49.25)	40 (19.9)	4 (1.99)
HIV-positive ( <i>n</i> = 26)	2 (7.69)	14 (53.85)	13 (50.00)	11 (42.31)	0 (0.00)
Total	9 (11.17)	61 (77.23)	112 (99.25)	51 (62.21)	4 (1.99)
<i>p</i> value	0.275	* <b>0.002</b>	1.000	* <b>0.022</b>	1.000

FIGURE 4: *Plasmodium* species population responsible for maternal and cord blood malaria infection among pregnant women.FIGURE 5: Relationship between parasitemia and the sulfadoxine-pyrimethamine dosages taken before delivery. The diagram shows the mean  $\pm$  SD parasitemia level between the three different groups. There was no statistical difference ( $p > 0.515$ ) in the parasitemia level across the three different doses of SP taken, between those who took a single dose and 2 doses ( $p > 0.999$ ), and between those who took two doses and those who took  $\geq 3$  doses ( $p > 0.366$ ) of sp.

**4.5. Proportion of Symptomatic and Asymptomatic Malaria Infection Detected by TFM, RDT, and PCR among HIV-Positive and HIV-Negative Pregnant Women.** The proportion of symptomatic malaria infection among the study population was extremely low, and most participants were asymptomatic carriers. The percentage of asymptomatic malaria detected by TFM, RDT, and PCR among the HIV-infected pregnant women was 2 (7.69%), 13 (50.00%), and 12 (46.15%), while among the HIV-negative pregnant women,

it was 6 (2.99%), 37 (18.41%), and 83 (41.29%), respectively (Table 5). The mean body temperature for the HIV-positive and HIV-negative groups was  $36.9 \pm 0.47$  (range: 36–38°C) and  $37.1 \pm 0.41$  (range: 36–39.1°C), respectively. There was a significant difference ( $p$ -value of 0.037) in the number of symptomatic and asymptomatic malaria-infected individuals detected by RDT in the HIV-negative group.

**4.6. Relationship between ART Regimens and Malaria Occurrence among the HIV-Positive Pregnant Women.** The population was stratified into two groups: those on tenofovir-lamivudine-dolutegravir regimen (TLD) which is the new treatment protocol introduced and those still on tenofovir-lamivudine-efavirenz regimen (TLE). Based on TFM, those on TLE recorded 2/26 (7.69%) cases of malaria infection. By RDT, 7/26 (26.92%) was recorded in both the groups, while based on PCR, those on TLD recorded 7/26 (26.92%) and those on TLE recorded 6/26 (23.08%) (Figure 6). No statistical difference was observed in the two groups using the three different diagnostic methods with  $p$  values of 0.063, 0.191, and 0.420, respectively.

**4.7. Comparing the Diagnostic Performance of Microscopy and SD-Bioline RDT Using Nested PCR as Reference Standard.** When using nested PCR as a reference standard, SD-bioline recorded a moderate agreement with nested PCR compared to TFM. RDT recorded a kappa value of 0.431, sensitivity of 49.00%, specificity of 90.55%, positive predictive value

TABLE 5: Prevalence of symptomatic and asymptomatic malaria infection among HIV-positive and HIV-negative pregnant women.

	HIV-positive ( <i>n</i> = 26)			HIV-negative ( <i>n</i> = 201)		
	TFM (%)	RDT (%)	PCR (%)	TFM (%)	RDT (%)	PCR (%)
Symptomatic malaria infection	0 (0.00)	1 (3.85)	1 (3.85)	1 (0.50)	10 (4.98)	16 (7.96)
Asymptomatic malaria infection	2 (7.69)	13 (50.00)	12 (46.15)	6 (2.99)	37 (18.41)	83 (41.29)
Total	2 (7.69)	14 (53.85)	13 (50.00)	7 (3.48)	47 (23.38)	99 (49.25)
<i>p</i> value	0.595	0.580	0.539	0.846	*0.037	0.083

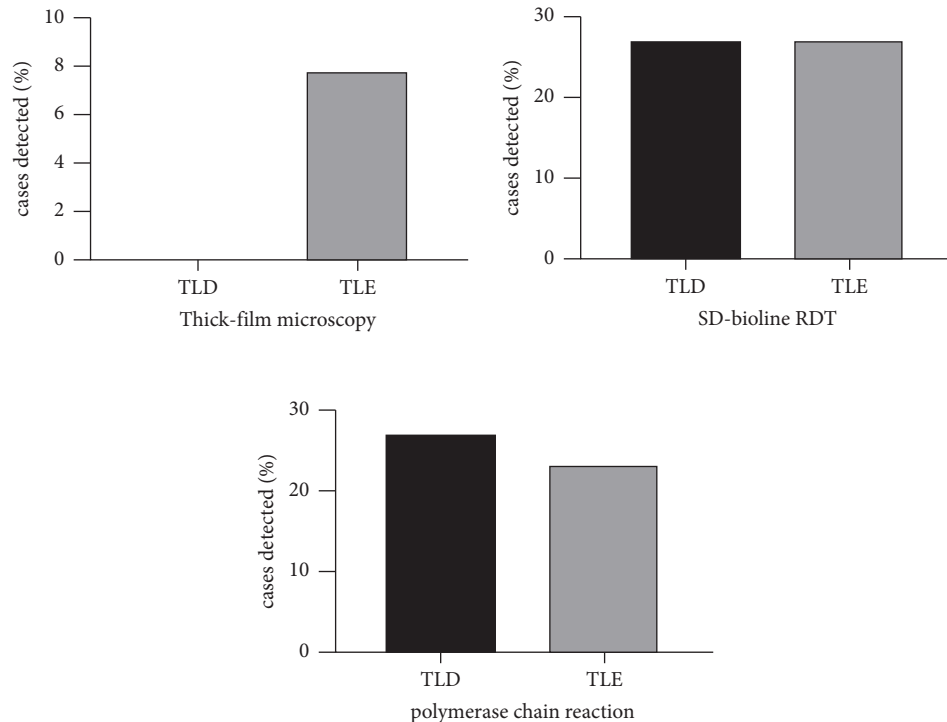


FIGURE 6: Bar charts showing the number of malaria-infected individuals detected among pregnant women on TLD and TLE using the three different diagnostic techniques.

(PPV) of 80.33%, negative predictive value (NPV) of 69.28%, and diagnostic accuracy of 72.25%, while TFM recorded a kappa value of 0.064, sensitivity of 7.21%, specificity of 99.14%, PPV of 88.89%, NPV of 52.75%, and diagnostic accuracy of 54.19% (Table 6).

**4.8. Diagnostic Performance Test of TFM, RDT, and PCR in the Diagnosis of Malaria Infection among Pregnant Women at Delivery Using Composite Test as Reference Standard.** Among the three methods used in the study to diagnose malaria, TFM recoded a sensitivity of 15.69% and a specificity of 99.43% with a positive predictive value (PPV) of 80.23% and a negative predictive value (NPV) of 88.89%. RDT recoded a sensitivity of 94.12% and a specificity of 93.18% with a PPV of 80.00% and an NPV of 98.20%. Finally, PCR recoded a sensitivity of 100% and a specificity of 65.34% with a PPV of 45.54% and an NPV of 100%. However, the three different test methods recorded a diagnostic accuracy of 80.62%, 93.39%, and 73.13% with kappa values of 0.213, 0.821, and 0.458, respectively. The closer the Kappa value is to one, the greater the level of agreement and efficacy of the

test procedure to the reference method. Among our study population, RDT recorded a better diagnostic performance, followed by PCR, while TFM recorded the lowest (Table 7).

**4.9. Percentage Discordance of the Different Test Methods Used in the Detection of Malaria Parasite Infection.** Out of the 227 participants enrolled, only 5 (2.20%) participants were detected by all three test methods used in the study. PCR detected 61 (26.87%) followed by RDT with 13 (5.72%) and TFM with just 1 (0.44%) only. Between PCR and RDT 43 (18.94%) were picked up by both methods followed by PCR and TFM just 3 (1.32%). The chances of detecting more positive cases are enhanced when more than one screening method is used (Figure 7).

## 5. Discussion

Accurate and early diagnosis of malaria infection remains the key to malaria elimination in malaria-endemic areas as early and effective treatment really on this aspect. Nevertheless, to achieve the zero-malaria elimination goal [17],

TABLE 6: Diagnostic performance of microscopy and SD-bioline RDT using nested PCR as reference standard.

	TFM	SD-bioline RDT
TP (nPCR = 112)	8	49
FP (nPCR = negative)	1	12
TN (nPCR = 115)	115	115
FN (nPCR = positive)	103	51
Sensitivity (95% CL)	7.21% (3.16–13.71%)	49.00% (38.86–59.20%)
Specificity (95% CL)	99.14% (95.29–99.98%)	90.55% (84.08–95.02%)
PPV (95% CL)	88.89% (50.49–98.44%)	80.33% (69.69–87.88%)
NPV (95% CL)	52.75% (51.39–54.11%)	69.28% (64.86–73.37%)
Accuracy (95% CL)	54.19% (47.46–60.79%)	72.25% (65.94–77.97%)
Cohen's kappa value	0.064	0.431

TABLE 7: Diagnostic performance test of TFM, RDT, and PCR in the detection of malaria infection among pregnant women at delivery.

	TFM	RDT	PCR
TP (CT = 51)	8	48	51
FP (CT = negative)	1	12	61
TN (CT = 176)	175	164	115
FN (CT = positive)	43	3	0
Sensitivity (95% CL)	15.69% (7.02–28.59%)	94.12% (83.76–98.77%)	100.00% (93.02–100%)
Specificity (95% CL)	99.43% (96.88–99.99%)	93.18% (88.39–96.43%)	65.34% (57.81–72.34%)
PPV (95% CL)	88.89% (50.60–98.42%)	80.00% (69.76–87.40%)	45.54% (40.57–50.60%)
NPV (95% CL)	80.28% (78.32–82.09%)	98.20% (94.80–99.39%)	100.00% (96.84–100%)
Accuracy (95% CL)	80.62% (74.87–85.55%)	93.39% (89.34–96.25%)	73.13% (66.86–78.78%)
Cohen's kappa value	0.213	0.821	0.458

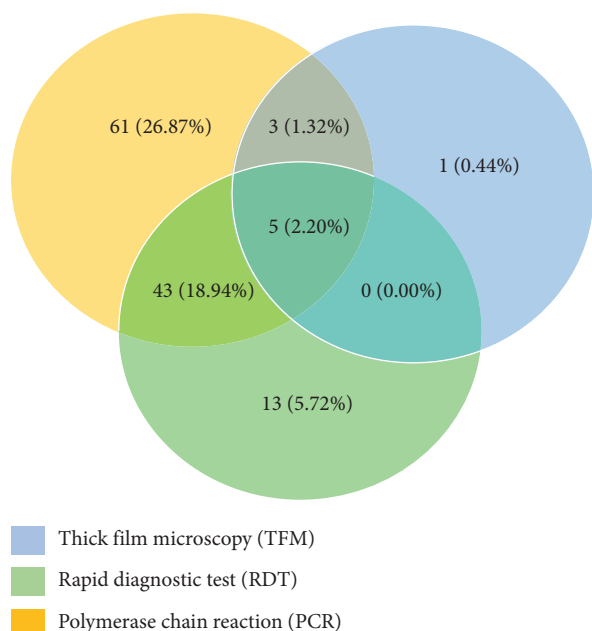


FIGURE 7: The percentage discordance of TFM, RDT, and PCR in the detection of malaria infection among pregnant women.

effective tools for malaria diagnosis are needed as clinical diagnosis of this infection remains the key, before making an informed choice of antimalarial medication. The current study screened pregnant women at delivery in Kumba health district area in the Southwest region of Cameroon to evaluate the diagnostic performances of TFM, RDT, and PCR in the detection of the malaria parasite. The study took place between March 2022 and September 2022.

In general, the study percentage of malaria detected by nested PCR was higher (99.25%) compared to the percentage by TFM (11.17%) and RDT (77.23%). This was in agreement with the well-known improved sensitivity of PCR for malaria detection [34–36], as well as establishing the presence of submicroscopic illnesses in the population. It can be seen in Table 4 that only 40/201, 19.9%, of the HIV-negative individuals and 11/26, 42.31%, of the HIV-positive group were positive for at least two test methods (composite positive). The SD-bioline rapid diagnostic test (RDT) used detects the presence of histidine rich protein 2 (HRP2) which is specific for *Plasmodium falciparum* and lactate dehydrogenase (PAN) which is indicative of the presence of the other species of *Plasmodium* [37]. Among the HIV-negative group, PCR detected the highest number of positive cases (49.25%) of malaria, while among the HIV-positive group, RDT detected a slight increase in the number of positive cases (53.85%) of malaria compared to PCR (Table 4). The presence of *pfhrp2* in blood does not necessarily indicate the presence of an active infection as studies have shown that these proteins can last over weeks in blood even after parasite clearance [38]. This must have contributed to the increased level of positive cases recorded by RDT in the HIV group (Table 4). Also, the discrepancy in the size of both (HIV+ and HIV-) population may have an influence on the results. However, in general, RDT detected 52 submicroscopic infections, while PCR detected 103. This means that 51 additional submicroscopic infections missed by RDT were detected by PCR. This confirms that the SD-bioline RDT and PCR are more sensitive in the detection of malaria than TFM even though both test methods have their limitations [36, 39].

A total of 4/201, 1.99%, of cord blood infection was recorded among the HIV-unexposed (HUE) cord blood, while none was recorded for the HIV-exposed (HE) cord blood by nPCR (Table 4). The ARTs received by these women may have an inhibitory effect on malaria parasite transmission as proven by a couple of studies [40, 41]. Furthermore, the maternal peripheral blood match for the positive cord blood samples was all negative for malaria. This confirms the findings of Leke Rose and collaborators who reported that pregnant women may test negative for malaria peripherally but positive at the level of the placenta if tested [42]. According to studies, parasites are significantly attracted to the placenta due to the presence of chondroitin sulfate A, which is concentrated at the placenta, and as a result, the parasite will sequester into the placenta. Their presence in the placenta may cause inflammation of the syncytiotrophoblast layer, allowing the parasites to pass the placenta into the umbilical cord, resulting in vertical transmission [43]. In general, malaria cases detected by all three methods were higher among the HIV-positive group as compared to the HIV-negative group (Table 4). According to a couple of research studies, HIV and AIDS promote immunosuppression, lowering the total immunological response to malaria parasitemia and hence increasing the frequency of clinical malaria attacks [5]. This is consistent with prior research conducted in Nigeria, where HIV-positive moms were more susceptible to malaria than HIV-negative mothers [8, 44]. However, this warrants further investigation looking at the prevalence of malaria in both groups with a much higher sample size as the sample size for the HIV population may have a contributing effect to the result.

Malaria parasite transmission in Cameroon varied, with high and perennial parasite transmission occurring in the forest, coastal, and humid savanna areas, and low and seasonal parasite transmission occurring in the highlands, Sahelian, and dry savanna areas [45]. Within these zones lie subzones with different malaria transmission dynamics. *Plasmodium falciparum* recorded the highest prevalence in this study population. HIV-positive cases recorded a slightly higher prevalence (50.00%) compared to the HIV-negative counterparts. The finding was in line with a study conducted in Malawi [46] and Nigeria [5]. Nevertheless, it was realized that malaria transmission in this area comprises only *P. falciparum* and *P. ovale* parasite. This is contrary to the findings of Kwenti and collaborators [47] who reported *P. falciparum* parasite as the only species responsible for malaria infection among children from all the five different strata of malaria transmission in Cameroon. Our finding may be due to evolutionary and behavioral changes of the parasite vector as well as environmental changes [48]. Much higher prevalence of *P. ovale* infection was observed among the HIV-positive individuals compared to their HIV-negative counterparts (Figure 4). The presence of HIV infection may have been a contributing factor to the increased prevalence of *P. ovale* infection among the HIV-positive group as HIV infection suppresses the immune system creating a conducive environment for several opportunistic infections [49].

In malaria-endemic areas where immunity against malaria is high, most individuals infected with malaria do not manifest any clinical signs and symptoms such as fever until after ascertaining a certain parasite load or immune suppression [13, 50]. In this study, a disproportionate share of the individuals was asymptomatic carriers of malaria infection. However, almost all of the individuals infected with malaria among the HIV-positive group were asymptomatic compared to their HIV-negative counterparts. Thick-film microscopy (TFM), RDT, and PCR detected 7.69%, 50.00%, and 46.15% positive cases (Table 5). This result was contrary to the results of previous studies reported in Nigeria [51, 52]. Nevertheless, this finding is concerning given that most of the participants testified of using mosquito bed nets (Table 6) and using antimalarial prophylaxes. Cotrimoxazole has been proven to reduce malaria prevalence by twofold; nevertheless, it is ineffective for malaria therapy [53, 54]. This could be related to inadequate adherence to prophylactic medication. Although the drugs are free for HIV patients and are collected during routine visits, low adherence among antimalarial patients has been extensively documented. Forgetfulness, perceived side effects, not seeing mosquitoes, and a lack of awareness about the repercussions of nonadherence have all been described as reasons for poor adherence [55, 56]. The increase in the proportion of asymptomatic infection is in line with other studies conducted in Nigeria and South Africa [57, 58] but contrary to a study conducted in Ethiopia [50]. Malaria parasitemia in HIV infection is worrisome because the patient's immune systems are already compromised, and the interaction of HIV and *Plasmodium* with the host's immune system results in complex activation of immune cells and dysregulated levels of antibodies and cytokine generation. Malaria has also been shown to promote HIV replication in vitro and in vivo [59].

The adoption of intermittent preventive treatment with sulfadoxine-pyrimethamine (IPTp-SP) for HIV-negative pregnant women and ART combined with cotrimoxazole for the HIV-positive pregnant women following the WHO recommendation was a wise move in reducing the burden of malaria among pregnant women [60]. However, the effectiveness of IPTp-SP is threatened by rising levels of parasite resistance in several countries across Africa [61]. Nevertheless, this study shows that those who took  $\geq 3$ -doses of IPTp-SP even though infected with malaria showed a very low level of parasitemia (Figure 5). This finding is in line with previous studies conducted in Mozambique [62], Tanzania [63], and Ghana [5] which showed a trend of decreasing parasite densities with an increasing number of IPTp-SP doses indicating a benefit of higher IPTp-SP uptake in reducing parasite density. However, a very high level of parasitemia ( $>5200$  parasites/ $\mu$ l of blood) was observed among those who took 2-doses of IPTp-SP compared to those who took just a single dose. This may be an indication of drug resistance strains as a result of inconsistency of IPTp-SP uptake. Studies have shown that the more pregnant women turn to attend ANC, the more they turn to adhere to their prophylactic treatment against malaria [37].

The number of malaria infected cases among pregnant women on TLD and TLE was evaluated, and there was no statistical difference in both the groups using the three different diagnostic test methods. TLD which was recently adopted in Cameroon [64] after the WHO recommendation shows high virological success despite prior exposure to efavirenz-based first-line ART. This drug has also been shown to be very effective in the prevention of MTCT of HIV infection due to its high efficiency in suppressing viral load. However, information on the effectiveness of this drug in preventing malaria infection is scanty. TLD is made of a combination of integrase strand transfer inhibitors (INSTIs) and nucleotide reverse transcriptase inhibitors (NRTIs). A study conducted in Uganda showed that children who received NNRTI-based regimen had more incidence of malaria compared to their counterparts who were on lopinavir-ritonavir regimen [65]. Other studies observed that protease inhibitors (PIs) of the human immunodeficiency virus (HIV) suppress erythrocytic stages of the human-malaria parasite *Plasmodium falciparum* in vitro and erythrocytic stages of the rodent-malaria parasite *Plasmodium chabaudi* in vivo. Although it is unknown how HIV PIs suppress parasite development, the effect shown in the erythrocytic stages is significant [66]. Even though our study attempted looking at the effectiveness of this ART regimen in malaria prevention, it recorded insignificant difference between the two ART-regimens (TLD and TLE) used. The small number of HIV-positive participants used may have contributed to the finding of this study. However, this finding will serve as a piece of baseline information for future studies as there is the need for further investigations with much larger sample size looking at the effectiveness of ART regimen in preventing malaria infection, especially among pregnant women.

When evaluating the diagnostic performance of TFM and RDT methods used for malaria diagnosis using the nPCR test as a reference standard, RDT recorded the best diagnostic performance with a moderate level of agreement (kappa 0.431). However, when nPCR was replaced with the composite test as the reference standard, the level of agreement was stronger (kappa 0.821). Even though PCR recorded a moderate level of agreement, it recorded a sensitivity of 100% (Table 7). This study is in line with a couple of studies [67–69] but is contrary to a study conducted by Innocent and collaborators [70] who reported Carestat RDT to be more sensitive than SD-bioline. The low level of specificity of PCR in this study may be due to the increased number of false-positive results due to the robust reference test method used. RDT detects either the presence of HRP2 or LDH which may not always signify the presence of an active infection as studies have shown that these proteins can last for weeks in blood after parasite clearance [71]. Nevertheless, because RDT missed a significant proportion of infections, it is insufficiently sensitive for mass screening programs, as recommended by WHO [72]. However, WHO recommends immediate treatment for individuals positive for either microscopy or RDT [73]. Therefore, routine screening for malaria among pregnant women at each ANC up to delivery using RDT is

recommended irrespective of the presence or absence of signs and symptoms of malaria, especially in areas where access to PCR is a problem.

The limitations of each of the different test methods can be seen from their percentage discordance (Figure 7). Even though each of the methods was able to detect a certain percentage of infected individuals, all the different test methods have their strength and weaknesses [69, 74, 75]. This can be explained by the fact that microscopy as previously mentioned relies on the technician's expertise as an experienced microscopist will only detect a minimum parasite density of between 40 and 100 parasite/ $\mu$ L of blood [2, 37]. Therefore, individuals with very low levels of parasitemia (submicroscopic infection) will be missed by microscopy [37]. RDT will detect p $fh$ rp2 which most often does not indicate the presence of an active infection and at the same time will report a false negative for individuals infected with mutant strains that have the deletion of p $fh$ rp2 gene responsible for the production of the histidine protein [76]. These individuals can only be detected by a highly sensitive molecular assay that will detect these individuals irrespective of the absence or presence of p $fh$ rp2 gene [2, 76].

The findings of this study are in line with similar observations of previous studies conducted in Saudi Arabia [77], India [78], and Ghana [79]. These studies also confirm that microscopy has a poor diagnostic performance in detecting malaria, especially among asymptomatic infected individuals with very low parasite levels. RDT and PCR had a superior diagnostic performance over microscopy in detecting malaria-infected individuals.

**5.1. Limitations of the Study.** The study did not analyze for placenta malaria as well as real-time PCR for a more precise quantification of the parasite load to properly evaluate the effectiveness of IPTp-SP on malaria parasite levels.

## 6. Conclusion

This study shows that PCR is more sensitive in the detection of malaria parasite followed by the SD-bioline RDT kit. However, in resource-limited settings where access to molecular diagnosis is a problem, RDT is more suitable for the diagnosis of malaria than microscopy and should be considered as an alternative. Nevertheless, TFM and SD-bioline RDT missed quite a good number of infected cases which were detected by nested PCR. It also revealed a high level of asymptomatic malaria infection circulating among pregnant women. Therefore, routine screening for malaria should be implemented in antenatal care visits irrespective of the presence or absence of clinical signs and symptoms of malaria. The use of insecticide-treated bed nets may not be sufficient enough in the fight against malaria. Therefore, it should be combined with other preventive measures such as good environmental hygiene, use of insecticide sprays, and use of mosquito repellants. Finally, there is a need to implement measures that will boost microscopy and RDT performance through refresher training and the establishment of quality-assured RDTs.



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