



## RESEARCH ARTICLE

# Efficacy of *Plasmodium falciparum* histidine-rich protein 2 (*Pfhrp 2*) rapid diagnostic test (RDT) and microscopy in the detection of falciparum malaria among symptomatic patients in Akure, Nigeria

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### ABSTRACT

Accurate diagnosis and prompt treatment are highly essential in the management of malaria, which is one of the deadliest infectious diseases worldwide, particularly in tropical and sub-tropical regions including Nigeria. This study was designed to evaluate the efficacy of malaria histidine-rich protein 2-based rapid diagnostic test (RDT) and microscopy in the diagnosis of falciparum malaria in Nigeria. This was a cross-sectional and hospital-based study. The standard method of microscopy was used as the gold standard. Giemsa stained thick and thin smears were prepared to count and detect malaria parasite species. Also, a malaria histidine-rich protein 2-based RDT was used to detect malaria parasites and diagnostic efficacy were determined through the measure of sensitivity, specificity, Positive Predictive Value (PPV), Negative Predictive Value (NPV), diagnostic accuracy and Youden Index (J). The result showed that out of the total 303 individuals examined, a total malaria prevalence of 67.0% and 68.0% were recorded for microscopy and RDT, respectively. Additionally, the sensitivity (95% C.I.), specificity (95% C.I.), PPV (95% C.I.), and NPV (95% C.I) of RDT compared to microscopy were 97.54 (94.36-98.94), 92.00 (85.00-95.89), 96.12 (92.53-98.02), and 94.85 (88.50-97.78), respectively. The diagnostic accuracy (95% C.I) and Youden Index (J) were 95.71 (92.77-97.70) and 0.89, respectively. Conclusively, our study revealed that RDT continues to remain efficacious. Thus, while malaria diagnosis by microscopy which is the gold standard remains the major method of malaria detection, it should be complemented by rapid diagnostic test (RDT), particularly in high malaria endemic regions where mean parasite density of patients are usually high.

**Keywords:** Diagnosis; microscopy; Nigeria; sensitivity; specificity.

### INTRODUCTION

Malaria remains a life-threatening infectious disease of major public health problem worldwide. Malaria accounts for significant morbidity and mortality rate among children and pregnant women. According to the World Health Organization (WHO), there were an estimated 229 million cases associated with 409 000 deaths reported in 2019 (WHO, 2020). Similarly, in Nigeria, malaria continues to pose serious public health challenge and accounts for approximately 27% of all malaria burden worldwide (WHO, 2019; Awosolu *et al.*, 2021).

Malaria diagnosis remains a significant problem in malaria control and elimination since the efficacy of diagnostic tools varies in different epidemiological settings (Mahende *et al.*, 2016). Despite this, early and accurate malaria diagnosis and treatment remains highly essential to malaria prevention and control in malaria endemic

countries (WHO, 2015, 2020). In order to ensure proper, accurate and fast malaria diagnosis for prompt treatment and management control, the World Health Organisation (WHO) recommended confirmatory diagnosis of malaria by microscopy which remains the gold standard and malaria rapid diagnostic test for point of care diagnosis prior to antimalarial treatment (WHO, 2013). Meanwhile, malaria histidine-rich protein 2 RDT is one of the major rapid diagnostic tests approved into the Nigeria national malaria treatment guideline for the diagnosis of malaria disease at the point of care in Nigeria, and there is a need for constant monitoring of its efficacy (Isiguzo *et al.*, 2014). Regarding the use of microscopy, it is burdensome as it requires a lot of work and energy coupled with the fact that it is time consuming and requires high expertise and training. Thus, microscopy is mostly used in major clinics and centres in Nigeria. However, RDT is fast and easy to use. Popular malaria antigens employ in malaria RDTs are histidine-rich

protein-2, parasite-specific lactate dehydrogenase (pLDH), and the aldolase (Bell *et al.*, 2006). One major difference is that histidine-rich protein-2 is a *P. falciparum*-specific antigen meanwhile parasite-specific lactate dehydrogenase (pLDH) can be used to detect pLDH from all four species of *Plasmodium*, but unable to specifically distinguish between *P. vivax*, *P. malariae* and *P. ovale* (Bell *et al.*, 2006; Abba *et al.*, 2011). RDTs that detect pLDH do not produce persistent positive results after malaria treatment as in the case of malaria histidine-rich protein 2 test.

In spite of the fact that RDT is rapid and easy to employ in malaria detection, it has been observed to be inefficacious in detecting malaria disease with low parasitaemia level. Also, false positive results have been documented as a result of malaria antigen persistence in the blood even after successful treatment. Similarly false negative has been noted due to *Plasmodium falciparum* histidine-rich protein 2 deletions (Kumar *et al.*, 2013; Pati *et al.*, 2018). Currently, the performance of malaria histidine-rich protein 2 RDT is largely unknown in Akure, Southwestern Nigeria. Thus, this study aimed to elucidate the efficacy of malaria histidine-rich protein 2 rapid diagnostic test (RDT) and blood smear microscopy in the detection of falciparum malaria in Akure, southwestern Nigeria.

## MATERIALS AND METHODS

### Ethical protocol

Prior to commencement of the study, ethical approval was obtained from Ondo State Ministry of Health with reference number OSHREC/21/08/2017/012. Written and oral informed consent were sought from each of the participants and caregiver or guardians after detailed explanation of the study protocol and procedures coupled with the risk and benefits of the study. All confirmed malaria positive individuals were treated according to the national guidelines.

### Study Area

The study was conducted at the Federal University of Technology, Akure Health Centre and Orita Obele Basic Health Centre which are located in Akure, Ondo State, Nigeria. Generally, Akure is doubling as a city and capital of Ondo State with a population projection of 665,524 in year 2020. Similarly, the climatic condition is typical of tropical regions which is made up of rainy and dry seasons that cut across from April to October and November to March, respectively. The average annual rainfall is 2378 mm, with temperatures ranging from 25.2°C to 28.1°C and relative humidity of about 80% (Geonames Geographical Database, 2021). Malaria disease is transmitted throughout the year. It is aided by the climatic condition which is typical of tropical region with appropriate temperature, rainfall and breeding sites for the development of mosquito vector which actively transmit malaria disease in the area.

### Study Design

A cross-sectional and hospital-based randomised study was carried out at the Federal University of Technology Akure Health Centre and Orita Obele Basic Health Centre. All symptomatic patients visiting the outpatient department of the health centre were included as potential participants once they meet the inclusion criteria. The inclusion criteria for selection include feelings of headache, fever with temperature  $\geq 37.5^\circ\text{C}$ , completion of questionnaires, willingness to submit blood samples and written or oral informed consent.

### Data collection and sample size determination

A structured questionnaire which has been pretested was given to each patient participating in study so as to obtain important data on their sex, age, and location. This information was obtained through a face-to-face interview with the participants. The sample size was obtained following the statistical formula given by Centres for Disease Control and Prevention (CDC, 2005). Using the previous malaria parasite prevalence of 84.20% and confidence interval (C.I) of 95% with a precision level of 5%, a minimum sample of 207 samples were required. However, in order to accommodate likely statistical error, 303 samples were examined in the study area.

### Blood sample collection and laboratory techniques

Venous blood samples were obtained with the assistance of a well-trained laboratory technician. Approximately 2-3mL venous blood was obtained through a sterile needle and syringe. The blood samples were transferred into an ethylenediaminetetraacetic acid (EDTA) bottle in order to protect and preserve the blood against coagulation. The blood samples were later taken to Department of Biology, Federal University of Technology, Akure for thick and thin smear preparation and staining. Meanwhile, the malaria rapid diagnostic test was conducted on the venous blood in the health centre to detect malaria disease using a common malaria rapid diagnostic test kit and following the guidelines provided by the manufacturer. The test kits were kept at optimal temperature between 2°C–30°C and the results were read after 20–25 min. Thereafter, thick and thin smears were made on slides that have been properly cleaned and disinfected. In order to fix the thin film, absolute ethanol was applied and subsequently, the thick and thin films were stained with 10% Giemsa stain and allowed to air dry in the laboratory for about 15 minutes. These slides were later examined under the light microscope using the x100 objective lens so as to count and identify *Plasmodium* species (Cheesbrough, 2006). Two different well-trained microscopists confirmed the malaria infection. Any discrepancy observed was reconfirmed by a senior expert microscopist and the final result recorded accordingly. Additionally, participants were recognised to have malaria disease when the positive RDT results were well confirmed positive by microscopy. Similarly, those who were positive by RDT but negative by microscopy were considered negative since RDT may lead to false positive results among participants due to presence of *Plasmodium* antigen in blood long after complete parasite clearance. In order to determine the level of parasitaemia that can be detected by RDT, the parasite density was classified to  $<100$  parasite/ $\mu\text{L}$  of blood and  $\geq 100$  parasite/ $\mu\text{L}$  of blood.

### Statistical analysis

Data entry and analyses were conducted through Statistical Package for Social Sciences (SPSS) version 22.0. The presence or absence of malaria disease was computed and the variance in the prevalence between categorical variables were calculated using chi-square at 95% confidence level. The sensitivity, specificity, Positive Predictive Value (PPV), Negative Predictive Value (NPV), diagnostic accuracy and Youden Index (J) were calculated by using the GraphPad Prism 8. *p*-values less than 0.05 were considered statistically significantly. Computations were carried out using the contingency table. The total accuracy was computed through the formula: Accuracy =  $(TP + TN)/(TP + FP + FN + TN)$ , where TP = true positive, TN = true negative, FN = false negative,

and FP = false positive. Youden's J index is the probability that a positive result gives the true condition or not and it combines sensitivity and specificity to form a single measure. The formula is given as Sensitivity + Specificity – 1, the value ranges between 0 to 1. When a test is perfect, Youden's J index =1.

## RESULTS

A total of 303 symptomatic individuals were examined, out of which males were 152 (50.20%) and females were 151 (49.80%). The age of the participants ranges from 10 years to 56 years with a mean of 23 years. Similarly, participants aged < 20 years were 120 (39.6%) while those ≥20 years were 183 (60.4%) (Table 1). The results showed that a total of 203 (67%) and 206 (68%) participants were positive of malaria disease by microscopy and RDT, respectively (Tables 2).

Figure 1 showed the venn comparing malaria prevalence by microscopy and RDT. The results showed that both microscopy and RDT detected a total malaria prevalence of 198 (65.34%) out of the total 303 participants. Similarly, while microscopy alone detected a malaria prevalence of 5 (1.65%), RDT alone detected a higher malaria prevalence of 8 (2.64%).

Table 3 details the diagnostic performance of RDT while microscopy was used as a reference standard. The result showed that malaria RDT detected a true positive of 96.11%, false positive of 3.88%, true negative of 94.84%, and false negative of 5.15%. Similarly, the sensitivity (95% C.I) and specificity (95% C.I) of RDT compared to microscopy were 97.54 (94.36-98.94) and 92.00 (85.00-95.89), respectively. Also, the Positive Predictive Value, PPV (95% C.I) and the Negative Predictive Value, NPV (95% C.I) were 96.12 (92.53-98.02) and 94.85 (88.50-97.78), respectively. Meanwhile, the accuracy (95% C.I) and Youden's j index were 95.71 (92.77-97.70) and 0.89, respectively. The relationship was statistically significant with  $p < 0.0001$ .

Table 4 details the malaria prevalence by microscopy and RDT with respect to parasite density classification based on <100 parasite/μL and ≥100 parasite/μL. Obviously, microscopy detected 5 sample that were <100 parasite/μL of blood and 198 samples that were ≥100 parasite/μL. Similarly, RDT detected 198 samples that were ≥100 parasite/μL of blood. However, RDT did not detect any sample that was <100 parasite/μL of blood in this study.

## DISCUSSION

This study provided one of the first baseline information on the performance of a common malaria histidine-rich protein 2 rapid diagnostic test (RDT) and microscopy in the detection of falciparum malaria in Ondo State, Nigeria. Our findings showed that the malaria prevalence by microscopy which is recognised worldwide as the gold standard was 67.0%. This is corroborated by the report of the World Health Organization that malaria continues to be a major public health burden in tropical regions particularly, in low- and middle-income countries and this requires a high burden to high impact (HBHI) country-led approach (WHO, 2020). The high malaria prevalence in this study is consistent with previous studies conducted in Akure and other parts of Nigeria (Awosolu *et al.*, 2021). The malaria prevalence of 67% by the gold standard method of microscopy recorded in this study is higher than the report of Oladosu and Oyibo (2013), Dada *et al.* (2016), Dawaki *et al.* (2016) and Ezedudu *et al.* (2016) who reported a malaria prevalence of 20.7% in Lagos, 20.7% in Akure, 60.6% in Kano and 20.0% in Newi, respectively. Similarly, other studies have reported lower

**Table 1.** General characteristics of the study participants (n=303)

Parameters	Number (N)	Percentage (%)
Age groups (years)		
<20	120	39.6
≥20	183	60.4
Sex		
Female	152	50.2
Male	151	49.8
Total	303	100

**Table 2.** Two by two table of malaria prevalence by RDT and microscopy

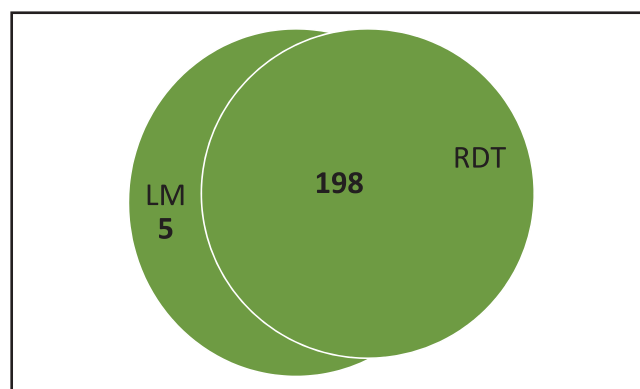
		Microscopy		Total
		Positive	Negative	
RDT	Positive	198 (97.5)	8 (8.0)	206 (68)
	Negative	5 (2.5)	92 (92.0)	97 (32)
Total		203 (67)	100 (33)	303 (100)

**Table 3.** Diagnostic accuracy of RDT using microscopy as standard

Test Variables	Rapid Diagnostic Test
TP (Microscopy=203)	198 (96.11%)
FP (Microscopy negative)	8 (3.88%)
TN (Microscopy=100)	92 (94.84%)
FN (Microscopy positive)	5 (5.15%)
Sensitivity (95% C.I)	97.54 (94.36–98.94)
Specificity (95% C.I)	92.00 (85.00–95.89)
PPV (95% C.I)	96.12 (92.53–98.02)
NPV (95% C.I)	94.85 (88.50–97.78)
Accuracy (95% C.I)	95.71 (92.77–97.70)
Youden Index (J)	0.89
p-value	<0.0001

**Table 4.** Malaria prevalence by microscopy and RDT with respect to parasite density classification based on <100 parasite/μL and ≥ 100 parasite/μL

	Parasite density classification	
	<100 parasite/μL	≥ 100 parasite/μL
Microscopy	5	198
RDT	0	198



**Figure 1.** Venn diagram comparing malaria prevalence by light microscopy (LM) and *Plasmodium falciparum* histidine-rich protein 2 rapid diagnostic test (*Pfhrp 2* RDT).

malaria prevalence within Nigeria and in other countries (Wang *et al.*, 2005; Uneke *et al.*, 2006; Baragatti *et al.*, 2009; Amala & Nwibani, 2015; Garba *et al.*, 2016). On the other hand, higher malaria prevalence has been documented by Nzeako *et al.* (2013), Udoh *et al.* (2013) and Ayogu *et al.* (2016) who reported a malaria prevalence of 72.5% in Rivers State, 71.4% in Cross River State and 85.7% in Enugu State, respectively. This difference in malaria prevalence could be attributed to the variation in climatic factors such as rainfall, temperature and humidity, presence or absence of mosquito breeding sites, low socio-economic status and disparity in malaria management, prevention and control interventions in these areas.

The malaria prevalence determined by RDT in this study was slightly higher than that of microscopy (67% vs 68%) without any statistically significant difference ( $p > 0.05$ ). This could be ascribed to false positive results of RDT obtained due to the presence of residual circulating malaria antigen which can remain in the blood up to 28 days after malaria parasites have been completely cleared among patients with recent history of malaria infection, and this is supported by many previous reports (Iqbal *et al.*, 2004; Maltha *et al.*, 2013; Girma *et al.*, 2019). Moreover, our findings revealed that RDT detected a high true positive and true negative of 96.11% and 94.84% which resulted in high sensitivity and specificity of 97.54% and 92.00%, respectively. Meanwhile, the RDT was observed to be more sensitive than being specific. This is consistent with the WHO recommendation of 95% sensitivity for malaria RDT, however, the specificity is lower than the recommended 97%. The sensitivity in our findings is higher than the sensitivity of 89% reported by Ishengoma *et al.* (2011) in Tanzania. Similarly, Landier *et al.* (2018), Hofmann *et al.*, (2018) and Girma *et al.* (2019) reported a lower sensitivity of 36.6% in Myanmar, 51% in Papua New Guinea and 33.9% in Ethiopia, respectively. The high sensitivity recorded in this study could be due to the fact that our study examined symptomatic patients with fever  $\geq 37.5^\circ\text{C}$  which is likely associated with high parasite density which are easily detectable by RDT. It has been confirmed by several reports that RDT can better detect high-density malaria infection but poor performance in detecting low-density malaria infection, particularly when parasite density is below 100 parasite/ $\mu\text{L}$  of blood (Abeku *et al.*, 2008; McMorrow *et al.*, 2011). Thus, low parasite density has greatly resulted in low performance of RDT. Interestingly, our findings further showed a low false positive of 3.88% and false negative of 5.15% RDT results. Thus, RDT could not detect 5 (5.15%) samples that were positive by microscopy and some factors could likely be responsible for this false negative results. Though, this study could not precisely ascertain the possibility of *P. falciparum* histidine-rich protein 2 (pfhrp 2) gene deletion, the available data revealed that such gene deletion is unlikely. Instead, this could be most likely ascribed to low parasite density whereby RDT could not detect malaria infection with low parasite density below 100 parasite/ $\mu\text{L}$  of blood and is in congruent with previous studies (Abeku *et al.*, 2008; McMorrow *et al.*, 2011). There is great implication associated with false positive and false negative RDT results in endemic regions. False positive RDT result could become a major public health problem as those who are negative for malaria infection may be treated, thereby jeopardising antimalarial drug and may lead to drug resistance. Moreover, false negative result could lead to non-treatment of malaria-infected individuals which could cause the malaria disease to become severe and subsequently lead to a fatal outcome if the malaria infection was not detected and treated early enough.

In view of our findings, it could be recommended that while RDT in this study had a high sensitivity detection level, it should be used along with the gold standard method of microscopy for better detection and treatment of malaria-infected individuals (FMOH 2015). However, RDT alone can be used in rural setting, particularly in low- and middle-income countries where state-of-the-art laboratory, electricity and experts for microscopy are lacking. This will give opportunity for prompt detection of malaria parasites and ultimately prevent drug abuse and parasite resistance. While RDT could be very useful for fast and easy detection of malaria parasites, some factors are known to reduce efficacy which need to be taken into consideration when RDT is being considered for local detection. These factors encompass the effect of temperature and humidity during transport and storage, quality assurance, parasite density and species, and recent history of malaria infection and treatment. Similarly, it has been reported that histidine-rich protein 2 deletion could also lead to false negative result which may lead to underdiagnosis and subsequently high morbidity and mortality rates (WHO, 2020). As such, there is constant need for monitoring the performance of RDT in order to ensure accurate diagnosis and prompt treatment, particularly in malaria endemic regions such as Nigeria. When all these are put into consideration, the goal of eliminating malaria by the year 2030 may not be far-fetched, ultimately leading to a malaria-free world.

## CONCLUSIONS

In conclusion, malaria remains a major public health problem. It is obvious from this current study that malaria histidine-rich protein 2 RDT employed in this study has a high sensitivity and specificity of 97.54% and 92.00%. As such, RDT continues to remain efficacious in high malaria-endemic countries including Nigeria. Thus, while malaria diagnosis by microscopy which is the gold standard method remains the major method of malaria detection, it should be complemented by rapid diagnostic test (RDT) for malaria detection, particularly in resource poor regions, in order to accomplish prompt and accurate malaria diagnosis and treatment. This will help to alleviate the morbidity and mortality effect of malaria disease worldwide.

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## Conflict of Interest

The authors declare that no conflict of interest exist.

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