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PERFORMANCE OF THE HISTIDINE RICH PROTEIN-2 (HRP-2) FOR DIAGNOSING *Plasmodium falciparum* AMONG PREGNANT WOMEN ATTENDING ANTENATAL CLINICS IN GASSOL LOCAL GOVERNMENT AREA (LGA), TARABA STATE, NIGERIA

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ABSTRACT

This study assessed the diagnostic performance of one step malaria antigen *P. falciparum* (HRP-2) among pregnant women attending antenatal clinics at First Referral Hospital and Baba Luka Clinic in Gassol Local Government Area, Taraba State, Nigeria. Three hundred and seventy-four (374) blood samples were collected from participants and tested for malaria parasitaemia using rapid diagnostic tests (RDTs) and microscopy. The HRP-2 diagnostic test had a high diagnostic accuracy, with 100% sensitivity and 99.64% specificity. The positive predictive value (PPV) was 98.97%, and the negative predictive value (NPV) was 100%. The overall accuracy was 99.73%, with an area under curve (AUC) of 99.0% making the HRP-2 a reliable test for malaria detection. This study showed that the one step malaria antigen *P. falciparum* (HRP-2) was a reliable tool in malaria diagnosis among pregnant women in Gassol LGA, Taraba State.

1. Introduction

Malaria is a parasitic disease caused by *Plasmodium* sp. It is one of the parasitic diseases that is of public health concern because it continuously poses a serious public health challenge in sub-Saharan Africa. It accounts for most cases of morbidity and mortality, particularly among pregnant women and young children who are vulnerable groups (WHO, 2022). Malaria remains endemic in Nigeria where *P. falciparum* is the most prevalent of all the species, accounting for roughly 95% of malaria cases (Federal Ministry of Health, 2021). Malaria infection is particularly dangerous in pregnant women due to its association with severe maternal anemia, stillbirth, low birth weight and increased risk of maternal and neonatal mortality (Satapathy *et al*., 2024).

The corner stone of effective malaria management is accurate diagnosis. The microscopic method has been the gold standard for malaria diagnosis, which allows for parasite detection and quantification. This method however requires expertise, electricity, and laboratory infrastructure. All of which may be inadequate in rural and resource limited settings (Wongsrichanalai *et al.*, 2007). Rapid diagnostic tests (RDTs) consequently, have emerged as an alternative. They offer the sole benefit of simplicity, speed, and ease of use, in peripheral health facilities and community settings (Moody, 2002). The Histidine - Rich Protein-2 (HRP-2) specifies to *Plasmodium falciparum,* and it is widely used because of its specificity (Martiáñez-Vendrell *et al.*, 2022).

Despite these advantages, growing evidence suggests that the diagnostic performance of HRP-2 based RDTs may be suboptimal in pregnant women, especially in endemic regions. The physiological and immunological changes associated with pregnancy, including altered immune responses and lower peripheral parasitaemia, may contribute to decreased test sensitivity (Bardají *et al.*, 2021). In particular, HRP-2 antigen levels may be below the detection threshold in women with placental malaria or chronic low-grade infections. Moreover, false positives can occur due to the persistence of HRP-2 antigen in the blood for up to several weeks following parasite clearance, leading to overtreatment and unnecessary exposure to antimalarials (Dalrymple *et al.*, 2021).

Another emerging concern is the detection failure due to HRP-2 gene deletions in *P. falciparum* strains. HRP-2 gene deletion leads to an inability of the parasite to produce the HRP-2 antigen, thereby rendering HRP-2 based RDTs ineffective in detecting such infections. Although the prevalence of HRP-2 deleted strains is relatively low in Nigeria.

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The World Health Organization has issued alerts calling for national surveillance and periodic evaluation of diagnostic tools in light of this growing threat (WHO, 2022). The lack of localized data on gene deletions or diagnostic accuracy further complicates the interpretation of RDT results and the planning of control interventions in Nigeria's diverse settings.

In Gassol LGA, where healthcare infrastructure is limited and malaria transmission remains high, healthcare providers rely heavily on RDTs such as the One-Step Malaria Antigen test for clinical decision-making during antenatal visits. However, there is a notable absence of data assessing the diagnostic performance of these RDTs among pregnant women in this region, making it difficult to gauge the reliability of the test results and to develop evidence-based clinical protocols. Without localized validation, there is a risk of underdiagnosis or overdiagnosis, both of which have significant consequences for maternal and neonatal health.

This study was essential in evaluating the diagnostic accuracy of the One-Step HRP-2 based RDT for *P. falciparum* among pregnant women attending ANC clinics at First Referral Hospital and Baba Luka Clinic in Gassol LGA.

2. Materials and methods

2.1 Study area

This study was conducted at the First Referral Hospital and Baba Luka Clinic in Gassol Local Government Area, Taraba State, Nigeria. Gassol LGA is situated at Latitude 8°61'67" N and Longitude 10°76'67" E. The region is characterized by a tropical climate marked by a rainy season, which fosters ideal breeding habitats for *Anopheles* mosquitoes the primary vectors responsible for malaria transmission. The hospitals in this area function as key healthcare institutions, delivering a broad spectrum of services, including antenatal care, to the surrounding population.

2.2 Study Design and Population

A cross-sectional research design was used to assess the diagnostic performance of the Histidine Rich Protein-2 (HRP-2) rapid diagnostic test among the pregnant women attending the antenatal care at First Referral Hospital and Baba Luka Clinic in Gassol Local Government Area, Taraba State, Nigeria.

2.3 Ethical Consideration

Ethical approval for this study was obtained from the Ethics Committee of the Taraba State Ministry of Health (TRSHREC/2024/041). Informed consent was obtained from all participants before their inclusion in the study, and they were assured of confidentiality and anonymity of their responses.

2.4 Inclusion and Exclusion Criteria

The inclusion criteria included all pregnant women who provided informed consent and were willing to participate in the study and the exclusion criteria included women who were not pregnant and those who were pregnant but not willing to fill the informed consent form. The study targeted women across all trimesters of pregnancy to capture a comprehensive picture of malaria prevalence and associated factors throughout pregnancy.

2.5 Sample Size Determination and Collection

The Cochran's formula was used to determine the sample size as follows:

$$\boldsymbol{n = \frac{z^2.p.(1-p)}{d^2}} \quad (1)$$

Where:

n = required sample size,
Z= standard normal deviate at a 95% confidence level (1.96),
P = estimated prevalence of malaria among pregnant women (50%),

d= margin of error (5%).

$$n = \frac{(1.96)^2.0.5.(1-0.5)}{(0.05)^2}$$

$$n = 384$$

2.6 Blood sample collection

Blood samples for both the Rapid Diagnostic Test (RDT) and the preparation of thick and thin blood smears were collected using a sterile lancet. The thumb of each patient was disinfected with alcohol swab and was pricked with a lancet; the blood oozing out was used for the RDT and preparation of blood film for microscopy.

2.7 Use of One step malaria antigen *P. falciparum* (HRP-2) as Rapid Diagnostic Tests

The blood samples were applied on the RDT cassette, about 2–3 drops of buffer solution were added to facilitate the flow of the blood sample across the test strip. They were allowed to stand for about 15–20 minutes. A positive result was indicated by two red lines (one in the control area and one in the test area), a negative result by a single red control line, and an invalid result by the absence of the red control line, in which case the test needed to be repeated with a new kit.

2.8 Preparation of the Thick Blood Film Smear

The blood samples were smeared onto clean grease free microscopic slides and allowed to air dry. The slides where then stained using a Giemsa stain and were observed using oil immersion ×100 objective lens under the microscope.

2.9 Data Analysis

Data from the questionnaires were transcribed into numerical and uploaded into Microsoft excel 2016. The Microsoft Excel data were transferred to MedCalc® Statistical Software version 20 (MedCalc Software Ltd, Ostend, Belgium) for analysis. The performance of the Rapid Diagnostic Tests used Sensitivity, Specificity, Positive Predictive Value (PPV), Negative Predictive Value (NPV) and Diagnostic accuracy. All analyses were done at the probability of $P \leq 0.05$.

3. Results

Table 1: Concordance of Rapid Diagnostic Tests (RDTs) to Microscopy among pregnant women attending antenatal clinics in Gassol Local Government Area, Taraba State, Nigeria

	Microscopy (%)					
RDT	Positive	Negative	Total	K	AUC (%)	*p-value*
				0.204	99.0	0.001
Positive	96 (99.0)	1 (0.4)	97 (25.9)			
Negative	0 (0.0)	277 (100.0)	277 (74.1)			
Total	96 (25.6)	278 (74.3)	374			

K= Cohen's Kappa index; AUC= Area Under the Curve

Table 1 shows the concordance of the Rapid Diagnostic Tests of malaria to microscopy among pregnant women attending antenatal clinics in Gassol Local Government Area, Taraba State, Nigeria. The results showed that malaria had an infection of 25.9% (97/374) with the RDT, while microscopy had 25.6% (96/374). There was a significant association between the two tests used in screening the pregnant women (k=0.204; p=0.001).

Table 2**:** Diagnostic Performance of Rapid Diagnostic Tests (RDTs) on pregnant women attending antenatal clinics in Gassol Local Government Area, Taraba State, Nigeria.

Parameters	Values (%)
Sensitivity	100.0
Specificity	99.6
Positive predictive value	98.9
Negative predictive value	100.0

The performance of the Rapid Diagnostic Tests is shown in Table 2. The test recorded a sensitivity of 100.0%, and specificity of 99.64% with positive and negative predictive values of 98.96% and 100.0% respectively.

4. Discussion

The result of this research showed that the One Step Malaria Antigen *P. falciparum* (HRP-2) rapid diagnostic tests demonstrated exceptional diagnostic performance among pregnant women attending antenatal clinics in Gassol Local Government Area, Taraba State, Nigeria. The present study is mesoendemic and with predominance among symptomatic pregnant women. This phenomenon enhanced the diagnostic performance of the RDTs used. It was mentioned that higher parasite density and symptomatic presentation do increase the probability of antigen detection by HRP-2 tests, thereby boosting sensitivity and PPV in such settings (Abdullahi *et al*., 2021).

The diagnostic performance of the RDTs had a good sensitivity, specificity, positive predictive value and negative predictive value, with a good Area Under the Curve and Cohen's Kappa index. The sensitivity observed in this study indicates that the RDTs successfully detected all individuals who were truly infected with *P. falciparum*, as confirmed by microscopy. This is a critical advantage in antenatal settings, where missed malaria infections can lead to serious consequences such as maternal anemia, miscarriage, low birth weight, and intrauterine growth retardation. This finding agrees with that of Oyeyemi *et al.* (2015), who reported a similarly high sensitivity of 100.0% with HRP-2 among pregnant women in a hospital-based study conducted in north-central Nigeria. Ochola *et al.* (2006) reported a similar sensitivity of 98.0% in Kenya which supports the current study. The HRP-2 are highly effective in high-transmission areas where parasite density is often sufficient for detection.

In terms of specificity, this study found that the RDT was able to correctly identify non-infected individuals with remarkable precision. This is consistent with earlier reports of Moody (2002), who found that HRP-2 typically yielded specificity ranging from 95.0% to 99.0%. In Nigeria, Uzochukwu *et al*. (2009) also reported high specificity (98.2%) in Primary Health Care settings, suggesting that HRP-2 have broad applicability across diverse health facility levels. The very low false-positive rate observed in this study further confirms the reliability of the test in minimizing unnecessary treatments.

The positive and negative predictive values obtained in this study further highlight the diagnostic strength. The positive predictive value implies that positive RDTs' results can be confidently acted upon, while an NPV of 100% assures clinicians that negative results are truly negatives. This contrasts with findings from studies conducted by Cohen *et al*. (2017) in low-transmission environments where the positive predictive values (PPVs) of HRP-2 based rapid diagnostic tests were markedly lower primarily due to reduced malaria prevalence, which tends to diminish diagnostic accuracy. In a hospital-based study in Eastern Sudan by Mohamed *et al*. (2013), RDTs had reasonable sensitivity (83.3%) but a relatively low PPV (57.7%) when PCR was used as the reference standard. Furthermore, a study in southern Senegal by Bougouma *et al*. (2023) reported very low sensitivity of RDTs among asymptomatic pregnant women in the dry season, largely attributed to low-density or submicroscopic parasitaemia, which impaired PPV and overall diagnostic accuracy.

The Cohen's Kappa index in this study was unexpectedly low, suggesting only slight agreement. This could be a statistical artifact resulting from the imbalanced distribution of positives and negatives, which can deflate the Kappa score despite high raw agreement. This phenomenon has been noted by McHugh (2012), and further supported by the works of Landis and Koch (1977) as well as Gwet (2014), who all emphasized the variability in inter-rater reliability depending on prevalence and bias indices. In contrast, the Area Under the Curve (AUC) affirms the test's exceptional discriminatory ability, aligning with the findings of Wongsrichanalai *et al*. (2007) who emphasized AUC as a more stable indicator of diagnostic accuracy than Kappa in unbalanced datasets. While the present findings corroborate the general consensus on the utility of HRP-2, they contrast with reports from regions where HRP-2 gene deletions are becoming more common. Previous studies highlighted the emergence of *P. falciparum* strains lacking the HRP-2 gene (Gamboa *et al.*, 2010; WHO, 2021) . The RDT performance in some endemic areas recorded false-negative results. Such deletions have not been widely reported in Nigeria, particularly in Taraba State, which may explain the adequate good performance of the HRP-2 in Gassol local Government Area.

These findings are indicative of a perfect diagnostic ability in correctly identifying both malaria-positive and malaria-negative cases.

5. Conclusion

The One Step Malaria Antigen *P. falciparum* (HRP-2) rapid diagnostic test (RDT) demonstrated excellent diagnostic performance among pregnant women attending antenatal clinics at the First Referral Hospital and Baba Luka Clinic in Gassol Local Government Area, Taraba State, Nigeria. The RDT achieved a sensitivity of 100.0% and a specificity of 99.64%, indicating its high accuracy in correctly identifying both malaria-positive and malaria-negative individuals. Additionally, the test showed a positive predictive value of 98.96% and a negative predictive value of 100.0%, highlighting its reliability in guiding clinical decision-making, particularly in low-resource antenatal care settings. The study recommends that the States and the Federal Ministry of Health in Nigeria and relevant healthcare authorities should integrate, and prioritize the use of RDTs in all primary and secondary health facilities for prompt and accurate

diagnosis, especially in resource-limited settings. The healthcare providers should undergo continuous training on proper RDTs usage, storage, and interpretation of results.

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