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RESEARCH **Open Access**

Comparative Performance of Four Rapid Diagnostic Tests Kits, Microscopy and PCR in Asymptomatic Malaria Detection among Children from Ilishan-Remo Community, Southwest Nigeria

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ABSTRACT

Background: Active malaria parasite detection is essential for effective malaria control and elimination in endemic settings. Asymptomatic malaria is often not detected and serves as a parasite reservoir for continuous malaria transmission. This study evaluated the comparative performance of four RDT kit brands, microscopy, and PCR to detect asymptomatic infection among children from the Ilishan-Remo community in Ogun state, southwest Nigeria.

Methods: A cross-sectional study design was carried out to actively detect asymptomatic parasitaemia in children aged 3 months to 10 years during the dry season (November and December, 2022). Finger prick blood samples were collected for rapid diagnostic tests using SD-BiolineTM, Micro-pointTM, CarestartTM, and First-ResponseTM. Thick blood smears were prepared for microscopy, and dried blood spots for PCR analysis. The performance of the diagnostic methods was compared.

Results: Of the 163 samples analysed, parasite positive rate as detected by PCR was 34 (20.9%), microscopy 38 (23.3%), SD-Bioline^{TM} 19 (11.7%), Micro-point^{TM} 20 (12.3%), CareStart^{TM} 25 (15.3%), and First-Response^{TM} 23 (14.1%) respectively. The Sensitivity was 45.5%, 50.0%, 56.2%, 51.5%, and 70.0% for SD-Bioline^{TM}, Micro-point^{TM}, CareStart^{TM}, First-Response^{TM}, and microscopy ($p = 0.000$), while specificity was 96.9%, 97.7%, 94.4%, 95.2% and 91.9% ($p = 0.116$) respectively. There was a significant statistical difference in the PPV ($p = 0.037$), while the NPV, accuracy, and kappa (k) were statistically not significant.

Conclusion: Microscopy has shown a higher sensitivity and positive predictive value than rapid RDTs in detecting asymptomatic parasitaemia compared with PCR. This underscores the need for developing ultra-sensitive, cost-effective, and point-of-care diagnostic tools. Such tools could significantly enhance the control and elimination of malaria by actively detecting asymptomatic infection and reducing the parasite reservoir and malaria burden in Nigeria.

Keywords: Asymptomatic malaria, Sensitivity, RDTs, Microscopy, PCR, Nigeria

1.0 INTRODUCTION

Malaria remains a major public health concern, particularly in sub–Saharan Africa, where over 90% of the global malaria burden occurs [1]. According to the 2022 World Malaria Report, Nigeria contributed the highest burden of global malaria, accounting for about 27% morbidity and 31% mortality rates [2]. In the WHO Africa region, epidemiology statistics also indicated that Nigeria contributes about 55% of malaria cases in West Africa [1]. Malaria transmission is all year-round in Nigeria, with seasonal variation between the southern and northern regions [3, 4]. *Plasmodium falciparum* is the predominant species, accounting for over 90% of all cases in Nigeria [5].

To scale up malaria intervention programs, the National Malaria Elimination Program (NMEP) initiated the High Burden High Impact (HBHI) approach to reduce the malaria burden in Nigeria to less than 50 deaths per 1,000 by 2025 [6]. However, progress towards achieving this projection is challenging with the burden of asymptomatic malaria and other associated factors, including weak health systems, the spread of drug-resistant parasites, and high levels of illiteracy [7–9]. Furthermore, asymptomatic malaria constitutes a hidden parasite reservoir, thus contributing to persistent malaria transmission in areas with high asymptomatic malaria burden [10, 11].

Malaria control and elimination programs require highly sensitive and cost-effective point-of-care tools [12, 13]. However, parasite-based diagnosis of malaria, particularly among asymptomatic carriers, poses a significant challenge due to low parasite density and limit of detection [14, 15]. Active parasite detection reduces the asymptomatic parasite reservoir [16]. Prompt and accurate parasite detection is important in all settings, as misdiagnosis may result in significant morbidity and disease progression to fatality [17]. WHO recommends parasite-based treatment of suspected malaria cases with microscopy or rapid diagnostic test [18]. Malaria RDTs are cost-effective, user-friendly, and field-applicable, ensuring parasite-based treatment of malaria, especially in resource-limited settings [19].

Microscopy is an essential tool and remains the gold standard for malaria diagnosis [20]. It can effectively distinguish Plasmodium species when handled by an expert microscopist. In addition, microscopy is used to quantify parasite density and monitor treatment outcomes during therapeutic efficacy clinical trials [21]. However, its diagnostic performance is limited by subjective parasite identification, erratic power supply in low resource settings, substandard reagents, and poor infrastructure [20].

The introduction and deployment of rapid diagnostic tests and its adoption in Nigeria has significantly reduced presumptive malaria treatment and disease burden [19, 22, 23]. In resource-limited settings, RDTs have become the primary tool for the parasitological-based diagnosis of malaria [24]. RDTs can provide quicker results (~ 15-20 min), are easy to perform, require little or no training, and have no infrastructural requirements compared to microscopy [25–27]. Thus, RDT serves as an alternative to microscopy in poor resource settings [28, 29]. Nevertheless, RDTs are challenged with the occurrence of false positive results due to persistence antigeneamia of histidine-rich protein-2 (HRP-2) antigens even after the clearance of asexual parasites and false negative results due to hrp-2 gene deletion [30, 31].

Polymerase chain reaction (PCR) is the most sensitive diagnostic method and can detect parasitaemia as low as 2–5 parasites/μL [32]. However, its field applicability is often limited due to high operational cost, complex methodology, and the need for expertise [32, 33].

There are several WHO pre-qualified RDT kits in the Nigerian markets with variable sensitivity and specificity [34, 35]. However, reports on the diagnostic performance of some of the most utilized RDTs in asymptomatic malaria detection are limited in the study location. Therefore, the study evaluated the performance of four brands of RDT kits (SD-BiolineTM, Micro-point^{TM}, Carestat^{TM}, and First-Response^{TM}), microscopy against PCR among asymptomatic children from Southwest Nigeria.

2.0 METHODOLOGY

2.1 Study area

The study was conducted at two selected Primary Health Centres (Ilishan Primary Health Center and Ago-Ilara Primary Health Center). Both facilities are in Ilishan-Remo, a town in Irepodun district in Ikenne Local Government Area (LGA) of Ogun State, Southwestern Nigeria. The coordinates of the study location comprise latitude 6^{o} 53’ 42” N, and Longitude 3^{o} 42’ 51’ E. Ikenne LGA is located along the transitional forest zone of southern Nigeria. About 47% of the inhabitants live in rural areas, and 67% are involved in agriculture. The mean annual rainfall ranges between 855 mm and 1500 mm, with a daily warm to hot temperature at about 28°C.

2.2 Study design

This was a cross-sectional community-based study conducted between November and December 2022. Sample collection was conducted every Thursday between 9 a.m. and 3 p.m. Malaria mass screening was conducted on children whose parents/guardians consented to participate.

2.3 Sample size calculation

The sample size was determined using a prevalence (p) of 46.8% for malaria in Southwest Nigeria; the maximum tolerable difference between the true population and sample incidence (d) of 5% is (Z1–α) equals 1.96 (at 95% confidence interval) [19]. Therefore, a minimum of 383 sample size was required for the study.

2.4 Study procedures

A well-structured questionnaire was administered to participants in layman's terms to capture socio-demographic and clinical information. Afterward, finger-prick blood was collected aseptically from the index finger and was used to perform rapid diagnostic tests. Thick blood smears were prepared on grease-free glass slides for microscopy examination and blotted on Whatmann™ (3MM) filter paper to make dried blood spots (DBS) for PCR analysis.

2.5 Laboratory investigation

2.5.1 Rapid diagnostic testing

Rapid diagnostic tests for the four RDT brands were carried out simultaneously according to manufacturers' instructions. Thick blood smears were prepared from peripheral blood after collection on a clean, grease-free microscope slide and allowed to air dry. The smears were stained with a 10% Giemsa stain for 10 minutes and allowed to dry at room temperature before an expert microscopist examined an oil immersion objective lens. A slide was declared negative only after observing 200 high -power fields. Two independent microscopists examined the slides, and each slide examined was taken as positive when there were concordant results from the two microscopists. When there was a discrepancy, a third microscopist assessed the slide. The microscopists were blinded to the RDT results.

2.5.2 PCR Amplification of *P. falciparum*

In a nested PCR protocol, the 18S ribosomal RNA (rRNA) gene of Plasmodium was amplified in the primary reaction using Plasmodium-specific primers (Plu-5 forward: 5′-CCTGTTGTTGCCTTAAACTTC-3′ and Plu-6 reverse: 5′-TTAAAATTGTTGCAGTTAAAACG-3′) as previously described [8, 36]. Briefly, a PCR mix was prepared, and 5µL of genomic DNA from each sample was used as a template into a PCR tube containing 5µL universal PCR master mix (5X FIREPol® Master Mix, Solis Biodyne, Estonia), 0.5µL each of primers and 9 µL nuclease-free PCR-grade water. The PCR thermal cycling conditions were as follows: Initial denaturation at 950C for 5 min, followed by 30 cycles of 94oC for 1 min (denaturation), 58oC for 2 min (annealing), and 72oC for 2 min (extension), and final extension at 72^0C for 5 min. The nested Plasmodium falciparum species–specific amplification was carried out with a second set of forward and reverse primers (Fal-1: 5′-TTAAACTGGTTTGGGAAAACCAAATATATT-3′ and Fal-2: 5′-ACACAATGAACTCAATCATGACTACCCGTC-3′). Two microlitres (2µL) of the primary PCR product were used as a template in a PCR mix as described in the primary reaction and under the same thermal cycling conditions. The final PCR products were loaded on a 1.5% prestained (EZ-vision blue dye) agarose gel and were allowed to run for 40 min at 100V on an electrophoretic tank. A molecular ladder of 100bp was used as a marker for fragment size determination. The final products were viewed under a UV-transilluminator and photographed and documented for analysis. The expected base pair size for P. falciparum is 205bp.

2.6 Ethical Consideration

The approval to conduct this study was obtained from Babcock University Health Research and Ethics Committee (BUHREC), with reference number BUHREC702/22. Participants were given a consent letter signed by their parents or guardians. The entire research was carried out following acceptable laboratory and clinical procedures. Patients who declined to participate were not denied access to the available healthcare services.

2.7 Statistical Analysis

Data was independently entered into a Microsoft Excel spreadsheet and IBM SPSS (version 23) for statistical analysis. The performance of the four malaria RDTs and microscopy was calculated against PCR, and the sensitivity, specificity, positive and negative predictive values, and accuracy were evaluated. The kappa (k) statistics was used to evaluate the level of diagnostic agreement with the reference standard. Kappa's value of 0.21 – 0.40 was considered fair agreement, 0.41 to 0.60 was moderate

agreement while 0.61 - 0.80 was substantial perfect, and 0.81 – 0.99 perfect agreement [5]. The level of statistical significance was considered at $\rho \leq 0.05$

3.0 RESULTS

3.1 Socio-demographic characteristics of the study population

One hundred and sixty-three of 383 participants (42.6%) enrolled in the study had complete and matched RDTs, microscopy, and PCR results. Of the 163 participants, 78 (47.8%) were from Ilishan Primary Health Centre (PHC) and 85 (52.2%) from Ago-Ilara Primary Health Centre (PHC). Participants' ages ranged between 3 months to 10 years. The majority of the participants were within the age 1–5 years, accounting for 77 (47.2%), followed by the age group greater ≥ 5 years, representing 69 (42.3%), and

the age group < 1 year with 17 (10.4%). Male participants were 73 (44.8%) while female participants accounted for 90 (55.2%) respectively. The majority of the participants were asymptomatic, accounting for 156 (95.7%), while 7 (4.3%) of the participants were symptomatic (Table 1).

Table 1. Demographics and Clinical Characteristics

Variable	Participants N = 163
Age (Years)	
<1	17
1-5	77
>5	69
Gender	
Male	73
Female	90
Temperature (°C)	
≤ 37.5	156
≥ 37.5	7
Weight (kg)	
<15	59
15-24	61
>24	43

Table 2. Comparative Performance of mRDTs, Microscopy Versus PCR

Variable	SD-Bioline	Micro-Point	Care Start	First Response	Micros-copy
True Positive	15	17	18	17	28
False Positive	4	3	7	6	10
False Negative	18	17	14	16	12
True Negative	125	126	117	118	113

N= 163; Invalid: SD-Bioline = 1, Carestat = 7, First Response = 6

3.2 Performance of RDTs and Microscopy against PCR

Of the 163 analysed results, the malaria-positive rate as detected by PCR was 34 (20.9%), microscopy, 38 (23.3%); SD-Bioline™, 19 (11.7%); Micro-point™, 20 (12.3%); CareStart™, 25 (15.3%); and First-Response™, 23 (14.1%) respectively. Out of the 34 PCR-confirmed (reference standard), the true positive detected by RDTs were: SD-Bioline™, 15 (9.2%); Micro-point™, 17 (10.4%); CareStart™, 18 (11.04%); and First-ResponseTM, 17 (10.4%) respectively. Microscopy detected 28 (17.2%) true positives. Similar false positive rates were recorded in the four RDTs (SD-Bioline™, Micro-point™, CareStart™, and FirstResponse™) as follows: 4 (2.4%), 3(1.8%), 7(4.3%), 6 (3.7%) and microscopy, 10 (6.1%). The positive predictive and negative predictive values were similar among the diagnostic methods. Although SD-Bioline™, CareStart™, and First-Response™ recorded 1, 7, and 6 invalid results, respectively (Table 2).

The prevalence of asymptomatic parasitaemia was similar for the four RDT brands and microscopy (p = 0.888). However, microscopy's sensitivity was statistically significant (p= 0.000) over the four RDTs, while the specificity was similar (p = 0.116). There was a statistically significant difference between microscopy and RDTs for positive predictive value (0.037), while negative predictive value, accuracy, and level of agreement (kappa) were similar (Table 3).

Table 3: Performance of the four mRDTs, Microscopy against PCR

Variable	SD- Bioline (%)	Micro-Point (%)	Carestat (%)	First-Response (%)	Microscopy (%)	p-value
Prevalence	20.4	20.9	20.5	21.0	24.5	0.888
Sensitivity	45.5	50.0	56.2	51.5	70.0	0.000*
Specificity	96.9	97.7	94.4	95.2	91.9	0.116
PPV	79.0	85.0	72.0	73.9	73.9	0.037*
NPV	87.4	88.1	89.3	88.1	90.4	0.922
Accuracy	86.4	87.7	86.5	86.0	86.5	0.994
Kappa (k)	0.50	0.56	0.48	0.46	0.50	1.000

3.3 Malaria-positive rate according to age group and parasite density

Eighty-one (49.7%) participants were under five years old. As detected by PCR and microscopy, the malaria-positive rate for this age group was 15 (18.5%) and 21 (25.9%), while the positive rate for the four RDTs was similar. However, children older than five years had a higher asymptomatic positive rate in all diagnostic methods. Most participants harbour parasite density less than 200 parasites/µL with a more positive rate than participants with parasite density greater than 200 parasites/µL (Table 4).

Table 4. Malaria-positive rate according to age group and parasite density

Variable	SD-Bioline n= 19, (%)	Micro-Point n=20 (%)	CareStat n= 25 (%)	First-Response n=23 (%)	Microscopy n= 38 (%)	PCR n= 34 (%)
Age (Years):						
< 5 (n = 81)	8 (9.9)	9 (11.1)	10 (12.3)	10 (12.3)	21 (25.9)	15 (18.5)
> 5 (n = 69)	11 (15.9)	11 (15.9)	15 (21.7)	13 (18.8)	17 (24.6)	19 (27.5)
PD (p/µL):						
≤ 200 (n = 146)	14(9.6)	16 (11.0)	19 (13.0)	17 (11.6)	23 (15.8)	27 (18.5)
≥ 200 (n = 19)	5 (26.3)	4 (21.1)	6 (31.6)	6 (31.6)	15 (78.9)	7 (36.8)

PD = Parasite density

The expected base pair size of the amplified *Plasmodium falciparum* gene after gel electrophoresis and documentation was 205bp (Figure 1).

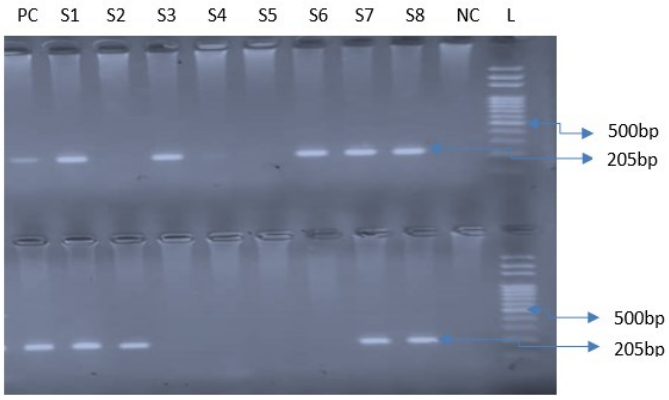

Figure 1. Gel electrophoresis of *P. falciparum* (*205bp*)
PC (Positive Control), S1 – S8 (Samples), NC (Negative Control) & L (100bp Molecular ladder)

4.0 DISCUSSION

The present study evaluated the comparative performance of four RDT kits and microscopy against PCR as the reference standard. Findings from the study show sub-optimal sensitivity of the four RDT kits compared to microscopy and PCR. This was unsurprising because of the low parasite density often associated with asymptomatic malaria. Several studies have reported low sensitivity of RDTs and sub-microscopic parasitaemia [37–39]. This underscores the need to develop cost-effective, ultra-sensitive, point-of-care diagnostic tools for actively detecting asymptomatic parasitaemia in peripheral settings where microscopy is not attainable.

Asymptomatic parasite reservoir plays a significant role in malaria transmission. Thus, a high population of asymptomatic individuals will hamper global malaria elimination efforts. Therefore, deliberate efforts and resource allocation are required to actively detect, treat, and reduce the reservoir of asymptomatic parasitaemia in endemic settings. Collaborative efforts to conduct intermittent mass screening and drug administration in malaria-endemic and high-risk populations are essential. A collaboration of the National Malaria Elimination Programs (NMEP) with state ministries of health, local authorities, and community leaders will be of immense value and impact to reducing asymptomatic malaria and the overall burden of malaria and its transmission.

In this study, the RDT's false positive rate was lower than that of microscopy, while microscopy had a higher true positive rate than RDTs. Malaria RDTs false positive is common in areas of high transmission, compromising its sensitivity. RDTs false positive is due to persistence antigenemia among other factors (including parasite, host, and storage) [40]. Several authors have reported persistence antigenemia from recently treated infections, which may persist in blood between 30 and 40 days post-treatment and clearance of asexual parasites [5, 41]. Another reason for the reduced performance of RDTs is low parasite density or biomass. Studies have shown that RDTs perform sub-optimally at low parasite densities (< 200 parasites/µL) [42], thus missing chronic latent infections in asymptomatic populations, particularly during the dry season where transmission is low with higher asymptomatic carriage [43, 44]. This study was conducted during the dry season (between November and December) among the asymptomatic population. Thus,

this study's low sensitivity of the evaluated RDTs was unsurprising. Although, RDTs targeting HRP-2 antigen are reported to have higher sensitivity compared to enzyme-based RDTs (those targeting Plasmodium-specific lactate dehydrogenase and aldolase) in symptomatic malaria diagnosis [45, 46]. In contrast, HRP2-only RDTs are reported to have lower sensitivity compared to the combination of HRP-2-based plus pan-specific RDTs in asymptomatic malaria detection [47]. The evaluated HRP -2-based RDTs in the study could not achieve 95% sensitivity as recommended [48]. However, RDT sensitivity is dependent on parasite density. Thus, RDTs are expected to have low sensitivity at low parasite densities, which may be attributable to low expression of the target antigen (HRP-2) in asymptomatic parasitaemia. However, both RDTs and microscopy demonstrated fair agreement with the reference standard (kappa = ~0.5) and were comparable with earlier reports [49].

The performance of malaria rapid diagnostic test kits and microscopy was comparable, demonstrating good agreement with the reference standard (PCR). However, to reduce the burden of malaria in endemic regions, ultrasensitive point-of-care diagnostic tools for active case detection of asymptomatic parasitaemia are needed.

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Conflict of interest

The authors declare that there is no conflict on interests.

Authors' Contributions

CCO, RIF conceived and designed the study, contributed to data collection, data analysis tools, analysis of data and manuscript writing. **WAH** contributed to data collection, data analysis tools and analysis of data. **OTO, HON, VUN, TB, AAA, ETO, GUO, OAS, AO, COA, NGE, ZEE, DEO, EOO, OO, EOI, OFA, CAO, AAO, CGO, OII, CJE** contributed to data collection. All authors approved the final copy of the manuscript.

REFERENCES

1. WHO. Report on malaria in Nigeria 2022. WHO | Regional Office for Africa. 2022. https://www.afro.who.int/countries/nigeria/publication/report-malaria-nigeria-2022. Accessed 12 Apr 2024.
2. Venkatesan P. The 2023 WHO World malaria report. Lancet Microbe. 2024;5:e214.
3. Funwei R, Umukoro P, Uyaiabasi G, Hammed W, Okangba C, Obaro M, et al. Sensitivity of Rapid Diagnostic Test, Microscopy and Pcr in Asymptomatic Malaria Detection in Rural Community from Southern Nigeria. CEOS Pediatr Child Health. 2023;1:1–8.
4. Oyibo W, Ntadom G, Uhomoibhi P, Oresanya O, Ogbulafor N, Ajumobi O, et al. Geographical and temporal variation in reduction of malaria infection among children under 5 years of age throughout Nigeria. BMJ Glob Health. 2021;6:e004250.
5. Orimadegun AE, Dada-Adegbola HO, Michael OS, Adepoju AA, Funwei RI, Olusola FI, et al. SD-Bioline Malaria Rapid Diagnostic Test Performance and Time to Become Negative After Treatment of Malaria Infection in Southwest Nigerian Children. Ann Afr Med. 2023;22:470 –80.
6. MI. U.S. President's Malaria Initiative: Nigeria Malaria Operational Plan FY 2023. 2023.
7. Chimezie RO. Malaria Hyperendemicity: The Burden and Obstacles to Eradication in Nigeria. J Biosci Med. 2020;8:165–78.
8. Funwei RI, Thomas BN, Falade CO, Ojurongbe O. Extensive diversity in the allelic frequency of Plasmodium falciparum merozoite surface proteins and glutamate-rich protein in rural and urban settings of southwestern Nigeria. Malar J. 2018;17:1.
9. Li J, Docile HJ, Fisher D, Pronyuk K, Zhao L. Current Status of Malaria Control and Elimination in Africa: Epidemiology, Diagnosis, Treatment, Progress and Challenges. J Epidemiol Glob Health. 2024. https://doi.org/10.1007/s44197-024-00228-2.
10. Andolina C, Rek JC, Briggs J, Okoth J, Musiime A, Ramjith J, et al. Sources of persistent malaria transmission in a setting with effective malaria control in eastern Uganda: a longitudinal, observational cohort study. Lancet Infect Dis. 2021;0.
11. Biruksew A, Demeke A, Birhanu Z, Golassa L, Getnet M, Yewhalaw D. Schoolchildren with asymptomatic malaria are potential hotspot for malaria reservoir in Ethiopia: implications for malaria control and elimination efforts. Malar J. 2023;22:311.
12. Fitri LE, Widaningrum T, Endharti AT, Prabowo MH, Winaris N, Nugraha RYB. Malaria diagnostic update: From conventional to advanced method. J Clin Lab Anal. 2022;36:e24314.

13. Hemingway J, Shretta R, Wells TNC, Bell D, Djimdé AA, Achee N, et al. Tools and Strategies for Malaria Control and Elimination: What Do We Need to Achieve a Grand Convergence in Malaria? PLoS Biol. 2016;14:e1002380.

14. Agbana HB, Rogier E, Lo A, Abukari Z, Jones S, Gyan B, et al. Detecting asymptomatic carriage of Plasmodium falciparum in southern Ghana: utility of molecular and serological diagnostic tools. Malar J. 2022;21:57.

15. Singh A, Singh MP, Bhandari S, Rajvanshi H, Nisar S, Telasey V, et al. Significance of nested PCR testing for the detection of low-density malaria infection amongst febrile patients from the Malaria Elimination Demonstration Project in Mandla, Madhya Pradesh, India. Malar J. 2022;21:341.

16. Shamseddin J, Ghanbarnejad A, Zakeri A, Abedi F, Khojasteh S, Turki H. Molecular Method Is Essential to Identify Asymptomatic Malaria Reservoirs: A Successful Experience in the Malaria Elimination Program in Iran. Diagnostics. 2022;12:3025.

17. Amexo M, Tolhurst R, Barnish G, Bates I. Malaria misdiagnosis: effects on the poor and vulnerable. The Lancet. 2004;364:1896–8.

18. WHO 2010. WHO releases new malaria guidelines for treatment and procurement of medicines. 2010.

19. Orimadegun AE, Funwei RI, Michael OS, Ogunkunle OO, Badejo JA, Olusola FI, et al. Comparative evaluation of three histidine-rich Protein-2 based rapid diagnostic tests, microscopy and PCR for guiding malaria treatment in Ibadan, Southwest Nigeria. Niger J Clin Pract. 2021;24:496.

20. Abeku TA, Kristan M, Jones C, Beard J, Mueller DH, Okia M, et al. Determinants of the accuracy of rapid diagnostic tests in malaria case management: evidence from low and moderate transmission settings in the East African highlands. Malar J. 2008;7:202.

21. Das D, Dahal P, Dhorda M, Citarella BW, Kennon K, Stepniewska K, et al. A Systematic Literature Review of Microscopy Methods Reported in Malaria Clinical Trials. 2021. 104 (3): 836-841 https://doi.org/10.4269/ajtmh.20-1219.

22. Falade CO, Orimadegun AE, Michael OS, Dada-Adegbola HO, Ogunkunle OO, Badejo JA, et al. Consequences of restricting antimalarial drugs to rapid diagnostic test-positive febrile children in south-west Nigeria. Trop Med Int Health TM IH. 2019;24:1291–300.

23. Okangba CC, Funwei RI, Elikwu CJ, Nwadike VU, Okangba KK, Osinowo AO, et al. Comparative Evaluation of BinaxNOWTM Malaria Rapid Diagnostic Test, Microscopy and Polymerase Chain Reaction for Plasmodium falciparum Detection among Individuals Suspected with Malaria in Lagos, Southwest Nigeria. Niger J Parasitol. 2022;43:324–34.

24. Boyce MR, O'Meara WP. Use of malaria RDTs in various health contexts across sub-Saharan Africa: a systematic review. BMC Public Health. 2017;17: 470

25. Azikiwe C, Ifezulike C, Siminialayi I, Amazu L, Enye J, Nwakwunite O. A comparative laboratory diagnosis of malaria: microscopy versus rapid diagnostic test kits. Asian Pac J Trop Biomed. 2012;2:307–10.

26. Batwala V, Magnussen P, Nuwaha F. Are rapid diagnostic tests more accurate in diagnosis of plasmodium falciparum malaria compared to microscopy at rural health centres? Malar J. 2010;9:349.

27. Mfuh KO, Achonduh-Atijegbe OA, Bekindaka ON, Esemu LF, Mbakop CD, Gandhi K, et al. A comparison of thick-film microscopy, rapid diagnostic test, and polymerase chain reaction for accurate diagnosis of Plasmodium falciparum malaria. Malar J. 2019;18:73.

28. Mbanefo A, Kumar N. Evaluation of Malaria Diagnostic Methods as a Key for Successful Control and Elimination Programs. Trop Med Infect Dis. 2020;5:102.

29. Mouatcho JC, Goldring JPD. Malaria rapid diagnostic tests: challenges and prospects. J Med Microbiol. 2013;62 Pt 10:1491–505.

30. 30. Funwei R, Nderu D, Nguetse CN, Thomas BN, Falade CO, Velavan TP, et al. Molecular surveillance of pfhrp2 and pfhrp3 genes deletion in Plasmodium falciparum isolates and the implications for rapid diagnostic tests in Nigeria. Acta Trop. 2019;196:121–5.

31. Orimadegun AE, Dada-Adegbola HO, Michael OS, Adepoju AA, Funwei RI, Olusola FI, et al. SD-Bioline Malaria Rapid Diagnostic Test Performance and Time to Become Negative After Treatment of Malaria Infection in Southwest Nigerian Children. Ann Afr Med. 2023;22:470 –80.

32. Berzosa P, de Lucio A, Romay-Barja M, Herrador Z, González V, García L, et al. Comparison of three diagnostic methods (microscopy, RDT, and PCR) for the detection of malaria parasites in representative samples from Equatorial Guinea. Malar J. 2018;17:333.

33. Valones MAA, Guimarães RL, Brandão LAC, de Souza PRE, de Albuquerque Tavares Carvalho A, Crovela S. Principles and applications of polymerase chain reaction in medical diagnostic fields: a review. Braz J Microbiol. 2009;40:1–11.

34. Aladenika S, Tatfeng M, Nwobu G, Osakue E, Ehiaghe A, Ehiaghe JI, et al. Sensitivity and specificity of five malaria rapid diagnostic kits used in south-south region of Nigeria. Open J Clin Diagn. 2012;2:70–5.

35. Cunningham J, Jones S, Gatton ML, Barnwell JW, Cheng

Q, Chiodini PL, et al. A review of the WHO malaria rapid diagnostic test product testing programme (2008–2018): performance, procurement and policy. Malar J. 2019;18:387.

36. Snounou G, Viriyakosol S, Zhu XP, Jarra W, Pinheiro L, do Rosario VE, et al. High sensitivity of detection of human malaria parasites by the use of nested polymerase chain reaction. Mol Biochem Parasitol. 1993;61:315–20.
37. Kaaya RD, Matowo J, Kajeguka D, Tenu F, Shirima B, Mosha F, et al. The Impact of Submicroscopic Parasitemia on Malaria Rapid Diagnosis in Northeastern Tanzania, an Area with Diverse Transmission Patterns. Infect Dis Rep. 2022;14:798–809.
38. Pava Z, Burdam FH, Handayuni I, Trianty L, Utami RAS, Tirta YK, et al. Submicroscopic and Asymptomatic Plasmodium Parasitaemia Associated with Significant Risk of Anaemia in Papua, Indonesia. PLOS ONE. 2016;11:e0165340.
39. van Eijk AM, Sutton PL, Ramanathapuram L, Sullivan SA, Kanagaraj D, Priya GSL, et al. The burden of submicroscopic and asymptomatic malaria in India revealed from epidemiology studies at three varied transmission sites in India. Sci Rep. 2019;9:17095.
40. Martiáñez-Vendrell X, Skjefte M, Sikka R,. Factors affecting the performance of HRP2-based malaria rapid diagnostic tests. Trop Med …. 2022.
41. Grandesso F, Nabasumba C, Nyehangane D, Page A-L, Bastard M, De Smet M, et al. Performance and time to become negative after treatment of three malaria rapid diagnostic tests in low and high malaria transmission settings. Malar J. 2016;15:496.
42. McMorrow ML, Aidoo M, Kachur SP. Malaria rapid diagnostic tests in elimination settings—can they find the last parasite? Clin Microbiol Infect. 2011;17:1624–31.
43. Coulibaly D, Travassos MA, Tolo Y, Laurens MB, Kone AK, Traore K, et al. Spatio-Temporal Dynamics of Asymptomatic Malaria: Bridging the Gap Between Annual Malaria Resurgences in a Sahelian Environment. 2017: 97 (6): 1761-1769. https://doi.org/10.4269/ajtmh.17-0074.
44. Opoku Afriyie S, Addison TK, Gebre Y, Mutala A-H, Antwi KB, Abbas DA, et al. Accuracy of diagnosis among clinical malaria patients: comparing microscopy, RDT and a highly sensitive quantitative PCR looking at the implications for submicroscopic infections. Malar J. 2023;22:76.
45. Alemayehu GS, Lopez K, Dieng CC, Lo E, Janies D, Golassa L. Evaluation of PfHRP2 and PfLDH Malaria Rapid Diagnostic Test Performance in Assosa Zone, Ethiopia. Am J Trop Med Hyg. 2020;103:1902–9.
46. Kayode TA, Addo AKA, Addison TK, Tweneboah A, Afriyie SO, Abass DA, et al. Welcome to the next generation of Malaria Rapid Diagnostic Tests: Comparative Analysis of NxTek Eliminate Malaria P.f, Biocredit Malaria Ag Pf, and SD Bioline Malaria Ag Pf for Plasmodium falciparum Diagnosis in Ghana. Res Sq. 2023;:rs.3.rs-3459263.
47. Agaba BB, Rugera SP, Mpirirwe R, Atekat M, Okubal S, Masereka K, et al. Asymptomatic malaria infection, associated factors and accuracy of diagnostic tests in a historically high transmission setting in Northern Uganda. Malar J. 2022;21:392.
48. WHO. New perspectives : malaria diagnosis : report of a joint WHO/USAID informal consultation, 25-27 October 1999. 2000