AFRICAN JOURNAL OF PARASITOLOGY, MYCOLOGY AND ENTOMOLOGY
Official journal of the African Society of Parasitology

Diagnostic Performance of NxTek™ Eliminate Malaria-Pf Test for the Detection of *Plasmodium falciparum* Infection in Pregnant Women from Bobo-Dioulasso, Burkina Faso

Running Title: Performance of NxTek™ Eliminate Malaria-Pf Test

Mamoudou Cissé, PhD [1,2,*], Constant Sirima, PhD [1], Michel K. Gomgnimbou, PhD [1,2], Isidore T. Traoré, PhD [1,2], Hamed Drabo, MD [1], Arnaud Q. Sanou, MSc [1], Edmond W. Silga, MSc [1], Alamissa Soulama, PhD [1], Victor E. Kornu, BSc [3], Felix Anshah, PhD [3], Arthur D. Djibougou, PhD [1], Ismael Diallo, MD [4,5], Adama Zida, PhD [4,5], Ibrahim Sangaré, PhD [1,2], Sanata Bamba, PhD [2,6], Yaw Aniweh, PhD [3], Issaka Zongo, PhD [7] and Gordon A. Awandare, PhD [3]

[1] Laboratoire de Recherche, Centre MURAZ, Bobo-Dioulasso 01 BP 390, Burkina Faso; constantsirima@yahoo.fr (C.S.); gomikir@yahoo.fr (M.K.G.); tiandiogo2002@yahoo.fr (I.T.T.); hameddrabo@yahoo.com (H.D.); arnaudsanou07@gmail.com (A.Q.S.); silgaedmond@gmail.com (E.W.S.); alamissas@yahoo.fr (A.S.); lynndjibougou@gmail.com (A.D.D.); babaibrasangare@yahoo.fr (I.S.)

[2] Institut Supérieur des Sciences de la Santé, Université Nazi BONI, Bobo-Dioulasso 01 BP 1091, Burkina Faso; hsanata@yahoo.fr

[3] West African Centre for Cell Biology of Infectious Pathogens, College of Basic and Applied Sciences, University of Ghana, Legon, Accra P.O. Box 54 LG, Ghana; kvictoredem@gmail.com (V.E.K.); ansahfelix66@yahoo.com (F.A.); yaniweh@ug.edu.gh (Y.A.); gawandare@ug.edu.gh (G.A.A.)

[4] Unité de Formation et de Recherche en Sciences de la Santé, Université Joseph KI-ZERBO, Ouagadougou 03 BP 7021, Burkina Faso; ismael.diallo@ujkz.bf (I.D.); zidaadama@live.fr (A.Z.)

[5] Centre Hospitalier Universitaire Yalgado OUEDRAOGO, Ouagadougou 03 BP 7022, Burkina Faso

[6] Centre Hospitalier Universitaire Sourô SANOU, Bobo-Dioulasso 01 BP 676, Burkina Faso

[7] Institut de Recherche en Sciences de la Santé, Direction régionale de l'Ouest, Bobo-Dioulasso 01 BP 545, Burkina Faso; zongoissaka08@gmail.com

* Corresponding author: cisse_m@yahoo.fr; Tel.: +226-71326769

Submitted: 8 December 2024, accepted: 29 April 2025, published: 26 June 2025

Abstract: Introduction: Knowledge gaps still exist in Burkina Faso regarding the diagnostic performance of the highly sensitive rapid diagnostic test (hsRDT) for the detection of *Plasmodium*

(P.) falciparum malaria infection in pregnant women during antenatal care visits in Bobo-Dioulasso city. **Methods:** A cross-sectional study including 288 pregnant women was conducted between October and December 2022. *P. falciparum* malaria infection in peripheral blood was detected using the hsRDT, conventional RDT (cRDT), microscopy, and an ultrasensitive quantitative polymerase chain reaction (qPCR). The hsRDT, cRDT, and microscopy performance were assessed using qPCR as the gold standard. Cohen's Kappa test was used to estimate the agreement between the different diagnostic tests. **Results:** The prevalence of *P. falciparum* infection was 59.72% (172/288) by qPCR. The sensitivity of the hsRDT, cRDT, and microscopy was 51.16% [95% CI (43.44–58.85)], 50.58% [95% CI (42.87–58.28)], and 32.56% [95% CI (25.62–40.11)], respectively. The specificities were 98.28% [95% CI (93.91–99.79)], 99.14% [95% CI (95.29–99.98)], and 99.14% [95% CI (95.29–99.98)] for the hsRDT, cRDT, and microscopy, respectively. The agreement between the hsRDT and qPCR was moderate (Kappa = 0.44; $p < 0.001$). For parasite density by qPCR below 100 parasites/μL, the hsRDT and cRDT had the same sensitivity of 28.81% [95% CI (20.85–37.87)] but higher than that of microscopy [5.93% (95% CI 2.42–11.84)]. **Conclusions:** The sensitivity of the hsRDT is similar to that of the cRDT but better than that of microscopy. These results highlight the need for further studies to better guide recommendations on using the hsRDT malaria control and elimination.

Study Registration Number: ClinicalTrials.gov NCT06572644 (retrospectively registered on 26 August 2024).

Keywords: malaria in pregnancy; rapid diagnostic tests; microscopy; diagnostic performance; qPCR; Burkina Faso

How to cite: Cissé, M.; Sirima, C.; Gomgnimbou, M.K.; Traoré, I.T.; Drabo, H.; Sanou, A.Q.; Silga, E.W.; Soulama, A.; Kornu, V.E.; Anshah, F.; et al. Diagnostic Performance of NxTek™ Eliminate Malaria-Pf Test for the Detection of *Plasmodium falciparum* Infection in Pregnant Women from Bobo-Dioulasso, Burkina Faso. *Afr. J. Parasitol. Mycol. Entomol.*, 2025, 3(1): 3; doi:10.35995/ajpme03010003.

1. Introduction

Malaria in pregnancy (MiP) is a public health concern in sub-Saharan Africa (sSA). In 2023, around 12.4 million pregnant women were exposed to malaria in this region [1]. In addition, MiP due to *Plasmodium* (*P.*) *falciparum* is one of the leading causes of maternal anemia, low birth weight, and fetal growth restriction, which are significant risk factors for neonatal and infant morbidity and mortality [1,2].

Today, intermittent preventive treatment in pregnancy with sulfadoxine/pyrimethamine (IPTp-SP) is a cornerstone of the World Health Organization's (WHO) recommended regimen for preventing MiP, and pregnant women have the opportunity to benefit from at least three doses of SP during scheduled ANC visits [3]. However, the low IPTp-SP coverage in most sSA countries [4] and the widespread resistance of *P. falciparum* to SP [5,6] may jeopardize the effectiveness of this strategy. Therefore, new alternative strategies to IPTp-SP are urgently needed.

A possible alternative strategy to IPTp-SP is the intermittent screening of malaria infection and treatment during pregnancy (ISTp) [7,8]. It involves intermittent antenatal screening for malaria with

RDTs and treating women with positive RDT results with antimalarials. Although the WHO does not currently recommend ISTp as an alternative strategy to IPTp-SP [4], it is being implemented in settings of low-level malaria transmission [9]. With widespread reductions in malaria transmission [10] and increasing resistance to SP [5,6], ISTp has the potential to offer an alternative to IPTp-SP for the prevention of MiP in malaria-endemic settings, including Burkina Faso. However, there are concerns regarding the ISTp approach because the conventional RDTs (cRDTs) used to screen for malaria infection have a detection limit of 100 parasites/μL and are not designed to detect low-density infections (subpatent infections) in asymptomatic pregnant women [8,9,11]. Therefore, low-cost and field-deployable highly sensitive RDTs (hsRDTs) that may improve the detection of malaria infection in these populations are needed.

Recently, a *P. falciparum* histidine-rich protein 2 (*Pf*HRP2)-based hsRDT (NxTek™ Eliminate Malaria Ag Pf) was prequalified by the WHO [12] and is a promising diagnosis tool as part of the ISTp strategy. Indeed, the hsRDT has an analytical sensitivity (i.e., detection threshold) ten times higher than cRDTs [13]. Under field conditions, it consistently outperformed the cRDT and microscopy in pregnant women from low, moderate, and high malaria transmission areas [13–18]. However, there are limited data on its performance in pregnant women, particularly in SSA [13,17,18].

Malaria is a serious public health problem in Burkina Faso, with pregnant women being one of the most high-risk groups [19]. The prevalence of falciparum malaria among pregnant women attending ANC visits was 18%, and IPTp-SP has been the main strategy used for MiP prevention since 2005 [20]. The first reported case of the quintuple mutation in the country in 2017 [21] suggests an urgent need for alternative strategies to IPTp-SP, such as ISTp. Notwithstanding, knowledge gaps still exist on the diagnostic performance of the hsRDT for detecting *P. falciparum* malaria infection among pregnant women. This, therefore, provided us with the singular opportunity to design the first study evaluating the performance of the hsRDT in pregnant women from Burkina Faso.

2. Methods

2.1. Study Setting, Design, and Period

This cross-sectional study was conducted from October to December 2022 at the Centre Médical Urbain (CMU) of Lafiabougou, located in the peri-urban area of Bobo-Dioulasso. Bobo-Dioulasso is the second largest city of Burkina Faso, located 365 km southwest of Ouagadougou, the capital of Burkina Faso. In 2019, this town had 214,824 households with 983,552 inhabitants [22]. Farming and trading are the main economic activities. The annual rainfall ranges from 1000 to 1200 mm. The study site is located in an area with a high malaria transmission season from May to November. *Anopheles arabiensis* is the major malaria vector in Bobo-Dioulasso city, with a sporozoite infection rate of 6.2% in the peri-urban area of the town [23]. The prevalence of *P. falciparum* parasitaemia reported among pregnant women in the study area was 18.1% [20]. Each month, an average of 400 pregnant women attend the CMU's maternity department for their ANC visits.

2.2. Study Population

Pregnant women self-presenting at the CMU de Lafiabougou for ANC visits and who met the following inclusion criteria were included in this study: being in the first, second, or third trimester of pregnancy, a resident of the study site for at least 6 months, and the provision of informed consent. The following exclusion criteria were used: past history of malaria or antimalarial drugs within the last 3 months, having tested positive for malaria by microscopy or the cRDT in any previous ANC visit, and symptoms and signs of severe malaria as defined by the WHO [24].

2.3. Sample Size and Sampling Technique

The sample size was calculated according to a previously published methodology [25] to obtain sensitivity and specificity estimates of the index test (hsRDT) with an acceptable width of a 95% confidence interval (CI) for the estimates of sensitivity and specificity of $\pm$10%. Assuming *P. falciparum* infection prevalence of 18.1% reported among pregnant women attending ANC visits in Burkina Faso [20], an expected sensitivity of the index test of 85.7% [14], and specificity of 90% when compared to the gold standard (ultrasensitive qPCR), the calculation resulted in a minimal target sample size of 262. Then, accounting for 10% of missing data, 288 pregnant women were included in this study. All eligible pregnant women were included until the final sample size was reached.

2.4. Socio-Demographic and Clinical Data and Blood Sample Collection

At enrolment, the selected pregnant women were given an individually structured questionnaire to collect their socio-demographic data, including age, education level, and occupation. In addition, obstetric history (gravidity, gestational age, and number of ANC visits) and body temperature were recorded. Venous blood samples (4 mL) were then collected in ethylenediaminetetraacetic acid (EDTA) tubes and taken to the Laboratory of Parasitology and Mycology of the Centre MURAZ for screening for malaria infection using the hsRDT, cRDT, and microscopy. A few drops of blood were then collected as dried blood spots (DBSs) on Whatman filter paper and stored in individual sterile plastic bags with desiccant at room temperature until processing with qPCR.

2.5. Diagnostic Test Procedures

2.5.1. Index Tests

hsRDT and cRDT

Both the hsRDT (NxTek Eliminate Malaria Pf, product code 05FK140, batch No. 05LDG008B, Alere/Abbott, Republic of Korea) and cRDT (AdvDx^{TM} Malaria Pf, product code 004ADFEF025KI-2, batch No. ADF77/0222, Advy Chemical, India) are two-band tests and qualitative and differential tests for the detection of *Pf*HRP2 in human whole blood. They were performed by two trained laboratory technicians according to the manufacturer's instructions. Briefly, 5 μL of venous blood was applied to the sample port of each test, followed by the application of four drops of assay diluent. Then, 15 (for cRDT) or 20 (for hsRDT) minutes after specimen application, the result was interpreted. To ensure the validity of the RDT results, two independent laboratory technicians read the results within the set timeframe. RDTs were positive if the antigen and control lines were visible. The result was negative when only the control line was visible. When the control line was not visible, the RDTs were invalid. In this case, the test was repeated. A third reader's opinion was sought in the case of a discrepancy between two readers.

Light Microscopy

Thin and thick slides were prepared and stained with Giemsa 10% for 10 min. The thin smears were fixed with methanol for 2 s before staining. Two qualified microscopists independently read thick and thin blood smears. Parasite density was calculated by counting the number of asexual malaria parasites per 200 leukocytes in the thick blood film, assuming 8,000 leucocytes/μL of blood [26]. A slide was considered negative if no parasite was found after counting 500 leukocytes. The final parasite density was calculated by averaging the two counts. Blood smears with discordant results (i.e., positive versus negative; parasitaemia difference > 50%; different species) were re-examined

by a third independent microscopist, and parasite density was calculated by averaging the two closest counts.

2.5.2. Reference Test

DNA was extracted from DBSs using the QIAamp DNA Mini Kit according to the manufacturer's instructions and stored at −20 °C until further use. Thereafter, *P. falciparum* DNA was detected by an ultrasensitive qPCR assay targeting the multicopy conserved var gene acidic terminal sequence (varATS) using the same primers and probe as previously described with minor adjustments in the qPCR reaction and cycling conditions [27]. Briefly, all reactions were performed in duplicates in a total volume of 15 µL containing 3 µL of DNA, 7.5 µL of 1X Luna® Universal Probe qPCR Master Mix, 0.27 µM of each primer, and 0.2 µM of FAM-labeled probe. Reactions were run on the QuantStudioTM 5 system (Applied Biosystems, Waltham, MA) with the following settings: 3 min at 95 °C, followed by 45 cycles of 15 s at 95 °C, 40 s at 50 °C, and 40 s at 60 °C. Standard curves of *P. falciparum* 3D7 culture (tenfold serial dilution steps) were used as positive controls, and negative controls (water) were included in every run in duplicate. The dilution series was used to estimate the parasite density. The actual parasite density of the tested sample by qPCR was then estimated from the calibration curve's y-intercept and slope. The limit of detection of the qPCR assay is approximately 0.2 parasites/µL of blood [27].

2.6. Statistical Analysis

Data were double-entered using EpiData 3.1, cleaned, and then analyzed using STATA version 12.0 (STATA Corporation, College Station, TX, USA).

Descriptive statistics (frequency, proportions, and means or median) were applied to describe the study participants' socio-demographic and clinical characteristics. Febrile women were those presenting fever (⩾37.5 °C axillary temperature), and afebrile women were those without fever.

The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of the index tests (hsRDT, cRDT, and microscopy) were estimated with 95% CIs, considering the qPCR as the gold standard (due to its higher sensitivity to detect low-density parasitaemia) using the MedCalc Software Ltd., diagnostic Test Evaluation Calculator, available at the following link: https://www.medcalc.org/calc/diagnostic_test.php (accessed on 1 August 2024). Cohen's Kappa values assessed the agreement between microscopy, cRDT, hsRDT, and qPCR. Kappa values < 0.2 were considered a poor agreement, 0.21–0.40 fair, 0.41–0.6 moderate, 0.61–0.8 very good, and 0.81–1.00 almost perfect [28]. McNemar's test was used to determine significant differences between the diagnostic performance of the tests. The Wilcoxon rank sum test and Kruskal–Wallis test were used for a comparison of the median parasite densities. A Venn diagram was generated with the InteractiVenn tool [29]. *P*-values of <0.05 were considered significant.

2.7. Ethics Statement

Written informed consent was obtained from all pregnant women prior to their enrolment in the study. For illiterate pregnant women, the informed consent administration process was witnessed by an impartial individual. In those cases, the informed consent form was signed with a thumbprint and a formal signature by the witness. The study protocol and documents were reviewed and approved by the Comité d'éthique institutionnel de l'Institut pour la Recherche en Sciences de la Santé, Direction régionale de l'Ouest (A-036-2022/CEIRES). This study was conducted according to the GCP and Declaration of Helsinki.

3. Results

3.1. Socio-Demographic and Clinical Characteristics of the Study Population

A total of 293 pregnant women were screened, and 288 (98.3%) were enrolled. Of the five study participants excluded, four had a history of malaria, and one refused to participate (Figure 1). The mean age of the participants was 25.25 $\pm$ 6.33 years, and the majority of women were illiterate (46.88%), afebrile (96.17%), and multigravida (41.40%). The median gestational age at enrolment (IQR) was 16 weeks (12–20), and approximately two-thirds of the participants (66.32%) were enrolled during the second trimester (Table 1).

Table 1: Socio-demographic and clinical characteristics of study participants.

Variables	Categories	Frequency	%
Age (years)	<20	54	18.75
	20–24	99	34.38
	>20	135	46.87
Occupation [a]			
	Housewife	216	76.33
	Others [b]	67	23.67
Educational status			
	Formal schooling	153	53.12
	No Formal schooling	135	46.88
Fever [c]			
	Yes	11	3.83
	No	276	96.17
Gravidity [d]			
	Primigravida	93	32.63
	Secundigravida	74	25.96
	Multigravida	118	41.40
Gestational age			
	1st trimester	96	33.33
	2nd trimester	191	66.32
	3rd trimester	1	0.35
Number of ANC visits			
	$\leqslant$1	224	77.78
	>1	64	22.22

a: 5 missing data b: Students, traders; c: 1 missing data; d: 3 missing data.

3.2. Prevalence of Malaria Infection as Determined by Different Diagnostic Methods

A total of 172 out of 288 (59.72%) *P. falciparum* malaria infections were detected by qPCR. Infected pregnant women showed parasite densities ranging from 0.2 to 17,661 parasites per microliter (p/μL) with a geometric mean of 9.83 p/μL (95% CI 5.7–16.8). Most infections (118, 40.97%) were of low density (<100 p/μL) as per the WHO [30].

The parasite density was higher in afebrile (median = 7.51 p/μL) women than in febrile women (median = 4.51 p/μL) (p = 0.84). Parasite densities tended to be higher in primigravida (median = 36.36 p/μL) compared to secundigravida (median = 23.03 p/μL) and multigravida (median = 2.98 p/μL) (p = 0.07) women. Likewise, infections detected during the first trimester of pregnancy (median

= 2.14 p/μL) showed lower densities than those occurring in the second (median = 16.68 p/μL) and third trimester (median = 197.39 p/μL) (p = 0.20).

Both the cRDT and hsRDT detected 30.56% (88/288) and 31.25% (90/288) of *P. falciparum* infection, respectively (Figure 2). Microscopy was positive for 19.79% (57/288) of women, with a geometric mean of parasite density of 650.84 trophozoites/μL (95% CI 463.07–914.77).

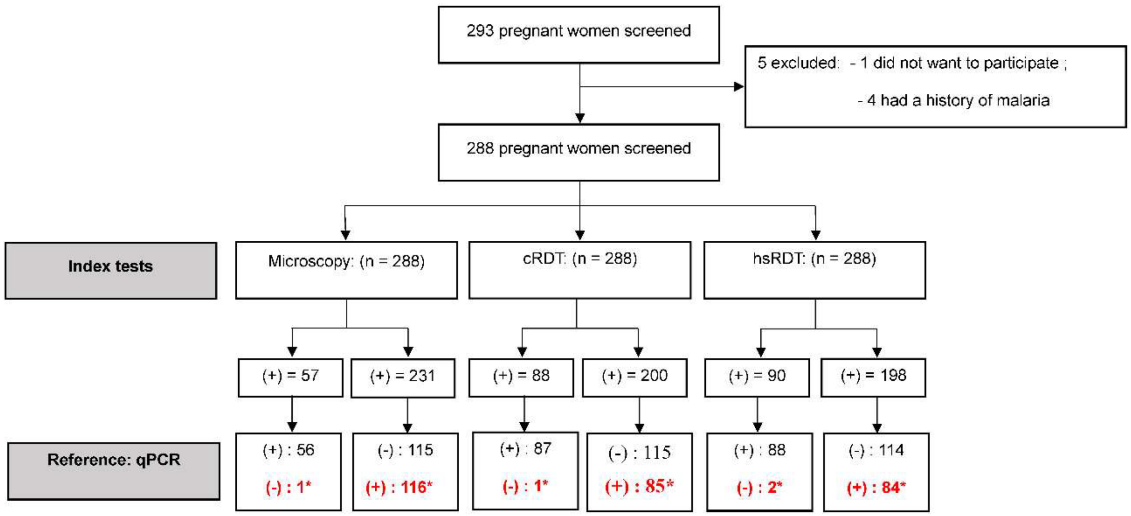

Figure 1: Study participants flowchart and testing results for *P. falciparum*. The chart shows the total number of pregnant women recruited and the overall number of *P. falciparum* infections detected by each test. *: Red and bold text: discrepant results when compared with the reference test. (+): positive; (−): negative; cRDT (conventional rapid diagnostic test); hsRDT (highly sensitive rapid diagnostic test); qPCR (quantitative polymerase chain reaction).

3.3. Performance of the Different Diagnostic Tests

All three index tests (i.e., microscopy, cRDT, and hsRDT) detected 54 of the 172 *P. falciparum* infections identified by qPCR (Figure 3). Likewise, microscopy, cRDT, and hsRDT detected 56, 87, and 88 of the *P. falciparum* infections confirmed by qPCR, respectively. Using qPCR as a reference method, the sensitivity of microscopy, cRDT, and hsRDT was 32.56% (95% CI 25.62–40.11), 50.58% (95% CI 42.87–58.28), and 51.16% (95% CI 43.44–58.85), respectively (Table 2). All three diagnostic methods showed specificity greater than 98%. Both the cRDT and hsRDT showed moderate agreement with the qPCR results (Kappa = 0.44, p < 0.001). On the other hand, the microscopy showed a fair level of agreement with the qPCR results (Kappa = 0.27, p < 0.001).

Regarding test performance in febrile pregnant women, both the cRDT and hsRDT had the same sensitivity [33.3% (95% CI 4.3–77.7)], which was two times greater than that of microscopy [16.7% (95% CI 0.4–64.1)]. Still, all methods showed the same specificity of 100% (Table 3). Among afebrile participants, the cRDT and hsRDT missed almost half of the infections detected by qPCR, resulting in sensitivities of 50.91% and 51.52%, respectively. Microscopy missed about two-thirds of positive afebrile cases (sensitivity of 33.3%).

The hsRDT performed better among primigravida and secundigravida participants (>57% sensitivity) than the cRDT and microscopy (Table 4).

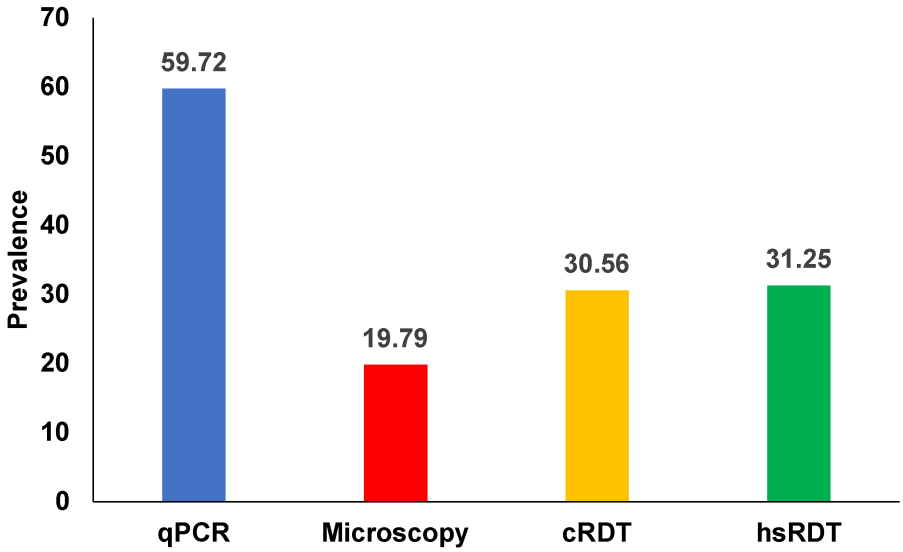

Figure 2: Prevalence of peripheral falciparum parasitaemia according to the test used. cRDT (conventional rapid diagnostic test); hsRDT (highly sensitive rapid diagnostic test); qPCR (quantitative polymerase chain reaction).

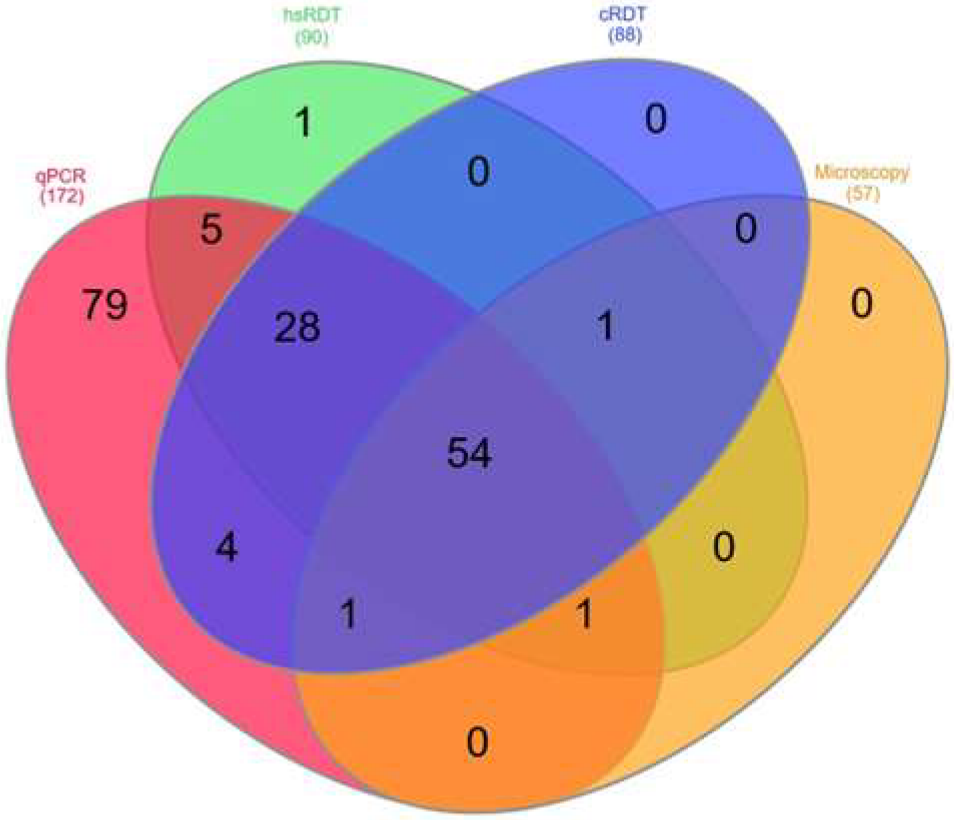

Figure 3: Venn diagram showing *P. falciparum* positivity by different diagnostic methods. cRDT (conventional rapid diagnostic test); hsRDT (highly sensitive rapid diagnostic test); qPCR (quantitative polymerase chain reaction). The number in brackets shows the total of positive samples detected by the test.

Table 2: Diagnostic performance of microscopy, cRDT, and hsRDT compared to qPCR as a reference test for the diagnosis of malaria.

Test		qPCR		Total	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	K
		(+)	(−)						
Microscopy	(+)	56	1	57	32.56 (25.62-40.11)	99.14 (95.29-99.98)	98.25 (88.72-99.75)	49.78 (47.16-52.41)	0.27
	(−)	116	115	231					
cRDT	(+)	87	1	88	50.58 (42.87-58.28)	99.14 (95.29-99.98)	98.86 (92.48-99.84)	57.50 (53.75-61.17)	0.44
	(−)	85	115	200					
hsRDT	(+)	88	2	90	51.16 (43.44-58.85)	98.28 (93.91-99.79)	97.78 (91.70-99.43)	57.58 (53.76-61.31)	0.44
	(−)	84	114	198					

cRDT (conventional rapid diagnostic test); hsRDT (highly sensitive rapid diagnostic test); qPCR (quantitative polymerase chain reaction); (+) (positive); (−) (negative); PPV (positive predictive value); NPV (negative predictive value).

Table 3: Diagnostic performance of microscopy, cRDT, and hsRDT among febrile and afebrile pregnant women.

	Test		qPCR		Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	K
Febrile (N = 11)	Microscopy		(+)	(−)					
		(+)	1	0	16.7 (0.4– 64.1)	100 (47.8–100)	100 (2.5–100)	50 (41.2–58.9)	0.15
		(−)	5	5					
	cRDT	(+)	2	0	33.3 (4.3–77.7)	100 (47.8–100)	100 (15.8–100)	55.6 (41.5–68.8)	0.31
		(−)	4	5					
	hsRDT	(+)	2	0	33.3 (4.3–77.7)	100 (47.8–100)	100 (15.8–100)	55.6 (41.5–68.8)	0.31
		(−)	4	5					
Afebrile (N = 276)	Microscopy	(+)	55	1	33.3 (26.2– 41.1)	99.10 (95.1–100)	98.2 (88.5– 99.8)	50 (47.3–52.7)	0.30
		(−)	110	110					
	cRDT	(+)	84	1	50.91 (43.0–58.8)	99.1 (95.1–100)	98.8 (92.2–99.8)	57.6 (53.7–61.4)	0.45
		(−)	81	110					
	hsRDT	(+)	85	2	51.52 (43.6–59.4)	98.20 (93.6–99.8)	97.70 (91.4–99.4)	57.67 (53.7–61.5)	0.46
		(−)	80	109					

cRDT (conventional rapid diagnostic test); hsRDT (highly sensitive rapid diagnostic test); qPCR (quantitative polymerase chain reaction); (+) (positive); (-) negative); PPV (positive predictive value); NPV (negative predictive value).

Table 4: Diagnostic performance of microscopy, cRDT, and hsRDT among primigravida, secundigravida, and multigravida.

	Test		qPCR		Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	K
Primigravida (N = 93)	Microscopy		(+)	(−)					
		(+)	25	1	48.1 (34.0–62.4)	97.6 (87.1–99.9)	96.2 (77.9–99.4)	59.7 (53.2–65.9)	0.43
		(−)	27	40					
	cRDT	(+)	28	1	53.9 (39.5–67.8)	97.6 (87.1–99.9)	96.6 (79.9–99.5)	62.5 (55.3–69.2)	0.49
		(−)	24	40					
	hsRDT	(+)	30	1	57.7 (43.2–71.3)	97.6 (87.1–99.9)	96.8 (81.0–99.5)	64.5 (56.9–71.5)	0.52
		(−)	22	40					
Secundigravida (N = 74)	Microscopy	(+)	14	0	31.1 (18.2–46.7)	100 (88.1–100)	100 (76.8–100)	48.3 (43.5–53.2)	0.26
		(−)	31	29					
	cRDT	(+)	25	0	55.6 (40.0–70.4)	100 (88.1–100)	100 (86.3–100)	59.2 (51.1–66.8)	0.50
		(−)	20	29					
	hsRDT	(+)	27	0	60 (44.3–74.3)	100 (88.1–100)	100 (87.2–100)	61.7 (53.0–69.7)	0.54
		(−)	18	29					
Multigravida (N = 118)	Microscopy	(+)	17	0	23.6 (14.4–35.1)	100 (92.3–100)	100 (80.5–100)	45.5 (42.4–48.7)	0.19
		(−)	55	46					
	cRDT	(+)	33	0	45.8 (34.0–58.0)	100 (92.3–100)	100 (89.4–100)	54.1 (48.8–59.3)	0.4
		(−)	39	46					
	hsRDT	(+)	30	1	41.7 (30.2–53.9)	97.8 (88.5–99.9)	96.8 (80.9–99.5)	51.7 (46.7–56.7)	0.34
		(−)	42	45					

cRDT (conventional rapid diagnostic test); hsRDT (highly sensitive rapid diagnostic test); qPCR (quantitative polymerase chain reaction); (+) (positive); (−) negative); PPV (positive predictive value); NPV (negative predictive value).

Among first-trimester participants, both the cRDT and hsRDT had a sensibility of 42.1% (95% CI 29.1–55.9) and 40.4% (95% CI 27.6–54.2), respectively. The two RDTs performed better among second-trimester women with comparable sensitivities (54.4% for cRDT versus 56.1% for hsRDT). An additional table shows this in more detail (see Additional file 1: Table S1).

Among pregnant women with low-density parasitaemia, the hsRDT and cRDT had the same sensitivity of 28.81% [95% CI (20.85–37.87)], but this was higher than that of microscopy [5.93% (95% CI 2.42–11.84)] (Additional file 1: Table S2). Between 100 and 200 p/μL, the sensitivity of the microscopy [62.50% (95% CI 24.49–91.48)] was still lower compared to both RDTs that showed the same sensitivity of 100% [95% CI (95% CI 63.06–100)]. At a parasite density > 200 p/μL, the hsRDT detected all 46 samples confirmed by qPCR, giving a sensitivity of 100% (95% CI 92.29–100). The microscopy and cRDT had a sensitivity of 95.65% (95% CI 85.16–99.47) and 97.83% (95% CI 88.47–99.94), respectively.

4. Discussion

To our knowledge, this is the first study to evaluate the performance of the hsRDT in detecting *P. falciparum* infection in pregnant women in Burkina Faso. The sensitivity of the hsRDT is similar to that of the cRDT but better than that of microscopy. In our study, the prevalence of falciparum malaria infection in pregnant women using qPCR (59.72%) was higher than that reported in Colombia (4.5%) [15], Ghana (10.54%) [31], Benin (25.49%) [13], Kenya (35.7%) [17], and Democratic Republic of Congo (DRC) (52.2%) [18]. An explanation for this observation could be a difference in study settings and the sensitivity of the molecular reference standards used. In addition, the proportion of women with low-density parasitaemia (40,97%) was higher than that reported in DRC (10%), where 50% of women had benefited from antimalarial drugs, either as IPTp or ISTp [18]. Malaria prevalence was found to be 19.79% by microscopy, which is similar to our previous study conducted in the same site (18.1%) [20] but slightly lower than that recently reported among women attending ANC in Africa (21.5%) [2]. However, in the latter study, diagnostic tests, including microscopy, PCR, and RDTs, were used to detect malaria during pregnancy [2]. Further studies are warranted to identify the factors involved in the persistence of the high prevalence of malaria in pregnant women in this part of Bobo-Dioulasso city.

The sensitivity of the hsRDT in our study (51.16%) was lower than that reported in studies that recruited both febrile and afebrile pregnant women ranging from 54.7 to 88% [18,32]. However, sensitivities of 19.6% and 53.8% were reported for the hsRDT in afebrile pregnant women from Indonesia [16] and Ghana [31], respectively. This difference could be attributed to variations in study settings (with malaria transmission intensities ranging from low to high), disease status (febrile versus afebrile), source of the samples used (fresh blood versus thawed blood), parasite density, and the reference test used [18,32].

The sensitivity of the hsRDT is similar to that of the cRDT in our study. This finding is consistent with that of Acquah et al., who showed that the hsRDT and cRDT had the same sensitivity of 53.8% in pregnant women from Ghana [31]. A plausible explanation for this observation could be that the cRDTs used had a lower detection limit than expected [32]. However, the hsRDT showed a slightly higher sensitivity than the cRDT in most studies conducted among pregnant women. Still, this difference was only statistically significant in the study conducted in Benin [32].

Both the hsRDT and the cRDT did detect high proportions of false negatives (48.84% versus 49.42%) when compared to qPCR. False negatives may be caused by certain factors, including low *Pf*HRP2 concentrations [13] and the prozone effect [33]. Low levels of *Pf*HRP2 may be due to low parasitaemia, the degradation of *Pf*HRP2 during storage, or deletions of the *Pfhrp2* and *Pfhrp3* genes [1,16,34]. Thus, the high proportions of false negatives observed in our study could be attributed to either low parasitemia or the circulation of malaria parasites carrying the *Pfhrp2* and *Pfhrp3* gene deletions. Indeed, for parasite density < 100 p/μL, the two RDTs had the same sensitivity of 28.81% but lower than those obtained for parasite density ⩾ 100 p/μL. In addition, our results highlight the

need to update the prevalence of *Pfhrp2* and *Pfhrp3* gene deletions, as malaria parasites harboring these mutations are circulating in regions neighboring Bobo-Dioulasso city [35].

The sensitivity of the hsRDT and cRDT was higher in afebrile-infected participants who carried high-density infections (median = 7.51 p/μL) compared to febrile women. This is likely because high-density infections among afebrile individuals may result in a high concentration of *Pf*HRP2 detected by both RDTs [12]. However, a recent review reported a high sensitivity of both tests among febrile women compared to afebrile ones, as was expected [32]. Indeed, those previous studies included a higher proportion of febrile women (ranging from 8.6 to 18.7%) [32] compared to our study (3.83%).

Our study did not find any trend of decreasing the hsRDT and cRDT sensitivity with increasing gravidity, as previously reported in Colombia [15], DRC [18], and Kenya [17]. Indeed, parasite densities tended to be higher in primigravida participants than in women with one or more previous pregnancies, although the difference was not significant.

The hsRDT and cRDT performed better among second-trimester women than first-trimester participants. This is consistent with the findings from high-transmission settings where both RDTs were more sensitive in later trimesters, although this difference was not significant [13,17]. However, in low-transmission settings, the highest sensitivity was observed in first-trimester women, but with no statistical difference [14,15]. This difference could be attributable to the lack of a clear pattern in the distribution of parasite densities by trimester [17].

The hsRDT, cRDT, and microscopy were highly specific and showed comparable specificity greater than 98%. The specificity of the hsRDT in our study (98.28%) was similar to that reported in Colombia and Indonesia, ranging from 98.2 to 99.9% [14–16], but higher than that found in Benin (93.5%) [13], DRC (94.4%) [18], and Kenya (95.8%) [17]. The lower specificity of the hsRDT observed in the latter studies could be attributed to false positive results that can be caused by several factors, including the persistence of *Pf*HRP2 in the blood for a while after the clearance of parasitaemia [36], cross-reactivity with rheumatoid factors [37], or inflammatory syndrome [38].

We acknowledge some limitations of this study. Firstly, we could not measure the concentration of *Pf*HRP2 in the blood samples collected due to a limited budget. Second, the small number of women in the third trimester did not allow us to estimate the performance of the tests in this group. Finally, the ultrasensitive qPCR used in our study may have overestimated parasite density due to variable numbers of copies of the amplification target and persistence of nucleic acid from non-viable parasites.

5. Conclusions

The sensitivity of the hsRDT is similar to that of the cRDT but better than that of microscopy. The hsRDT, cRDT, and microscopy are highly specific with similar values. There was a moderate agreement between RDTs and qPCR, whereas microscopy showed a fair agreement with the qPCR results. Further studies on the performance of the hsRDT, including the measurement of PfHRP2 concentration and assessment of the Pfhrp2 and Pfhrp3 gene deletions, particularly among false negatives, are warranted to better guide recommendations on its use for malaria control and elimination.

Abbreviations ANC: antenatal care; CI: confidence interval; CMU: Centre Médical Urbain; cRDT: conventional rapid diagnostic test; DRC: Democratic Republic of Congo; EDTA: ethylenediaminetetraacetic acid; hsRDT: highly sensitive rapid diagnostic test; IQR: interquartile range; IPTp-SP: intermittent preventive treatment in pregnancy with sulfadoxine/pyrimethamine; ISTp: intermittent screening of malaria infection and treatment during pregnancy; MiP: Malaria in pregnancy; NPV: negative predictive value; *Pf*HRP2: *Plasmodium falciparum* histidine-rich protein 2; PPV: positive predictive value; qPCR: quantitative polymerase chain reaction; sSA: sub-Saharan Africa; varATS: var gene acidic terminal sequence.

Supplementary Materials: The following are available online at www.xxx.com/xxx/s1, **Additional file 1: Table S1:** Additional file 1 shows the performance of microscopy, cRDT, and hsRDT among women in the first and second trimesters. **Additional file 2: Table S2:** Additional file 1 compares microscopy, cRDT, and hsRDT sensitivity stratified by parasite density as determined by qPCR.

Author Contributions: M.C., C.S., M.K.G., and I.T.T. conceived and designed the study. M.C. conducted the field study. H.D., A.Q.S., and E.W.S. performed field data collection under M.C.'s supervision. A.Q.S. and E.W.S. performed the molecular analyses under F.A., V.E.K., and M.K.G.'s supervision. M.C. analyzed the data and drafted the manuscript. A.S., A.D.D., I.D., A.Z., I.S., S.B., Y.A., I.Z., and G.A.A. critically revised the manuscript. All authors have read and agreed to the published version of this manuscript.

Funding: This work was supported by a World Bank African Centres of Excellence grant (ACE02-WACCBIP) as part of a regional partnership between Centre MURAZ and the University of Ghana. The funder had no role in the study design, data collection and analysis, decision to publish, or manuscript preparation.

Acknowledgments: We would like to thank all the pregnant women for their excellent cooperation, and we are very grateful to the staff of the CMU of Lafiabougou.

Conflicts of Interest: The authors declare no conflicts of interest.

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