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Original article

Performance and cost comparison of a rapid diagnostic test for malaria and microscopy: A cross-sectional diagnostic study in Gusau, Nigeria

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Abstract

Background: The shift from traditional microscopy to rapid diagnostic tests (RDTs) for malaria diagnosis is gaining popularity, especially in resource-limited settings where electricity supply is unstable and expertise is limited. However, trust issues arise from inconsistent performance across different RDT kits.
Objectives: This study aimed to identify variations in the performance and cost of an RDT brand for malaria among different population demographics, including pregnant women, young children, and adults, compared with microscopy as a gold standard.
Methods: The research was conducted in northwestern Nigeria and involved 360 participants who were stratified into three groups: children (5–17 years), adults (≥ 18 years), and pregnant women. Samples were examined using RDT and microscopy, and cost data were gathered from retrospective study expenses.
Results: The RDT exhibited higher sensitivity (86.1%) and a positive predictive value of 87.9% in children, but lower sensitivity (59.5%) and specificity (54.3%) in pregnant women. Logistic regression analysis indicated that the likelihood of testing positive decreased by 2.0% for each year of age ($P = 0.046$) and by 10.0% for each unit increase in body mass index (BMI) ($P < 0.001$) and was twice that of positivity in those who experienced vomiting ($P = 0.004$). Moreover, RDT had lower labor costs (₦61) than microscopy (₦746), which requires higher technical expertise.
Conclusion: RDTs offer a rapid diagnostic option for malaria, which may be suitable in resource-constrained settings for certain populations, such as children or adults. However, their limitations in pregnant women and the impact of age and BMI on accuracy necessitate supplementary tests where resources permit.

Keywords: Cost, microscopy, performance, rapid diagnostic tests, resource-limited.

Malaria continues to pose a considerable threat to human health, especially in sub-Saharan Africa, including Nigeria.[1,2] Despite efforts set by the Global Technical Strategy for Malaria 2016–2030 to eradicate malaria, the World Health Organization's (WHO) targets for 2025 are still far from being met. In 2023, the malaria incidence rate was three times higher than the target, and the mortality rates were more than twice the desired levels.[3] Nigeria has the highest global malaria burden, contributing to approximately 25.9% of malaria cases and 30.9% of malaria-related deaths worldwide, with vulnerable groups such as children under the age of 5 years, pregnant women, and the immunocompromised being most at risk.[4] In the 2022 malaria report, Zamfara state contributed 1.7 million malaria cases out of the total 68 million malaria cases recorded nationwide[5].Due to the high prevalence of malaria in Africa as well as high annual economic impact, policymakers in most African nations have implemented several preventive measures, including campaigns on environmental

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hygiene, the use of insecticide-treated nets (ITNs), seasonal malaria chemoprevention, and intermittent preventive therapy (IPT) for pregnant women using sulfadoxine-pyrimethamine (SP).[6, 7] However, because of inadequate resources, the sustainability of these preventive measures remains a challenge.[8] The prevalence of malaria in Nigeria is aggravated by poverty, conflicts, and climate change, which intensify the transmission dynamics, hamper control efforts, and create obstacles to accessing proper care.[9] Climate change has contributed to the increasing occurrences and severity of unusual weather events, such as heavy rainfall and flooding, which create breeding grounds for mosquitoes.[10]

Recent advancements, such as the malaria vaccines RTS, S, and R21, are promising but still face limitations, including reduced efficacy over time and only focusing on young children, thereby omitting susceptible individuals, including pregnant women and immunocompromised older children, who may incur severe consequences.[11] Recently, researchers and private pharmaceutical companies have been working to create point-of-care biosensor devices, similar to glucometers, capable of detecting and measuring the *Plasmodium falciparum* histidine-rich protein 2 (HRP-2) antigen for malaria diagnosis. Unlike typical rapid diagnostic tests (RDTs), these devices are designed to evaluate the antigen levels, which will aid in assessing the parasite burden and predicting clinical effects.[12] If these devices become widely accessible and affordable in malaria-endemic regions, they could effectively complement the efforts to eradicate malaria in sub-Saharan Africa.

For malaria diagnostics, high-quality microscopy remains the superior method, providing precise quantification of parasites and accurate species identification.[13] However, microscopy is time-consuming and requires well-trained, experienced personnel, as well as regular microscope maintenance.[14] While PCR has the potential to be the gold standard, it has not yet received general recognition as a diagnostic tool. The reluctance to implement this technology is primarily due to obstacles in adapting it to the harsh environment of malaria-endemic locations and its high cost.[15] Furthermore, doctors are often reluctant to perform diagnostic tests and instead use their clinical judgment to diagnose and treat malaria.[16] However, because of the limitations of microscopy, as well as recent WHO guidelines that emphasize the confirmation of parasites in blood through testing before initiating therapy and the introduction of RDTs for malaria, diagnostic practices have shifted toward relying on RDTs. These tests require only a small amount of blood and minimal expertise, and they produce results quickly, typically within 5–15 min. However, trust issues regarding their performance and acceptance in field settings persist.

Given these challenges, it is essential to assess the diagnostic accuracy of RDTs under real-world conditions and examine the economic feasibility of these methods, especially in low-resource settings. This research aimed to fill gaps in understanding the comparative performance and costs of RDTs for malaria and microscopy tools in vulnerable populations, such as pregnant women and young children, to improve prompt diagnosis and treatment outcomes in Gusau, Nigeria.

Materials and methods

This was a cross-sectional study, and the research was conducted over eight months, from August 2024 to March 2025, at Yarima Bakura Specialist Hospital and Daula Hospital and Maternity Home, both of which are located in Gusau, Zamfara State, Nigeria. The region is malaria-endemic, and most of the people are poor, which makes a study regarding the cost and performance of malaria diagnostic tests more applicable.

Participants and sampling

Consecutive sampling was used to enroll all patients who met the inclusion criteria, which included young children (5–17 years), adults (≥ 18 years), and pregnant women who visited the facilities, were clinically suspected of having malaria, and consented to participate. Children under 5 years of age were excluded, as this group has been studied in a similar context. [17] Moreover, patients who declined consent or those already on malaria treatment were excluded.

Sample size calculation

The sample size was estimated based on the malaria prevalence of 46.0% among pregnant women and the general population, with a 0.07 margin of error.[17, 18] A precision level of 0.05 was set, targeting 92.5% sensitivity and specificity. The sample size for each group (pregnant women, children 5–17 years, and adults ≥ 18 years) was calculated to be 120 for sensitivity and 101 for specificity. Therefore, to explore the diagnostic ability of the RDT, a total of 360 participants were included, with 120 participants per group.

Data collection

Clinical and sociodemographic data were collected after informed consent was obtained. Participants meeting the inclusion criteria were interviewed face-to-face by a research assistant using a semi-structured questionnaire that was designed based on previous studies and the study's objectives.[18, 19] Each questionnaire had a unique code, which was used on the RDT kit and the microscopy request form. The questionnaire included five sections that were tailored to the study's goals. After completing the questionnaire, the research assistant performed an RDT test for malaria. Samples were then collected for microscopy, which was performed by a laboratory scientist who was blinded to the RDT result. Once both test results were obtained and interpreted, the participants were informed of their malaria status, and appropriate treatment was provided. Cost data were collected from the provider's perspective (consumables, cost incurred due to invalid tests, and labor cost) and reported in Nigerian Naira (₦), using sources such as procurement records, retrospective research expenses, local pharmacies, and the Federal Government's salary scale.

Malaria diagnosis by RDT

The AdvDx™ Malaria *P. falciparum* HRP2 Ag RDT kit was selected for this study because it is available in the study area. This brand is manufactured in India, and the leaflet reported sensitivity and specificity of 96.7% and 98.4%, respectively. After explaining the procedure to the participant and obtaining informed consent, a fingertip was cleaned with 70.0% ethyl alcohol and pricked with a sterile lancet. Three drops of blood were placed on the RDT cassette using a capillary tube, followed by two drops of the kit-provided buffer solution. Results were observed after 10–15 min, as per the producer's guidelines. In addition, the time at which the lines first appeared was noted, as this aligns with real-life clinical practice. A positive result was indicated by the test and control lines both appearing, while a negative result showed only the control line. If only the test line appeared or no lines showed up, the result was deemed invalid, and the test was repeated. All invalid results were valid after repetition.

Malaria diagnosis by microscopy

After explaining the procedure and obtaining informed consent, the blood sampling site was cleaned with 70% alcohol for asepsis. An experienced laboratory technician collected 0.2 mL of blood in an ethylenediaminetetraacetic acid container, spreading it over a glass slide to create a thick blood film. The slide was air-dried, stained with 0.1% Giemsa solution, and rinsed with phosphate buffer. The slide was then examined under a light microscope with a 100× objective lens. At least 100 oil-immersion fields were examined to confirm a negative result.[20, 21] The diagnosis was considered positive if *Plasmodium* developmental stages were detected and negative if they were not observed.

Ethical approval and consent to participate

Ethical review and approval were obtained from the Nigerian Institute of Medical Research (NIMR) (IRB/24/036) and the Institute Review Boards of Chulalongkorn University, Bangkok, Thailand (0803/67). After approval was received, the participants were informed about the study's objectives, risks, and potential benefits. Only those who provided written informed consent themselves or through their parents (in the case of minors) were enrolled. Furthermore, verbal assent was obtained from participants under the age of 18, and to maintain confidentiality, the participants' names were not requested or documented.

Statistical analysis

After collecting data in the case record forms, it was entered into Microsoft Excel 16 and cleaned before being transferred to STATA 18.0 SE for analysis. Descriptive statistics were used to examine the baseline features, including normality and missing data. The sociodemographic data and other variables were presented as the mean ± standard deviation (SD) in tables and charts. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), prevalence, and positive and negative likelihood ratios (LR+ and LR") were calculated for pregnant women, children, and adults, as well as for the overall diagnostic parameters. In addition, subgroup analysis was conducted among pregnant women who used IPT with SP and those who did not. Binary logistic regression was applied to evaluate the effect of age, height, weight, preventive measures (e.g., drugs, ITNs, and insecticides), symptoms (e.g., fever, vomiting, body weakness, and poor appetite), and pregnancy status on the RDT outcomes. The difference in time taken to run RDT and microscopy was not normally distributed and was thus analyzed using the Wilcoxon Signed-Rank Test. Costs (i.e., expenditure costs) were analyzed descriptively, and the key patterns were presented in tables. A 95% confidence interval (CI) was used, and $P < 0.05$ was considered statistically significant throughout the analyses.

Results

Baseline features of the participants

Figure 1 illustrates the flowchart of the recruitment process and the tests performed. The participants' baseline characteristics varied across the groups (**Table 1**). Nearly 80.0% of the total participants were recruited from Daula Hospital, while the remaining 20.0% (pregnant women) were recruited from Yarima Bakura Specialist Hospital. Notably, one of the pregnant participants was 17 years old. Participants reported different forms of malaria presentation and used various preventive measures. Fever was the most common symptom, which was reported by nearly all participants in the three groups, while ITN use was the most widely practiced preventive measure (90.0%–93.0% across groups), and 28.3% of pregnant women took SP for malaria IPT. The most common occupation among adults was civil service (40.8%), while a higher proportion of pregnant women were housewives (50.8%), and most children (98.3%) had fathers who were civil servants (**Table 1)**.

Prevalence of malaria and RDT performance

The median time taken to run the RDT (5.9 min) was significantly faster than that of microscopy (47.8 min), as confirmed by the Wilcoxon Signed-Rank Test ($P < 0.001$). For prevalence, among the children aged 5–17 years, 82.5% were positive by RDT, compared to 84.2% by microscopy. Furthermore, 54.2% of pregnant women had positive RDT results, while a positive microscopy result was confirmed in 61.7%. Among adults ≥ 18 years old, 51.7% tested positive with RDT, while microscopy confirmed a positive rate of 70.0% **(Table 2).**

Binary logistic regression analysis

The potential influence of age, height, weight, preventive measures (e.g., drugs, ITNs, and insecticides), symptoms (e.g., fever, vomiting, body weakness, and poor appetite), and pregnancy status on the RDT outcomes was assessed using binary logistic regression analysis. Correlation analysis revealed that height and weight were highly correlated, with a Pearson's coefficient of 0.82 ($P < 0.001$). Therefore, when the weight and height were combined into the body mass index (BMI), the BMI, along with age and vomiting, were the main predictors of RDT positivity, as indicated by their significant *P*-values. However, pregnancy was not significant in the multivariable model; hence, it was removed. The model, as shown in **Table 3**, revealed that the likelihood of testing positive decreased by 2.0% with each unit increase in age ($P = 0.046$), 10.0% with each unit increase in BMI ($P < 0.001$), and twice the chance of getting a positive result in those who presented with vomiting ($P = 0.004$). The overall model was significant ($P < 0.001$), which suggests that age, BMI, and vomiting contributed to variations in RDT positivity. The pseudo-R^2 value was 0.1185, indicating that the model explained approximately 11.85% of the variability in RDT positivity. Moreover, the goodness-of-fit test ($P = 0.357$) suggested that the model fitted the data well.

Cost data

Costs were categorized as shared or individual. The shared costs comprise expenses related to consultation services, reception, logistics for the supply of consumables/RDT kits, and laboratory facilities, which were used similarly by patients who opted for either RDT or microscopy tests. Individual costs, specific to RDT or microscopy, were divided further into direct and indirect/labor medical costs (**Table 4**). The consumables comprise all the essential items required to perform the microscopy or RDT tests. For RDT, nearly all these consumables came in the kit package, and there are 25 RDT kits per package at ₦ 10,000. Therefore, one RDT cassette costs ₦ 400. The consumables for microscopy tests, including their corresponding prices, amount to ₦ 292 per test.

Labor cost refers to the expenses incurred by healthcare staff in conducting these tests. On average, RDT requires 5.9 min to perform, compared to the 47.8 min for microscopy. The laboratory technician performing RDT earns an average salary of ₦100,000 per month, whereas the laboratory scientist performing microscopy earns an average salary of ₦150,000 per month. Thus, the per-minute labor charges are ₦ 10.42 for RDT and ₦15.63 for microscopy, assuming fully employed personnel in Nigeria work 40 hours a week (9,600 min/month).

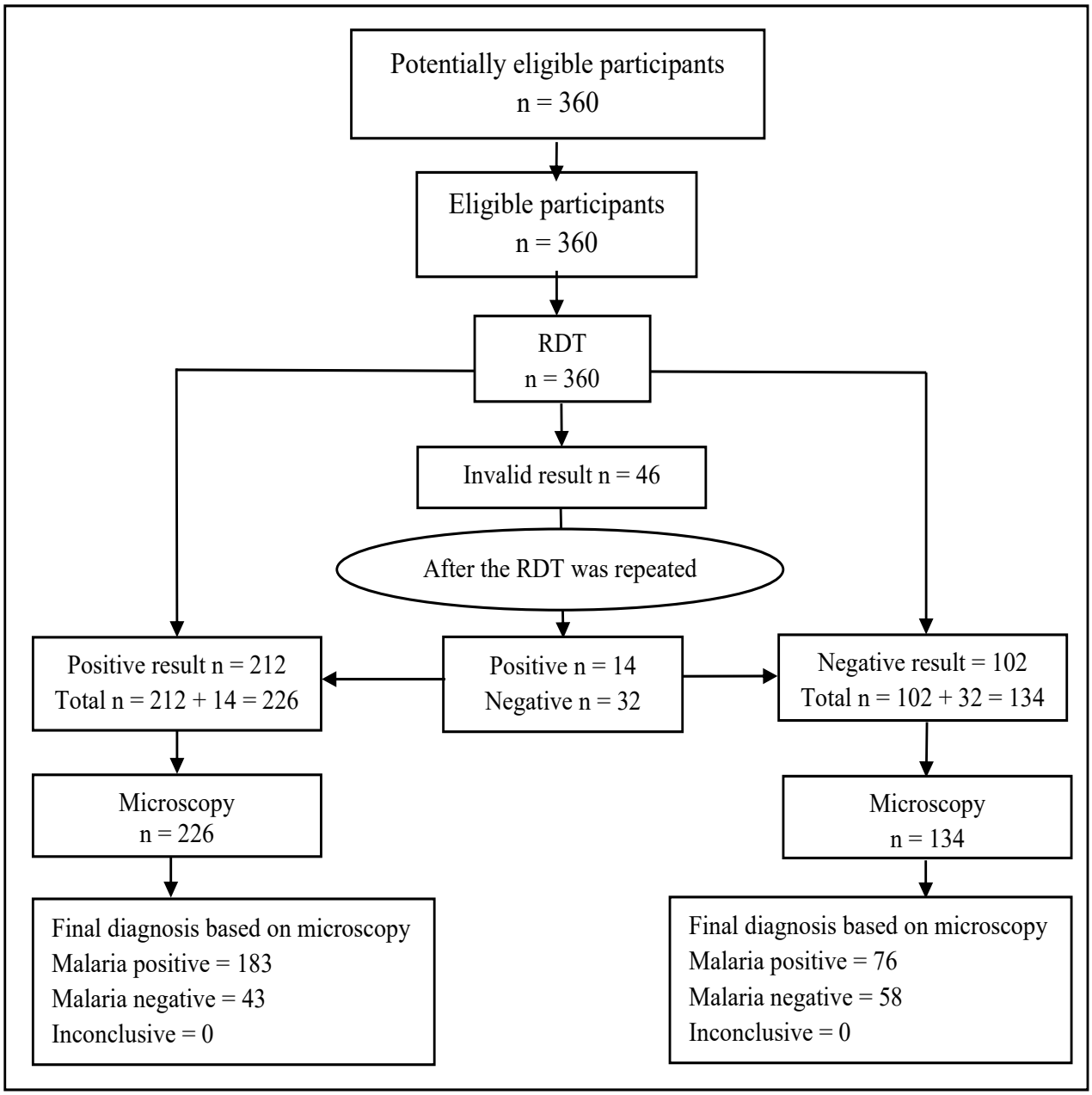

Figure 1. Flow chart showing a summary of the recruitment and test processes. An invalid test result is an RDT result that cannot be classified as positive or negative. In contrast, an inconclusive test refers to a microscopy test for which the laboratory scientist cannot categorize it as positive or negative. n, number of participants; RDT, rapid diagnostic test.

Table 1. Baseline features of the three participant categories

Baseline features		**5–17 years**	**Pregnant women**	**≥ 18 years**
Facility	Daula n (%)	120 (100)	40 (66.7)	120 (100)
	Yarima Bakura n (%)	0 (0)	80 (33.3)	0 (0)
Gender	Female n (%)	55 (45.8)	120 (100)	70 (58.3)
	Male n (%)	65 (54.2)	0 (0)	50 (41.7)
Age (years)	Range	6–17	17–41	18–62
	Mean ± SD	10.6 ± 2.8	27.5 ± 5.5	34.6 ± 10.6
Weight (kg)	Range	12–65	39–90	31–86
	Mean ± SD	26.6 ± 9.7	60.8 ± 8.0	57.6 ± 12.0
Height (cm)	Range	69–173	137–175	142–203
	Mean ± SD	131.3 ± 18.3	159.1 ± 8.1	161.4 ± 9.6
BMI (kg/m²)	Range	11.1–29.4	16.5–33.9	14.3–39.3
	Mean ± SD	15.0 ± 2.6	24.1 ± 3.3	22.1 ± 4.4
Signs and symptoms		**5–17 years**	**Pregnant women**	**≥ 18 years**
Fever n (%)	Yes	120 (100)	113 (94.2)	118 (98.3)
	No	0 (0)	7 (5.8)	2 (1.7)
Vomiting n (%)	Yes	62 (51.7)	84 (70)	30 (25)
	No	58 (48.3)	36 (30)	90 (75)
Poor appetite n (%)	Yes	120 (100)	101 (84.2)	119 (99.2)
	No	0 (0)	19 (15.8)	1 (0.8)
Body weakness n (%)	Yes	118 (98.3)	92 (76.7)	117 (97.5)
	No	2 (1.7)	28 (23.3)	3 (2.5)
Forms of prevention		**5–17 years**	**Pregnant women**	**≥ 18 years**
Drugs n (%)	Yes	0 (0)	34 (28.3)	1 (0.8)
	No	120 (100)	86 (71.7)	119 (99.2)
Insecticides n (%)	Yes	11 (9.2)	2 (1.7)	10 (8.3)
	No	109 (90.8)	118 (98.3)	110 (91.7)
ITN n (%)	Yes	109 (90.8)	112 (93.3)	108 (90)
	No	11 (9.2)	8 (6.7)	12 (10)
Occupations		**5–17 years**	**Pregnant women**	**≥ 18 years**
Civil servants n (%)		118 (98.3)†[f]	32 (26.7)	49 (40.8)
Self-employed n (%)		2 (1.7)†[f]	19 (15.8)	20 (16.7)
Private employment n (%)		-	8 (6.7)	2 (1.7)
Housewife n (%)		-	61 (50.8)	39 (32.5)
Student n (%)		-	-	10 (8.3)

BMI, body mass index; ITN, insecticide-treated net; SD, standard deviation
†[f] Father's occupation for children aged 5–17 years

Table 2. Performance of rapid diagnostic test (RDT) for malaria across the samples and subgroups

Diagnostic parameters	5–17 years	Pregnant women			≥ 18 years	Overall
		Those on IPT	Those not on IPT	Overall		
Sensitivity % (95% CI)	86.1 (77.8–92.2)	64.7 (38.3–85.8)	57.9 (44.1–70.9)	59.5 (47.4–70.7)	61.9 (50.7–72.3)	70.7 (64.7–76.1)
Specificity % (95% CI)	36.8 (16.3–61.6)	58.8 (32.9–81.6)	51.7 (32.5–70.6)	54.3 (39–69.1)	72.2 (54.8–85.5)	57.4 (47.2–67.2)
PPV% (95% CI)	87.9 (79.8–93.6)	61.1 (35.7–82.7)	70.2 (55.1–82.7)	67.7 (54.9–78.8)	83.9 (72.3–92)	81 (75.2–85.9)
NPV% (95% CI)	33.3 (14.6–57)	62.5 (35.4–84.8)	38.5 (23.4–55.4)	45.5 (32–59.4)	44.8 (31.7–58.5)	43.3 (34.8–52.1)
LR+	1.36 (0.96–1.94)	1.57 (0.81–3.06)	1.20 (0.77–1.86)	1.3 (0.9–1.9)	2.2 (1.3–3.9)	1.66 (1.31–2.11)
LR-	0.38 (0.18–0.81)	0.60 (0.28–1.28)	0.81 (0.51–1.30)	0.75 (0.5–1.1)	0.5 (0.4–0.7)	0.51 (0.4–0.66)
Accuracy (%)	78 (70.9–85.8)	62 (45.4–78.1)	56 (45.3–66.3)	58 (48.6–66.4)	65 (56.5–73.5)	70 (61.9–71.6)
TP	87	11	33	44	52	183
TN	7	10	15	25	26	58
FP	12	7	14	21	10	43
FN	14	6	24	30	32	76
Prevalence % (95% CI)	84.2 (76.4–90.2)	50.0 (32.4–67.6)	66.3 (55.3–76.1)	61.7 (52.4–70.4)	70 (61–78)	71.9 (67–76.5)

CI, confidence interval; FN, false-negative; FP, false-positive; IPT, intermittent preventive therapy; LR+, positive likelihood ratio; LR-, negative likelihood ratio; NPV, negative predictive value; PPV, positive predictive value; TN, true-negative; TP, true-positive.

Table 3. Binary logistic regression analysis

Variables	Univariate analysis			Multivariate analysis		
	Odds ratio	95% CI	*P*-value	Odds ratio	95% CI	*P*-value
Age (years)	0.95	0.93–0.97	<0.001	0.98	0.95–0.99	0.046
BMI (kg/m^2)	0.87	0.83–0.91	<0.001	0.90	0.84–0.96	0.001
Drugs	0.60	0.30–1.20	0.147			
ITNs	1.91	0.91–4.00	0.087			
Insecticides	0.76	0.32–1.78	0.522			
Vomiting	1.82	1.18–2.82	0.007	2.04	1.25–3.32	0.004
Fever	3.48	0.86–14.17	0.081			
Body weakness	2.87	1.38–6.00	0.005	2.19	0.98–4.89	0.055
Poor appetite	1.74	0.71–4.30	0.229			
Pregnancy	0.58	0.37–0.91	0.017			

BMI, body mass index; CI, confidence interval; ITNs, insecticide-treated nets.

Table 4. Direct and indirect medical costs per participant

Tests	Medical costs (₦)			Total costs (₦)
	Direct		Indirect/labor	
	Consumables	Invalid		
RDT	400.0	80.0	61.5	541.5
Microscopy	292.0	–	746.9	1,038.9

₦, symbol of the Nigerian currency (Nigerian Naira).

Discussion

This study aimed to evaluate the diagnostic performance and cost of an RDT for malaria compared with microscopy across different population groups in Nigeria. The study included 360 participants across three groups, namely pregnant women, young children (5–17 years), and adults (≥18 years). The performance of the RDT was assessed in each of the three groups, and the overall accuracy was assessed. The results revealed that across all participants, the sensitivity, PPV, and overall accuracy exceeded 70%. The sensitivity was higher than the specificity, which often led to more false positives and raised concerns about unnecessary treatment and potential economic burden. This pattern of suboptimal specificity with relatively high sensitivity is consistent with the findings from previous studies.[22, 23]

In the subgroup analysis, the RDT exhibited higher sensitivity in children. However, the test has a low specificity, as seen in the overall performance. The higher PPV of 88.0% in this age group should be interpreted with caution, as PPV can be influenced by disease prevalence, which is highest in this subgroup at 84.0%. However, the low NPV in children meant that negative results could not be fully trusted. Therefore, positive RDT results can be acted on immediately with treatment, whereas a confirmatory microscopy test should follow negative results. The sensitivity of the RDT among pregnant women was the lowest across the subgroups, indicating that the test was less effective at detecting malaria in this vulnerable group. This is concerning, as the early diagnosis and treatment of malaria during pregnancy are essential to prevent severe complications for the mother and fetus.[24] In addition, the specificity and

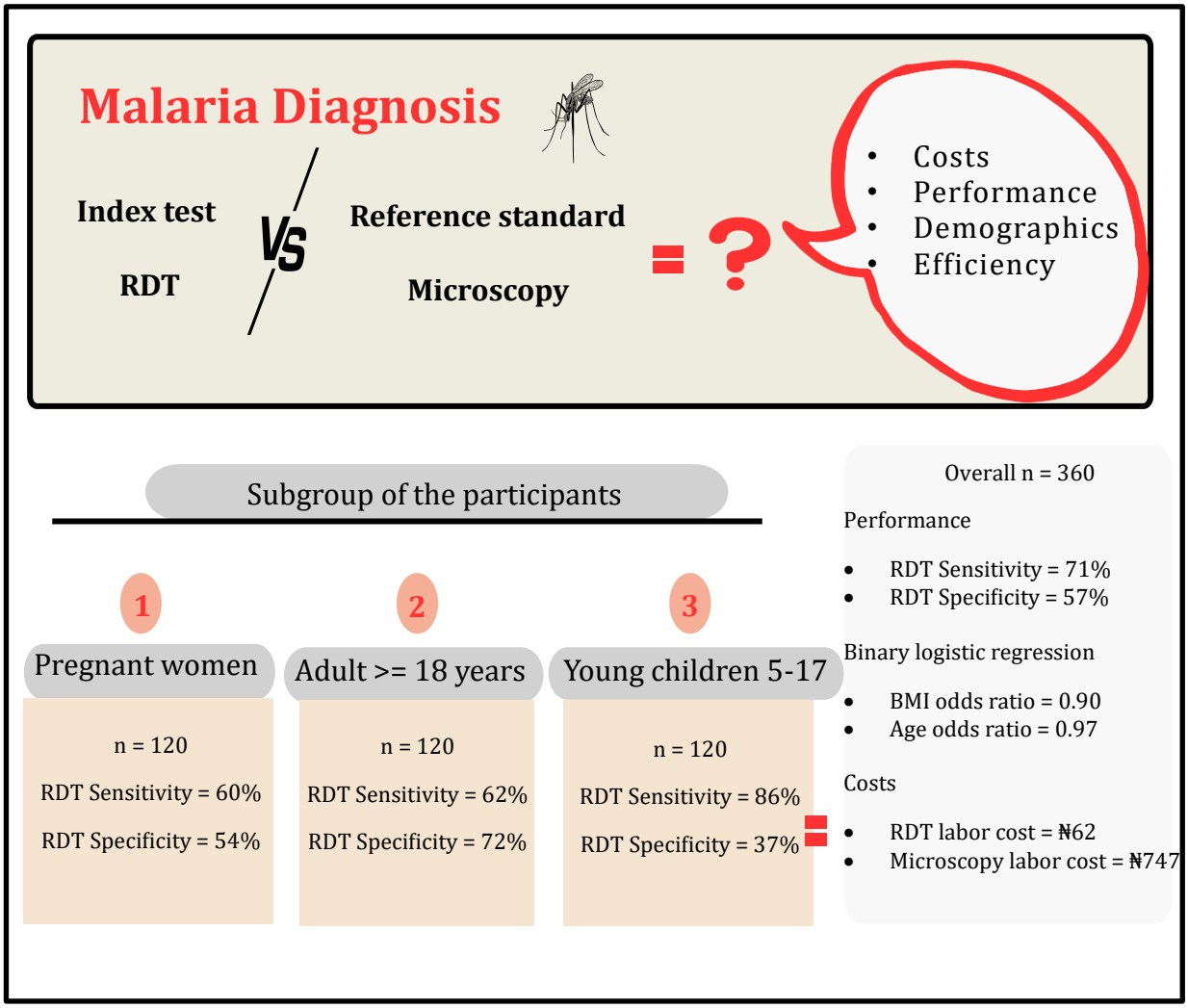

Figure 2. Graphical abstract summarizing the key method and findings. BMI, body mass index; n, number of participants; RDT, rapid diagnostic test.

PPV are relatively low, which indicates a higher chance of false-positive results in this group, and nearly half of the pregnant women could be exposed to unnecessary treatment, which further raises concerns regarding the development of multidrug-resistant malaria and additional economic burden in an already strained healthcare system. Furthermore, the subgroup of pregnant women who were on IPT exhibited better performance with the RDT. However, it is unclear how IPT with SP for pregnant women improves RDT performance. In remote settings that use the same brand of our RDT and where microscopy is not available, we recommend referral to tertiary centers as the ideal and safest practice for pregnant women. Adults had moderate sensitivity and PPV, falling between the two groups, but had the highest specificity.

The performance variability of RDTs can be attributed to biological and methodological factors. For instance, residual *P. falciparum* HRP-2 antigens, which remain in the bloodstream after infected red blood cells rupture, could be detected by the RDT even after the parasite has been cleared from the body by the immune response or previous treatments. This persistence of antigens may contribute to false-positive results, thus lowering the RDT's specificity.[25] Another possible explanation is the parasite density in the blood. At low parasitemia, less experienced laboratory scientists may struggle to visualize the parasite under a microscope. Meanwhile, the antigen in the blood may be sufficient to be taken up by the monoclonal antibodies and visualized on the malaria RDT. In addition, the HRP-2/3 gene deletion occurs in some *P. falciparum* strains, which may contribute to false negatives, as it can easily evade detection by the RDT tools.[26, 27] Furthermore, the lack of thin-film preparation prevented the identification of the parasite species, which may have contributed to this dilemma and is a limitation of this study. The RDT kit used in this study detects the HRP-2 protein of *P. falciparum*, which is identified as the primary parasite that causes malaria in approximately more than 90.0% of cases in the study area and worldwide.[10, 21] Therefore, a few patients who may have been infected with other variants of *Plasmodium* will be missed by the RDT kit. However, in real-life practice, this limitation is often overlooked or rarely comes to mind for physicians. One of the key benefits of this study is to provide stakeholders with a real-world reflection of clinical practice, thereby enabling them to prepare effective countermeasures.

The study also examined predictors of RDT positivity, including age, BMI, and vomiting. Regression analysis revealed that for every 1-year increase in age, the odds of testing positive for malaria decreased by 2.0% after adjusting for BMI and vomiting. In comparison, each unit increase in BMI reduced the odds of a positive result by 10% after adjusting for age and vomiting. However, a patient who is experiencing vomiting has twice the odds of testing positive for malaria after adjusting for age and BMI. The fact that children are more likely to test positive for malaria suggests that they are at higher risk.[28] Several reasons may be attributed to this, including increased exposure because of behaviors such as playing outdoors, lower immunity, and possibly higher parasitemia. This emphasizes the need for age-targeted prevention, such as seasonal malaria chemoprevention in children. Individuals with a higher BMI may have lower circulating parasite densities, possibly due to the dilution effect from increased blood volume or body fluid. A similar finding was reported in a study conducted in Thailand by Wilairatana, *et al*.[29] Further studies should be performed, aiming to uncover the hidden factors that influence how age, BMI, and pregnancy status may influence malaria diagnosis.

An economic evaluation was also conducted to compare the costs associated with RDTs and microscopy. The cost data was collected from the provider's perspective to provide policymakers with insight into implementing this intervention in public healthcare settings. Individual costs were further divided into direct and indirect medical costs. Direct medical costs for RDT per patient include ₦400.0 for consumables (a unit of RDT cassette). Based on our study, approximately 5 RDT cassettes out of 25 in a pack are likely to yield an invalid result, and the cost of invalid tests, spread over the 25 cassettes, amounts to ₦80.0. Similarly, the direct microscopy expense is primarily due to consumables, which cost ₦292.0 per patient. Indirect medical costs reflect the labor costs (average minutes to run a test multiplied by charges per minute), which are ₦61.5 for performing RDT and ₦ 746.9 for the microscopy test **(Figure 2)**. The difference in labor costs is due to variations in the time spent performing each test, and microscopy requires more technical expertise, where the scientist earns a higher salary. For instance, in our case, approximately 50.0% of RDT results were ready for interpretation within 5 min, and the results remained unchanged even after

waiting an additional 10 min (the producer's recommended time). In clinical settings, most physicians interpret RDT results within 3 min and treat the patients accordingly. However, it is unclear whether such a practice may negatively affect overall patient care.

The total cost for RDT (₦ 541.5) and microscopy (₦1,038.9) could be partially recovered through charges to the patients or their health insurance companies. Based on local hospital data, the charges for diagnostic services using microscopy are ₦ 1,000.0 per test and, for RDT, ₦ 500.0 per test. Given fixed factors such as time, salary, and expenses, the cost per test would be ₦ 41.5 for RDT and ₦ 38.9 for microscopy. Nevertheless, when factors vary, such as salary increments, the model in this study can be used to calculate the predicted total cost per test. The affordability of these diagnostic tests may be challenging for providers and recipients. Nigeria, with a population of more than 237 million, faces high patient turnover, which puts pressure on healthcare providers and requires substantial investment.[30] In addition to outpatient visits, malaria contributes to approximately 30.0% of hospital admissions, and healthcare services often rely on external funding.[31] From the patient's perspective, affordability is also a concern with the current minimum wage in Nigeria at ₦70,000/month.[32]For example, a household earning minimum wage would spend about 2.9% of their monthly income on two microscopy tests, which is an estimate the cost of one kilogram of rice. Most of these expenses are paid out-of-pocket, as fewer than 10.0% of Nigerians have health insurance. While the 2022 National Health Insurance Authority Act aims to provide mandatory coverage, especially for vulnerable groups, challenges remain, particularly in rural areas and the informal work sector.[33]

The findings from this study have important implications for public health policy and practice in Nigeria. The Nigerian Federal Ministry of Health (FMOH) can use this evidence to refine the national malaria diagnostic algorithms by integrating RDTs with microscopy as a confirmatory tool and to project implementation costs. Moreover, it is essential to support training programs for laboratory staff on the storage conditions and accurate interpretation of RDT results, as improper handling can result in false results and subsequently inappropriate treatment. The FMOH should consider procurement policies that prioritize high-specificity RDTs that are validated for local *Plasmodium* strains. Such policies would improve the accuracy of malaria diagnostics and reduce the unnecessary economic burden on individuals and the healthcare system. While this study provides valuable insights into the use of RDTs for malaria diagnosis, it does have some limitations that warrant attention in future research. The lack of thin film analysis prevented species identification, which could have provided further context for understanding the false negatives that were obtained, as well as the limitations of the RDT. The cost analysis focused on individual costs specific to microscopic or RDT diagnosis, leaving the shared costs, such as facilities, consultations, and treatments, for further research. In addition, the sample size was relatively small, and molecular confirmatory techniques such as PCR were not employed, which may have enhanced the accuracy and reliability of the findings. Future studies should incorporate these methods, increase the sample size, and consider regional strain diversity to better understand how local variations in *Plasmodium* species affect RDT performance. Finally, the outcomes of this study apply to the region where the use of this RDT brand is the most predominant. Malaria transmission and economic status are also integral factors. In northern Nigeria, the climate, malaria endemicity, and economic status are fairly similar among the states. However, these factors may differ slightly in the southern part of the country, where most of the revenue is generated. The Southern part of the country is occupied with commercial industries, and that is where the country's crude oil reserves are located. Therefore, this influences the differences in job opportunities and socioeconomic status between the two regions. [34]

Conclusion

This study assessed the diagnostic accuracy of malaria RDTs compared with microscopy across three groups, namely children (5–17 years), pregnant women, and adults (≥ 18 years). RDTs demonstrated high sensitivity and PPV in children but had low specificity, thus raising concerns about the possibility of increased false positives. In pregnant women, sensitivity and specificity were both lower, indicating a higher risk of potential missed diagnoses and the need for complementary diagnostic tools. In addition, age and BMI influenced RDT outcomes, with older individuals and those with a higher BMI being less likely to test

positive. Although RDTs offer cost and time advantages over microscopy, especially in low-resource settings, their diagnostic limitations, particularly in pregnancy, warrant cautious use. Future studies should aim to enhance RDT accuracy, explore novel biomarkers, and assess the integrated cost strategies for combined diagnostic approaches.

Author contributions

ASI and YC contributed to the study conception and design, data acquisition, literature review, and data analysis and interpretation. HUI and AAL contributed to data acquisition. All authors contributed to drafting and critically revising the manuscript, approved the final version, and accept responsibility for the content of the published article.

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Conflict of interest statement

All authors have completed and submitted the International Committee of Medical Journal Editors Uniform Disclosure Form for Potential Conflicts of Interest. All authors declare that they have no conflicts of interest.

Data sharing statement

All data generated or analyzed in the present study are included in the published article. Further details are available for non-commercial purposes from the corresponding author upon reasonable request.

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