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Diagnostic Performance of Rapid Tests and Microscopy Compared with PCR in the Diagnosis of Malaria among Children Under Five Years of Age in Kisangani, Democratic Republic of the Congo

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Abstract **Original Research Article**

Introduction: Malaria remains one of the leading causes of pediatric morbidity and mortality in sub-Saharan Africa. The diagnostic performance of rapid diagnostic tests (RDTs) and microscopy remains limited in high-endemic settings because of submicroscopic infections and low parasite densities. This study aimed to evaluate the diagnostic performance of RDTs and microscopy using PCR as the reference method among children under five years of age in Kisangani. ***Methods:*** This was a multicenter cross-sectional study with prospective data collection conducted from June 15 to December 30, 2025, in several healthcare facilities in Kisangani. Children aged 0–59 months presenting with fever or a recent history of fever were included. Each child underwent an RDT, microscopy, and PCR. Diagnostic performance was assessed through calculation of sensitivity, specificity, predictive values, and the Kappa coefficient. ***Results:*** A total of 417 children were included. The overall prevalence of malaria according to PCR was 64.5%. The proportion of submicroscopic infections was estimated at 27.8%. RDT sensitivity was low compared with PCR, whereas specificity was high. PCR also identified several mixed infections not detected by microscopy. ***Conclusion:*** PCR proved significantly more effective than conventional methods for malaria diagnosis among children under five years of age in Kisangani. Submicroscopic infections appear frequent and may contribute to the persistence of malaria transmission.

Keywords: Malaria; PCR; rapid diagnostic test; microscopy; child; submicroscopic infections; Kisangani; Democratic Republic of the Congo.

INTRODUCTION

Malaria remains one of the leading causes of morbidity and mortality among children in sub-Saharan African countries. According to the World Health Organization (WHO) World Malaria Report, the African region accounted for nearly 94% of global malaria cases and more than 95% of malaria-related deaths in 2022, more than three-quarters of which occurred among children under five years of age [1].

The Democratic Republic of the Congo (DRC) is among the countries most affected worldwide and represents a substantial proportion of the global malaria burden [2]. In Tshopo Province, transmission is intense and stable throughout the year due to equatorial climatic conditions favorable to vector development [3].

Biological diagnosis of malaria mainly relies on microscopy and rapid diagnostic tests (RDTs) [4]. Microscopy enables identification of Plasmodium species and estimation of parasite density, but its performance strongly depends on laboratory technical quality and microscopist expertise [5,6].

RDTs are widely used in low-resource settings due to their simplicity and rapidity. However, several studies have shown a significant reduction in sensitivity in the presence of low parasitemia, mixed infections, or non-falciparum species [7–9].

In high-endemicity settings such as Kisangani, submicroscopic infections are frequent and represent a silent reservoir likely to sustain malaria transmission over time [10].

Molecular methods, particularly polymerase chain reaction (PCR), are recognized as the most sensitive diagnostics techniques for detecting malaria infections with low parasite density [11,12].

Despite their importance, little information is available in eastern DRC regarding the comparative performance of RDTs and microscopy when evaluated against PCR [13].

This study therefore aimed to assess the diagnostic performance of RDTs and microscopy compared with PCR among children under five years of age in Kisangani.

MATERIALS AND METHODS

Study Setting

The study was conducted in Kisangani, the capital city of Tshopo Province, located in the northeastern part of the Democratic Republic of the Congo.

The healthcare facilities included the University Clinics of Kisangani, the Makiso-Kisangani, Kabondo and Lubunga General Referral Hospitals, as well as the Alabul and Nouveau Village de Pédiatrie hospital centers.

Study Design and Period

This was a multicenter cross-sectional study with prospective data collection conducted from June 15 to December 30, 2025.

Study Population

The study population consisted of children aged 0–59 months attending the selected healthcare facilities for a febrile episode or clinical suspicion of malaria.

Inclusion Criteria

The following were included:

- children aged 0–59 months;
- presenting with a temperature ≥ 37.5 °C or a recent history of fever;
- whose parents or guardians had provided written informed consent.

Exclusion Criteria

The following were excluded:

- children who had received antimalarial treatment within the two weeks preceding inclusion;
- children with insufficient blood sample volume;
- children with incomplete biological data.

Sample Size

The minimum sample size was calculated according to Schwartz's formula for prevalence studies:

$n = Z^2 \times p\,(1-p) / d^2$

Considering an expected prevalence of 50%, a precision of 5%, and an alpha risk of 5%, the minimum required sample size was estimated at 384 children. After increasing this value by 10% to account for missing data, the minimum retained sample size was 422 subjects.
A total of 417 children were included.

Data Collection

Sociodemographic, clinical, and anthropometric data were collected using a structured pretested questionnaire. A capillary blood sample was obtained from each child for RDT, microscopy, and PCR analyses.

Biological Analyses

Rapid Diagnostic Tests

The SD Bioline Malaria Ag Pf® (Abbott) test was used according to the manufacturer's recommendations.

Microscopy

Thick and thin blood smears were prepared and stained with 10% Giemsa. Slides were independently read by two experienced microscopists. In cases of discordance, a third reading was performed.

PCR

PCR was considered the reference method for evaluating diagnostic performance. Parasite DNA was extracted from dried blood spots collected on Whatman 903 filter paper.

A PCR targeting the mitochondrial **cox3** gene was performed, followed by a nested PCR allowing identification of *Plasmodium* species.

Statistical Analysis

Data were analyzed using R software version 4.3.1.

Qualitative variables were expressed as proportions with their 95% confidence intervals.

The diagnostic performance of RDTs and microscopy was evaluated through calculation of sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and Cohen's Kappa coefficient.

Missing data were mainly related to insufficient biological samples or technical difficulties during molecular analyses.
The threshold for statistical significance was set at $p < 0.05$.

Ethical Considerations

This study was conducted according to the ethical principles of the Declaration of Helsinki concerning research involving human subjects.

Approval was obtained from the Ethics Committee of the University of Kisangani (Ref.:

UNIKIS/CE/KGB/004/07/2025). Written informed consent was obtained from parents or legal guardians before inclusion of the children.

RESULTS

Table 1: Sociodemographic and Anthropometric Characteristics

Characteristics	n = 417[1]	95% CI
Sex		
Female	202 (48%)	44%, 53%
Male	215 (52%)	47%, 56%
Age of child (months)	16 (9,36)	21,24
Child weight (kg)	10.0 (8.0,13.0)	10,11
Height (cm)	76 (68,89)	77,81
Mid-upper arm circumference (mm)	130 (122,138)	130,133
Nutritional status ≥6 months		
SAM (MUAC <115 mm)	36 (9.8%)	7.1%,13%
MAM (MUAC 115–124 mm)	68 (19%)	15%,23%
Normal (≥125 mm)	263 (72%)	67%,76%
Area of residence		
Makiso	212 (51%)	
Kabondo	67 (16%)	
Tshopo	54 (13%)	
Kisangani	29 (7.0%)	
Lubunga	26 (6.2%)	
Mangobo	23 (5.5%)	
Peripheral area	6 (1.4%)	

[1] n (%); Median (Q1, Q3)
Abbreviation: CI = Confidence Interval

The study included 417 children under five years of age. Sex distribution showed a slight male predominance, with 215 boys representing 52% (95% CI: 46–56%) and 202 girls representing 48% (95% CI: 43–53%).

The median age of children was 16 months (IQR: 9–36). Regarding anthropometric characteristics, the median weight was 10.0 kg (8.0–13.0), while the median height was 76 cm (68–89).

Among children older than six months, a substantial proportion presented acute malnutrition: 9.8% (n=36) suffered from severe acute malnutrition (MUAC <115 mm), and 19% (n=68) had moderate acute malnutrition (MUAC between 115 and 124 mm), whereas 72% (n=263) had normal nutritional status (MUAC ≥125 mm).

Regarding geographical distribution, the majority of children originated from Makiso municipality (51%), followed by Kabondo municipality (16%).

Prevalence of Malaria According to Four Diagnostic Approaches

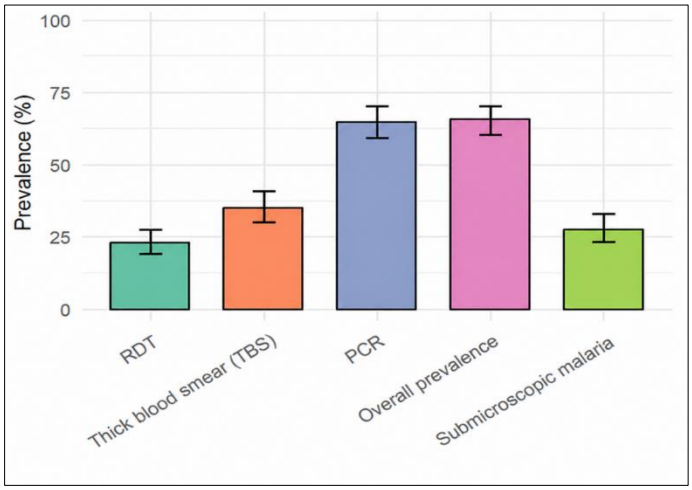

Figure 1: Prevalence of malaria according to four diagnostic approaches

Figure 1 highlights substantial differences in malaria prevalence according to the diagnostic methods used.

Overall prevalence, defined by positivity of at least one test, was estimated at 64.5% (95% CI: 59.7–69.1), reflecting a high malaria burden in the study population.

A marked variation was observed according to diagnostic techniques. PCR showed the highest prevalence at 64.5% (95% CI: 59.7–69.1), followed by thick smear microscopy at 34.8% (95% CI: 30.2–39.6), whereas RDT displayed the lowest prevalence at 22.8% (95% CI: 18.9–27.2).
Furthermore, the proportion of submicroscopic malaria was estimated at 27.8% (95% CI: 23.4–32.7).

PCR identified five additional malaria cases among children younger than one month compared with thick smear microscopy, which detected only one case, and six additional cases compared with RDT, which detected none.

Table 2: Plasmodium Species According to Diagnostic Tests (Smear and PCR)

Species	Smear	PCR
Plasmodium falciparum	129 (89%)	185 (75.8%)
Plasmodium malariae	11 (7.6%)	30 (12.3%)
Plasmodium ovale	5 (3.4%)	15 (6.1%)
Mixed infections	0%	5.8%

The most represented *Plasmodium* species was *Plasmodium falciparum* (89% by smear and 75.8% by PCR), followed by *Plasmodium malariae* (7.6% by smear and 12.3% by PCR).

PCR made it possible to identify mixed infections that were not detected by blood smear microscopy.

Table 3: Concordance Between RDT and PCR

Diagnostic test	PCR (+)		PCR (-)		Kappa [IC 95%]	Accuracy [IC 95%]	P
	n	%	n	%			
RDT (+)	74	30,3	1	0,7	23	55,2	
RDT (-)	170	69,7	136	93,3	[16,8-29,9]	[50,1-60,1]	<0,001
Total	244	100,0	137	100,0			

The analysis included 381 complete observations after exclusion of 8.6% missing PCR data. Missing values were mainly related to insufficient biological samples or technical problems during molecular analyses.

RDT showed weak concordance with PCR (κ = 0.233; 95% CI: 0.168–0.299; $p < 0.001$) and an overall accuracy of 55.2% (95% CI: 50.1–60.1). According to the Landis and Koch classification, this value corresponds to weak agreement.

Table 4: Concordance Between Thick Smear Microscopy and PCR

Diagnostic test	PCR (+)	%	PCR (-)	%	Kappa [IC 95%]	Accuracy [IC 95%]	P
	n	%	n	%			
Thick smeat (+)	125	51,2	0	0,7	43,0	62,8	
(-)	119	48,8	137	93,3	[34,8-51,3]	[58,1-67,3]	<0,001
Total	244	100,0	137	100,0			

The analysis included 381 complete observations after exclusion of 8.6% missing PCR data. Missing values were mainly related to insufficient biological samples or technical problems during molecular analyses.

Thick smear microscopy showed moderate concordance with PCR (κ = 43.0; 95% CI: 34.8–51.3; $p < 0.001$) and an overall accuracy of 62.8% (95% CI: 58.1–67.3).

Key Performance Parameters of RDT and Thick Smear Microscopy

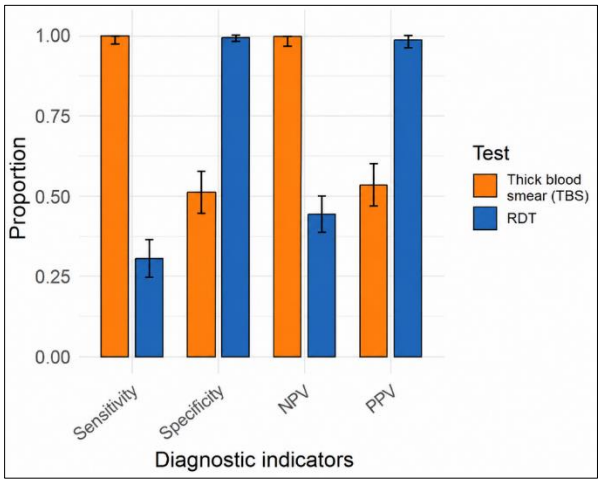

Figure 2: Key performance parameters of RDT and thick smear microscopy

Using PCR as the reference method, RDT showed a sensitivity of 30.3% [24.5–36.1], specificity of 99.3% [98.0–100], PPV of 98.7% [96.1–100], NPV of 44.4% [38.8–50.0], FPR of 0.7% [0–2.0], and FNR of 69.7% [63.9–75.5].

Thick smear microscopy showed a sensitivity of 100.0% [97.3–], specificity of 51.2% [44.7–57.6], PPV of 53.5% [47.0–60.0], and NPV of 97.4% [94.8–100.0].

ROC Curve of RDT Compared with PCR

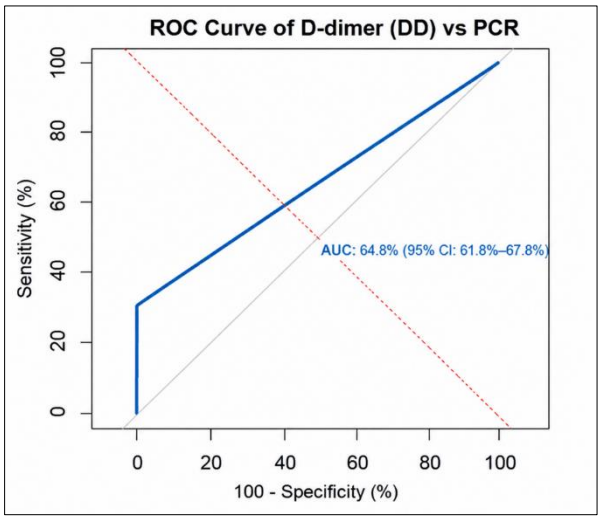

Figure 3: ROC curve of RDT compared with PCR

The ROC curve of RDT compared with PCR showed an area under the curve of 64.8% (95% CI: 61.8%–67.8%), with a curve close to the diagonal.

DISCUSSION

Our study highlights substantial variability in malaria prevalence according to the diagnostic methods used. PCR identified a significantly higher prevalence compared with rapid diagnostic tests (RDTs) and microscopy, confirming its superior diagnostic performance for detecting low-density parasitic infections [14–18].

The high prevalence observed in our study reflects the intense malaria transmission occurring in Kisangani, a humid equatorial region favorable to the proliferation of *Anopheles* vectors [1–3]. These findings are consistent with the World Malaria Report and several studies conducted across sub-Saharan Africa, indicating that the Democratic Republic of the Congo remains one of the countries bearing the greatest malaria burden worldwide [1,2,19].

The high proportion of submicroscopic infections observed in our study constitutes a particularly important epidemiological finding. These infections represent a silent parasite reservoir capable of sustaining malaria transmission despite ongoing control strategies [10,13,20]. In high-transmission settings, repeated exposure to parasites contributes to the gradual

acquisition of semi-protective immunity, allowing infected individuals to maintain low-density parasitemia that often remains undetectable by conventional diagnostic methods [20–22].

Similar findings were reported in Cameroon by Kojom Foko *et al.,* who demonstrated a high frequency of submicroscopic infections in areas with intense malaria transmission [10]. Likewise, Fila-Fila *et al.,* in the Republic of Congo reported that molecular methods identified a substantial number of infections missed by microscopy [9]. In Tanzania, Budodo *et al.,* also demonstrated significantly better performance of qPCR compared with RDTs and microscopy in hyperendemic villages [14].

This situation raises important implications for malaria control and elimination programs. Undiagnosed submicroscopic infections likely constitute a hidden reservoir maintaining parasite circulation despite vector-control interventions and the widespread use of artemisinin-based combination therapies [23–25].

The poor sensitivity of RDTs observed in our study may be explained by several mechanisms. First, low parasite densities associated with submicroscopic infections reduce the quantity of detectable HRP2 antigen available for rapid tests [7,8,16]. Second, mixed infections and non-*falciparum* species may compromise the performance of RDTs specifically designed to detect *Plasmodium falciparum* [12,15]. Finally, several recent African studies have identified deletions of the *pfhrp2/pfhrp3* genes, leading to false-negative results despite the presence of parasites [17,18,26].

Agaba et al., in a systematic review conducted in Africa, reported an increasing prevalence of *pfhrp2/pfhrp3* gene deletions across several regions of the continent [17]. In Ethiopia, Alemayehu *et al.,* found a high prevalence of these deletions significantly compromising HRP2-based RDT performance [18]. Similar findings have also been reported in Sudan, Eritrea, and Rwanda [27–29].

Our findings are also in agreement with those reported by Osun *et al.,* in Nigeria, who observed significantly lower sensitivity of RDTs compared with PCR among children living in areas of intense malaria transmission [7]. Ahmad *et al.,* in India similarly reported limited RDT performance in highly endemic regions [12]. Furthermore, a recent Ethiopian meta-analysis by Feleke *et al.,* confirmed the superior diagnostic performance of molecular approaches compared with conventional techniques [16].

Microscopy demonstrated better diagnostic performance than RDTs but remained inferior to PCR in detecting submicroscopic infections. This finding is consistent with WHO reports indicating that microscopy performance strongly depends on parasite density, staining quality, and the expertise of microscopists [5,6]. In resource-limited settings, deficiencies in laboratory quality-control procedures may substantially reduce diagnostic sensitivity [9,30].

The poor agreement observed between RDTs and PCR in our study further confirms the limitations of rapid diagnostic tests in hyperendemic settings. According to the Landis and Koch classification, the observed Kappa coefficient corresponds to weak agreement, reflecting substantial diagnostic discordance between the two methods [31].

The predominance of *Plasmodium falciparum* observed in our study is consistent with epidemiological patterns reported throughout Africa [1,2]. However, PCR additionally identified *Plasmodium malariae*, *Plasmodium ovale*, and several mixed infections that were not detected by microscopy [19,32].

These findings emphasize the value of molecular approaches in identifying non-*falciparum* species that are frequently underestimated in highly endemic settings [10,15,32]. Recent studies suggest that non-*falciparum* species may contribute more substantially than previously believed to malaria transmission in Central Africa [32–34].

The identification of mixed infections carries major clinical and epidemiological significance. Certain *P. malariae* and *P. ovale* infections may be associated with chronic anemia, recurrent disease episodes, and prolonged complications [35]. Underestimation of these infections may therefore influence treatment quality and surveillance strategies.

The analysis of our ROC curve showed an area under the curve (AUC) of 64.8% (95% CI: 61.8–67.8), with a curve close to the diagonal line, indicating a low discriminative ability of the rapid diagnostic test (RDT) compared with PCR. Indeed, an AUC between 0.6 and 0.7 reflects poor to acceptable diagnostic performance, which remains far below the values expected for a reliable test (>0.8). The fact that the curve is close to the diagonal suggests that the RDT has a limited ability to correctly distinguish infected from non-infected subjects when compared with PCR, considered the molecular reference method in our study.

These findings are consistent with those reported in a meta-analysis conducted in Nigeria among children, which showed a low RDT AUC close to 0.5–0.6, thereby confirming the limited performance of RDTs. Recent studies conducted in Africa have also reported similar AUC values (0.70–0.85), confirming the moderate performance of RDTs. [16]

The poor performance of the RDT observed in our study may be explained by the inability of the RDTs used to detect low-parasitemia infections, which are very

common among children under 59 months of age in endemic areas such as the city of Kisangani and often remain below the detection threshold of RDTs. In addition, the RDTs used in our study targeted only *Plasmodium falciparum*, thereby failing to detect mixed and non-*falciparum* infections. Furthermore, the presence of *P. falciparum* parasites carrying HRP2/3 gene deletions may also have contributed to false-negative results, although the extent of these deletions was not determined in the present study. Moreover, a prozone effect related to an excess of HRP2 antibodies or antigens may also contribute to these false-negative results. [17]

The public health implications of our findings are considerable. The high frequency of submicroscopic infections suggests that the true malaria burden may be significantly underestimated in surveillance systems relying exclusively on RDTs and microscopy [20,24]. Such underestimation may limit the effectiveness of national control programs and complicate malaria elimination strategies [23,24,36].

Strengths and Limitations of the Study

This study has several important methodological strengths, including its multicenter design, prospective approach, and the integration of molecular methods enabling highly sensitive detection of malaria infections.

In addition, the relatively large sample size and the simultaneous use of rapid diagnostic tests, microscopy, and PCR provide a comprehensive comparative assessment of malaria diagnostic performance in a high-transmission setting.

However, several limitations should be considered.

First, PCR remains a costly method that is difficult to implement routinely in resource-limited settings.

Second, this study did not assess *pfhrp2/pfhrp3* gene deletions, which may significantly influence the performance of HRP2-based rapid diagnostic tests.

Third, the hospital-based design may limit the generalizability of findings to the broader pediatric population of Kisangani.

Finally, the absence of precise molecular quantification of parasite density represents another important methodological limitation.

CONCLUSION

This study demonstrates a high prevalence of malaria among children under five years of age in Kisangani, together with a substantial proportion of submicroscopic infections undetected by conventional diagnostic methods.

PCR proved significantly more sensitive than microscopy and rapid diagnostic tests for the detection of malaria infections, particularly low-density infections and mixed-species infections.

The limited performance of rapid diagnostic tests observed in our study highlights the need for regular monitoring of their diagnostic accuracy in high-transmission settings.

In the context of the Democratic Republic of the Congo, PCR could represent an important complementary tool for epidemiological surveillance, evaluation of conventional diagnostic approaches, and optimization of malaria control strategies.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Authors' Contributions

All authors contributed to study conception, data collection, analysis of findings, and manuscript preparation. All authors read and approved the final version of the manuscript.

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