Nigerian Medical Journal
NIGERIA MEDICAL ASSOCIATION
Print ISSN 0300-1652, E-ISSN 2229-774X

Original Article

Comparison of diagnostic accuracy of ultra-sensitive and conventional rapid diagnostic tests for malaria in asymptomatic pregnant women at a tertiary hospital in North-Central Nigeria

Ushakuma Michael Anenga[1], Terrumun Zaiyol Swende[1], Samuel Kuma Hembah-Hilekaan[1]

[1]Department of Obstetrics and Gynaecology, College of Health Sciences, Benue State University, Makurdi, Nigeria

Abstract

Background: Malaria in pregnancy continues to be a major public health problem in sub-Saharan Africa. Pregnant women are at a higher risk because asymptomatic malaria infections may lead to detrimental pregnancy outcomes. Early diagnosis is the main strategy to reduce these adverse outcomes; however, conventional rapid diagnostic tests (RDTs) are inadequate for detecting low-density infections. There are prospects that ultra-or highly sensitive RDTs may improve the detection of malaria in these populations. The objective of this study is to assess and compare the diagnostic accuracy of ultrasensitive and conventional RDTs for malaria in asymptomatic pregnant women at the Benue State University Teaching Hospital, Makurdi.

Methodology: This was a cross-sectional comparative study conducted in the antenatal clinic of Benue State University Teaching Hospital, Makurdi, Nigeria. It involved 107 asymptomatic pregnant women whose peripheral venous blood samples were obtained and tested with conventional RDT (co-RDT) and ultra-sensitive RDT (us-RDT) to detect Plasmodium falciparum in peripheral blood. The results from microscopy were used as the reference standard. Results were categorised as positive and negative.

Results: The overall prevalence rate of P. falciparum was 14% using microscopy, followed by us-RDT (12.1%) and co-RDT (2.8%). The us-RDT showed a higher sensitivity (66.7% vs. 13.3%), positive predictive value (83.3% vs. 50.0%), negative predictive value (94.7% vs. 87.4%), and test accuracy (93.5% vs. 86.0%) compared to co-RDT. In comparison, the specificity of both tests was similar (97.8%). There was a significant difference in diagnostic performance between the two RDTs (p=0.002).

Conclusion: The study suggests that ultrasensitive RDTs are a more effective test for detecting P. falciparum infection in asymptomatic pregnant women compared to conventional RDTs. This may have important implications for clinical decision-making and the management of malaria in pregnancy.

Keywords: Conventional Rapid Diagnostic Tests; Diagnostic Accuracy; Malaria in Pregnancy; Sensitivity; Specificity. Ultrasensitive Rapid Diagnostic Tests.

***Correspondence:** Anenga UM. Department of Obstetrics and Gynaecology, College of Health Sciences, Benue State University, Makurdi, Nigeria **Email:**uanenga@yahoo.com

How to cite: Anenga UM, Swende TZ, Hembah-Hilekaan SK. Comparison of diagnostic accuracy of ultra-sensitive and conventional rapid diagnostic tests for malaria in asymptomatic pregnant women at a tertiary hospital in North-Central Nigeria. Niger Med J 2025; 66 (2):489-499.https://doi.org/10.71480/nmj.v66i2.638.

Quick Response Code:

Introduction

Malaria continues to cause unacceptably high levels of disease and death, as documented in successive editions of the World Malaria Report; it affects the lives of almost 40 per cent of the world's population, and the high-risk groups are pregnant women and young children (under 5 years of age).[1,2] In 2022, the WHO African Region reported that 36% of pregnancies in 33 moderate and high transmission countries were exposed to malaria infection during pregnancy.[3]Sub-Saharan Africa still accounts for 96% of the global malaria burden, out of which four countries accounted for almost half of all cases: Nigeria (26.6%), the Democratic Republic of the Congo (12.3%), Uganda (5.1%) and Mozambique (4.1%).[4] Nigeria accounts for 31.3% of malaria cases in Sub-Saharan Africa and has high prevalence rates of malaria in pregnancy, ranging from 19.7% to 72.0%, and 11% of maternal deaths are attributed to the disease.[4,5,6]Research conducted in the study area, Makurdi, reports the prevalence of malaria in pregnancy as 49%.[7]

Of all the species of Plasmodium, P. falciparum causes the most virulent cases of human malaria; it is of major importance in sub-Saharan Africa.[8] Its adverse effects in pregnancy include spontaneous abortion, intrauterine growth restriction (IUGR), low birth weight, foetal and neonatal death, poor development, behavioural problems, short stature, and neurological deficits. Maternal complications of malaria include severe anaemia, hypoglycaemia, acute pulmonary oedema, cerebral oedema, renal failure, puerperal sepsis, postpartum haemorrhage, and increased risk of death.[9]

Accurate diagnosis of malaria in pregnancy is thus essential for appropriate clinical management. Microscopy is regarded as the gold standard in malaria diagnosis and serves as the method of reference for other malaria diagnostic tests, however, it is operator-dependent and requires initial and continuous training to maintain a high quality of testing. Such quality assurance practices are often difficult to implement in resource-poor countries. [10,11] Owing to these challenges, the World Health Organisation (WHO) recommended rapid diagnostic tests (RDTs) to enhance the diagnosis and management of cases, prevent complications of delayed treatment, prolong survival, and monitor treatment.[12]

The current generation of conventional rapid diagnostic tests (co-RDTs) for malaria has a limit of detection of 100 parasites/µl and is limited in detecting low-density infections in asymptomatic pregnant women.[13] In 2019, the WHO prequalified a new RDT called the ultra or highly sensitive rapid diagnostic test (us-RDT) which is said to have tenfold improved analytical sensitivity as compared to the average conventional RDTs.[14] There are prospects that this new RDT may improve the detection of malaria in asymptomatic pregnant women.[14]Early and precise detection of low-density P. falciparum infections can improve clinical decision-making, enhance timely treatment, and reduce adverse pregnancy outcomes. This study is therefore aimed at assessing and comparing the diagnostic accuracy of ultrasensitive RDTs and conventional RDTs for malaria in asymptomatic pregnant women attending antenatal care at a tertiary hospital in North-central Nigeria.

Methods

Study area

This study was conducted in the antenatal clinic of Benue State University Teaching Hospital (BSUTH), which is in Makurdi, the state capital of Benue State. Benue State is one of the North Central states in Nigeria. The study area has a tropical sub-humid climate, with two distinct seasons, namely wet and dry seasons. The wet season lasts from April to October, with an annual rainfall in the range of 1120 to 1500 mm. The dry season begins in November and ends in March. The climate is characterised by a high annual temperature regime, ranging from 27-38^{0}C. [15]

Study design

This was a cross-sectional comparative study.

Study population

It consisted of all pregnant women who presented at the antenatal clinic at BSUTH during the study period, gave informed consent, and met the inclusion criteria.

Study period

The study was conducted from 1st January to31st March 2023.

Inclusion and exclusion criteria

The inclusion criteria for the study involved pregnant women attending antenatal care at BSUTH who freely consented to participate, either through signed informed consent or thumbprint in the presence of a witness. Exclusion criteria included women with recent or current severe malaria, as well as those with other serious chronic diseases or medical emergencies during pregnancy.

Sample size determination

Buderer's formula for diagnostic accuracy studies, aiming for a 95% confidence interval with 10% precision, was used.[16] With a prevalence of malaria in pregnancy at 49.0% from Amali et al[7] and expected sensitivity and specificity of the us-RDT at 85% and 90% respectively, the final sample size was calculated to be 107 women after considering a 10% dropout rate.

Sampling method

A purposive sampling method was employed, recruiting consecutive pregnant women presenting at the routine antenatal clinic who met the eligibility criteria and provided consent until the sample size was achieved.

Outcome measures

The outcome measures were the measures of diagnostic accuracy, which include estimates of sensitivity, specificity, negative predictive value, positive predictive value, and test accuracy of us-RDT and co-RDT with microscopy as a reference test.

Data collection

A structured proforma was used to collect participant data, including basic characteristics like age, socio-economic status, parity, and gestational age. The socio-economic class was determined using the Olusanya classification model.[17]

Laboratory procedures

Preparation and staining of the thick and thin blood smear:

The process began by cleaning the woman's antecubital area with 70% ethyl alcohol and allowing it to dry. Using a 3 ml syringe, approximately three millilitres of blood were drawn from the antecubital vein and dispensed into pre-labelled EDTA sample bottles. These samples were promptly transferred to the medical microbiology laboratory for smearing.

To prepare the thick blood film, two drops of the blood sample were placed on a slide and stirred in a circular motion, ensuring it wasn't too thick, and allowed to dry. After drying, the spot was stained with 10% Giemsa solution for 20 minutes, washed in buffered water, and air-dried vertically.

For the thin blood film, a drop of blood was spread on a slide at a 45° angle using a spreader slide, fixed with absolute methanol, stained with Giemsa solution, washed, and air-dried vertically. Both thick and thin blood films were examined under a microscope with a ×100 oil immersion lens, reviewing at least 200 high-power fields for each film. Approximately 15 blood smears were randomly selected and re-read by a different microscopist for quality assurance.

Rapid Diagnostic Tests (RDTs)

The NxTek™ Ultra-sensitive Malaria Ag P. falciparum RDT and the SD Bioline™ Malaria Ag P. falciparum RDT were employed to detect malaria HRP2 Pf in patient blood samples following the manufacturer's instructions. A micro-pipette was used to collect about 5 µl of blood, which was added to the "S" well, followed by 60 µl of assay buffer solution added to the "A" well. Results were read after 20 minutes for NxTek™ and 15 minutes for SD Bioline™. A line in the 'C' window indicated a valid test, with a positive result if a line appeared in the P. falciparum-line (Pf-line) window. An adjacent line to "C" indicated a negative result. If no line appeared adjacent to "C", the test was considered invalid and repeated using a new cassette. The presence of a Pf-line was confirmed by two independent assistants, with a third person consulted in case of uncertainty. The person conducting the RDTs was blinded to the reference test results. RDT kits were stored at the recommended temperature (<40°C), and the validity of each kit was certified by laboratory scientists before use.

Data analysis

The data collected was entered into Statistical Product and Service Solutions (SPSS) version 25.0 for analysis. Univariate analysis was conducted to summarise categorical variables, while bivariate analysis was performed to test hypotheses comparing malaria diagnosis between co-RDT and us-RDT using McNemar's ($\chi 2$) test of proportion. A P-value of ≤ 0.05 was considered statistically significant. Additionally, Sensitivity, Specificity, Positive Predictive Value (PPV), Negative Predictive Value (NPV), and overall test accuracy of co-RDT and us-RDT were calculated using MedCalc® software version 20.218.

Ethical considerations

Approval of this study was obtained from the Health Research Ethics Committee of the BSUTH, Makurdi. Written informed consent was obtained from the participants before enlistment into the study, and they were informed of their right to withdraw at any time after initial consent. Privacy was ensured for the study population, and all information supplied was handled confidentially. Ethical considerations in this study were based on the Helsinki Declaration guidelines as applicable to human subjects; these are respect for people, beneficence, non-maleficence, and justice.[18]

Results

A total of 126 pregnant women were assessed for eligibility during the study period, of whom 107 participated in the study. Table 1 shows the distribution of participants across different age groups, ethnicities, socio-economic classes, parity, and gestational ages. The average age was 30.0 ± 5.4 years, with most participants within the age range of 25-34 years (69.2%) and belonging mainly to the Tiv ethnic group (71.0%). Socioeconomic class distribution was relatively balanced across upper, middle, and lower classes. Most participants had a parity of 1-4 (68.2%) and were in the third trimester of pregnancy (47.7%).

Table 2 presents the prevalence of P. falciparum using microscopy, ultrasensitive RDT (us-RDT), and conventional RDT (co-RDT) in a sample size of 107. Microscopy detected P. falciparum in 15 cases (14.0%), followed by us-RDT in 13 cases (12.1%), and co-RDT in 3 cases (2.8%).

Table 3 presents the diagnostic parameters and their values for co-RDT and us-RDT. The results showed that the us-RDT had a higher sensitivity (66.7%, 95% CI = 38.4 – 88.2) compared to co-RDT (13.3%, 95% CI = 1.7 - 40.5). The us-RDT also had a higher test accuracy (93.5%, 95% CI = 87.0 - 97.3) compared to co-RDT (86.0%, 95% CI = 77.9 – 91.9). The positive predictive value and negative predictive value of us-RDT (83.3%, 95% CI = 54.8 – 95.4 and 94.7%, 95% CI = 89.8 – 97.4, respectively) were also higher compared to co-RDT (50.0%, 95% CI = 13.2 - 86.8 and 87.4%, 95% CI = 85.0 – 89.4, respectively). The specificity of both tests was similar (97.8%, 95% CI = 92.4 - 99.7).

Table 4 displays the comparison of the diagnostic performance of the co-RDT and us-RDT. The results of the McNemar's test showed that there was a significant difference between the two methods (p=0.002).

Table 1. Sociodemographic and obstetric characteristics of participants (n=107)

Variable	Frequency	Percentage (%)
Age (years)		
15-19	4	3.7
20-24	9	8.4
25-29	34	31.8
30-34	40	37.4
35-39	16	15.0
40-44	4	3.7
Ethnicity		
Tiv	76	71.0
Idoma	15	14.0
Igede	4	3.7
Others	12	11.2
Socio-economic class		
Upper (I and II)	30	28.0
Middle (III)	30	28.0
Lower (IV and V)	47	43.9
Parity		
0	31	29.0
1-4	73	68.2

≥ 5	3	2.8
Gestational Age		
First trimester	11	10.2
Second trimester	45	42.1
Third trimester	51	47.7

Table 2: Prevalence of P. falciparum using microscopy, us-RDT, and co-RDT (n=107)

Variable	Frequency	Percentage (%)
Conventional RDT	3	2.8
Ultrasensitive RDT	13	12.1
Microscopy	15	14.0

Table 3: Determination and comparison of the Sensitivity, Specificity, Positive predictive value (PPV), Negative predictive value (NPV) and Test Accuracy (TA) between co-RDT and us-RDT (n=107)

Diagnostic parameter	co-RDT		us-RDT	
	Value (%)	95% CI	Value (%)	95% CI
Sensitivity	13.3	1.7 - 40.5	66.7	38.4 – 88.2
Specificity	97.8	92.4 - 99.7	97.8	92.4 - 99.7
Positive Predictive Value	50.0	13.2 - 86.8	83.3	54.8 – 95.4
Negative Predictive Value	87.4	85.0 – 89.4	94.7	89.8 – 97.4
Test Accuracy	86.0	77.9 – 91.9	93.5	87.0 - 97.3

Table 4: Comparison of diagnostic performance between co-RDT and us-RDT (n=107)

		Conventional RDT		P-value
		Positive	Negative	
Ultrasensitive RDT	Positive	3	10	0.002
	Negative	0	94	

Discussion

The study included 107 pregnant women with an average age of 30.0 ± 5.4 years, most of whom were 25– 29 years and 30 – 34 years (31.8% and 37.4% respectively). In a study by Briand et al,[19] women between the ages of 23-30 formed 76.3% of the participants, with only 4.9% above the age of 30. The high number of women below the age of 30 years in both studies is understandable, as those are peak reproductive years. Studies have shown that when a woman is younger than 30, she has the highest chance (85%) of conceiving within 1 year.[20] However, the high percentage of women above 30 years in our study could be explained by the fact that women in our setting complete their reproductive careers late due to high parity. Those aged 19 years or less are less likely to be married and pregnant, while those 40 years or more are less likely to get pregnant.[20] A majority of participants were of Tiv ethnicity, which is consistent with the fact that Tiv is the predominant ethnic group in the study area. The finding that the majority of participants were in the middle and upper socioeconomic class contrasts with findings by Briand et al [19] in which participants in the upper and lower socioeconomic class were the majority (41.2% and 34.2%, respectively). This may be due to the location of the health facilities where these studies were carried out. The current study was carried out in an urban area, hence the status of the patients.

The majority were multigravidae (71%), which is consistent with findings by Oyeyemi et al (68.8%),[21] Vasquez et al (79.5%),[22] and Briand et al (92.3%).[19] This may be due to factors such as early marriage, shorter birth intervals, or higher fertility rates of the study population. Also, a little less than half of the participants were in their second trimester (42.1%) and third trimester (47.7%), which aligns with the findings of previous studies by Vasquez et al. [22] and Oyeyemi et al[21], where most of the participants fell within the second trimester (42.5%). One possible explanation for the higher representation of women in the second trimester and probably the third trimester is that this period is often considered a relatively stable phase of pregnancy.

The overall prevalence of malaria in the studied population was highest when diagnosed using microscopy (14%), followed by us-RDT (12.1%), and co-RDT (2.8%). These findings are consistent with previous studies conducted by Vasquez et al [22] and Briand et al [19] where microscopy consistently reported higher prevalence rates compared to RDTs. Vasquez et al. [22] reported prevalence rates of 2.4%, 3%, and 2.7% using co-RDT, us-RDT, and microscopy, respectively. On the other hand, Briand et al [19] found higher prevalence rates of 11.6%, 16.2%, and 3.2% using co-RDT, us-RDT, and microscopy, respectively. These variations in prevalence rates could be attributed to the differences in the sensitivity and specificity of the diagnostic methods used in the respective studies. Each diagnostic method has its own limitations and performance characteristics, which can contribute to slight variations in prevalence estimates.

The results of the present study showed that the us-RDT had a higher sensitivity (66.7%) compared to the co-RDT (13.3%). This finding is consistent with previous studies, such as the one conducted by Vásquez et al [22], which reported a sensitivity of 64.1% for us-RDT and 53.8% for co-RDT and Briand et al, where the sensitivity of us-RDT (60.5%) was more compared to that of co-RDT (42.5%). Another study by Unwin et al. [23] found lower sensitivity for both tests, with us-RDT at 19.6% and co-RDT at 22.8%. A possible explanation for the higher sensitivity of us-RDT is its unique design and composition. The us-RDT utilises biotinylated and carboxyl-modified latex fragment antibodies (FAbs) along with polystreptavidin in the test line.[24] These components enhance the test's ability to detect the Plasmodium falciparum-specific histidine-rich protein 2 (HRP2) in whole blood. The us-RDT's improved sensitivity allows for the detection of even lower concentrations of the target antigen, resulting in a higher proportion of true positive cases being correctly identified. In contrast, the co-RDT, which uses an immunochromatographic membrane strip test, lacks the same level of sensitivity as the us-RDT. This may be attributed to differences in the composition and detection mechanisms employed by the two

tests.[24] The co-RDT may have a lower limit of detection, resulting in a higher number of false negative results when the parasite density in the blood is relatively low. The ability of the us-RDT to detect lower levels of malaria parasites has significant implications for the diagnosis and management of malaria, particularly in regions with low parasite densities.

The specificity of both tests in our study was similar, with values of 97.8%. This is consistent with findings by Vásquez et al [22] (100% vs 99.9%), Briand et al[19] (93.6% vs 95.7%), and Unwin et al[23] (95.5% vs 98.2%). The specificities of both tests were within a close range and above 95% in those studies as well. The similar specificities observed between the us-RDT and co-RDT in our study and previous studies suggest that both tests are effective in correctly identifying individuals who are not infected with malaria. A specificity of 97.8% indicates that the tests have a low rate of false-positive results. False-positive results can occur for various reasons, including the persistence of the histidine-rich protein antigen (HRP2) in the bloodstream after successful treatment for malaria. HRP2 can persist in the blood for several weeks, leading to false-positive results even when the individual is no longer infected. Another factor that can contribute to false-positive results is the presence of rheumatoid factor, which can bind to immunoglobulin G and yield erroneous positive results.[25]

In the present study, us-RDT showed a PPV of 83.3%while co-RDT had a lower PPV of 50.0%.This agrees with Unwin et al, who found PPV higher in us-RDT (93.9%) compared to co-RDT (87.8%) and contrasts with the findings of Vasquez et al.[22]On the other hand, us-RDT exhibited an NPV of 94.7% while co-RDT had an NPV of 87.4%, which is consistent with Briand et al,who have also reported higher NPV for us-RDT. The NPV found in Vásquez et al[22]and Unwin et al[23] were comparable for both RDTs. A higher value of PPV indicates a lower likelihood of false-positive results, while a higher NPV indicates a lower likelihood of false-negative results. The higher PPV and NPV of us-RDT can be attributed to its ultrasensitive nature, which allows for the detection of lower levels of malaria parasites in the blood.

Regarding overall test accuracy, us-RDT demonstrated higher accuracy (93.5%, 95% CI = 87.0 - 97.3) compared to co-RDT (86.0%, 95% CI = 77.9 – 91.9) in our study. This result aligns with previous studies by Vásquez et al[22]and Briand et al[19]that have also reported higher accuracy for us-RDT. The wider confidence interval observed for co-RDT suggests greater variability in its accuracy, which can potentially lead to misdiagnosis and inappropriate treatment. On the other hand, the higher accuracy of us-RDT indicates its potential to improve the management of malaria in pregnant women and contribute to reducing the overall burden of the disease.[26]

The present study revealed a statistically significant difference in the diagnostic performance between the ultrasensitive rapid diagnostic test (us-RDT) and the conventional rapid diagnostic test (co-RDT) for detecting Plasmodium falciparum infections during pregnancy (p-value = 0.002). This finding contrasts with the results of a study conducted by Unwin et al[23], where they reported a non-significant difference in performance between us-RDT and co-RDT (p-value = 0.169). Similarly, a study by Vásquez et al[27] in 2018 found no significant differences in sensitivity between the two tests. These results suggest that us-RDT may be more effective in detecting P. falciparum infections in asymptomatic pregnant women.

The study is limited by the useof microscopy as the reference test, however, researchers in recent times have used Polymerase Chain Reaction (PCR)-based techniques which have proven to be one of the most specific and sensitive diagnostic methods, as the reference standard when comparing the diagnostic performance of malaria RDTs, however, it is expensive and not readily available.

Conclusion

The study found that microscopy had the highest prevalence rate for P. falciparum, followed by us-RDT and co-RDT. This study also suggests that us-RDT may offer a more sensitive and accurate means of predicting P. falciparum infections during pregnancy compared to co-RDT. Further, multicentre studies should be conducted to verify these findings and assess the feasibility of utilising ultrasensitive rapid diagnostic tests for routine screening of asymptomatic pregnant women for malaria in endemic areas.

Conflict of interest: Authors declare no conflict of interest.

References

1. Monroe A, Williams NA, Ogoma S, Karema C, Okumu F. Reflections on the 2021 World Malaria Report and the future of malaria control. Malar J. 2022 May 27;21(1):154.doi: 10.1186/s12936-022-04178-7.

2. Joseph Omang, Antor O Ndep, Dominic Offiong, Fidelis Otu, Kenneth Onyejose. Malaria in pregnancy in Nigeria: A literature review. IHRJ. 2020 Feb 19;3(11):346–8.doi: 10.26440/IHRJ/0311.02315.

3. Reddy V, Weiss DJ, Rozier J, Ter Kuile FO, Dellicour S. Global estimates of the number of pregnancies at risk of malaria from 2007 to 2020: a demographic study. Lancet Glob Health. 2023 Jan;11(1):e40–7.doi: 10.1016/S2214-109X(22)00431-4.

4. World Health Organization. Malaria in the African Region. WHO Regional Office for Africa; 2022. Available from: https://www.afro.who.int/health-topics/malaria.

5. Ita Ita O. The Pattern of Severe Malaria in Plateau State of Nigeria: A Five-Year Review of Severe Malaria Case-Based Surveillance Data from 2013 to 2017. CAJPH. 2019;5(1):52.doi: 10.11648/j.cajph.20190501.18.

6. Agomo CO, Oyibo WA, Anorlu RI, Agomo PU. Prevalence of malaria in pregnant women in Lagos, South-West Nigeria. Korean J Parasitol. 2009 Jun;47(2):179–83.doi: 10.3347/kjp.2009.47.2.179.

7. Amali O, Okwori GO, Awodi N. Malaria and anaemia among pregnant women in Makurdi, Benue State. Niger J Parasitol. 2011;32(2):193–196.

8. Fana SA, BunzaMDA, Anka SA, Imam AU, Nataala SU. Prevalence and risk factors associated with malaria infection among pregnant women in a semi-urban community of north-western Nigeria. Infect Dis Poverty. 2015 Apr 24;4:24.doi: 10.1186/s40249-015-0054-0.

9. Ejike BU, Ukpai OM, Ihemanma CA, Ajuga MU, Eme GF. Impact of malaria infection on the haematological profile of pregnant women in south-eastern Nigeria. Nig J Para. 2022 May 28;43(1):78–84.doi: 10.4314/njpar.v43i1.11.

10. World Health Organization, UNICEF/UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases. Microscopy for the detection, identification and quantification of malaria parasites on stained thick and thin blood films in research settings (version 1.0): procedure: methods manual. Geneva: World Health Organization; 2015. Available from: https://iris.who.int/handle/10665/163782.

11. Mbanefo A, Kumar N. Evaluation of malaria diagnostic methods as a key for successful control and elimination programs. Trop Med Infect Dis. 2020 Jun 19;5(2): 102-117.doi: 10.3390/tropicalmed5020102.

12. Soniran OT, Mensah BA, Cheng NI, Abuaku B, Ahorlu CS. Improved adherence to test, treat, and track (T3) malaria strategy among Over-the-Counter Medicine Sellers (OTCMS) through interventions implemented in selected rural communities of Fanteakwa North district, Ghana. Malar J. 2022 Nov 5;21(1):317.doi: 10.1186/s12936-022-04338-9.

13. Acquah FK, Donu D, Obboh EK, Bredu D, Mawuli B, Amponsah JA, et al. Diagnostic performance of an ultrasensitive HRP2-based malaria rapid diagnostic test kit used in surveys of afebrile people living in Southern Ghana. Malar J. 2021 Mar 2;20(1):125.doi: 10.1186/s12936-021-03665-7.

14. Slater HC, Ding XC, Knudson S, Bridges DJ, Moonga H, Saad NJ, et al. Performance and utility of more highly sensitive malaria rapid diagnostic tests. BMC Infect Dis. 2022 Feb 4;22(1):121.doi: 10.1186/s12879-021-07023-5.

15. Anthonia AN, Muyiwa O, Ogah TA, Bello YA. Analysis of Climate Variables and Resource Conflict in Benue State, Nigeria. World Journal of Innovative Research 2020; 9(5): 6-13.

16. Buderer NM. Statistical methodology: I. Incorporating the prevalence of disease into the sample size calculation for sensitivity and specificity. Acad Emerg Med. 1996 Sep;3(9):895–900.doi: 10.1111/j.1553-2712.1996.tb03538.x.

17. Olusanya O. The importance of social class in voluntary fertility control in developing country. West Afr J Med. 1985;4:205–12.

18. Council for International Organizations of Medical Sciences. International ethical guidelines for biomedical research involving human subjects. Bull Med Ethics. 2002 Oct;(182):17–23.

19. Briand V, Cottrell G, Tuike Ndam N, Martiáñez-Vendrell X, Vianou B, Mama A, et al. Prevalence and clinical impact of malaria infections detected with a highly sensitive HRP2 rapid diagnostic test in Beninese pregnant women. Malar J. 2020 May 24;19(1):188.doi: 10.1186/s12936-020-03261-1.

20. Delbaere I, Verbiest S, Tydén T. Knowledge about the impact of age on fertility: a brief review. Ups J Med Sci. 2020 May;125(2):167–74.doi: 10.1080/03009734.2019.1707913.

21. Oyeyemi OT, Sode OJ, Adebayo OD, Mensah-Agyei GO. Reliability of rapid diagnostic tests in diagnosing pregnancy and infant-associated malaria in Nigeria. J Infect Public Health. 2016 Aug;9(4):471–7.doi: 10.1016/j.jiph.2015.11.012.

22. Vásquez AM, Vélez G, Medina A, Serra-Casas E, Campillo A, Gonzalez IJ, et al. Evaluation of highly sensitive diagnostic tools for the detection of P. falciparum in pregnant women attending antenatal care visits in Colombia. BMC Pregnancy Childbirth. 2020 Jul 31;20(1):440.doi: 10.1186/s12884-020-03114-4.

23. Unwin VT, Ahmed R, Noviyanti R, Puspitasari AM, Utami RAS, Trianty L, et al. Use of a highly-sensitive rapid diagnostic test to screen for malaria in pregnancy in Indonesia. Malar J. 2020 Jan 16;19(1):28.doi: 10.1186/s12936-020-3110-6.

24. Das S, Peck RB, Barney R, Jang IK, Kahn M, Zhu M, et al. Correction to: Performance of an ultra-sensitive Plasmodium falciparum HRP2-based rapid diagnostic test with recombinant HRP2, culture parasites, and archived whole blood samples. Malar J. 2019 Feb 4;18(1):33.doi: 10.1186/s12936-019-2669-2.

25. Das S, Jang IK, Barney B, Peck R, Rek JC, Arinaitwe E, et al. Performance of a High-Sensitivity Rapid Diagnostic Test for Plasmodium falciparum Malaria in Asymptomatic Individuals from Uganda and Myanmar and Naive Human Challenge Infections. Am J Trop Med Hyg. 2017 Nov;97(5):1540–50.doi: 10.4269/ajtmh.17-0245.

26. Mahende C, Ngasala B, Lusingu J, Yong T-S, Lushino P, Lemnge M, et al. Performance of rapid diagnostic test, blood-film microscopy and PCR for the diagnosis of malaria infection among febrile children from Korogwe District, Tanzania. Malar J. 2016 Jul 26;15(1):391.doi: 10.1186/s12936-016-1450-z.

27. Vásquez AM, Medina AC, Tobón-Castaño A, Posada M, Vélez GJ, Campillo A, et al. Performance of a highly sensitive rapid diagnostic test (HS-RDT) for detecting malaria in peripheral and placental blood samples from pregnant women in Colombia. PLoS ONE. 2018 Aug 2;13(8):e0201769.doi: 10.1371/journal.pone.0201769.