OPEN ACCESS

Orapuh Journal

ORIGINAL ARTICLE

Journal of Oral & Public Health

Diagnostic performance of the SD Bioline Malaria Ag Pf® rapid diagnostic test for malaria screening among school-aged children in Mbujimayi, Democratic Republic of the Congo

Yamba, M. A.[1,2], Kabongo-Tshibaka, A.[3,4,5], Kalala-Tshituka, N.[2,6,7], Ndjibu-Mpoji, F.[1,2], Lufuluabu, M. A.[3,7], Cindibu, K. F.[3,6,7], Ngombo, K. B.[3], Cimuanga-Mukanya, A.[3], Losimba, J. L.[1,2], Tshibangu-Kabamba, E.[3,6,7], & Kayiba, K. N.[2,8]

[1]*Department of Public Health, Faculty of Medicine, University of Kisangani, Kisangani, Democratic Republic of the Congo* [2]*Department of Public Health, Faculty of Medicine, Pharmacy and Public Health, University of Mbujimayi, Mbujimayi, Democratic Republic of the Congo* [3]*Department of Internal Medicine, Faculty of Medicine, Pharmacy and Public Health, University of Mbujimayi, Mbujimayi, Democratic Republic of the Congo* [4]*Department of Molecular Infection Dynamics, Institute of Tropical Medicine, Nagasaki University, Nagasaki, Japan* [5]*Infection Biochemistry, Institute of Tropical Medicine, Nagasaki University, Nagasaki, Japan* [6]*Research Center for Infectious Disease Sciences, Graduate School of Medicine, Osaka Metropolitan University, Osaka, Japan* [7]*Departments of Virology and Parasitology, Graduate School of Medicine, Osaka Metropolitan University, Osaka, Japan* [8]*Research Institute of Health and Society, Catholic University of Louvain, Brussels, Belgium*

ARTICLE INFO

Received: *15 October 2025*
Accepted: *18 December 2025*
Published: *24 January 2026*

Keywords:
Malaria, school-aged children, diagnostic performance

Peer-Review: *Externally peer-reviewed*

Correspondence to:
Prof Nadine Kayiba Kalenda
nadinekayiba@yahoo.fr

To cite:
Yamba, M. A., Kabongo-Tshibaka, A., Kalala-Tshituka, N., Ndjibu-Mpoji, F., Lufuluabu, M. A., Cindibu, K. F., Ngombo, K. B., Cimuanga-Mukanya, A., Losimba, J. L., Tshibangu-Kabamba, E., & Kayiba, K. N. (2026). Diagnostic performance of the SD Bioline Malaria Ag Pf® rapid diagnostic test for malaria screening among school-aged children in Mbujimayi, Democratic Republic of the Congo. *Orapuh Journal, 7*(1), e1409 https://dx.doi.org/10.4314/orapj.v7i1.9

ISSN: *2644-3740*

Published by Orapuh, Inc. (info@orapuh.org)

Editor-in-Chief: Prof. V. E. Adamu
Orapuh, UMTG PMB 405, Serrekunda, The Gambia, editor@orapuh.org.

ABSTRACT

Introduction
School-aged children in malaria-endemic regions often harbour asymptomatic and low-density infections, constituting a hidden reservoir that complicates malaria control efforts. Although rapid diagnostic tests (RDTs) provide a practical screening tool, data on their diagnostic accuracy in this population remain limited.

Purpose
This study aimed to assess the diagnostic accuracy of the SD Bioline Malaria Ag Pf® RDT among school-aged children and to generate evidence to support malaria surveillance and control strategies in Mbujimayi.

Methods
A cross-sectional survey was conducted in February 2023 among 494 students aged 5–12 years from two primary schools in Mbujimayi, Democratic Republic of the Congo. Capillary blood samples were collected for thick and thin blood smears and SD Bioline Malaria Ag Pf® testing. Sensitivity, specificity, positive and negative predictive values (PPV and NPV), Cohen's kappa coefficient, and receiver operating characteristic (ROC) curve analysis were used to evaluate RDT performance relative to microscopy.

Results
The median age of the children was 10 years (range: 4–15 years). The prevalence of malaria was 27.3% (95% CI: 23.5–31.4) by both thick blood smear microscopy and the SD Bioline Malaria Ag Pf® RDT. *Plasmodium falciparum* accounted for 97.0% of infections. Agreement between the two diagnostic methods was fair (κ = 0.38; p = 0.001). The RDT demonstrated a sensitivity of 57.3% and a specificity of 87.1%, with a PPV of 47.2% and an NPV of 91.1%. The area under the ROC curve was 0.715 (95% CI: 0.663–0.767). The optimal parasite density threshold for detection was 202 parasites/µL, corresponding to a sensitivity of 52% and a specificity of 91%.

Conclusion
Among school-aged children, the SD Bioline Malaria Ag Pf® RDT demonstrated moderate diagnostic performance, reliably detecting moderate-to-high parasitaemia but missing a substantial proportion of low-density infections. Complementary diagnostic approaches may therefore be required to accurately identify malaria infections in this age group.

INTRODUCTION

Malaria remains a major public health challenge in the Democratic Republic of the Congo (DRC), which ranks as the second most affected country worldwide (World Health Organization [WHO], 2024). Transmission is intense and stable across most provinces, sustaining a high burden of disease throughout the year (WHO, 2023). Although malaria control efforts have historically focused on children under five years of age, recent surveys indicate that school-aged children experience the highest infection rates, reaching up to 62% (Nankabirwa et al., 2014; National Malaria Control Programme [PNLP], 2022; Sarpong et al., 2015).

In highly endemic settings, school-aged children frequently harbour asymptomatic and low-density *Plasmodium* spp. infections due to partially acquired immunity (Snow & Marsh, 2002). These infections are associated with chronic anaemia, impaired cognitive development, poor school performance, and increased absenteeism (Nankabirwa et al., 2013), while also sustaining malaria transmission by acting as a community reservoir (Walldorf et al., 2015). Targeted malaria screening among school-aged children is therefore critical for effective malaria control (Staedke & Maiteki-Sebuguzi, 2023).

In the DRC, malaria diagnosis relies primarily on light microscopy, the reference method capable of identifying *Plasmodium* species and quantifying parasitaemia, with a detection threshold of approximately 5–20 parasites/µL (Ohrt et al., 2002). However, its use is limited in many settings by logistical constraints, the need for skilled personnel, and laboratory infrastructure requirements (Mukadi et al., 2016). Consequently, rapid diagnostic tests (RDTs) are now widely used in routine clinical practice nationwide (Moody, 2014).

Among these, the SD Bioline Malaria Ag Pf® RDT, which detects the histidine-rich protein 2 (HRP2) antigen of *Plasmodium falciparum*, is extensively deployed under the recommendations of the National Malaria Control Programme (PNLP). This test provides a practical option for malaria screening in settings such as schools, where microscopy is not readily available (Berzosa et al., 2018; Kavanaugh et al., 2021). However, its diagnostic performance among school-aged children remains insufficiently assessed. This gap is particularly important given the predominance of asymptomatic and low-density infections in this population, which may reduce RDT sensitivity and lead to underdiagnosis (Nundu et al., 2022). Assessing the accuracy of this RDT in primary school settings is therefore essential to determine its suitability for school-based screening strategies in high-transmission areas.

Accordingly, the present study aimed to evaluate the performance of the SD Bioline Malaria Ag Pf® test for malaria screening among school-aged children in Mbujimayi. Specifically, it assessed malaria burden and the diagnostic accuracy of this RDT relative to standard microscopy, with the goal of generating locally relevant evidence to inform malaria surveillance and school-based control interventions.

METHODS

Study setting

This study was conducted from 20 to 28 February 2023 in Mbujimayi, the main city of Kasai-Oriental Province, one of the provinces recording the highest prevalence of malaria in the DRC (PNLP, 2023). The study was carried out in two primary schools: Kankolongo wa Bondo Institute, located in the Muya health district, and Sainte-Trinité School Complex, located in the Lukelenge health district.

Together, the two schools enrolled 1,112 students in 2023 (534 at Kankolongo wa Bondo and 578 at Sainte-Trinité), mostly aged 5–12 years and originating from low- to middle-income families. The region bears a high burden of infectious diseases, including malaria, acute respiratory infections, diarrhoeal diseases, and intestinal parasitic infections (Toma, 2018).

Study design

This was a cross-sectional study conducted among school-aged children from two primary schools in Mbujimayi, Democratic Republic of the Congo.

Study population

The study population comprised students enrolled in the selected schools. Eligible participants were students whose parents or legal guardians provided written informed consent and who assented to blood sampling. Students who were absent during the survey period were excluded.

Sample size determination

The sample size was calculated to estimate malaria prevalence and assess the diagnostic performance (sensitivity and specificity) of the SD Bioline Malaria Ag Pf® test. The Cochran formula, adjusted for finite populations, was applied using an expected prevalence of 50%, a margin of error of 5%, and a 95% confidence level. This yielded adjusted sample sizes of 225 and 232 students for the two schools. A 10% increase was applied to account for non-response, resulting in target sample sizes of 236 and 244 students, respectively, for a total of 480

participants. Ultimately, 494 students were included, allowing for precise estimation of malaria prevalence and diagnostic parameters in each school.

Sampling technique

The two schools were selected based on accessibility. Within each school, a comprehensive sampling approach was applied to maximise representativeness, with all enrolled students considered using class registration lists as the sampling frame.

Data collection tools

Twelve final-year medical students from the University of Mbujimayi, fluent in the local language and familiar with the urban context, were trained in participant selection, interviewing, RDT performance and interpretation, administration of weight-based artemether–lumefantrine, blood sample collection, and appropriate handling of sampling equipment. Following administrative approvals and parental consent, classroom information sessions were conducted prior to interviews, clinical assessments, RDTs, and blood sampling.

Data were collected using Android devices through a web-based form developed with KoboToolbox. The form captured sociodemographic variables (age, sex, grade, academic performance), clinical data (body temperature and antimalarial use within the preceding three days), and laboratory results (RDT and microscopy findings). Data entries were continuously supervised, verified, and corrected to ensure quality. Children with positive RDT results were treated with weight-based artemether–lumefantrine in accordance with national malaria treatment guidelines. Capillary blood samples were used for both RDTs and preparation of thick and thin blood smears for microscopy.

Laboratory methods

Rapid diagnostic test for malaria

Malaria screening was performed on site using the SD Bioline® Malaria Ag Pf HRP2 test (Standard Diagnostics, South Korea), which targets the *P. falciparum*-specific HRP2 antigen. This WHO-prequalified test was supplied free of charge by the PNLP in line with WHO quality assurance standards (Srivastava et al., 2023). Testing was conducted according to the manufacturer's instructions, with results read after 15 minutes. Two investigators independently interpreted the results, and a high-resolution photograph of each test was taken for quality control. Tests were considered positive when both control and test bands appeared, negative when only the control band was visible, and invalid when the control band was absent, necessitating immediate repetition. Discrepancies between readers were resolved by a third reader.

Microscopic examination for malaria

For each participant, both thick and thin blood smears were prepared from fresh capillary blood following standard procedures (WHO, 2014). Microscopic examination was conducted by two independent, qualified microscopists who were blinded to the RDT results. Parasite density was expressed as parasites per microlitre (parasites/µL), calculated based on the parasite-to-leukocyte ratio per 200 leukocytes, assuming a standard leukocyte count of 8,000/µL, in accordance with WHO recommendations (WHO, 2014).

Statistical analysis

Data were entered into Microsoft Excel (Microsoft Office 365) and analysed using R software (version 4.5.1; R Foundation for Statistical Computing, Vienna, Austria). Categorical variables were summarised as frequencies and percentages, while continuous variables were described using medians and interquartile ranges (IQR).

SD Bioline Malaria Ag Pf® results were compared with microscopy, the reference standard. Diagnostic performance measures, including sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV), were calculated. Agreement between diagnostic methods was assessed using Cohen's kappa (κ) coefficient and interpreted as follows: no agreement ($\kappa < 0$), poor ($\kappa = 0.01–0.20$), fair ($\kappa = 0.21–0.40$), moderate ($\kappa = 0.41–0.60$), substantial ($\kappa = 0.61–0.80$), and almost perfect ($\kappa = 0.81–1.00$) (McHugh, 2012).

Receiver operating characteristic (ROC) curve analysis was used to determine the optimal parasite density threshold for detection by the SD Bioline Malaria Ag Pf® test and to assess overall diagnostic accuracy based on the area under the curve (AUC) (Çorbacıoğlu & Aksel, 2023).

Associations between categorical variables were examined using Pearson's chi-square test or Fisher's exact test, as appropriate (Kim, 2017). The Shapiro–Wilk and Levene's tests were applied to assess normality and homogeneity of variances, respectively. Given non-parametric distributions, median comparisons between two groups were conducted using the Mann–Whitney U test (Ledolter et al., 2020). Statistical significance was set at $p < .05$, with analyses conducted at a 95% confidence level.

Operational definition

Symptomatic malaria was defined as a positive RDT and/or microscopy result in a student who experienced

fever or related symptoms on the day of the survey or within the preceding three days.

Ethical considerations

The study was conducted in accordance with the Declaration of Helsinki and national ethical guidelines for research involving human participants in the Democratic Republic of the Congo. Ethical approval was obtained from the Institutional Ethics Committee of the University of Mbujimayi (Approval No. 03/CEI/UM/ESU/2022), and administrative authorisation was granted by the Provincial Health Division of Kasai-Oriental. Written informed consent was obtained from parents or legal guardians, and assent was obtained from each participant prior to enrolment.

RESULTS

Participant characteristics

The median age of the participants was 10 years (range: 4–15 years), and 57.5% were male. Only 3.8% of the children presented with fever at the time of the survey, while 6.1% reported having received antimalarial treatment within the three days preceding data collection **(Table 1)**.

Table 1:
Sociodemographic and clinical characteristics of school-aged children (n = 494)

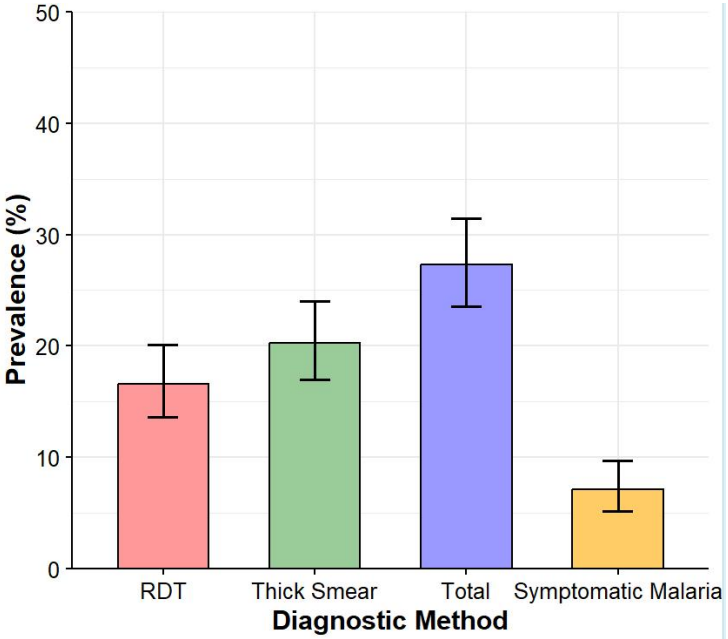
Characteristic	n	%
Primary school		
Kankolongo wa Bondo Institute	267	54.0
Sainte-Trinité School Complex	227	46.0
Age (years)		
≥ 8 years	401	81.8
< 8 years	93	18.2
Sex		
Female	210	42.5
Male	284	57.5
School grade		
Beginner (1st–2nd)	89	18.0
Intermediate (3rd–4th)	205	41.5
Final (5th–6th)	200	40.5
Body temperature		
Normal (36.0–37.4 °C)	475	96.2
Fever (≥ 37.5 °C)	19	3.8
Antimalarial treatment within 3 days prior to survey		
No	464	93.9
Yes	30	6.1

Note. Median age (minimum–maximum) = 10 (4–15) years.

Malaria prevalence and Plasmodium species involved

The overall malaria prevalence was 27.3% (95% CI: 23.5–31.4). Prevalence was 20.2% (95% CI: 17.0–24.0) by thick blood smear microscopy and 17.0% (95% CI: 13.5–20.1) by the SD Bioline Malaria Ag Pf® RDT. Symptomatic malaria was observed in 7.1% (95% CI: 5.1–9.7) of children **(Figure 1)**.

Figure 1:
Malaria prevalence among school-aged children by diagnostic method



Malaria diagnosis was determined using SD Bioline Malaria Ag Pf® RDT, thick blood smear microscopy, and combined prevalence (at least one positive test). Clinical malaria was defined by the presence of symptoms (fever, chills, body pain, or headache) within three days preceding data collection.

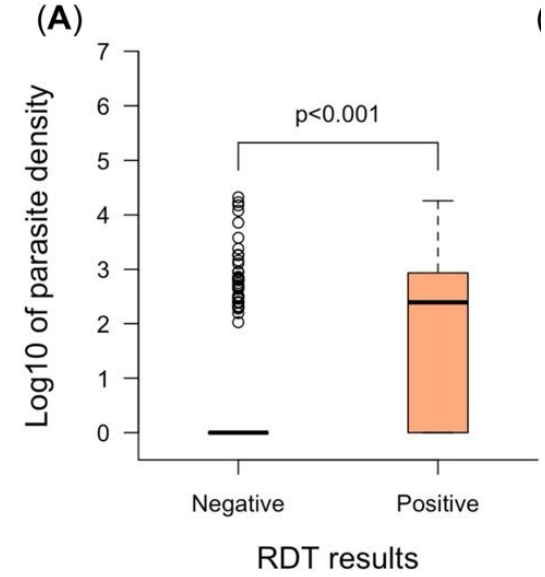
Thin blood smear analysis identified *Plasmodium falciparum* in 97.0% of microscopy-confirmed infections and *Plasmodium malariae* in 3.0%, with no mixed infections detected **(Table 2)**.

Table 2:
Comparison of thin smear species identification with thick smear microscopy results (n = 99)

***Plasmodium* species**	**Thick smear positive**	**Thick smear negative**	**Total**
	n (%)	n (%)	n (%)
P. falciparum	92 (100.0)	4 (57.1)	96 (97.0)
P. malariae	0 (0.0)	3 (42.9)	3 (3.0)
Total	92 (100.0)	7 (100.0)	99 (100.0)

The mean parasite density was significantly higher among RDT-positive children (1,023.3 parasites/µL) than among RDT-negative children (250.3 parasites/µL; $p < .001$) **(Figure 2A)**. The proportion of positive RDT results increased with increasing parasite density **(Figure 2B)**.

Figure 2:
Distribution of SD Bioline Malaria Ag Pf® RDT results by parasite density



(A) Comparison of mean parasite density between RDT-positive and RDT-negative children. Log_{10} transformation was applied to parasite density values to facilitate visual comparison.

(B) Frequency of RDT-positive and RDT-negative results across parasite density categories.

Diagnostic performance of the RDT

Comparison between SD Bioline Malaria Ag Pf® RDT and microscopy showed fair but statistically significant agreement (κ = 0.38, p = .001) **(Table 3)**.

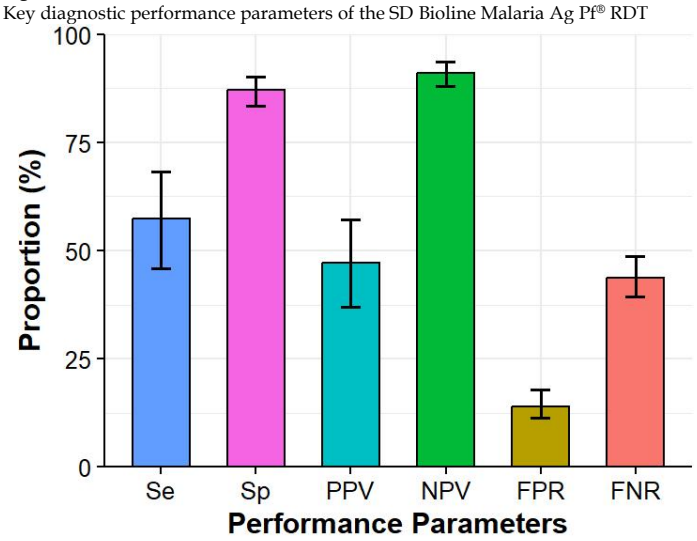
Table 3:
Agreement between SD Bioline Malaria Ag Pf® RDT and microscopy (n = 494)

RDT result	Microscopy positive	Microscopy negative	κ (95% CI)	Accuracy % (95% CI)	p
	n (%)	n (%)			
Positive	47 (43.9)	35 (9.0)	0.38 (0.31–0.48)	80.8 (77.3–84.3)	< .001
Negative	60 (56.1)	352 (91.0)			
Total	107 (100.0)	387 (100.0)			

Note. RDT = SD Bioline Malaria Ag Pf®; microscopy includes thick and thin blood smears.

Using microscopy as the reference standard, the RDT demonstrated a sensitivity of 57.3% (95% CI: 45.9–68.2) and a specificity of 87.1% (95% CI: 83.5–90.2). The negative predictive value (NPV) was high at 91.1% (95% CI: 87.9–93.7), whereas the positive predictive value (PPV) was moderate at 47.2% (95% CI: 36.9–57.2). The false-positive rate was 13.9% (95% CI: 11.4–17.9), and the false-negative rate was 43.7% (95% CI: 39.4–48.6) **(Figure 3)**.

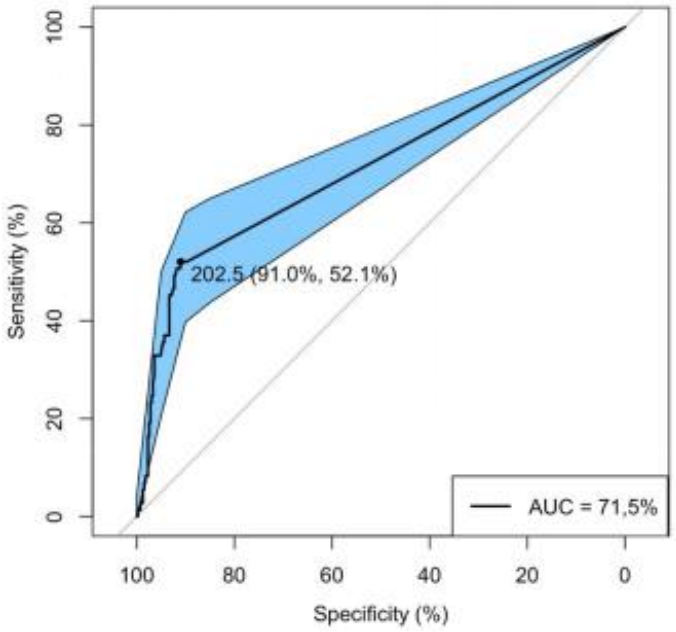
Figure 3:
Key diagnostic performance parameters of the SD Bioline Malaria Ag Pf® RDT



Se = sensitivity; Sp = specificity; PPV = positive predictive value; NPV = negative predictive value; FPR = false-positive rate; FNR = false-negative rate.

Receiver operating characteristic (ROC) curve analysis yielded an area under the curve (AUC) of 0.72, indicating moderate diagnostic accuracy. The optimal parasite density threshold for RDT detection was approximately 202 parasites/μL, corresponding to a sensitivity of 52% and a specificity of 91% **(Figure 4)**.

Figure 4:
ROC curve of the SD Bioline Malaria Ag Pf® RDT



The ROC curve plots sensitivity against specificity relative to microscopy and illustrates overall diagnostic accuracy, as reflected by the AUC and optimal detection threshold.

DISCUSSION

This study assessed malaria prevalence and the diagnostic performance of the SD Bioline Malaria Ag Pf® rapid diagnostic test (RDT) compared with microscopy among school-aged children in Mbujimayi, a high-transmission setting in the Democratic Republic of the Congo (DRC). Three key findings emerged: (i) a high prevalence of malaria in this population; (ii) a strong dependence of RDT performance on parasite density; and (iii) moderate agreement between the RDT and microscopy.

The high malaria prevalence (27%) observed in this study confirms that school-aged children are heavily infected despite being largely asymptomatic and constitute a significant reservoir for malaria transmission and persistence in the study area. This finding is consistent with reports from other malaria-endemic African settings, where prevalence among school-aged children may reach 50% or higher (Igbawua et al., 2024; Kambou et al., 2024; Sagna et al., 2024). Variations in prevalence across regions likely reflect ecological, seasonal, and methodological differences. Several studies have reported higher malaria incidence during the rainy season (Acquah et al., 2021; Moussa et al., 2023; Yimam et al., 2022), whereas the present survey was conducted at the end of the short dry season, a period that may be associated with relatively lower transmission in Mbujimayi.

In line with the epidemiology of malaria in the DRC, microscopy identified *Plasmodium falciparum* as the dominant species (97%), with *Plasmodium malariae* accounting for only 3% of infections. This contrasts with a previous PCR-based study among adults in the same region, which reported 53% *P. falciparum* and 24% *P. malariae* infections (Kayiba et al., 2024). Although differences in study populations limit direct comparison, this discrepancy highlights the limitations of microscopy in species identification, particularly due to morphological overlap among *Plasmodium* species.

RDT performance was strongly associated with parasite density, with a marked increase in positivity above 200 parasites/µL. This indicates that the SD Bioline Malaria Ag Pf® RDT reliably detects moderate-to-high parasitaemia but is likely to miss many low-density infections. This finding is consistent with previous reports demonstrating a sharp decline in RDT sensitivity below 100 parasites/µL, a threshold well above the detection limit of PCR (5–10 parasites/µL) (Joste et al., 2021). Consequently, asymptomatic children with low-density infections may remain undetected, thereby sustaining malaria transmission within the community. These results underscore the tendency of RDTs to underestimate true malaria prevalence and highlight the need for complementary diagnostic approaches, such as PCR, particularly in elimination-oriented school-based programmes.

Notably, even at high parasite densities, some infections were not detected by the SD Bioline Malaria Ag Pf® RDT. This may be explained by the prozone effect, whereby excess antigen interferes with test reactivity (Gillet et al., 2009). Other possible explanations include *pfhrp2* gene deletions or mutations and infections with non-*falciparum* species, which are not targeted by this RDT (Martínez-Vendrell et al., 2022; Parr et al., 2017).

The observed moderate agreement between the SD Bioline Malaria Ag Pf® RDT and microscopy likely reflects the combined effects of low parasite densities in asymptomatic children and the RDT's specificity for *P. falciparum*, despite the circulation of non-*falciparum* species in Mbujimayi (Kayiba et al., 2024). Similar levels of agreement ($\kappa \approx 0.39$) have been reported in Uganda (Kabbale et al., 2025), supporting the complementary use of microscopy and RDTs in settings characterised by low-density parasitaemia.

Furthermore, the RDT demonstrated moderate sensitivity and positive predictive value, falling below the 95% sensitivity threshold recommended by the World Health

Organization (WHO, 2020) and the 71–96% range reported in studies from Burkina Faso, Gabon, and Cameroon (Joste et al., 2021; Sayang et al., 2009; Sorgho et al., 2015). In addition to low parasitaemia, reduced sensitivity may have resulted from factors such as heat-related degradation of test kits during transportation or storage (Yimam et al., 2022). In contrast, specificity and negative predictive value were high, indicating strong performance of the SD Bioline Malaria Ag Pf® RDT for ruling out malaria in children who tested negative. These findings are consistent with reports from Djibouti and Nigeria (Moussa et al., 2023; Orimadegun et al., 2023), although slightly lower than values reported in Uganda and Mauritania (Ba et al., 2017; Kabbale et al., 2025).

False-positive RDT results (13.9%) may be attributable to persistence of HRP2 antigen in the bloodstream following recent antimalarial treatment (Iqbal et al., 2004). Cross-reactivity with other infections, including hepatitis Cleishmaniasis, toxoplasmosis, schistosomiasis, dengue, and human African trypanosomiasis, may also contribute to false positivity (Haberichter et al., 2017; WHO, 2017).

Receiver operating characteristic curve analysis demonstrated moderate overall diagnostic accuracy of the SD Bioline Malaria Ag Pf® RDT. These results are consistent with findings from Cameroon, where sensitivity ranged from 45% to 52% alongside high specificity (Ngalame et al., 2025). In contrast, studies conducted in Ethiopia and the Central African Republic reported sensitivities exceeding 85% at higher parasite densities (>500 parasites/μL) (Djallé et al., 2014; Tinto et al., 2015). Such variations likely reflect differences in parasite density distributions, reference standards, and contextual factors influencing test performance.

Overall, this study provides valuable evidence on the performance of the SD Bioline Malaria Ag Pf® RDT among school-aged children and supports the integration of targeted school-based interventions to strengthen malaria control efforts in endemic settings.

Limitations

This study has several limitations. First, microscopy was used as the reference standard and may have missed low-density infections detectable by PCR, potentially leading to underestimation of true malaria prevalence. Second, *hrp2/hrp3* gene deletions were not assessed, limiting interpretation of false-negative RDT results. Third, the study was conducted in a single city and included only two schools, which may limit generalisability to other regions or school-aged populations. Finally, seasonal variation in malaria transmission was not captured due to the cross-sectional design and the timing of data collection.

CONCLUSION

This study confirms that malaria remains highly prevalent among school-aged children in Mbujimayi, with a substantial burden of asymptomatic and low-density infections. The SD Bioline Malaria Ag Pf® RDT demonstrated moderate diagnostic accuracy, reliably detecting moderate-to-high parasitaemia but likely missing many low-density infections. These findings highlight the need for complementary diagnostic approaches, such as PCR, to support targeted school-based interventions aimed at reducing malaria transmission in endemic settings.

Acknowledgments: The authors sincerely thank the twelve final-year medical students from the University of Mbujimayi (Kabengele Kabengele Kary, Kalenda Musasa Marie France, Kapena Mulamba Ambroise, Mbowa Tula Dorcas, Mbuyi Mpoyi Marie Paul, Mitongo Ilunga Aicha, Nsongolo Tshibangu Jonathan, Ntumba Ciunza Joël, Kabeya Mpoyi Vincent, Kasunyi Tshibanda Harmonie, Kabala Luwambwa Zoé, and Kalambo Mutombo Josué) for their dedication and invaluable contribution to fieldwork. Their commitment and local expertise were essential to the successful completion of this study.

Author Contributions: A.Y.M., E.T.K., and N.K.K. designed the study and developed the study protocol. A.Y.M., E.T.K., and A.K.T. performed data analyses. A.Y.M., A.K.T., and N.K.K. drafted the initial manuscript. N.K.T., F.N.M., A.L.M., F.C.K., A.C.M., A.K.T., J.L.L., E.T.K., and N.K.K. contributed to manuscript revision. All authors read and approved the final manuscript.

Ethical Approval: Ethical approval was obtained from the Institutional Ethics Committee of the University of Mbujimayi (Approval No. 03/CEI/UM/ESU/2022), and administrative authorisation was granted by the Provincial Health Division of Kasai-Oriental.

Conflicts of Interest: None declared.

ORCID iDs:

Yamba, M. A.[1,2]: https://orcid.org/0009-0005-9605-9019
Kabongo-Tshibaka, A.[3,4,5]: https://orcid.org/0000-0001-9519-5653
Kalala-Tshituka, N.[2,6,7]: https://orcid.org/0009-0003-1401-1683
Ndjibu-Mpoji, F.[1,2]: https://orcid.org/0009-0002-7037-6444
Lufuluabu, M. A.[3,7]: https://orcid.org/0009-0009-7359-1466
Cindibu, K. F.[3,6,7]: https://orcid.org/0009-0003-6736-5303
Ngombo, K. B.[3]: https://orcid.org/0009-0001-7368-0006
Cimuanga-Mukanya, A.[3]: https://orcid.org/0000-0002-9255-3627
Losimba, J. L.[1,3]: https://orcid.org/0000-0002-2110-4488
Tshibangu-Kabamba, E.[3,6,7]: https://orcid.org/0000-0002-4339-229X
Kayiba, K. N.[2,8]: https://orcid.org/0000-0001-5557-304X

REFERENCES

Acquah, F. K., Donu, D., Obboh, E. K., Bredu, D., Mawuli, B., Amponsah, J. A., Quartey, J., & Amoah, L. E. (2021). Diagnostic performance of an ultrasensitive

HRP2-based malaria rapid diagnostic test kit used in surveys of afebrile people living in Southern Ghana. *Malaria Journal*, *20*(1), 1–11. https://doi.org/10.1186/s12936-021-03665-7

Ba, H., Ahouidi, A. D., Duffy, C. W., Deh, Y. B., Diedhiou, C., Tandia, A., Diallo, M. Y., Assefa, S., Lô, B. B., Elkory, M. B., & Conway, D. J. (2017). Evaluation du test de diagnostic rapide du paludisme OptiMal-IT® pLDH à la limite de la distribution de Plasmodium falciparum en Mauritanie. *Bulletin de La Societe de Pathologie Exotique*, *110*(1), 31–37. https://doi.org/10.1007/s13149-017-0541-y

Berzosa, P., Lucio, A. De, Barja, M. R., Herrador, Z., González, V., García, L., Martínez, A. F., Morales, M. S., Ncogo, P., Valladares, B., Riloha, M., & Benito, A. (2018). Comparison of three diagnostic methods (microscopy , RDT , and PCR) for the detection of malaria parasites in representative samples from Equatorial Guinea. *Malaria Journal*, 1–12. https://doi.org/10.1186/s12936-018-2481-4

Çorbacıoğlu, Ş. K., & Aksel, G. (2023). Receiver operating characteristic curve analysis in diagnostic accuracy studies: A guide to interpreting the area under the curve value. *Turkish Journal of Emergency Medicine*, *23*(4), 195–198. https://doi.org/10.4103/tjem.tjem_182_23

Djallé, D., Gody, J. C., Moyen, J. M., Tekpa, G., Ipero, J., Madji, N., Breurec, S., & Manirakiza, A. (2014). Performance of ParacheckTM-Pf, SD Bioline malaria Ag-Pf and SD Bioline malaria Ag-Pf/pan for diagnosis of falciparum malaria in the Central African Republic. *BMC Infectious Diseases*, *14*(1). https://doi.org/10.1186/1471-2334-14-109

Gillet, P., Mori, M., Van Esbroeck, M., Van Den Ende, J., & Jacobs, J. (2009). Assessment of the prozone effect in malaria rapid diagnostic tests. *Malaria Journal*, *8*(1), 1–7. https://doi.org/10.1186/1475-2875-8-271

Haberichter, K. L., Johnson, P. C., Chittick, P. J., Millward, P., Robinson-Dunn, B., & Boyanton, B. L. (2017). The Brief Case: False-Positive Rapid Malaria Antigen Test Result in a Returned Traveler. *Journal of Clinical Microbiology*, *55*(8), 2294–2297. https://doi.org/10.1128/JCM.02347-16

Iqbal, J., Siddique, A., Jameel, M., & Hira, P. R. (2004). Persistent Histidine-Rich Protein 2, Parasite Lactate Dehydrogenase, and Panmalarial Antigen Reactivity after Clearance of *Plasmodium falciparum* Monoinfection. *Journal of Clinical Microbiology*, *42*(9), 4237–4241. https://doi.org/10.1128/JCM.42.9.4237-4241.2004

Isaac Nyiayem Igbawua, Yakubu Boyi Ngwai, David Ishaleku, & Jibril Egwu Owuna. (2024). Prevalence of malaria and comparative diagnostic performance of malaria rapid diagnostic test against microscopy in Nasarawa-west senatorial District, Nasarawa, Nigeria. *GSC Advanced Research and Reviews*, *18*(1), 062–070. https://doi.org/10.30574/gscarr.2024.18.1.0486

Joste, V., Bailly, J., Hubert, V., Pauc, C., Gendrot, M., Guillochon, E., Madamet, M., Thellier, M., Kendjo, E., Argy, N., Pradines, B., Houzé, S., Garabedian, C., Domergue, A., Clauser, S., Agnamey, P., Damiani, C., de Gentile, L., Pihet, M., … Caraux-Paz, P. (2021). Plasmodium ovale wallikeri and P. ovale curtisi Infections and Diagnostic Approaches to Imported Malaria, France, 2013-2018. *Emerging Infectious Diseases*, *27*(2), 372–384. https://doi.org/10.3201/eid2702.202143

Kabbale, K. D., Nsengimaana, B., Semakuba, F. D., Kagurusi, B. A., Mwubaha, C., Wiringilimaana, I., Katairo, T., Kiyaga, S., Mbabazi, M., Gonahasa, S., Kamya, M. R., Tukwasibwe, S., Nsobya, S. L., Asua, V., Jjingo, D., Agaba, B., Maiteki-Sebuguzi, C., Opigo, J., Hilton, K., … Briggs, J. (2025). Field evaluation of the Bioline Malaria Ag P.f/Pan rapid diagnostic test: causes of microscopy discordance and performance in Uganda. *Malaria Journal*, *24*(1), 138. https://doi.org/10.1186/s12936-025-05379-6

Kambou, S. A. E., Millogo, K. S., Sondo, P., Kabore, B., Kouevi, A. F. C., Bouda, I., Rouamba, T., Derra, K., Tahita, M. C., Ilboudo, H., Rouamba, E., Tougri, G., Otienoburu, S. D., Dhorda, M., Bamba, S., Guerin, P. J., & Tinto, H. (2024). Prevalence of asymptomatic parasitaemia among household members of children under seasonal malaria chemoprevention coverage and comparison of the performance of standard rapid diagnostic tests versus ultrasensitive RDT for the detection of asymptomatic pa. *Parasitology Research*, *123*(11), 1–10. https://doi.org/10.1007/s00436-024-08380-1

Kavanaugh, M. J., Azzam, S. E., & Rockabrand, D. M. (2021). Malaria rapid diagnostic tests: Literary review and recommendation for a quality assurance, quality control algorithm. *Diagnostics*, *11*(5). https://doi.org/10.3390/diagnostics11050768

Kayiba, N. K., Nitahara, Y., Tshibangu-Kabamba, E., Mbuyi, D. K., Kabongo-Tshibaka, A., Kalala, N. T., Tshiebue, B. M., Candray-Medina, K. S., Kaku, N., Nakagama, Y., Speybroeck, N., Mumba, D. N., Disashi, G. T., Kaneko, A., & Kido, Y. (2024). Malaria infection among adults residing in a highly endemic region from the Democratic Republic of the Congo. *Malaria Journal*, *23*(1), 1–14. https://doi.org/10.1186/s12936-024-04881-7

Kim, H.-Y. (2017). Statistical notes for clinical researchers: Chi-squared test and Fisher's exact test. *Restorative Dentistry & Endodontics*, *42*(2), 152–155. https://doi.org/10.5395/rde.2017.42.2.152

Ledolter, J., Gramlich, O. W., & Kardon, R. H. (2020). Parametric Statistical Inference for Comparing Means and Variances. *Investigative Ophthalmology & Visual Science*, *61*(8), 25. https://doi.org/10.1167/iovs.61.8.25

M**artiáñez-Vendrell**, X., Skjefte, M., Sikka, R., & Gupta, H. (2022). Factors Affecting the Performance of HRP2-Based Malaria Rapid Diagnostic Tests. *Tropical Medicine and Infectious Disease*, *7*(10). https://doi.org/10.3390/tropicalmed7100265

McHugh, M. L. (2012). Interrater reliability: the kappa statistic. *Biochemia Medica*, *22*(3), 276–282.

Moody, A. (2014). Rapid diagnostic tests for malaria. *BMJ (Online)*, *348*(1), 66–78. https://doi.org/10.1136/bmj.g3846

Moussa, R. A., Papa Mze, N., Arreh, H. Y., Hamoud, A. A., Alaleh, K. M., Omar, A. R. Y., Abdi, W. O., Guelleh, S. K., Abdi, A. I. A., Aboubaker, M. H., Basco, L. K., Khaireh, B. A., & Bogreau, H. (2023). Molecular investigation of malaria-infected patients in Djibouti city (2018–2021). *Malaria Journal*, *22*(1), 1–14. https://doi.org/10.1186/s12936-023-04546-x

Mukadi, P., Lejon, V., Barbé, B., Gillet, P., Nyembo, C., Lukuka, A., Likwela, J., Lumbala, C., Mbaruku, J., Veken, W. Vander, Mumba, D., Lutumba, P., Muyembe, J. J., & Jacobs, J. (2016). Performance of microscopy for the diagnosis of malaria and human African trypanosomiasis by diagnostic laboratories in the democratic Republic of the Congo: Results of a nation-wide external quality assessment. *PLoS ONE*, *11*(1), 1–15. https://doi.org/10.1371/journal.pone.0146450

Nankabirwa, J., Brooker, S. J., Clarke, S. E., Fernando, D., Gitonga, C. W., Schellenberg, D., & Greenwood, B. (2014). Malaria in school-age children in Africa: an increasingly important challenge. *Tropical Medicine & International Health*, *19*(11), 1294–1309. https://doi.org/10.1111/tmi.12374

Nankabirwa, J., Wandera, B., Kiwanuka, N., Staedke, S. G., Kamya, M. R., & Brooker, S. J. (2013). Asymptomatic Plasmodium Infection and Cognition among Primary Schoolchildren in a High Malaria Transmission Setting in Uganda. *The American Society of Tropical Medicine and Hygiene*, *88*(6), 1102–1108. https://doi.org/10.4269/ajtmh.12-0633

Ngalame, A. L., Watching, D., Kibu, O. Z., Zeukóo, E. M., & Nsagha, D. S. (2025). Assessment of the diagnostic performance of the SD Bioline Malaria antigen test for the diagnosis of malaria in the Tombel health district, Southwest region of Cameroon. *PLoS ONE*, *20*(3 March), 1–15. https://doi.org/10.1371/journal.pone.0298992

Nundu, S. S., Simpson, S. V., Arima, H., Muyembe, J. J., Mita, T., Ahuka, S., & Yamamoto, T. (2022). It Is Time to Strengthen the Malaria Control Policy of the Democratic Republic of Congo and Include Schools and School-Age Children in Malaria Control Measures. *Pathogens*, *11*(7). https://doi.org/10.3390/pathogens11070729

Ohrt, C., Purnomo, Sutamihardja, M. A., Tang, D., & Kain, K. C. (2002). Impact of Microscopy Error on Estimates of Protective Efficacy in Malaria-Prevention Trials. *The Journal of Infectious Diseases*, *186*(4), 540–546. https://doi.org/10.1086/341938

OMS. (2014). *Techniques de base pour le diagnostic microscopique du paludisme*. https://iris.who.int/server/api/core/bitstreams/391850f1-97e3-44d0-aef6-a65e196f8885/content

OMS. (2017). *Mise en œuvre des programmes de paludisme pendant la grossesse dans le contexte des recommandations de l'Organisation Mondiale de la Santé (OMS) concernant les soins prénatals pour que la grossesse soit une expérience positive*. https://iris.who.int/server/api/core/bitstreams/2ccd6d04-3429-4476-97bc-c9403536431a/content

Orimadegun, A. E., Dada-Adegbola, H. O., Michael, O. S., Adepoju, A. A., Funwei, R. I., Olusola, F. I., Ajayi, I. O. O., Ogunkunle, O. O., Ademowo, O. G., Jegede, A. S., Baba, E., Hamade, P., Webster, J., Chandramohan, D., & Falade, C. O. (2023). SD-Bioline malaria rapid diagnostic test performance and time to become negative after treatment of malaria infection in Southwest Nigerian Children. *Annals of African Medicine*, *22*(4), 470–480. https://doi.org/10.4103/aam.aam_220_21

Parr, J. B., Verity, R., Doctor, S. M., Janko, M., Carey-Ewend, K., Turman, B. J., Keeler, C., Slater, H. C., Whitesell, A. N., Mwandagalirwa, K., Ghani, A. C., Likwela, J. L., Tshefu, A. K., Emch, M., Juliano, J. J., & Meshnick, S. R. (2017). Pfhrp2 -Deleted Plasmodium falciparum Parasites in the Democratic Republic of the Congo: A National Cross-sectional Survey. *Journal of Infectious Diseases*, *216*(1), 36–44. https://doi.org/10.1093/infdis/jiw538

PNLP. (2022). *Rapport annuel des activités de lutte contre le paludisme en RDC*. https://pnlprdc.org/wp-content/uploads/2024/04/Rapport-annuel-2022-des-activites-de-lutte-contre-le-Paludisme-1.pdf

PNLP. (2023). *Rapport annuel des activités de lutte contre le paludisme en RDC*. https://pnlprdc.org/wp-

content/uploads/2024/11/Rapport-annuel-2023-des-activites-de-lutte-contre-le-Paludisme.pdf

Sagna, A. B., Gebre, Y., Vera-Arias, C. A., Traoré, D. F., Tchekoi, B. N., Assi, S. B., Koffi, A. A., Rogier, C., Remoue, F., & Koepfli, C. (2024). High prevalence of asymptomatic and subpatent Plasmodium falciparum infections but no histidine-rich protein 2 gene deletion in Bouaké, Côte d'Ivoire. *Scientific Reports*, *14*(1), 1–11. https://doi.org/10.1038/s41598-024-70215-x

Sarpong, N., Owusu-Dabo, E., Kreuels, B., Fobil, J. N., Segbaya, S., Amoyaw, F., Hahn, A., Kruppa, T., & May, J. (2015). Prevalence of malaria parasitaemia in school children from two districts of Ghana earmarked for indoor residual spraying: a cross-sectional study. *Malaria Journal*, *14*(1), 260. https://doi.org/10.1186/s12936-015-0772-6

Sayang, C., Soula, G., Tahar, R., Basco, L. K., Gazin, P., Moyou-Somo, R., & Delmont, J. (2009). Use of a histidine-rich protein 2-based rapid diagnostic test for malaria by health personnel during routine consultation of febrile outpatients in a peripheral health facility in Yaoundé, Cameroon. *American Journal of Tropical Medicine and Hygiene*, *81*(2), 343–347. https://doi.org/10.4269/ajtmh.2009.81.343

Snow, R., & Marsh, K. (2002). The consequences of reducing transmission of Plasmodium falciparum in Africa. In *Advances in Parasitology Volume 52* (pp. 235–264). Elsevier. https://doi.org/10.1016/S0065-308X(02)52013-3

Sorgho, H., Douamba, K. R. D., Valéa, I., & Tinto, H. (2015). Performance diagnostique de SD Bioline malaria antigen P . f ® pour la détection des infections palustres chez la femme enceinte au troisième trimestre de grossesse au Burkina Faso. *Science et Technique, Sciences de La Santé*, *40*(2), 81–87.

Srivastava, B., Sharma, S., Ahmed, N., Kumari, P., Gahtori, R., Sinha, S., Kumar, S., Sanalkumar, M., Mahale, P., Swarnkar, D., & Anvikar, A. R. (2023). Quality assurance of malaria rapid diagnostic tests: An aid in malaria elimination. *The Indian Journal of Medical Research*, *157*(1), 23–29. https://doi.org/10.4103/ijmr.ijmr_2262_21

Staedke, S. G., & Maiteki-Sebuguzi, C. (2023). Targeting malaria control to schoolchildren. *The Lancet Global Health*, *11*(8), e1156–e1157. https://doi.org/10.1016/S2214-109X(23)00238-3

Tinto, H., Sombié, O., Valea, I., Ferdinand, L., Sandrine, Y., Lompo, P., Tahita, M. C., Kazienga, A., Ouédraogo, S., Sorgho, H., Maaike, D. K., & Ravinetto, R. (2015). Field evaluation of SD Bioline Malaria Antigen P.f® for Plasmodium falciparum malaria diagnosis in Nanoro, Burkina Faso. *African Journal of Parasitology Research*, *2*(11), 161–165.

Toma, I. A. (2018). *Kasai: The forgotten province of DRC – gender assessment*. https://doi.org/10.21201/2017.1657

Walldorf, J. A., Cohee, L. M., Coalson, J. E., Bauleni, A., Nkanaunena, K., Kapito-Tembo, A., Seydel, K. B., Ali, D., Mathanga, D., Taylor, T. E., Valim, C., & Laufer, M. K. (2015). School-Age Children Are a Reservoir of Malaria Infection in Malawi. *PLOS ONE*, *10*(7), e0134061. https://doi.org/10.1371/journal.pone.0134061

WHO. (2020). *Protocole type pour la surveillance des délétions des gènes Pfhrp2 et Pfhrp3*. https://iris.who.int/server/api/core/bitstreams/e34f9186-49f0-45cc-bdca-5e21327d26cb/content

WHO. (2023). *World Malaria Report 2023*. https://www.who.int/teams/global-malaria-programme/reports/world-malaria-report-2023

WHO. (2024). *World malaria World report 2024*. https://www.who.int/teams/global-malaria-programme/reports/world-malaria-report-2024

Yimam, Y., Mohebali, M., & Afshar, M. J. A. (2022). Comparison of diagnostic performance between conventional and ultrasensitive rapid diagnostic tests for diagnosis of malaria: A systematic review and meta-analysis. *PLoS ONE*, *17*(2 February), 97–100. https://doi.org/10.1371/journal.pone.0263770