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Original paper

Assessment of the performance of five malaria rapid diagnostic tests in health facilities in Abidjan (Côte d'Ivoire)

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ABSTRACT. Regular monitoring of malaria rapid diagnostic tests (RDTs) for the management of uncomplicated malaria in healthcare facilities is a key factor in improving diagnostic quality and ensuring better case management. This study aimed to assess the performance of five RDTs (Standard Q Malaria P.f Ag and Standard Q Malaria P.f/Pan (SD Biosensor, Korea), One Step Malaria HRP2/pLDH (P.f/Pan) (Guangzhou Wondfo Biotech Co., Ltd., China), Malaria Pf/Pan (B&O Pharm, France), and Malaria test P.f/pan (Das Labor, Germany)) in two healthcare facilities in Abidjan. This cross-sectional study was conducted between September and October 2022. Overall, 250 patients suffering from uncomplicated malaria were included with a predominance of female patients (56.6%). The mean age was 22.3 years (SD = 20.6; range, 0.17–73). Of the patients tested, forty-six (46) tested positive for thick smears, reflecting a prevalence of 18.5%. *Plasmodium falciparum* was the most commonly detected species (93.5%). The geometric mean parasitemia was 6,111.80 parasites/µl (SD = 80,026.93) (range: 116–412461). The sensitivity ranged from 95.24% to 95.65%, whereas the specificity ranged from 93.07 to 94.09% for all five tests evaluated. The false positive rate of the tests was less than 10%. No invalid test results were reported. Two-thirds of *P. malariae* cases detected by microscopy showed also positive results with all the RDTs. All five RDTs showed 100% sensitivity at low parasitemia levels (< 1,000 parasites/µl blood) including three cases of parasites < 200 parasites/µl blood. This study demonstrated the importance of monitoring the performance of RDTs in clinical samples.

Keywords: malaria, RDTs, HRPII, panLDH

Introduction

Malaria remains a major public health concern, particularly in Côte d'Ivoire, where it accounts for 33% of all outpatient visits and one-third of reported deaths in healthcare facilities [1]. Globally, the World Health Organization (WHO) reported an estimated 249 million malaria cases with approximately 608,000 cases of deaths in 2022. A total of 29 countries, including Côte d'Ivoire, accounted for 96% of malaria cases and malaria deaths globally in 2022 (World Malaria Report 2023). In 2020, the incidence rate of malaria in Côte d'Ivoire was 173.43 per 1000 in the general population and 440.97 per 1000 in children under 5 years of age [3].

Faced with this burden, the WHO has listed ensuring universal access to malaria prevention, diagnosis, and treatment as the first pillar of the Global Technical Strategy for Malaria Control

2016–2030 [4]. Thus, malaria diagnosis is a key factor in its elimination in endemic areas and should precede any specific treatment [5]. Diagnosis is performed either by rapid diagnostic testing (RDT) or microscopy [6]. Microscopy is the gold standard for this purpose; however, it has several limitations. Microscopy requires well-trained personnel and the availability of both equipment and reagents, making RDTs an attractive alternative diagnostic method, especially in countries where malaria is endemic [6]. The use of RDTs prior to treatment reduces the incidence of incorrect prescription of antimalarial drugs and enables the early diagnosis of non-malarial illnesses, thereby reducing treatment costs and morbidity and mortality rates [7]. In Côte d'Ivoire, the biological diagnosis of malaria in primary health facilities relies mainly on RDTs because of the widespread unavailability of microscopy [8] hence, it is important to ensure the quality of RDTs.

The regular monitoring of the performance of malaria RDTs for the diagnosis of malaria in healthcare facilities is a key factor in improving diagnostic quality and ensuring better case management. Although the procurement of RDTs used in public health facilities to diagnose clinical malaria in the country is WHO-prequalified, studies using clinical samples are highly informative from a test performance perspective in routine usage [9]. In Côte d'Ivoire, the assessment of any medical products including RDTs is coordinated by the National Public Health Laboratory (In French: Laboratoire National de Santé Publique (LNSP)) under the intended authority of the Ivorian Pharmaceutical Regulatory Authority (In French: Autorité Ivoirienne de Régulation Pharmaceutique (AIRP)). The primary mission of the AIRP is to guarantee access to effective pharmaceutical products, laboratory tests, and reagents of proven quality. This study was part of this regular assessment.

This study aimed to assess the performance of five RDTs intended for the diagnosis of uncomplicated malaria in Abidjan.

Materials and Methods

Study sites

The study was conducted between September and October 2022 at two healthcare facilities in Abidjan: the General Hospital of Adjamé and the Health Center El-Rapha of Abobo. Abidjan is the economic capital of Côte d'Ivoire. It is located on the coast and experiences a humid equatorial-type climate with two rainy seasons (April–July and October–November) and two dry seasons (December–March and August– September). The temperature varies from 25°C to 30°C, and the relative humidity varies from 80% to 90%. The transmission of malaria in Abidjan and across Côte d'Ivoire occurs year-round, with peaking during the rainy season. Malaria cases in Côte d'Ivoire are predominantly caused by *P. falciparum* (95–99%) [8,10]. All biological analyses were performed at the Malaria Research and Control Center (MRCC) located at the National Public Health Institute.

Study design and sample collection

This cross-sectional study focused on outpatients with symptoms of indicative of uncomplicated malaria. The inclusion criteria were as follows: patients regardless of their age and sex with fever (axillary temperature ≥37.5°C) or history of fever within the last 24 hours. Patients with signs or evidence of severe malaria [11] or symptoms of malnutrition were not included. Before inclusion, the study protocol was explained to patients, and written informed consent was obtained from the patient or the legal guardians in the case of minors.

The sample size was not calculated. The number of patients included was a reasoned choice based on the number of tests available for the evaluation.

Blood collection

Venous whole blood was collected from each of the patients in a violet-capped collection tube using EDTA. The samples were then promptly sent in triple packaging to the MRCC for analysis.

Microscopy analysis

To confirm *Plasmodium* carriage and to determine parasitemia, thick and thin blood smears were performed on each sample. The parasitemia was determined by counting the number of asexual parasites for 200 white blood cells per µl; i.e., number of parasites × 8,000/200 assuming a white blood cell mean of 8,000 cells per µl, as recommended by the WHO when the patient's exact white blood cell count is not available [12]. Double-check readings were performed for all slides. A negative result was determined only after evaluating at least 100 microscopic fields. A discrepancy in the results was indicated by a difference of 10% in

Table 1. Characteristics of evaluated tests

	Rapid Diagnostic Tests evaluated				
	Standard Q Malaria P.f Ag (SD Biosensor)	Standard Q Malaria P.f/Pan Ag (SD Biosensor)	One Step Malaria HRP-2/ pLDH (Pf/Pan Ag) (Wondfo)	Malaria test P.f/pan (Das Labor)	Malaria Pf/Pan (B&O Pharm)
Batch number	50734C2AC	51034M1AC	W05420202	24054-A	2111368
Antigen detected	*HRP2*	*HRP2* *pLDH*	*HRP2* *pLDH*	*HRP2* *Aldolase*	*HRP2* *pLDH*
Species detected	*P. falciparum*	*P. falciparum* *P. ovale* *P. malariae* *P. vivax*	*P. falciparum* *P. ovale* *P. malariae* *P. vivax*	*P. falciparum* *P. ovale* *P. malariae* *P. vivax*	*P. falciparum* *P. ovale* *P. malariae* *P. vivax*
Expiration date	11/04/2024	10/09/2023	17/02/2024	10/2023	11/2023
Storage temperature	2–40°C	2–40°C	4–30°C	2–30°C	2–30°C

parasite density. In the case of a discrepancy, a third reading was made using a third microscopist.

Analysis using RDTs

Five malaria RDTs were evaluated in this study: Standard Q Malaria P.f/Pan Ag (SD Biosensor, Korea), Standard Q Malaria P.f/Ag (SD Biosensor, Korea), One-Step Malaria HRP2/pLDH (P.f/Pan) (Guangzhou Wondfo Biotech Co., Ltd., China), Malaria Pf/Pan (B&O Pharm, France), and Malaria test P.f/pan (Das Labor, Germany).

RDTs are qualitative tests based on the immunochromatography method [13]. The Standard Q Malaria P. f. Ag (SD Biosensor, Korea) test detects only Histidine-Rich Protein II (HRP-II), a specific antigen of *P. falciparum*. The other four RDTs identified antigens of *P. falciparum* and three other *Plasmodium* species (*P. malariae*, *P. ovale*, and *P. vivax*). The RDTs were evaluated according to the manufacturer's instructions.

The RDTs and storage conditions are listed in Table 1.

Data collection

A questionnaire was used to collect patient information. The questionnaire was completed during interviews conducted by the research team. Sociodemographic, clinical, and therapeutic data were collected from the patients.

End points

In this study, blood smears were considered the reference or 'gold standard' test [12]. The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of each RDT were determined. Sensitivity is defined as the percentage of patients with infection who will have a positive result in the test under evaluation, determined from the result of the reference or 'gold standard' test. Specificity is defined as the percentage of patients without infection who will have a negative result in the test under evaluation, determined from the result of the reference or 'gold standard' test.

The variable measures included the number of true positives (TP), true negatives (TN), false positives (FP), and false negatives (FN). Sensitivity was calculated as TP/(TP + FN), specificity as TN/(TN + FP), PPV as TP/(TP + FP), and NPV as TN/(TN + FN) [9].

Apart from sensitivity and specificity, some technical criteria [14] were included in the evaluation, such as the simplicity of use (reconstruction required), presentation of kits (≤25/Box), storage temperature (>6°C out of fridge), migration time (≤15 min), ease of interpretation (colored band), the presence of all components, and instructions in French (the official local language).

Ethical considerations

This study was conducted under the authority of the Ivorian Pharmaceutical Regulatory Authority. Written informed consent was obtained from patients or their legal guardians before inclusion.

Table 2. Socio-demographic characteristics of patients

	Positive microscopic result n (%) N = 46	Negative microscopic result n (%) N = 203	Total n (%) N=249	*P-value
Sex				
Male	26 (24.1)	82 (75.9)	108 (43.4)	0.0498
Female	20 (14.2)	121 (85.8)	141 (56.6)	
Sex-ratio	0.77			
Age (years)				
0–5	11 (12.5)	77 (87.5)	88 (35.3)	<0.0001
6–15	14 (48.3)	15 (51.7)	29 (11.6)	
>15	21 (15.9)	111 (84.1)	132 (53.0)	
Moyenne	22.3			
Minimum	0.17			
Maximum	73			

Explanation: * Fisher's exact test

Table 3. *Plasmodium* species found in the study

Species	Frequency (n)	%
P. falciparum monoinfection	38	82.6
P. malariae monoinfection	3	6.5
Mixed infections *P. falciparum* + *P. malariae*	3	6.5
Mixed infections *P. falciparum* + *P. ovale*	1	2.2
Mixed infections *P. falciparum* + *P. malariae* + *P. ovale*	1	2.2
Total	46	100

Table 4. Performance of Standard Q Malaria P.f Ag (HRP II) in the detection of *P. falciparum*

		Standard Q Malaria P.f Ag (HRP II)		
		Positive	Negative	Total
Microscopic examination results	Positive	40	2	42
	Negative	13	189	202
	Total	53	191	244

Statistical analyses

Statistical analysis of the data was conducted using EPI Info 6.04 (CDC, Atlanta, USA). Chi-squared (χ^2) and Fisher's exact tests were used to assess significant variation in the data, with $P<0.05$ considered statistically significant.

Results

A total of 250 patients were included in the RDTs evaluation. However, only 249 samples were validated because of a manipulation error in performing smears, leading to a lack of interpretation in one case.

Table 5. Detection of *Plasmodium* species by Standard Q Malaria P.f/Pan Ag (SD Biosensor) and One Step Malaria HRP-2/ pLDH (Pf/Pan Ag) (Wondfo)

	Standard Q Malaria P.f/Pan Ag (SD Biosensor) N = 249					One Step Malaria HRP-2/ pLDH (Pf/Pan Ag) (Wondfo) N = 247				
Microscopic examination results	Negative	HRPII Positive	HRPII/Pan LDH Positive	Pan LDH Positive	Total	Negative	HRPII Positive	HRPII/Pan LDH Positive	Pan LDH Positive	Total
P. falciparum mono infection	1	12	25	0	38	1	11	25	0	37
P. malariae monoinfection	1	0	1	1	3	1	0	1	1	3
Mixed infections *P. falciparum* + *P. malariae*	0	0	3	0	3	0	0	2	1	3
Mixed infections *P. falciparum* + *P. ovale*	0	0	0	1	1	0	0	0	1	1
Mixed infections *P. falciparum* + *P. malariae* + *P. ovale*	0	0	1	0	1	0	0	1	0	1
Total positive	2	12	30	2	46	2	11	29	3	45
Negative	190	11	2	0	203	189	11	2	0	202
Total	192	23	32	2	249	191	22	31	3	247

Table 6. Detection of *Plasmodium* species by Malaria test P.f/pan (Das Labor) and Malaria Pf/Pan (B&O Pharm)

	Malaria test P.f/pan (Das Labor) N = 248					Malaria Pf/Pan (B&O Pharm)N = 247				
Microscopic examination results	Negative	HRPII Positive	HRPII/Pan LDH Positive	Pan LDH Positive	Total	Negative	HRPII Positive	HRPII/Pan LDH Positive	Pan LDH Positive	Total
P. falciparum mono infection	1	12	24	0	37	1	10	26	0	37
P. malariae monoinfection	1	0	1	1	3	1	0	1	1	3
Mixed infections *P. falciparum* + *P. malariae*	0	0	3	0	3	0	0	3	0	3
Mixed infections *P. falciparum* + *P. ovale*	0	0	0	1	1	0	0	0	1	1
Mixed infections *P. falciparum* + *P. malariae* + *P. ovale*	0	0	1	0	1	0	0	1	0	1
Total positive	2	12	29	2	45	2	10	31	2	45
Negative	191	10	2	0	203	188	12	2	0	202
Total	193	22	31	2	248	190	22	33	2	247

Table 7. Performance and praticability of evaluated RDTs

	Standard Q Malaria P.f Ag (HRP II)	Standard Q Malaria P.f/Pan Ag (SD Biosensor)	One Step Malaria HRP-2/ pLDH (Pf/Pan Ag) (Wondfo)	Malaria test P.f/pan (Das Labor)	Malaria Pf/Pan (B&O Pharm)
Sensitivity	95.24	95.65	95.56	95.56	95.56
Specificity	93.56	93.6	93.56	94.09	93.07
Positive-predictive value	75.47	77.19	76.79	78.18	75.44
Negative-predictive value	98.95	98.96	98.95	98.96	98.95
Simplicity of use (no reconstruction required)	Yes	Yes	Yes	Yes	Yes
Presentation (≤25/Box)	Yes	Yes	Yes	Yes	Yes
Storage temperature (>6°C (out of fridge)	Yes	Yes	Yes	Yes	Yes
Migration time (≤15 min)	Yes	Yes	Yes	Yes	**No**
Easy interpretation (colored band)	Yes	Yes	Yes	Yes	Yes
Presence of all components	Yes	Yes	***No**	***No**	Yes
Instructions in French (Official local language)	Yes	Yes	Yes	Yes	Yes

Explanation: *No: No alcohol swabs and sterile lancets

Patients' data

The majority of the patients surveyed were female (56.6%; sex ratio, 0.77). Their mean age was 22.3 years (SD = 20.6; range: 0.17–73). Patients aged >15 years accounted for 53.0% of the study population. The sociodemographic characteristics of the patients are detailed in Table 2.

Forty-six (46) patients were tested positive for thick smears, representing a prevalence of 18.5%. *Plasmodium falciparum* was the most commonly detected species (93.5%). Five cases of mixed infections were observed (Table 3). The geometric mean parasitemia was 6,111.80 parasites/µl (SD = 80,026.93) (range 116–412461). A history of fever was the most frequent symptom in patients (94%), followed by headache (63.9%). A total of 45 patients (18.1%) were taking antimalarial drugs before consultation. In most cases, these were combinations of artemether/lumefantrine. Most antimalarial drugs were prescribed were prescribed at an appropriate dose (93.3%).

Biological data

The sensitivity ranged from 95.24–95.65%, whereas the specificity ranged from 93.07–94.09% for all five tests evaluated. The false positive rate of the tests was less than 10%. No invalid test results were reported. Positive case profiles were the same for all RDTs that detected HRPII and panLDH. *P. falciparum* monoinfections were detected mainly by both HRPII and panLDH bands. Most mixed infections were detected using both HRPII and panLDH bands. The four (4) RDTs detecting both the HRPII and pLDH antigens showed the same profile for the detection of *P. malariae*. Two-thirds of *P. malariae* cases detected by microscopy also displayed a positive result with all five RDTs: one positive for the pLDH band and the second positive for both bands (HRPII and pLDH). The details are presented in Tables 4 to 7. All five RDTs showed 100% sensitivity at low parasitemia levels (<1,000 parasites/µl blood) including three cases of <200 parasites/µl blood. Seven (7) cases of *P. falciparum*

gametocyte carriage were identified, including one case of unique carriage. This single case was positive with all five tests.

Not all the necessary components to carry out the test were available in the One-Step Malaria HRP-2/pLDH (Pf/Pan Ag) (Wondfo) or Malaria test P. f/pan (Das Labor) kits. These kits lacked alcohol swabs or sterile lancets required to carry out the tests.

Discussion

Microscopy and rapid diagnostic tests (RDTs) are recommended by the WHO for confirmation of diagnosis in suspected malaria patients [2].

In Côte d'Ivoire, the large-scale use of RDTs for the diagnosis of malaria in peripheral public health facilities has improved the overall confirmation rate of suspected cases of malaria from 66% in 2012 to 75% in 2013 [8]. Globally, the detection of suspected malaria cases has steadily increased in the public sector since 2010; the most pronounced increase has been observed in the WHO African region, where the screening rate increased from 36% in 2010 to 87% in 2016 [15].

Because *Plasmodium falciparum* is predominant in Côte d'Ivoire [10], RDTs that detect only this species are generally preferable [16]. One of the tests, the Standard Q Malaria P.f Ag (SD Biosensor was manufactured to detect *P. falciparum* only, while the other four (Standard Q Malaria P.f/Pan Ag (SD Biosensor), One Step Malaria HRP-2/ pLDH (Pf/Pan Ag) (Wondfo), Malaria test P.f/pan (Das Labor), and Malaria Pf/Pan (B&O Pharm)) are capable of detecting *P. falciparum* and the *Plasmodium* species in two separate bands.

According to WHO recommendations, RDTs should show greater than 95% sensitivity [9] associated with a specificity above 90% [14] to be useful and efficient diagnostic tools. In this study, all five tests showed a sensitivity of >95%. The highest sensitivity was shown by the Standard Q Malaria P.f/Pan Ag (SD Biosensor) test. The false-positive rate was less than 10% for all tests evaluated. False-negative (FN) results are defined as RDT-negative but microscopy-positive results that lead to the misdiagnosis of malaria infections [17]. False negative (FN) results may have been caused by several factors. The first factor is operator-dependent. This can occur if the RDT test bands are misinterpreted or if the result is read before or after the recommended incubation period [18]. The second factor is the species detected. A typical example is that non-*P. falciparum* malaria is not detected by the commonly used PfHRP2-based RDTs [16]. The third factor was the deletion of pfhrp2/3 gene [17]. The fourth factor concerns the RDT itself. Poor quality of RDTs caused by improper storage, including prolonged exposure to hot or humid conditions [19], a variation between lots [13], and parasite density being below the RDT's limit of detection (LOD), typically in the range of 200 parasites/µl [20]. In this study, all five RDTs showed 100% sensitivity at low parasitemia levels (<1,000 parasites/µl blood) including three cases of parasites <200 parasites/µl blood.

In addition, HRP2, pLDH, and aldolase are produced by gametocytes (immature stages produce HRPII, whereas mature stages produce the enzymes pLDH and aldolase) [16]. Gametocytes are not pathogenic, and gametocytes of *P. falciparum* can persist following chemotherapy without implying drug resistance. Their presence in the blood could lead to a positive RDT result and can thus lead to erroneous interpretations (false positives) and unnecessary treatment of people not suffering from malaria. RDTs that detect antigens produced by gametocytes (such as pLDH) can yield positive results in infections in which only gametocytes are present [14]. In this study, a single case of gametocyte carriage without trophozoites was positive in all five tests.

Little is known about the performance of RDTs in diagnosis of non-*P. falciparum* malaria infections. In previous studies conducted in Côte d'Ivoire, most malaria cases were caused by *P. falciparum* (95–99%), followed by *P. malariae* (3–4.2%), and *P. ovale* (0.3–0.7%) [8,10], showing that *P. malariae* is the second-most predominant species after *P. falciparum*. The sensitivity of RDTs can vary according to *Plasmodium* species. Generally, RDT sensitivity is good for *P. falciparum* but only moderate for other species [21]. Data on *P. malariae* are even more limited, both in terms of the studies and samples tested [21]. In this study, only three cases of *P. malariae* monoinfection were observed by microscopy. The four RDTs detecting both the HRPII and pLDH antigens showed the same profile for the detection of *P. malariae*. Two-thirds of the *P. malariae* cases detected by microscopy also showed positive results for all five RDTs. One case was detected only by the pLDH band and the other by both bands (HRPII and pLDH). In any case, to make more concrete

conclusions on this, a greater number of non-*P. falciparum* cases is required.

Malaria occurs more frequently among vulnerable populations, such as pregnant women and children under the age of five (World Health Organization, 2023). Contrary to most studies conducted in Côte d'Ivoire [22,23], our study population mainly comprised patients under 15 years of age.

Parasitological diagnosis of malaria based on RDTs could improve the therapeutic management of patients and reduce instances of incorrect treatment with antimalarial drugs [8], which only increases the risk of the genesis of chemoresistant *P. falciparum* strains. In the present study, most antimalarial drugs were prescribed at an appropriate dosage (93.3%). This high rate highlights the good knowledge of the National Malaria Control Program recommendations concerning the therapeutic management of malaria cases in Côte d'Ivoire.

In conclusions, currently rapid diagnostic tests are the cornerstone of malaria diagnosis. This study assessed the performance of five RDTs in the diagnosis of uncomplicated malaria in Abidjan. The results showed high sensitivity (>95%) and specificity (>90%) for the five tests. Additionally, relatively good detection of *P. malariae* was observed across all kits. Additionally, some kits should be improved, notably by including sterile lancets and alcohol swabs. This study demonstrated the importance of monitoring the performance of RDTs in clinical samples.

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