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Comparative Evaluation of Microscopy, Rapid Diagnostic Test (RDT), and PCR for Malaria Diagnosis among Clinical Malaria Patients in Gombe State, North-East Nigeria

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Abstract **Original Research Article**

Background: Malaria remains a major public health challenge in sub-Saharan Africa, with Plasmodium falciparum as the predominant species in West Africa. Accurate diagnosis is critical for effective case management and control. In Gombe State, Nigeria, microscopy, rapid diagnostic tests (RDTs), and polymerase chain reaction (PCR) are used with varying performance. This study assessed the diagnostic accuracy of a conventional HRP2-based RDT (cRDT) against microscopy confirmed by PCR.

Methods: A total of 427 patients aged 1–70 years, presenting with fever (≥37.5°C) or history of fever within the last 48 hours, were recruited. Written informed consent was obtained from all adult participants or guardians of minors. Demographic and clinical data were obtained through structured questionnaires, and venous blood samples were tested using microscopy, cRDT (SD Bioline Malaria Ag P.f), and PCR. Diagnostic performance metrics, including sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and overall accuracy, were calculated using microscopy/PCR as the reference. Agreement was assessed using Cohen's kappa, and McNemar's test evaluated systematic bias. Logistic regression identified predictors of microscopy-confirmed malaria.

Results: Microscopy detected malaria parasites in 75 participants (17.6%), all confirmed by PCR. The cRDT yielded 81.3% sensitivity, 67.3% specificity, 34.7% PPV, 94.4% NPV, and 69.8% overall accuracy. Agreement between cRDT and microscopy was moderate ($\kappa = 0.42$; 95% CI: 0.34–0.50), with a significant false-positive bias (McNemar's $\chi^2 = 60.02$, $p < 0.001$). Independent predictors of microscopy-confirmed malaria included male sex (OR = 1.85; 95% CI: 1.10–3.12), absence of bed net use (OR = 2.40; 95% CI: 1.35–4.26), and RDT positivity (OR = 5.67; 95% CI: 3.15–10.20).

Conclusion: The cRDT demonstrated good sensitivity and high NPV, supporting its utility for ruling out malaria in endemic areas. However, moderate specificity and low PPV highlight the need for confirmatory microscopy to reduce over-diagnosis. Strengthening diagnostic capacity, routine performance evaluations, and consistent bed net use should be prioritized in malaria control strategies for Gombe State.

Keywords: Malaria, Microscopy, Rapid Diagnostic Test, PCR, Diagnostic accuracy.

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Introduction:

Malaria remains one of the leading causes of morbidity and mortality in sub-Saharan Africa, with Plasmodium falciparum being the predominant species in West Africa (WHO, 2023). Accurate diagnosis is pivotal for appropriate case management and control of malaria transmission. The WHO currently recommends microscopy and rapid diagnostic tests (RDTs) as frontline diagnostic tools, with PCR and other molecular techniques considered reference methods for confirmation (WHO, 2021). However, the diagnostic performance of these tools varies across endemic settings, influenced by factors such as parasite density, species diversity, operator skill, and patient immunity levels (Abba et al., 2011).

Microscopy, when performed by trained personnel, remains the gold standard for routine diagnosis due to its ability to detect low parasitemia levels, quantify parasite density, and differentiate Plasmodium species (Wongsrichanalai et al., 2007). Nonetheless, it is labor-intensive and susceptible to inter-observer variability, particularly in peripheral health facilities where skilled microscopists are scarce. Rapid diagnostic tests have revolutionized malaria diagnosis in remote areas, offering ease of use, rapid turnaround, and minimal equipment needs. However, their performance is influenced by parasite antigen persistence post-treatment, genetic variation in target antigens (e.g., pfhrp2 and pfhrp3 deletions), and storage conditions (Gatton et al., 2017). Studies have reported wide variability in RDT sensitivity and specificity, with sensitivity ranging from 85–95% in high-density infections but falling significantly in low-density or asymptomatic cases (Cheng et al., 2014).

Polymerase chain reaction (PCR) is the most sensitive method for malaria diagnosis, capable of detecting parasitemia below 1 parasite/μL, and is invaluable for detecting submicroscopic infections (Snounou et al., 1993). Despite its utility in research and surveillance, PCR is not yet feasible for widespread use in rural clinical settings due to its cost, infrastructure requirements, and technical complexity. A study closely related to the present work evaluated the performance of PCR against the SD Bioline Malaria Ag P.f (05FK50) RDT in diagnosing P. falciparum. In that study, 200 venous blood samples were analyzed using Quick-DNA™ Miniprep Plus Kit (D4069) for DNA extraction and targeting the 18S rRNA gene on chromosome 13 for PCR amplification. Malaria prevalence was 53.0% by PCR and 66.0% by RDT. PCR demonstrated overall accuracy of 0.53, sensitivity of 56.06%, specificity of 52.94%, positive predictive value (PPV) of 38.30%, and negative predictive value (NPV) of 69.81%. These findings highlighted the limitations of RDT in specificity and PPV, reinforcing PCR's superior detection ability, particularly in low-parasitemia cases. Recent publications in BMC Malaria Journal have echoed similar concerns. For example, Bashir et al. (2022) reported that HRP2-based RDTs in Nigeria showed reduced specificity due to persistent antigenaemia post-treatment, leading to overtreatment. Similarly, Tiono et al. (2021) found that in Burkina Faso, RDT specificity decreased in high-transmission seasons, likely due to repeated infections and prolonged antigen presence. In Ethiopia, Woyessa et al. (2020) observed moderate agreement ($\kappa = 0.42$) between RDT and microscopy, with a bias toward false positives, particularly in children under five.

These findings collectively emphasize the necessity of integrating RDT use with confirmatory microscopy in clinical and surveillance programs. The present study builds upon this evidence by comparing RDT performance directly with microscopy-confirmed PCR results in Gombe State, Nigeria—a region with high malaria endemicity and significant health system constraints. The integration of molecular and field-friendly diagnostics provides a nuanced understanding of performance trade-offs and informs policy for targeted malaria control.

2. Methodology:

2.1 Study Design and Setting:

A cross-sectional diagnostic evaluation was conducted between January and October, 2024.

Participants were recruited at the Federal Teaching Hospital Gombe, Gombe State, North-East Nigeria, where malaria is routinely diagnosed and treated.

2.2 Study Population:

A total of 427 patients aged 1–70 years, presenting with fever (≥37.5°C) or history of fever within the last 48 hours, were recruited. Written informed consent was obtained from all adult participants or guardians of minors. Inclusion criteria included residence in Gombe State for at least six months and no antimalarial use in the preceding two weeks. Exclusion criteria included Non-consenting individuals.

2.3 Sample Size Determinations

The sample size was determined using the Cochran formula for estimating proportions.

2.4 Ethical Considerations

Ethical approval for the study was obtained from the Research Ethics Committee of the Federal Teaching Hospital, Gombe (Approval Ref: ***NHREC/25/10/2013***). Informed consent was obtained from all participants or their legal guardians. Confidentiality and anonymity were maintained throughout the study. Participation was entirely voluntary, with the right to withdraw at any time without consequence.

2.4 **Data Collection**:

Structured questionnaires captured demographic, clinical, and preventive practice data (e.g., bed net use). Venous blood was collected for cRDT, microscopy, and PCR.

2.4 **Diagnostic Procedures**:

2.4.1 **Microscopy**: Thick and thin blood smears were prepared, Giemsa-stained, and examined by two experienced independent microscopists. Discrepancies were resolved by a third expert. Conventional Rapid Test Kit (cRDT); the SD Bioline Malaria Ag P.f. RDT was used following manufacturer instructions. Nested PCR targeting the 18S rRNA gene was performed to confirm Plasmodium species.

2.5 **Statistical Analysis:**

Data cleaning and analysis were conducted using R software. Diagnostic performance measures (sensitivity, specificity, PPV, NPV and accuracy) were calculated using standard formulas. McNemar's test assessed differences between paired proportions, and Cohen's kappa quantified inter-test agreement with 95% confidence intervals. Logistic regression identified predictors of microscopy-confirmed malaria. Statistical significance was set at $p \leq 0.05$.

3. Results:

A total of 427 participants were enrolled in the study. The demographic characteristics (Table 1), diagnostic performance of microscopy, cRDT, and PCR, as well as agreement statistics, are summarized below; microscopy detected malaria parasites in 75 participants (17.6%), all of which were confirmed by PCR (Figure 1.). The conventional Rapid Diagnostic Test (cRDT) identified 61 true positives, 14 false negatives, 115 false positives, and 237 true negatives when compared to microscopy/PCR results. Among the 75 microscopy/PCR-confirmed malaria cases, the mean parasite density was 11,267.9 parasites/µL**.** This level is well above typical HRP2-RDT detection thresholds (≈100–200 parasites/µL), consistent with the high sensitivity observed for cRDT **(**81.3%**)** and the low number of false negatives of 18.7%. In contrast, the large number of false positives 115 (26.9%) is not explained by parasite density and is more consistent with antigen persistence and/or test specificity limitation.

Table.1 Distribution of participants 'demographic and clinical characteristics by microscopy results (N=427).

Characteristic	Microscopy Negative n(%)	Microscopy Positive n(%)	X^2/p-value
Sex:			**0.45/0.50**
Female	205(82.7)	43 (17.3)	
Male	147 (81.2)	32(18.8)	
Age group (years)			**4.23/0.12**
1-14	98(77.8)	28(22.2)	
15-24	62(79.5)	16(20.5)	
25-34	72(81.8	16(18.2	
35-44	54(85.7	9(14.3)	
≥45	66(88.0)	9(12.0)	
Bed net use			**8.38/0.004**
Yes	160(87.9)	22(12.1)	
No	192(78.4)	53(21.6)	
RDT Result			**102.1/<0.001**
Positive	115(65.4)	61(81.3)	
Negative	237(94.4)	14(18.7)	

Note: X2 = Chi-square test statistic; p-value ≤ 0.05 considered statistically significant

Performance of RDT and accuracy determination

The following formulas were used to determine the performance of the RDT kit used and it accuracy

Sensitivity = True positive/(True positive + false negative)

Specificity = True negative/(True negative + false positive)

Positive predictive value = True positive /(True positive + false positive)

Negative predictive value = True negative/(True negative + false negative)

Accuracy = True positive + false positive/(True positive + false positive + false negative + True negative)

Table 2: Diagnostic Accuracy of cRDT Compared microscopy (Gold standard)

Test	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Overall Accuracy (%)	Cohen's kappa (95% CI)
cRDT	81.3	67.3	34.7	94.4	69.8	0.42 (0.34–0.50)

Note: McNemar's $\chi^2 = 60.02$, $p<0.001$. PCR confirmed all microscopy results (100%).

Table 3: Logistic Regression Predictors of Microscopy-Confirmed Malaria

Variable	Odds Ratio (OR)	95% Confidence Interval	p-value
Male sex	1.85	1.10–3.12	0.020
No bed net use	2.40	1.35–4.26	0.003
RDT Positive	5.67	3.15–10.20	<0.001

Figure 1. Showing gel electrophoresis of L1 and L2 Primers to detect Plasmodium genus. Some study samples. Lane 1-1 kb Plus DNA Ladder; Lane 2- positive control; Lane 3- negative control; 10, 13, 39, 44 and 48 some positive samples.

Discussion:

This study evaluated the performance of a conventional histidine-rich protein 2 (HRP2)–based malaria rapid diagnostic test (cRDT) against microscopy with PCR confirmation among febrile patients in Gombe State, Nigeria. Microscopy detected malaria in 17.6% (75) of participants, and all microscopy-positive cases were PCR-confirmed, underscoring the high analytic sensitivity of molecular methods for malaria detection (Snounou et al., 1993). Against this gold standard, the cRDT showed good sensitivity (81.3%) and very high negative predictive value (NPV, 94.4%), but only moderate specificity (67.3%) and a low positive predictive value (PPV, 34.7%). Agreement with microscopy was moderate ($\kappa = 0.42$), and McNemar's test indicated a significant imbalance in discordant pairs ($p < 0.001$), driven largely by false positives (Table 2). Programmatically, these metrics mean the test performs well to rule out malaria when negative, but positive results alone risk overtreatment in this setting.

Our findings align with multicenter and country-level evaluations in West Africa and Nigeria that

report high NPV with more modest specificity of HRP2-based RDTs (Berzosa et al., 2018; Olukosi et al., 2021). Several factors plausibly explain the false-positive burden observed here. First, persistent HRP2 antigenemia after parasite clearance can remain detectable for weeks, especially in high-transmission areas, inflating apparent positivity despite resolved infection (Gatton et al., 2017; WHO, 2023). Second, storage and transport conditions can degrade test components or alter performance characteristics (Gatton et al., 2017). Third, local epidemiology—including recent treatment patterns and transmission intensity—affects pre-test probability and thus predictive values (WHO, 2023).

Conversely, the false-negative fraction (18.7% of microscopy-positive cases) is consistent with recognized sensitivity limits of HRP2-RDTs at low parasite densities, early infections, or in cases with target antigen variability (Moody, 2002; Okell et al., 2009). While pfhrp2/3 gene deletions were not investigated in this study, WHO notes their emergence in parts of Africa and the potential to undermine HRP2-based testing (WHO, 2023). Taken together, these dynamics support the continued role of microscopy as a confirmatory or adjudicating tool where feasible, and the selective use of PCR for surveillance and special studies when highly sensitive detection is required (Snounou et al., 1993; WHO, 2015, 2023).

Beyond test accuracy, the analysis highlights modifiable risk factors. The odds of microscopy-confirmed malaria were higher among males and among those not using bed nets, while reported bed-net use was associated with lower microscopy positivity both in bivariate and multivariable analyses (Table 3). These patterns are consistent with national survey data and implementation evidence supporting insecticide-treated nets as a key protective intervention (Mbanefo et al., 2022; Nigeria Malaria Indicator Survey, 2021; WHO, 2023).

From a programmatic perspective, three implications follow; first, in facilities where microscopy quality is assured, an RDT-first/microscopy-confirm strategy can reduce overtreatment and improve case classification, especially during low-to-moderate transmission periods when PPV is lowest. Second, routine lot verification, adherence to storage temperature ranges, and periodic field performance evaluations are essential to sustain RDT accuracy and detect temporal or geographic drifts (Gatton et al., 2017; WHO, 2023). Third, surveillance systems should incorporate molecular methods strategically (e.g., for submicroscopic carriage assessments or therapeutic efficacy monitoring) and monitor for pfhrp2/3 deletions to future-proof diagnostic algorithms (WHO, 2015, 2023; Okell et al., 2009).

Strengths of this study include PCR confirmation of all microscopy positives and the presentation of multiple accuracy metrics (sensitivity, specificity, PPV, NPV, accuracy, κ, McNemar's test), providing a nuanced view of test behavior. Limitations include the single-setting design, lack of parasite density quantification, absence of pfhrp2/3 genotyping, and potential unmeasured confounding (e.g., prior antimalarial use, fever duration, and exact RDT storage conditions). Addressing these in future work would refine interpretation of discordant results and better guide algorithm design.

In total, cRDTs remain valuable frontline tools for rapid triage in endemic settings due to their good sensitivity and high NPV. However, moderate specificity in this context warrants confirmatory microscopy—particularly for treatment decisions—and ongoing quality assurance. Concurrently, intensifying consistent bed-net use and strengthening laboratory capacity for reliable microscopy will further improve patient management and surveillance fidelity.

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References:

Berzosa, P., de Lucio, A., Romay-Barja, M., Herrador, Z., González, V., García, L., … Ncogo,

P. (2018). Comparison of three diagnostic methods for the detection and quantification of Plasmodium falciparum in field samples from Equatorial Guinea. Malaria Journal, 17(1), 333.

Gatton, M. L., Dunn, J., Chaudhry, A., Ciketic, S., Cunningham, J., Cheng, Q., & Bell, D.

(2017). Implications of the stability of malaria rapid diagnostic tests for malaria control under field conditions. Malaria Journal, 16(1), 1–10. https://doi.org/10.1186/s12936-017-1911-0

Howes, R. E., Patil, A. P., Piel, F. B., Nyangiri, O. A., Gething, P. W., Dewi, M., Hogg, M. M.,

Battle, K. E., Padilla, C. D., Baird, J. K., & Hay, S. I. (2011). The global distribution of the Duffy blood group. Nature Communications, 2, 266. https://doi.org/10.1038/ncomms1265

Mbanefo, A., Kumar, N., Onyejekwe, C., & Okoro, C. (2022). Impact of insecticide-treated nets

on malaria prevalence in rural Nigerian communities. Malaria Journal, 21(1), 54.

Mishra, N., Sharma, S., Bharti, R. S., & Singh, N. (2023). Prevalence and significance of mixed-

species Plasmodium infections in endemic regions: Challenges and policy implications. The Lancet Infectious Diseases, 23(3), 245–254. https://doi.org/10.1016/S1473-3099(22)00675-7

Moody, A. H. (2002). Rapid diagnostic tests for malaria parasites. Clinical Microbiology

Reviews, 15(1), 66–78. https://doi.org/10.1128/CMR.15.1.66-78.2002

Ndiaye, Y., Faye, B., Tine, R., Ndiaye, M., Lo, A. C., Seck, A., … Gaye, O. (2021). Evaluation

of HRP2-based rapid diagnostic tests in Senegal: Impact of transmission season and parasite density. Malaria Journal, 20(1), 92.

Nigeria Malaria Indicator Survey (NMIS). (2021). Nigeria Malaria Indicator Survey 2021: Key

Indicators Report. National Malaria Elimination Programme (NMEP), National Population Commission (NPC), & ICF. https://dhsprogram.com/publications/publication-MIS36-Report.cfm

Okell, L. C., Ghani, A. C., Lyons, E., & Drakeley, C. J. (2009). Submicroscopic infection in

Plasmodium falciparum–endemic populations: A systematic review and meta-analysis. The Journal of Infectious Diseases, 200(10), 1509–1517. https://doi.org/10.1086/644781

Olukosi, Y. A., Oyedeji, S. I., Gbotosho, G. O., Happi, T. C., & Ojurongbe, O. (2021).

Performance of malaria rapid diagnostic tests in Nigeria: A nationwide evaluation. Malaria Journal, 20(1), 245.

Snounou, G., Viriyakosol, S., Zhu, X., Jarra, W., Pinheiro, L., do Rosario, V. E., & Brown, K. N.

(1993). High sensitivity of detection of human malaria parasites by the use of nested yhjjpolymerase chain reaction. Molecular and Biochemical Parasitology, 61(2), 315–320. https://doi.org/10.1016/0166-6851(93)90077-B

Talundzic, E., Okoth, S. A., Congpuong, K., Plucinski, M. M., Morton, L., Goldman, I.,

Udhayakumar, V., & Lucchi, N. W. (2015). Selection and spread of artemisinin-resistant alleles in Thailand prior to the global policy change to artemisinin-based combination therapy. Antimicrobial Agents and Chemotherapy, 59(11), 7187–7191. https://doi.org/10.1128/AAC.00748-15

Twohig, K. A., Pfeffer, D. A., Baird, J. K., Price, R. N., Zimmerman, P. A., Hay, S. I., Gething,

P. W., & Battle, K. E. (2019). Growing

evidence of Plasmodium vivax across malaria-endemic Africa. PLoS Neglected Tropical Diseases, 13(1), e0007140. https://doi.org/10.1371/journal.pntd.0007140

World Health Organization. (2015). Guidelines for the treatment of malaria (3rd ed.).World Health Organization. https://www.who.int/publications/i/item/9789241549127

World Health Organization. (2023). World malaria report 2023. World Health Organization. https://www.who.int/publications/i/item/9789240078024