



# Evaluating Prevalence and Misdiagnosis of *Plasmodium* Using Microscopy Compared With Polymerase Chain Reaction Technique in Two Tertiary Care Hospitals in Rivers State, Nigeria

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## Abstract

**Background:** The accurate diagnosis of etiologic agents of diseases, including *Plasmodium* species, is a major challenge to effective control programs in sub-Saharan Africa. Malaria misdiagnosis hinders prompt treatment of infected patients, which increases malaria morbidities and mortalities.

**Objectives:** A study to evaluate the prevalence and misdiagnosis of *Plasmodium* species using microscopy and polymerase chain reaction (PCR) technique in two tertiary care hospitals in Rivers State was conducted.

**Methods:** A cross-sectional randomized study involving 2,000 participants (age and sex were noted) was conducted from January 2016 to December 2017, and only patients arriving in the Outpatient Clinic of the selected tertiary care hospitals were recruited for this study. Intravenous blood samples (5 mL) were collected from all study participants and analyzed for the presence of *Plasmodium* species using Giemsa-microscopy and Real-time PCR technique. All data generated were analyzed using analysis of variance (ANOVA) and Chi-square test. A  $P < 0.05$  was considered statistically significant.

**Results:** The only species of *Plasmodium* observed in this study was *Plasmodium falciparum* (*P. falciparum*), and the overall prevalence in the study was 37.65% and 34.0% for microscopy and PCR, respectively ( $P > 0.05$ ). Microscopy had a 3.5% variation and misdiagnosis of 5.5% compared with PCR. *P. falciparum* misdiagnosis according to age was 6.6%, 4.8%, 3.2%, 7.3% and 6.7% for age groups 0-10, 11-20, 21-30, 31-40, and  $> 40$ , respectively while males and females had *P. falciparum* misdiagnosis of 6.9% and 4.5%, respectively ( $P > 0.05$ ). Microscopy had sensitivity, specificity, and diagnostic accuracy of 95.8%, 94.3%, and 94.9%, respectively.

**Conclusions:** Microscopy remains the gold standard for *Plasmodium* species diagnosis, and suspected malaria cases should be confirmed with an efficient laboratory diagnosis before treatment to prevent misdiagnosis or antimalarial drug resistance.

**Keywords:** Prevalence, Misdiagnosis, *Plasmodium*, microscopy, PCR

## 1. Background

Malaria is one of the most severe public health challenges globally, it is the leading cause of morbidities and mortalities in many developing countries with young children (who have not developed partial acquired immunity to malaria), and pregnant women (whose immunities are decreased by pregnancy) are the most vulnerable groups (1). Malaria is a parasitic disease caused by *Plasmodium* species (*P. falciparum*, *P. vivax*, *P. ovale*, *P. malariae* and *P. knowlesi*) and transmitted mainly via the bites of infected *Anopheles* female mosquitoes but other minor forms of transmission are by sharing sharp objects, congenital malaria (mother to fetus), transfusion of infected blood and infected organ transplantation (2). According

to the World Malaria Report, 228 million malaria cases occurred globally in 2018, and Nigeria accounted for 57 million (25%) cases (3). Also, there were 405,000 estimated malaria mortalities globally, with 97,000 (24%) occurring in Nigeria (3). The World Health Organization (WHO) recommends that a prompt, accurate laboratory diagnosis and treatment by skilled professionals is the most efficient strategy in curtailing asymptomatic malaria cases from progressing into severe cases and deaths (3). Laboratory diagnosis of malaria is mainly conducted by detecting *Plasmodium* species in clinical samples by microscopy, rapid diagnostic tests (RDTs) kits, and polymerase chain reaction (PCR) technique. Microscopic diagnosis of *Plasmodium* species in blood smears is the globally accepted “gold standard” for laboratory malaria diagnosis (4). How-

ever, low sensitivity or diagnostic accuracy, especially at low parasitemia, unavailable trained microscopists, absence of electricity and diagnostic equipment (such as microscopes, slides, etc.) are the major challenges to microscopy (5). The low diagnostic accuracy of microscopy, when conducted by individuals with little expertise, had led to malaria misdiagnosis globally (6). PCR technique can examine large clinical samples and detect mixed infections as well as has high specificity and sensitivity rates (7). Various PCR techniques have high levels of reliability in *Plasmodium* species diagnosis such as Nested PCR, Real-time PCR, and Reverse transcription PCR (8) but are not regularly used in most developing countries because they are expensive, complex to operate, and unavailable quality control. Several studies have been conducted in different countries, which highlight the importance of microscopy and PCR in detecting *Plasmodium* species; Nigeria (9), Tanzania (10), Indonesia (11), Saudi Arabia (12), and Mali (13). Misdiagnosis has led to wrong usage of anti-malarial drugs and increased cases of malaria morbidities/mortalities globally.

## 2. Objectives

This study was conducted to evaluate the prevalence and misdiagnosis of *Plasmodium* using microscopy compared with PCR technique in two tertiary care hospitals in Rivers State, Nigeria.

## 3. Methods

### 3.1. Study Area and Population

A cross-sectional study was conducted in Rivers State University Teaching Hospital (RSUTH) and the University of Port Harcourt Teaching Hospital (UPTH). In fact, RSUTH is located at latitude 4°46'49"N and longitude 7°0'50"E in Port Harcourt Local Government Area (LGA), while UPTH is located at latitude 4°53'58"N and longitude 6°55'43"E in Obio-Akpor LGA (14). Port Harcourt and Obio-Akpor LGAs are in Rivers State, Nigeria. A sample size of 2,000 randomly selected participants (participants were of varying ages and both sexes) attending the Outpatients Clinics of the two selected healthcare facilities were recruited for this study (from January 2016 to December 2017). The inclusion criteria were informed consent to participating in the study, intermittent fever, abdominal pains, reduced/loss of appetite, and general body weakness, while the exclusion criteria were refusal to give informed consent, undergoing malaria treatment, and the presence of other febrile diseases.

### 3.2. Data Collection

**Sample Preparation for Data Collection:** Intravenous blood samples were collected by trained laboratory scientists and stored in Ethylene Diamine Tetra Acetate bottles to prevent coagulation.

**Microscopic Diagnosis:** Blood films (thick and thin) were prepared, stained (using Giemsa stains) from the collected blood samples, and visualized using X100 objective power lens of a microscope to detect the forms of *Plasmodium* present (15). Thick blood smears were used to quantify parasites (15).

**DNA Extraction:** The DNA from the blood sample of each study subject was first extracted using a Quick DNA Universal Kit (Zymo Research, USA). Blood sample (100  $\mu$ l) from each study subject was put in separate microcentrifuge tubes, 100  $\mu$ l of Bio-Fluid and Cell buffer and 10  $\mu$ l of Proteinase K were added to each microcentrifuge tubes. The solution in the tubes were mixed and incubated for 10 minutes at 55°C to digest the blood samples. Genomic binding buffer (210  $\mu$ l) was added to each of the digested blood samples and thoroughly mixed. The mixtures were transferred to Zymo-Spin Columns in collection tubes, centrifuged for 1 minute at 10,000 g, and the supernatant was discarded. About 20  $\mu$ l of DNA Pre-Wash Buffer was added to the remaining mixture in new collection tubes, the columns were centrifuged at 10,000 g for 1 minute, and the supernatants in the collection tubes were discarded. About 350  $\mu$ l of g-DNA Wash Buffer was added, centrifuged for a minute at 10,000 g, and the supernatants in the collection tubes were discarded. The remaining contents of the Zymo-Spin columns were transferred to clean centrifuged tubes; 50  $\mu$ l of DNA Elution Buffer was added and centrifuged for 30 seconds at 10,000 g to elute the purified DNA in the supernatant. The ultra-pure DNA was preserved at < 0°C. Nanodrop 1,000 Spectrophotometer was used to quantify the extracted genomic DNA from each study subject.

**Screening by Real-time PCR:** Malaria diagnosis using the extracted DNA was conducted by Real-time PCR (ABI 9700 Applied Biosystems Thermal cycler). A universal primer that detected the four human species of *Plasmodium* was used; PL 1473 F (5'-TAA CGA ACG AGA TCT TAA-3') and PL 1679 R (5'-GTT CCT CTA AGA AGC TTT-3') (16). The cocktail mix (25  $\mu$ l) for each PCR reaction contained 12.5  $\mu$ l Dream taq Master mix by Inqaba, South Africa (taq polymerase, DNTPs, MgCl), 0.4  $\mu$ l of PL 1473 F, 0.4  $\mu$ l of PL 1679 R, 4  $\mu$ l of extracted DNA and 7.7  $\mu$ l of PCR water. Reaction conditions for the amplification were initial denaturation (10 minutes at 95°C), denaturation (40 cycles for 10 seconds at 95°C), annealing (11 cycles for 5 seconds at 60°C with a touchdown at 0.5°C), extension (20 seconds at 75°C), final extension (2 minutes at 95°C), and cooling (30 seconds at 68°C).

PCR Product Analysis by Agarose Gel Electrophoresis: The amplification products were viewed under UV transilluminator using agarose stained with 2% ethidium bromide at 120 V for 15 minutes (Figure 1).

### 3.3. Data Analysis

The data obtained for microscopy were evaluated using the PCR technique as the gold standard to determine variation, misdiagnosis, sensitivity, specificity, and diagnostic accuracy. Data were analyzed using the Chi-square test, and a P value of less than 0.05 was considered statistically significant.

## 4. Results

The only species of malaria parasite identified in this study by microscopy and with confirmation from polymerase chain reaction technique was *P. falciparum*. An overall prevalence of 38.0% and 34.0% were recorded for microscopy and PCR, respectively ( $P > 0.05$ ) (Table 1).

Table 1. Overall Prevalence of Malaria

Healthcare Facility	Number Examined	Number Infected, (%)	
		Microscopy	PCR
RSUTH	1000	322 (32.0)	283 (28.0)
UPTH	1000	431 (43.0)	397 (40.0)
TOTAL	2000	753 (38.0)	680 (34.0)

An overall prevalence variation of 73 (4.0%) was recorded for microscopy compared with PCR and an overall misdiagnosis (overdiagnosis) of 5.5% was recorded in this study ( $P > 0.05$ ) (Table 2). Overall malaria misdiagnosis for age groups 0-10, 11-20, 21-30, 31-40, and  $> 40$  was 6.3%, 4.7%, 3.1%, 6.9% and 6.5%, respectively ( $P > 0.05$ ) (Table 3). Overall malaria misdiagnosis for males and females was 6.5% and 4.5%, respectively ( $P > 0.05$ ) (Table 3).

In the overall study participants, *P. falciparum* was diagnosed in 73 (9.7%) by microscopy alone (not detected by PCR) and 680 (90.3%) were diagnosed as positive samples by microscopy and confirmed by PCR (Table 4). The overall diagnostic efficiency values in the study for sensitivity, specificity and diagnostic accuracy for microscopy when compared to PCR were 95.8%, 94.3%, and (94.9%), respectively (Table 5).

## 5. Discussion

*Plasmodium falciparum* was the only species of malaria parasite recorded in this study. This finding is in agreement with reports from other studies that observed only

Table 2. Diagnostic Prevalence Variation and Misdiagnosis of Microscopy

Healthcare Facility (N = 2)	Number Examined	Number Infected Variation (%)
RSUTH	1000	39 (4.0)
UPTH	1000	34 (3.0)
TOTAL	2000	73 (4.0)
<b>RSUTH</b>		
Overdiagnosis, %	5.4	
Underdiagnosis, %	0.0	
<b>UPTH</b>		
Overdiagnosis, %	5.6	
Underdiagnosis, %	0.0	
<b>TOTAL</b>		
Overdiagnosis, %	5.5	
Underdiagnosis, %	0.0	

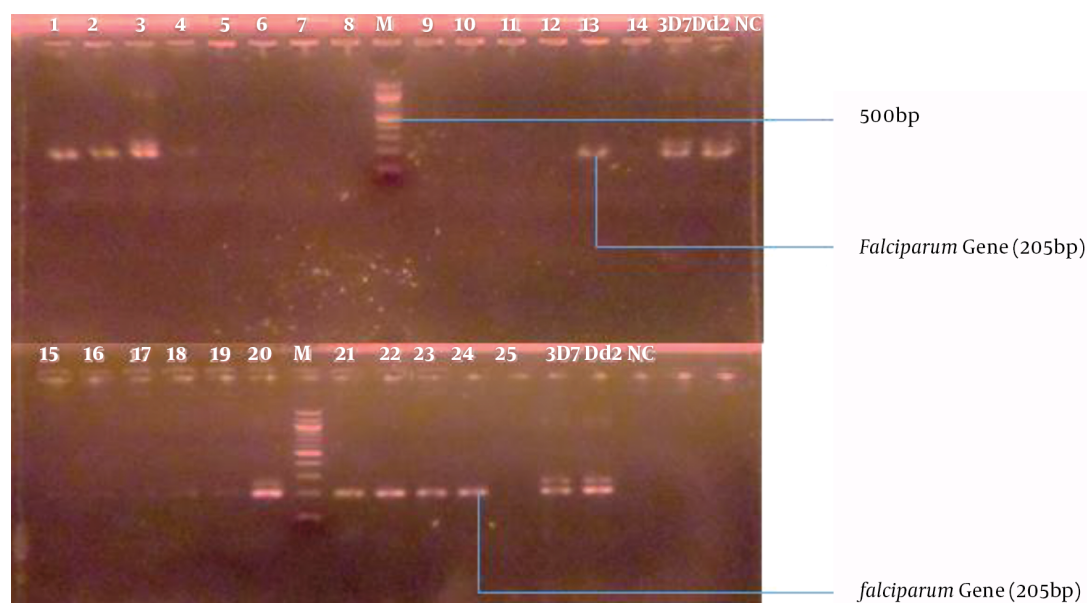
Table 3. Overall Microscopy Diagnostic Variation According to Age Groups and Sex

Variable	Misdiagnosis	
	Overdiagnosis, %	Underdiagnosis, %
<b>Age Groups (N = 5)</b>		
0 - 10	6.3	0.0
11 - 20	4.7	0.0
21 - 30	3.1	0.0
31 - 40	6.9	0.0
$> 40$	6.5	0.0
TOTAL	5.5	0.0
<b>Sex (N = 2)</b>		
Male	6.5	0.0
Female	4.5	0.0
TOTAL	5.5	0.0

Table 4. Positive Parasite Diagnosis by Microscopy

Positive Parasite Diagnosis	Number Infected, (%)		Grand Total, (%)
	RSUTH	UPTH	
Microscopy alone (not confirmed by PCR)	39 (12.1)	34 (7.9)	73 (9.7)
Microscopy and confirmed by PCR	283 (87.9)	397 (92.1)	680 (90.3)
TOTAL	322	431	753

*P. falciparum* (17-19). Malaria is endemic in most regions of sub-Saharan Africa and *P. falciparum* is responsible for 99.7% of estimated malaria cases in WHO African regions



**Figure 1.** Agarose Gel electrophoresis of *Plasmodium falciparum* gene isolated from positive and negative malaria samples. Lanes 1-14 and 15-25 represent the samples. Lane M is the 100 bp molecular ladder; 3D7 and Dd2 are the positive controls, while NC is the negative control.

**Table 5.** Diagnostic Efficiency of Microscopy

Diagnostic Parameters	RSUTH (n = 1000) <sup>a</sup>	UPTH (n = 1000)	TOTAL (n = 2000)
Sensitivity	95.6	95.9	95.8
Specificity	94.5	94.2	94.3
Diagnostic accuracy	94.8	94.9	94.9

<sup>a</sup>n = number of study subjects.

(where this study was conducted), 50.0% of cases in the WHO South-East Asia regions, 71.0% of cases in the East Mediterranean and 65.0% in the Western Pacific. The overall malaria prevalence of 38.0% in this study is comparable to 36.0% reported in Abia and 36.6% in Plateau (20), 40.8% in Rivers (21), 40.8% in Sokoto (22), 34.5% in Ogun (23), 38.7% in Kano (24), 31.6% in India (25), 35.7% in Kaduna (26) and 39.5% in Benue (27) but lower than 46.6% in Zamfara (28), 60.6% in Kano (29), 71.4% in Cross Rivers (30), 72.5% in Rivers (31), 78.0% in Southern Tanzania (32), 66.8% in Ogun (33), and 67.5% in Rivers (34). The study prevalence (38.0%) is higher than 14.7% reported in Lagos (35), 4.1% in Ethiopia (36), 20.7% in Lagos (37), 15.9% in North-west Angola (38) and 11.4% in India (39). The low prevalence of malaria in this study, compared with higher prevalence values of previous malaria studies in Rivers State, might be attributed to increased public awareness and usage of insecticide treated nets as well as increased laboratory tests

and prompt treatment of confirmed malaria-infected individuals. However, the malaria prevalence recorded in this study is of public health significance and it could be attributed to several factors in the study area such as environmental conditions (the temperature, humidity and altitude support the thriving of *Anopheles* species), blocked drainage systems (flooding and accumulation of stagnant water, especially after heavy rainfall, increases the competence levels and breeding of *Anopheles* mosquitoes) and over-crowded settlements (increases the chances of contracting malaria due to frequent physical contact between humans and mosquitoes). The overall diagnostic variation of microscopy was low when compared with the malaria diagnostic values of PCR. The diagnostic variations by microscopy in the study could be attributed to varying expertise among microscopists and errors in preparing or reading stained slides. The overall misdiagnosis of 5.5% in this study was relatively low; it was not statistically significant among age groups and sexes. The low value of misdiagnosis could be attributed to improved diagnosis and quality equipment in the tertiary hospitals as well as the successful efforts of the Rivers State Ministry of Health and Management of the studied healthcare facilities in improving the efficiency of malaria diagnosis by microscopy as one of the several tools in effective malaria control. Microscopists should be trained regularly to achieve high proficiency but PCR technique can further strengthen microscopy when *Plasmodium* identification is difficult. The

overall positive sample diagnosis by microscopy and confirmed by PCR shows that microscopy remains the gold standard for malaria diagnosis when properly conducted but PCR diagnosis is also vital in sub-microscopic parasite densities.

### 5.1. Conclusion

The study provided data on *Plasmodium* species prevalence and misdiagnosis, which is necessary for effective malaria control strategies in endemic countries. The study also validated the use of microscopy by well-trained experts as a reliable *Plasmodium* diagnostic tool.

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### Footnotes

**Authors' Contribution:** MNW and EOO designed the study, acquired data, performed the study analysis and wrote the first draft of the manuscript. Both authors read and approved the final manuscript.

**Conflict of Interests:** The authors declare that there is no competing interest in this study.

**Ethical Approval:** Ethical approval was obtained from the Rivers State Ministry of Health before the commencement of this study.

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**Informed Consent:** Informed consent was obtained from all study participants; the parents/guardians gave the consent for study participants below 18 years.

### References

1. CDC. *Parasites - Malaria*. 2020, [cited May 02]. Available from: [https://www.cdc.gov/malaria/malari\\_worldwide/impact.html](https://www.cdc.gov/malaria/malari_worldwide/impact.html).
2. Owusu-Ofori AK, Betson M, Parry CM, Stothard JR, Bates I. Transfusion-transmitted malaria in Ghana. *Clin Infect Dis*. 2013;**56**(12):1735–41. doi: [10.1093/cid/cit130](https://doi.org/10.1093/cid/cit130). [PubMed: [23463635](https://pubmed.ncbi.nlm.nih.gov/23463635/)].
3. WHO. *World malaria report 2019*. Geneva: WHO Malaria Publications; 2019, [cited 4 December 2019]. Available from: <https://www.who.int/malaria/publications/world-malaria-report-2019/en/>.
4. Bharti AR, Patra KP, Chuquiyaui R, Vinetz JM, Kosek M, Gilman RH, et al. Polymerase Chain Reaction Detection of Plasmodium vivax and Plasmodium falciparum DNA from Stored Serum Samples: Implications for Retrospective Diagnosis of Malaria. *Am J Trop Med Hyg*. 2007;**77**(3):444–6. doi: [10.4269/ajtmh.2007.77.444](https://doi.org/10.4269/ajtmh.2007.77.444).
5. Erdman LK, Kain KC. Molecular diagnostic and surveillance tools for global malaria control. *Travel Med Infect Dis*. 2008;**6**(1-2):82–99. doi: [10.1016/j.tmaid.2007.10.001](https://doi.org/10.1016/j.tmaid.2007.10.001). [PubMed: [18342279](https://pubmed.ncbi.nlm.nih.gov/18342279/)].
6. Ugah UI, Alo MN, Owolabi JO, Okata-Nwali OD, Ekejindu IM, Ibeh N, et al. Evaluation of the utility value of three diagnostic methods in the detection of malaria parasites in endemic area. *Malar J*. 2017;**16**(1):189. doi: [10.1186/s12936-017-1838-4](https://doi.org/10.1186/s12936-017-1838-4). [PubMed: [28477621](https://pubmed.ncbi.nlm.nih.gov/28477621/)]. [PubMed Central: [PMC5420404](https://pubmed.ncbi.nlm.nih.gov/PMC5420404/)].
7. Hawkes M, Kain KC. Advances in malaria diagnosis. *Expert Rev Anti Infect Ther*. 2007;**5**(3):485–95. doi: [10.1586/14787210.5.3.485](https://doi.org/10.1586/14787210.5.3.485). [PubMed: [17547512](https://pubmed.ncbi.nlm.nih.gov/17547512/)].
8. Mlambo G, LeBlanc R, Kumar N, Vasquez Y, Sullivan D. A Filter Paper Method for the Detection of Plasmodium falciparum Gametocytes by Reverse Transcription-Polymerase Chain Reaction. *Am J Trop Med Hyg*. 2008;**78**(1):114–6. doi: [10.4269/ajtmh.2008.78.114](https://doi.org/10.4269/ajtmh.2008.78.114).
9. Ayogu EE, Ukwe CV, Nna EO. Assessing the reliability of microscopy and rapid diagnostic tests in malaria diagnosis in areas with varying parasite density among older children and adult patients in Nigeria. *J Postgrad Med*. 2016;**62**(3):150–6. doi: [10.4103/0022-3859.183167](https://doi.org/10.4103/0022-3859.183167). [PubMed: [27241807](https://pubmed.ncbi.nlm.nih.gov/27241807/)]. [PubMed Central: [PMC4970340](https://pubmed.ncbi.nlm.nih.gov/PMC4970340/)].
10. Mahende C, Ngasala B, Lusingu J, Yong TS, 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;**15**(1):391. doi: [10.1186/s12936-016-1450-z](https://doi.org/10.1186/s12936-016-1450-z). [PubMed: [27459856](https://pubmed.ncbi.nlm.nih.gov/27459856/)]. [PubMed Central: [PMC4962396](https://pubmed.ncbi.nlm.nih.gov/PMC4962396/)].
11. Fransisca L, Kusnanto JH, Satoto TB, Sebayang B, Andriyan E; Supriyanto, et al. Comparison of rapid diagnostic test Plasmodium Malaria-3, microscopy, and quantitative real-time PCR for diagnoses of Plasmodium falciparum and Plasmodium vivax infections in Mimika Regency, Papua, Indonesia. *Malar J*. 2015;**14**:103. doi: [10.1186/s12936-015-0615-5](https://doi.org/10.1186/s12936-015-0615-5). [PubMed: [25890368](https://pubmed.ncbi.nlm.nih.gov/25890368/)]. [PubMed Central: [PMC4356117](https://pubmed.ncbi.nlm.nih.gov/PMC4356117/)].
12. Abdel-Wahab MM, Ismail KA, El-Sayed NM. Laboratory diagnosis of malaria infection in clinically suspected cases using microscopic examination, optimal rapid antigen test and PCR. *Parasitol United J*. 2012;**5**(1):59–66.
13. Koita OA, Doumbo OK, Ouattara A, Tall LK, Konare A, Diakite M, et al. False-negative rapid diagnostic tests for malaria and deletion of the histidine-rich repeat region of the hrp2 gene. *Am J Trop Med Hyg*. 2012;**86**(2):194–8. doi: [10.4269/ajtmh.2012.10-0665](https://doi.org/10.4269/ajtmh.2012.10-0665). [PubMed: [22302847](https://pubmed.ncbi.nlm.nih.gov/22302847/)]. [PubMed Central: [PMC3269266](https://pubmed.ncbi.nlm.nih.gov/PMC3269266/)].
14. Wogu M, Nduka F, Wariso K. Prevalence of Uncomplicated and Severe Malaria in Outpatients of a Tertiary Hospital in Rivers State, Nigeria. *J Appl Life Sci Int*. 2017;**15**(3):1–5. doi: [10.9734/jalsi/2017/37370](https://doi.org/10.9734/jalsi/2017/37370).
15. Cheesbrough M. *District laboratory practice in tropical countries, part 2*. Cambridge university press; 2005.
16. Mangold KA, Manson RU, Koay ES, Stephens L, Regner M, Thomson RJ, et al. Real-time PCR for detection and identification of Plasmodium spp. *J Clin Microbiol*. 2005;**43**(5):2435–40. doi: [10.1128/JCM.43.5.2435-2440.2005](https://doi.org/10.1128/JCM.43.5.2435-2440.2005). [PubMed: [15872277](https://pubmed.ncbi.nlm.nih.gov/15872277/)]. [PubMed Central: [PMC1153761](https://pubmed.ncbi.nlm.nih.gov/PMC1153761/)].
17. Wogu MN, Nduka FO. Evaluating Malaria Prevalence Using Clinical Diagnosis Compared with Microscopy and Rapid Diagnostic Tests in a Tertiary Healthcare Facility in Rivers State, Nigeria. *J Trop Med*. 2018;**2018**:3954717. doi: [10.1155/2018/3954717](https://doi.org/10.1155/2018/3954717). [PubMed: [29849665](https://pubmed.ncbi.nlm.nih.gov/29849665/)]. [PubMed Central: [PMC5937386](https://pubmed.ncbi.nlm.nih.gov/PMC5937386/)].
18. Abah AE, Temple B. Prevalence of malaria parasite among asymptomatic primary school children in Angiama community, Bayelsa State, Nigeria. *Trop Med Surg*. 2015.
19. Pondei K, Epidor L, Eno N. Prevalence of the malaria parasite in screened blood in a tertiary health centre in the malaria-endemic Niger-Delta region of Nigeria. *Glob Adv Res J Microbiol*. 2012;**1**(11):188–93.
20. Noland GS, Graves PM, Sallau A, Eigege A, Emukah E, Patterson AE, et al. Malaria prevalence, anemia and baseline intervention coverage prior to mass net distributions in Abia and Plateau States, Nigeria. *BMC Infect Dis*. 2014;**14**:168. doi: [10.1186/1471-2334-14-168](https://doi.org/10.1186/1471-2334-14-168). [PubMed: [24669881](https://pubmed.ncbi.nlm.nih.gov/24669881/)]. [PubMed Central: [PMC3994282](https://pubmed.ncbi.nlm.nih.gov/PMC3994282/)].
21. Amala SE, Nwibani CP. Malaria in pregnancy and its association with ABO blood group and haemoglobin genotype. *Int J Dev Res*. 2015;**5**(8):5317–20.

22. Sani UM, Jiya NM, Ahmed H. Evaluation of a malaria rapid diagnostic test among febrile children in Sokoto, Nigeria. *Int J Med Sci*. 2013;**3**(5):434–40.
23. Olufunmilayo A, Oluwatosin M, Ayodele S. Assessment of Accuracy and Effectiveness of Rapid Diagnostic Test for Malaria Diagnosis at Primary Health Centres in Abeokuta Nigeria. *Int J Trop Dis Health*. 2015;**8**(3):90–7. doi: [10.9734/ijtdh/2015/17725](https://doi.org/10.9734/ijtdh/2015/17725).
24. Mohammed Y, Dabo NT, Kawo AH, Yakubu A. Comparison of microscopic examination and rapid diagnostic tests used to diagnose malaria among pregnant women in Kano, North-Western, Nigeria. *Int J Sci Technol*. 2015;**5**(2):47–52.
25. Naz R, Khan S, Farooqui MK, Girotra R, Malik AK. Comparison of Microscopic Determination and Rapid Diagnostic Tests (RDTs) in the Detection of Plasmodium Infection. *Scholars J Appl Med Sci*. 2016;**4**(7):2539–43. doi: [10.21276/sjams.2016.4.7.49](https://doi.org/10.21276/sjams.2016.4.7.49).
26. Umaru ML, Uyaiabasi GN. Prevalence of malaria in patients attending the general hospital Makarfi, Makarfi Kaduna-State, North-Western Nigeria. *Am J Infect Dis Microbiol*. 2015;**3**(1):1–5.
27. Houmsou RS, Amuta EU, Sar TT, Adagba AH. Malarial infection among patients attending a Nigerian semi-urban based hospital and performance of HRP-2 pf Rapid diagnostic Test (RDT) in screening clinical cases of Plasmodium falciparum malaria. *Transl Biomed*. 2011;**2**(1).
28. Garba B, Muhammad A, Musa A, Edem B, Yusuf I, Bello N, et al. Diagnosis of malaria: A comparison between microscopy and rapid diagnostic test among under-five children at Gusau, Nigeria. *Sub-Saharan Afr J Med*. 2016;**3**(2):96. doi: [10.4103/2384-5147.184371](https://doi.org/10.4103/2384-5147.184371).
29. Dawaki S, Al-Mekhlafi HM, Ithoi I, Ibrahim J, Atroosh WM, Abdulsalam AM, et al. Is Nigeria winning the battle against malaria? Prevalence, risk factors and KAP assessment among Hausa communities in Kano State. *Malar J*. 2016;**15**:351. doi: [10.1186/s12936-016-1394-3](https://doi.org/10.1186/s12936-016-1394-3). [PubMed: [27392040](https://pubmed.ncbi.nlm.nih.gov/27392040/)]. [PubMed Central: [PMC4938925](https://pubmed.ncbi.nlm.nih.gov/PMC4938925/)].
30. Udoh EE, Oyo-Ita AE, Odey FA, Eyong KI, Oringanje CM, Oduwole OA, et al. Malariometric Indices among Nigerian Children in a Rural Setting. *Malar Res Treat*. 2013;**2013**:716805. doi: [10.1155/2013/716805](https://doi.org/10.1155/2013/716805). [PubMed: [23533951](https://pubmed.ncbi.nlm.nih.gov/23533951/)]. [PubMed Central: [PMC3603376](https://pubmed.ncbi.nlm.nih.gov/PMC3603376/)].
31. Nzeako SO, Nduka FO, Origie OA. Prevalence of Malaria in Pregnant Women Attending Ante Natal Care at University of Port Harcourt Primary Health Care Centre Aluu, Port Harcourt, Rivers State, Nigeria. *Int J Sci Res Environ Sci*. 2013;**1**(10):268–72. doi: [10.12983/ijres-2013-p268-272](https://doi.org/10.12983/ijres-2013-p268-272).
32. Harchut K, Standley C, Dobson A, Klaassen B, Rambaud-Althaus C, Althaus F, et al. Over-diagnosis of malaria by microscopy in the Kilombero Valley, Southern Tanzania: an evaluation of the utility and cost-effectiveness of rapid diagnostic tests. *Malar J*. 2013;**12**:159. doi: [10.1186/1475-2875-12-159](https://doi.org/10.1186/1475-2875-12-159). [PubMed: [23663437](https://pubmed.ncbi.nlm.nih.gov/23663437/)]. [PubMed Central: [PMC3689637](https://pubmed.ncbi.nlm.nih.gov/PMC3689637/)].
33. Oyeyemi OT, Ogunlade AF, Oyewole IO. Comparative Assessment of Microscopy and Rapid Diagnostic Test (RDT) as Malaria Diagnostic Tools. *Res J Parasitol*. 2015;**10**(3):120–6. doi: [10.3923/rjp.2015.120.126](https://doi.org/10.3923/rjp.2015.120.126).
34. Wariso KT, Oboro IL. Diagnosis of Malaria among Blood Donors in Port Harcourt, Nigeria: Microscopy or Rapid Diagnostic Tests? *Adv Microbiol*. 2015;**5**(5):358–63. doi: [10.4236/aim.2015.55037](https://doi.org/10.4236/aim.2015.55037).
35. Aina OO, Agomo CO, Olukosi YA, Okoh HI, Iwalokun BA, Egbuna KN, et al. Malariometric survey of ibeshe community in ikorodu, lagos state: dry season. *Malar Res Treat*. 2013;**2013**:487250. doi: [10.1155/2013/487250](https://doi.org/10.1155/2013/487250). [PubMed: [23766924](https://pubmed.ncbi.nlm.nih.gov/23766924/)]. [PubMed Central: [PMC3676961](https://pubmed.ncbi.nlm.nih.gov/PMC3676961/)].
36. Endeshaw T, Gebre T, Ngondi J, Graves PM, Shargie EB, Ejigsemahu Y, et al. Evaluation of light microscopy and rapid diagnostic test for the detection of malaria under operational field conditions: a household survey in Ethiopia. *Malar J*. 2008;**7**:118. doi: [10.1186/1475-2875-7-118](https://doi.org/10.1186/1475-2875-7-118). [PubMed: [18598344](https://pubmed.ncbi.nlm.nih.gov/18598344/)]. [PubMed Central: [PMC2474640](https://pubmed.ncbi.nlm.nih.gov/PMC2474640/)].
37. Oladosu OO, Oyibo WA. Overdiagnosis and Overtreatment of Malaria in Children That Presented with Fever in Lagos, Nigeria. *ISRN Infect Dis*. 2013;**2013**:1–6. doi: [10.5402/2013/914675](https://doi.org/10.5402/2013/914675).
38. Fancony C, Sebastiao YV, Pires JE, Gamboa D, Nery SV. Performance of microscopy and RDTs in the context of a malaria prevalence survey in Angola: a comparison using PCR as the gold standard. *Malar J*. 2013;**12**:284. doi: [10.1186/1475-2875-12-284](https://doi.org/10.1186/1475-2875-12-284). [PubMed: [23941281](https://pubmed.ncbi.nlm.nih.gov/23941281/)]. [PubMed Central: [PMC3751255](https://pubmed.ncbi.nlm.nih.gov/PMC3751255/)].
39. Ranjan P, Ghoshal U. Utility of nested polymerase chain reaction over the microscopy and immuno-chromatographic test in the detection of Plasmodium species and their clinical spectrum. *Parasitol Res*. 2016;**115**(9):3375–85. doi: [10.1007/s00436-016-5098-y](https://doi.org/10.1007/s00436-016-5098-y). [PubMed: [27147091](https://pubmed.ncbi.nlm.nih.gov/27147091/)].