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Prevalence of *Plasmodium* Parasitaemia and Diagnostic Accuracy of Malaria Rapid Diagnostic Tests in Sabongidda-Ora, Edo State, Nigeria

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ABSTRACT

Accurate, timely diagnosis underpins effective case management wherever malaria is endemic, yet many rural health facilities in sub-Saharan Africa lack the trained personnel and equipment that microscopy requires. Rapid diagnostic tests (RDTs) offer an alternative, but their field performance can vary by brand, storage conditions, and the population tested. We evaluated a Histidine-Rich Protein 2 (HRP2)-based RDT against thick blood-film microscopy in 100 febrile adults attending the Michael Imoudu Comprehensive Health Care Centre in Sabongidda-Ora, Edo State, Nigeria, over May–June 2025, a period of peak transmission — a rural setting for which diagnostic performance data have not previously been reported. *Plasmodium* parasitaemia was detected in 59 of the 100 participants by RDT and in 56 of the 100 participants by microscopy. Taking microscopy as the reference standard, the RDT missed none of the 56 confirmed cases (sensitivity 100%) and correctly cleared 41 of 44 uninfected participants (specificity 93.2%), yielding a positive predictive value of 94.9%, a negative predictive value of 100%, and an overall agreement of 97% with microscopy. These findings indicate that, in a high-transmission, symptomatic adult population, HRP2-based RDTs can detect malaria as reliably as microscopy at the point of care. Given that specificity fell short of 100%, we recommend that microscopy be retained alongside RDTs for confirmatory testing and surveillance, particularly where treatment decisions carry downstream costs. Because these results come from a single facility during one transmission season, their generalisability to low-transmission or asymptomatic populations should be tested in further studies.

Keywords: Malaria, *Plasmodium* Parasitaemia, Rapid Diagnostic Test, Microscopy, Diagnostic Accuracy, Prevalence

INTRODUCTION

Malaria is an infectious disease caused by protozoan parasites of the genus *Plasmodium* transmitted to humans through the bite of an infective female *Anopheles* mosquito (Talapko *et al.*, 2019; Braimah *et al.*, 2024). Of the five *Plasmodium* species known to infect humans – *P. falciparum, P. vivax, P. malariae, P. ovale,* and *P. knowlesi* – *P. falciparum* is responsible for the majority of malaria cases of illness and deaths in sub-Saharan Africa (World Health Organisation, 2022b; Venkatesan, 2024). The burden of this disease is no stranger to human history, as it is believed to have contributed to the collapse of multiple ancient civilisations and is attributed to the death of historical personalities such as Alexander the Great (Talapko *et al.*, 2019).

The burden of this disease remains global; in 2022, the WHO estimated 249 million cases of malaria worldwide and 608,000 deaths, with the majority in the African Region (Audu *et al.*, 2024; Venkatesan, 2024). Nigeria carries the biggest burden globally, with about a quarter of global cases and nearly a third of global deaths (Bassey *et al.*, 2024; World Health Organisation, 2022a, 2022b). The malaria burden varies within Nigeria, depending on the geopolitical zone, and the diagnosis becomes complicated when malaria co-exists with other febrile illnesses, such as typhoid fever, that present similar symptoms (Olowolafe *et al.*, 2024).

Definitive diagnosis of malaria is achieved through the demonstration of parasites or their antigens in the blood. The gold standard for diagnosis is light microscopy of a Giemsa- or Fields-stained blood film, which enables identification of the species as well as parasite density estimation (Moody, 2002; Talapko *et al.*, 2019). However, microscopy is labour-intensive and requires well-trained microscopists and functional laboratory infrastructure, which are frequently unavailable in resource-limited, rural health facilities across sub-Saharan Africa (World Health Organisation, 2024). Malaria rapid diagnostic tests (RDTs), which detect parasite antigens such as histidine-rich protein 2 (HRP2), *Plasmodium* lactate dehydrogenase (pLDH) or aldolase, were developed to overcome these limitations and now form the backbone of malaria case management at the point of care (Moody, 2002; Adebola *et al.*, 2023).

Though user-friendly, RDTs can produce false-negative results at low parasite densities and false-positive results due to persistent antigenaemia or cross-reactivity, and may have variable sensitivity and specificity due to batch-to-batch variability, inappropriate storage, or *hrp2/3* deletions in some *P. falciparum* populations (World Health Organisation, 2024; Apinjoh *et al.*, 2024). Therefore, quality control and quality assurance using a second, confirmatory test such as microscopy is recommended (World Health Organisation, 2024). Although several Nigerian studies have evaluated malaria prevalence and RDT performance (Akinbo *et al.*, 2009; Okonko *et al.*, 2010; Ilesanmi *et al.*, 2017; Iwuafor *et al.*, 2018; Adebola *et al.*, 2023; Braimah *et al.*, 2024; Adeyemi *et al.*, 2025), data specific to Sabongidda-Ora, a rural community in Owan West Local Government Area of

Edo State, remain scarce. This study therefore aimed to determine the prevalence of *Plasmodium* parasitaemia and evaluate the performance of HRP2-based malaria RDTs, compared with microscopy, among febrile adults presenting at the Michael Imoudu Comprehensive Health Care Centre, Sabongidda-Ora, Edo State, Nigeria.

MATERIALS AND METHODS

Study Area

The study area is the Michael Imoudu Comprehensive Health Care Centre (MICH), the major secondary health facility located in Sabongidda-Ora (Ebviobe-Ora in the native dialect), the headquarters of Owan West Local Government Area (LGA) of Edo State, Nigeria. Sabongidda-Ora is located at Latitude 6.90231° North and Longitude 5.93117° East (Mapcarta, 2024), and the landmass of Owan West LGA is approximately 732 km² (National Population Commission, 2006). It has a tropical climate with two distinct seasons – a rainy season that spans from April to October, and a dry season that spans from November to March (harmattan); mean annual rainfall of about 1,700 mm; mean temperature ranging from 25-31°C; and a mean relative humidity of 75-85% (Amaechi *et al.*, 2025). The population of Owan West LGA as of the national population census of 2006 was 97,388 (National Population Commission, 2006).

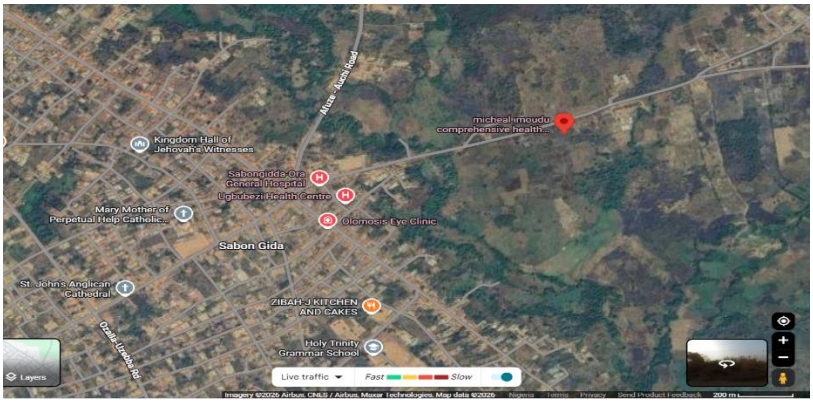


Figure 1: Map of Edo State showing the location of the study area, Sabongidda-Ora, Owan West Local Government Area

Study Population

The target population for this study was adults aged 18 years and above who presented with febrile symptoms and attended MICH in Sabongidda-Ora, Edo State, between May 2025 and June 2025.

Inclusion and Exclusion Criteria

Inclusion Criteria

Adults aged 18 years and above who attended MICH during the study period, had febrile illness (axillary temperature of ≥37.5°C, or reported fever within the last 48 hours), and provided informed consent were eligible for inclusion in the study.

Exclusion Criteria

Those who had taken antimalarial medication within the last two weeks prior to recruitment, pregnant women, and those who did not provide consent were excluded from the study.

Sample Size

A Cochran formula for sample size estimation was used to determine the minimum sample size for this study with a confidence level of 95% (Ajireloja, 2023):

$n = Z^2p\ (1-p) / e^2$

where n is the minimum sample size; Z is the standard normal deviate at the 95% confidence level (1.96); p is the estimated proportion of the attribute of interest in the population, assumed at 50% (0.5) in the absence of a locally available prevalence estimate, so as to obtain the maximum sample size variance; q is 1 - p (0.5); and e is the desired margin of error, set at 10% (0.1).

Substituting these values:

$n = (1.96)^2 \times 0.5 \times 0.5 / (0.1)^2 = 3.8416 \times 0.25 / 0.01 = 96.04 \approx 96$

The minimum calculated sample size was therefore 96. This was rounded up to 100 participants to compensate for possible sampling errors, incomplete records or dropouts.

Sampling Technique

Participants were recruited using consecutive sampling until the desired sample size of 100 participants was reached. All febrile adults attending MICH during the study period who fulfilled the inclusion criteria were invited to participate in the study. This method has less potential for selection bias compared to convenience sampling.

Ethical Approval

Ethical approval to conduct the study was sought and obtained from the Health Research Ethics Committee (HREC) of Ambrose Alli University, Ekpoma (HREC number 150/24).

Informed Consent

Prior to enrolment and sample collection, written informed consent was obtained from all participants. The details of the study, including the purpose, procedures, and potential risks and benefits, were explained in either English or the local dialect, and only those individuals who agreed to participate and provided their consent with a signature (or thumbprint) on the consent form were enrolled in the study.

Research Design

The study design selected for this study was a cross-sectional study design to assess the prevalence of malaria as well as to assess the sensitivity and specificity of the frequently used rapid diagnostic test kit (RDT) for the diagnosis of Malaria in Sabongidda-Ora, Edo State, Nigeria.

Sample and Data Collection
Four millilitres (4 mL) of venous blood were collected from each participant by venipuncture using a sterile Vacutainer needle and evacuated tube system into a labelled, five-millilitre (5 mL) ethylenediaminetetraacetic acid (EDTA) container. Each sample was gently mixed by inversion to prevent clotting and labelled with the participant's identification number and the date of collection. Demographic data such as age and gender were collected from sampled participants.

Analytical Method for Rapid Diagnostic Test Kit
The RDT was performed within one hour of blood collection, following the manufacturer's instructions. One drop (approximately 5 µL) of venous blood was dispensed into the sample well of the RDT cassette using the dropper provided with the kit, followed by four drops (approximately 60 µL) of the assay buffer to facilitate the lateral migration of the blood across the nitrocellulose test strip. The test was allowed to stand for 15-20 minutes before the result was read.
Results were interpreted according to the manufacturer's recommendations for this type of lateral-flow malaria cassette: the appearance of both the control line and test line (even if faint) was considered a positive result for malaria; the absence of a test line in the presence of a control line was considered a negative result for malaria. Any cassette that failed to produce a control line was considered to be a faulty cassette, and the test was repeated on a new cassette. Used cassettes were disposed of according to biohazard waste disposal procedures at the facility.

Analytical Method for Thick Blood Film
A thick blood film was prepared from a drop of the venous blood sample on a grease-free microscope slide. It was left unfixed, allowed to air-dry and then stained with Field's stain by dipping the slide in Field's stain A for 1-2 seconds, rinsed gently in clean water and dipped in Field's stain B for 1 second, rinsed again and air-dried in an upright position.
Stained slides were examined by an experienced microscopist using a binocular compound light microscope (x100 oil-immersion objective), and all positive and 10% of negative slides were re-examined by a second microscopist for quality control purposes. A slide was considered negative after examining at least 100 high-power fields, and no parasite was seen.

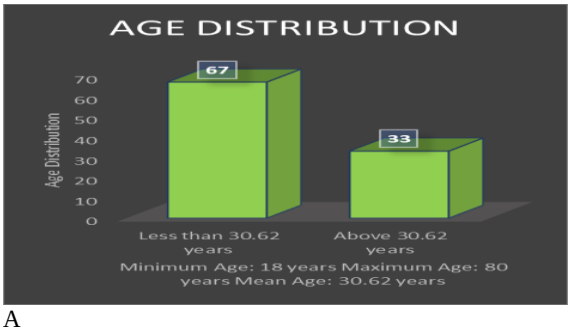
Test Principle and Quality Control
The malaria RDTs used in this study were immunochromatographic lateral-flow assays that detect *Plasmodium falciparum* histidine-rich protein 2 (HRP2), one of the antigens most commonly targeted by commercial malaria RDT kits, alongside *Plasmodium* lactate dehydrogenase (pLDH) and aldolase, which are targeted by other kit formats (Moody, 2002). To ensure quality control, the expiry date on each kit was checked before use, as expired kits can yield unreliable results. The kits were stored at room temperature away from direct sunlight and humidity according to the manufacturer's recommendations, as exposure to these conditions could affect the reagents and compromise the tests.
The thick film concentrates blood and makes it easier to detect the presence of parasitaemia. Field staining is a modification of Romanowsky stain in which polychromatic stain stains the nucleic acids and cytoplasmic elements of the parasite, enabling *Plasmodium* spp. to be recognised under the microscope (blue-purple colouration) (Moody, 2002; Talapko *et al.,* 2019).

Diagnostic Accuracy
To evaluate the performance of the RDT, sensitivity, specificity, positive predictive value, negative predictive value, and the overall accuracy of the RDT were calculated using microscopy as a comparison method.

Statistical Analysis
Statistical Package for the Social Sciences (SPSS 21) software was used to analyse and process the data. The prevalence of infection was determined as the number of positive individuals per total individuals examined multiplied by 100. The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy of the RDT were estimated in comparison to microscopy results. To evaluate the discriminatory performance of the RDT, the area under the curve (AUC) was calculated using a receiver operating characteristic (ROC) curve analysis. The association between RDT and microscopy results was determined using a chi-square ($\chi 2$) test. Statistical significance was defined as a probability value of < 0.05. Demographic data were evaluated using descriptive statistics.

RESULTS AND DISCUSSION

Demographic Characteristics of the Study Population
A total of 100 participants were enrolled. Figure 2 shows the age and gender distribution of the study participants.
The age range was between 18 and 80 years, with a mean age of 30.62 years. More than two-thirds (67%) of the population were under 30.62 years old, and one-third (33%) of the population was over 30.62 years of age, indicating that the sample was predominantly a younger population (Figure 2A). The gender distribution comprised 33 males (33%) and 67 females (67%); more than two-thirds of the population (67%) were female, while one-third (33%) were male (Figure 2B).



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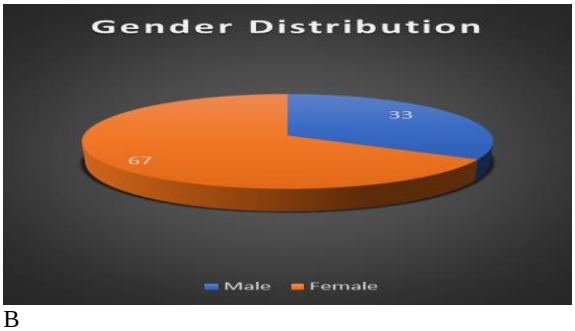

B

Figure 2: (A) Age distribution among participants. (B) Gender distribution among participants

Prevalence of *Plasmodium* Parasitaemia according to Diagnostic Method

Table 1 shows that malaria prevalence was 59% by rapid diagnostic test (RDT) and 56% by microscopy. A significant association was observed between RDT and microscopy results ($\chi^2 = 88.44$, $p < 0.001$), indicating strong agreement and high diagnostic reliability of the RDT compared with microscopy.

Table 1 presents the prevalence of *Plasmodium* parasitaemia among the study participants as detected by RDT and microscopy.

Table 1: Prevalence of *Plasmodium* Parasitaemia according to Diagnostic Method

Diagnostic Method	Number Tested	Number Positive	Number Negative	Prevalence (%)	X^2	df	p-value
Malaria RDT	100	59	41	59	88.44	1	0.000
Microscopy	100	56	44	56	-	-	-

Diagnostic Accuracy of the Malaria RDT Compared with Microscopy

Table 2 presents the cross-tabulation of RDT and microscopy results, together with the derived diagnostic indices of the RDT.

The malaria RDT showed an excellent diagnostic performance compared to microscopy (Table 2). RDT had a sensitivity of 100%, detecting 56 positive samples with no false negatives (Table 2; Figure 3), indicating that the RDT performed well as a screening test for malaria infection, as it did not miss any positive samples (malaria cases). RDT also had a high specificity of 93.2% in identifying negative samples.

The RDT had a positive predictive value (PPV) of 94.9%, indicating that a positive test result was likely to be truly positive, and a negative predictive value (NPV) of 100%, indicating that a negative test result could be interpreted with full confidence as a true negative. The overall diagnostic accuracy was 97%, indicating a high level of agreement between the RDT and microscopy results.

Receiver-Operating Characteristic (ROC) curve analysis was performed to further evaluate the diagnostic accuracy of the RDT (Figure 4). The RDT showed excellent accuracy in discriminating between malaria-positive and malaria-negative samples, with an area under the curve (AUC) of 0.95. A value of 1.0 for the area under the ROC curve represents a perfect test. The difference was statistically significant ($p < 0.001$).

Table 2: Cross-tabulation of RDT and Microscopy Results, and Diagnostic Indices of the RDT

	RDT Positive	RDT Negative	Total
Microscopy Positive	56	0	56
Microscopy Negative	3	41	44
Total	59	41	100
Sensitivity	Specificity	PPV	NPV
100%	93.2%	94.9%	100%
True Positive	True Negative	False Positive	False Negative
56	41	3	0
Overall diagnostic accuracy: 97%			

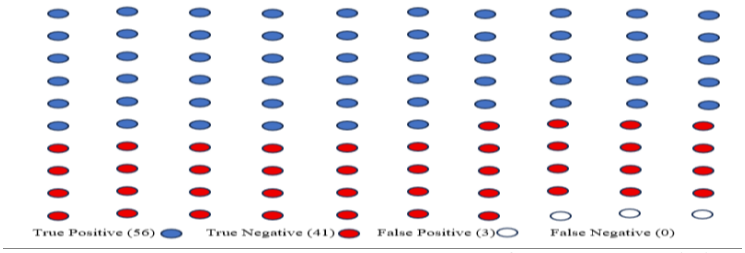

Figure 3: Personograph showing the distribution of participants' Malaria Parasite Test results

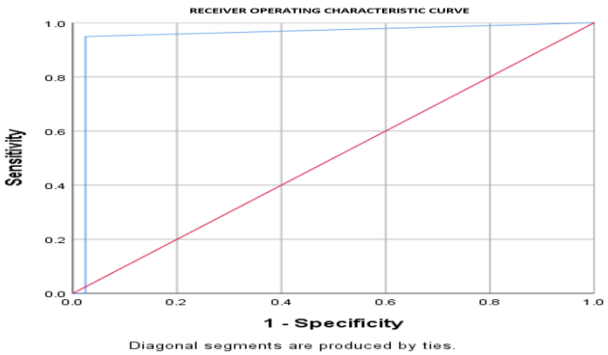

Figure 4: Receiver Operating Characteristic (ROC) curve of the RDT Malaria Parasite Test against Microscopy

Discussion

This study revealed a malaria prevalence of 56% by microscopy and 59% by RDT among symptomatic adults attending a health care facility in Sabongidda-Ora, indicating high transmission intensity and a massive reservoir of malaria parasites in the community. It is similar to the results obtained among adults in high-burden communities in Nigeria during the rainy season (Okonko *et al.*, 2010; Odikamnoro *et al.*, 2018), a period when conditions for *Anopheles* mosquito breeding are optimal.

But the prevalence reported by Akinbo *et al.* (2009) among children under five years old with febrile illness in Benin City was higher (75.8%), since younger children have less immunity and higher parasitaemia. Contrarily, Braimah *et al.* (2024) recorded a much lower prevalence of 15.5% in a wider age range of mostly asymptomatic participants from Edo North; hence, a comparison of prevalence rates must factor in age group, symptomatology, setting, and seasonality. This current study targeted febrile adult patients at the peak transmission season and therefore may be the closest representation of the burden of clinically significant malaria in this rural resource-poor community.

Results obtained by microscopy and RDT showed minimal disagreement, which is possibly due to two counteracting factors: RDT can remain positive up to two weeks after parasite clearance due to residual HRP2 antigen following successful treatment (Apinjoh *et al.,* 2024), whereas microscopy may fail to detect very low levels of parasitemia in semi-immune adults with repeated and lifelong exposure to malaria. Thus, semi-immune adults with lifelong exposure may harbour sub-microscopic infections that may only be detected by RDT, highlighting the value of highly sensitive diagnostic tools at the point of care in this population.

None of the 56 microscopy-confirmed cases was missed by the RDT, resulting in a sensitivity of 100%. Results from nearby regions show similar detection rates >99% in South Sudan (Lynch *et al.*, 2022) and southwestern Nigeria (Adebola *et al.,* 2023). Much lower sensitivities (as low as 50% and 51.4%) were reported among febrile infants in other studies (Ilesanmi *et al.*, 2017; Iwuafor *et al.*, 2018). RDTs are more likely to miss infections of low parasite density, which are observed more among infants than adults (Maina *et al.*, 2010), so our sample of adult symptomatic patients may contribute to the higher sensitivity observed in this study. In a context like Sabongidda-Ora where a missed case could rapidly progress to severe illness and death without detection, this index of sensitivity is the most clinically relevant of the four presented here.

Specificity was 93.2%, indicating the RDT incorrectly identified approximately 3 of 100 malaria-negative individuals as positive for malaria infection. This is similar to specificity values for other HRP2-based RDTs evaluated in Nigeria and other parts of West Africa (Adebola *et al.*, 2023; Lynch *et al.*, 2022). False positive test results likely reflect persistent antigenemia among patients recently treated for malaria and seeking care for febrile illness, which is common in endemic areas (Apinjoh *et al.*, 2024). Given that the RDT specificity is less than 100%, careful use of microscopic diagnosis is recommended for patients who test RDT-positive to avoid unnecessary antimalarial drug use among those who may be falsely positive, particularly if symptoms persist and treatment failure is suspected.

The positive predictive value suggests that 94.9% of positive test results are true positives, while the negative predictive value indicates that a negative result is an accurate reflection of the absence of infection in this high-prevalence population. The authors emphasise that these results, obtained when the prevalence of malaria was 59%, cannot be generalised to low-transmission settings. The area under the curve for the ROC and the overall accuracy of the rapid diagnostic test were 0.95 and 97%, indicating excellent overall performance of the RDT in distinguishing positive from negative malaria infection compared to the traditional gold standard, which aligns with other point-of-care test assessments (Lynch *et al.*, 2022).

Taken together, these findings highlight the usefulness of quality-assured HRP2-based RDT for malaria case detection at the primary health care level.

CONCLUSION

This study investigated the prevalence and diagnostic accuracy of *Plasmodium* parasitaemia detection among symptomatic adults residing in Sabongidda-Ora, Edo State. The findings point to a significant burden of malaria in this rural, resource-limited community, with a prevalence of 59% by RDT and 56% by microscopy, underscoring malaria's role as a common cause of febrile illness in the area. The malaria RDT demonstrated excellent sensitivity, specificity and overall diagnostic accuracy relative to microscopy, supporting its continued use as a reliable point-of-care screening tool within the primary healthcare system, while microscopy should be retained for confirmatory testing and quality assurance. Because these results come from a single facility during one transmission season, their generalisability to low-transmission or asymptomatic populations should be tested in further studies.

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