MIKAILALSYS
Journal of Multidisciplinary Sciences

e-ISSN : 2987-2286
p-ISSN : 2987-3924

Index : Dimensions, Scilit, Crossref, Garuda, Semantic, Lens,Google Scholar,Base, etc

https://doi.org/10.58578/mikailalsys.v3i2.5442

A Study on the Comparative Diagnostic Performance of Polymerase Chain Reaction, Rapid Diagnostic Technique and Microscopy in the three Northern Local Government Area of Taraba State, Nigeria

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Article Info:

Submitted:	Revised:	Accepted:	Published:
Mar 21, 2025	Apr 5, 2025	Apr 17, 2025	Apr 22, 2025

Abstract

This study is conducted to compare diagnostic performance of Polymerase Chain Reaction, Rapid Diagnostic Techniqu`e and Microscopy. Nested PCR, RDT and Microscopy methods were used to screen for *P. falciparum* in the study populations. From site Jalingo, 85(75.9%), 70(62.5%) and 97(86.6%) samples were positive using nested PCR, RDT and Microscopy, while 27(24.1%), 42(37.5%) and 15(13.4%) were negative respectively. For site Zing, 85(73.3%) were positive for PCR, while 31(26.7%) were negative; 81(69.8) were positive for RDT and 35(30.1) were negative, while 104(89.7%) were positive with Microscopy and 12(10.3%) negative. Also, 82(75.2%), 50(45.9%) and 103(94.5%) were positive respectively for nested PCR, RDT and Microscopy, while 27(24.8%), 59(54.9%) and 6(5.5%) were negative respectively for the methods from site Lau. In all, microscopy method record the highest number of positive samples. The number of True Positive (TP) and True Negative (TN) recorded are 85 and 11 for PCR and 75 and 5 for

Microscopy in Jalingo, 85 and 14 for PCR and 77 and 4 for Microscopy in Zing and 82 and 9 for PCR and 82 and 6 for Microscopy in Lau. The specificity and sensitivity of RDT from Jalingo, Zing and Lau are respectively 47.8% & 95.5%, 51.8% & 95.5% and 50.0% & 96.9%. Also, the specificity and sensitivity of Microscopy are 18.55% & 88.2%, 12.9% & 90.6% and 22.2% and 100% respectively**.** Owing to the higher sensitivity of the PCR method compared to Microscopy and RDT, *P. falciparum* detection by PCR was used as the reference method. From study site Jalingo, 22 (19.6%) and 5 (4.5%) false positive results Microscopy and RDT were negative for PCR and 10 (4.5%) and 20 (9.0%) false negatives Microscopy and RDT. This shows that Microscopy has a four-fold false positive detection error rate than RDT, while RDT has a two-fold false negative detection error rate. 56 (50%) were positive for the three methods. From study site Zing, the number of false positive for Microscopy and RDT are respectively 27 (23.3%) and 8 (6.9%), while PCR corrected that were false negatives were 8 (6.9%) and 12 (10.3%) respectively. Also from site Lau, 21 (19.3%) false positives were recorded for Microscopy, while only 2 (1.8%) were recorded for RDT. Microscopy had 0 (0%) false negatives while RDT has 34 (31.2%) that were nested PCR corrected. The same trend in the number of false positives and false negatives was observed in all the three sites, while Microscopy generally has higher false positive rate, RDT has a higher false negative rate. This study underscores the clinical utility of hematological and biochemical parameters in malaria management, particularly in resource-limited settings like Nigeria. The findings highlight the importance of comprehensive diagnostic approaches and suggest integrating these adjunct tools into malaria treatment protocols to enhance patient care and outcomes.

Keywards: Polymerase Chain Reaction, Rapid Diagnostic Technique, Microscopy, Northern Local Government Area, Taraba State, Nigeria

INTRODUCTION

Several Species of intracellular protozoa of the genus *Plasmodium* cause malaria in humans. Among them are *Plasmodium ovale*, *Plasmodium vivax, Plasmodium falciparum*, and *Plasmodium malariae.* (Joshi *et al*., 2008; WHO, 2019) and more recently, *P. knowlesi* (World Malaria Report, 2018). *P. falciparum* and *P. vivax* cause the most serious forms of the disease (World Health Organization, 2010; Elhassan *et al*., 2009; Idonije *et al*., 2011; WHO, 2022). Humans contract diseases from the bite of female Anopheles mosquitoes, which spread the disease around the world. These disease cuasing organisms have a complicated life history that requires a vertebrate host for the asexual cycle and female *Anopheles* mosquitoes for

completion of the sexual cycle. Malaria poses a threat to public health with 80 to 90% of morbidity and mortality occurring in Africa, afflicting both young and old (Federal Ministry of Health, 2005; Adeneye *et al*., 2007; Kamau *et al*., 2015). In addition, reports showed that malaria could be transmitted by transfusion of infected blood (Ali & Kadaru, 2005; WHO, 2010), sharing needles (Tracy and Webster, 2001) and congenital transmission (Ezechukwu *et al*., 2004).

In 2020, there were 241 million cases of malaria, compared to 227 million cases in 2019, according to the most recent World Malaria Report. An estimated 627 000 people died from malaria in 2020, an increase of 69 000 over the year before. A recent modification in WHO's technique for measuring malaria mortality accounts for one third of these deaths (22,000), while about two thirds of these deaths (47 000) were caused by interruptions during the COVID-19 pandemic (irrespective of COVID-19 disruptions). 32 sub-Saharan African nations, which account for almost 93% of all malaria fatalities worldwide, were subjected to the new cause-of-death methodology (World Malaria Report, 2021). Applying the methodology revealed that malaria has taken a considerably higher toll on African children every year since 2000 than previously thought (WHO, 2022). The WHO African Region continues to carry a disproportionately high share of the global malaria burden. In 2020, the Africa region was home to 95% of all malaria cases and 96% of deaths. About 80% of all malaria deaths in the region were in children under the age of five. Just over half of all malaria deaths globally occurred in four African countries: Mozambique (3.8%), the Democratic Republic of the Congo (13.2%), Nigeria (31.9%), and the United Republic of Tanzania (4.1%) (WH0, 2022).

Approximately 24% of Nigerians reside in low transmission zones, while 76% of the country's population lives in high transmission areas. Malaria is contagious across the country. The transmission season can last all year round in the south and is about 3 months or less in the northern part of the country.

With its elevated morbidity and mortality, malaria continues to be one of the most common parasite diseases in the tropics. In sub-Saharan Africa, *Plasmodium falciparum* is the most common malarial parasite; outside of Africa, *Plasmodium vivax* is the most common parasite and accounts for over 30% of cases in South-East Asia (World Malaria Report, 2018). According to Khuraiya *et al.* (2016), there is typically a significant morbidity and mortality rate in instances of severe malaria as well as those with changed haematological

and biochemical parameters, which typically result in frequent malaria sequelae. However, infection with *P. falciparum* represented more than 90% of the global malaria mortality and thus it is still a relevant global threat to public health (Snow, 2015). Furthermore, an estimated of 781,000 deaths and 225 million cases of *falciparum* malaria were recorded annually (WHO, 2010). Moreover, *P. falciparum* infection is frequently correlated to cerebral malaria and mortality that promote neurological sequelaes (Schiess *et al.*, 2020). Although clinical presentations are common in malaria diagnosis particularly in poor countries, however, those symptoms of fever are reasonable for malaria diagnosis due to lack of specificity as it is common with other illnesses making clinical diagnosis more confront (Erhart *et al.*, 2004). Furthermore, this results in an overuse of antimalarial medications, decreasing their specificity and lowering the standard of treatment provided to patients (Reyburn *et al.*, 2007). While, microscopic diagnosis continues to be the gold standard, most economical technique for malaria diagnosis, but its sensitivity and specificity for parasite detection are still weak particularly in endemic areas in Africa, as this technique needs a qualified technical expertise, consumes more time and deficient for a satisfactory health care issues (Reyburn *et al.*, 2007; WHO, 2009; Maina *et al.*, 2010).

During the hepatic stage of the malaria parasite's life cycle, when malaria sporozoites transform into merozoites, the liver is a crucial organ. One frequent side effect that typically follows a malaria infection is liver damage. Studies have shown that persons with malaria may experience a sudden elevation in liver enzyme levels, which may be a sign of liver malfunction (Onyesom and Onyemakonor, 2011). This could be as a result of the invasion of liver cells by the sporozoite during malaria parasite life cycle. The changes caused in the hepatic cell by sporozoite can lead to the leakage of parenchymal and membranous enzymes of the liver into the circulatory system, which can be responsible for the increase in liver enzymes (Chidoka *et al.*, 2013). Liver is one of the important organs of the body. It plays a vital role for the proper function of the body. Liver enzyme tests are broadly defined as tests useful in the evaluation and treatment of patients with hepatic dysfunction. They also help to monitor therapy and assess prognosis. As a result of high level of complications and death of children due to malaria infection, there is need to evaluate the extent of hepatic dysfunctions in malaria cases so that there will be proper management of malaria infection and its associated complications (Ogbadoyi and Tsado, 2009).

METHODS

Study Area

Taraba state is the third largest state in terms of landmass in Nigeria. It is located in the Southern part of northeastern Nigeria along the eastern borderland between Nigeria and Cameroon. The state lies roughly between latitude 6° 25'N and 9° 30'N and between longitude 9°30'E and 11° 45'E. It is bordered on the west by Nasarawa and Plateau States, to the north by Bauchi and Gombe States and by Adamawa State to the northeast. It also shares its southwestern boundary with Benue State. Taraba State is bounded on the south and southeast by the Republic of Cameroon (an international boundary). The state covers a land area of about 60,291 km2 with a population of about 2,300,736 people according to the 2006 census. Taraba state is made up of 16 Local Government Areas (LGAs) and 3 Senatorial districts (northern Taraba, southern Taraba, and Taraba central). Northern Taraba consists of six LGAs, Ardo Kola, KarimLamido, Lau, Jalingo, Yorro and Zing.

Study Sites

The study was conducted in three centres of three Local Government Areas selected randomly in Taraba State which include Federal Medical Centre in Jalingo, General Hospital in Lau and General Hospital, Zing. The residents of the Local Governments are predominantly Mumuye, Yandang, with few minor tribes viz Jenjo, Fulani and Hausa among others and they are mostly farmers. The climate in the area can be described as tsub-humid type with two distinct (wet and dry seasons). It has an average rainfall of 7 months annually with total range between 1,200mm and 2000mm in the months of April and October. The temperature is relatively high throughout the year averaging 28-32°C with occasional peak at 44.0 °C between March and April.

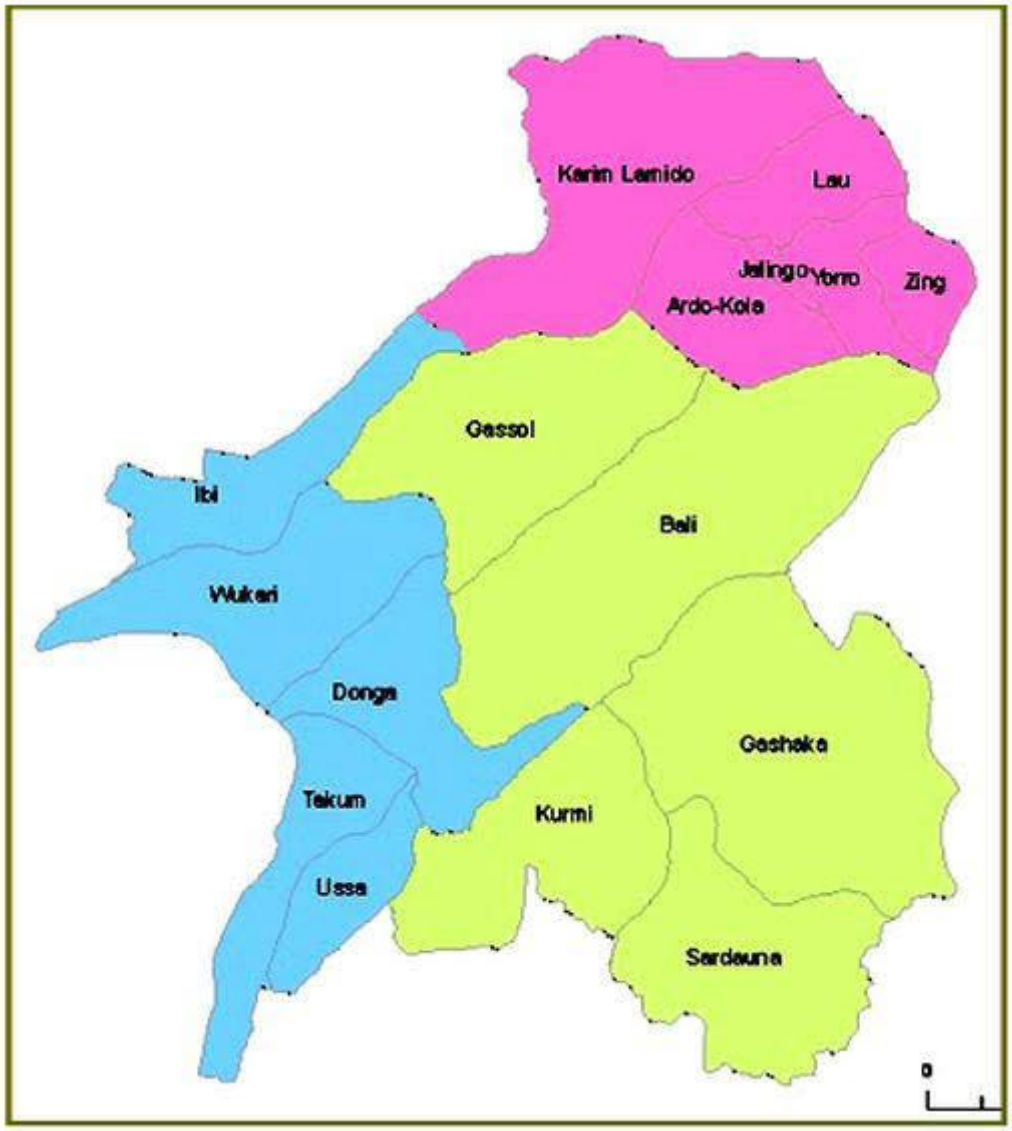

Fig. 1. Map of Taraba State showing the study area

Study population and Selection of subjects

A total of 1,500 patients who were diagnosed for the presence of *Plasmodium species*, by the routine laboratory methods, that is, giemsa stained blood film examination, as per WHO protocol (WHO, 2018) and Immunochromatography was included in this study. A total of 500 participants was selected randomly from each centre in the three Local Government Areas chosen for the study.

Inclusion criteria

Only subjects who consent to the study were included. Only individuals with *P. falciparum* infection were included as test subjects

Exclusion criteria

Subjects who refuse to give consent were excluded. Subjects who are infected with or who have co-infection with other types of *Plasmodium* species were excluded. Subjects with underlying medical condition(s) inimical to biochemical or haematological indices including liver and kidney problems were excluded as well. Children below the age of 1 year were excluded as well.

Research Design

This is a prospective cross-sectional study involving subjects with *Plasmodium falciparum* (*P. falciparmn*) malaria infection serving as tests while *P. falciparum* negative subjects serving as controls.

Data Collection

Sample collected was given a coded number without the patient's name and labelled appropriately taking note of the centre of collection, Local Government Area, gender, age group, marital status, educational status etc. Data were collected for a period of 12 months concurrently in the three study sites.

Sample Handling and Disposals

All the samples collected for this research work was handled discretely by professional Laboratory Technicians or scientist who determine the infection status of participants. After testing, the samples were incinerated with the aid of Kerosene. The Data collected was kept confidential and only use for research purpose only. The test result of each individual was made known to them following request.

Sample Size Estimation

The sample size was determined from a z-test formula for the calculation of sample size.

Sample size (n)

$$\frac{(Z_{1-a})^2 \, x \, P(1-P)}{d^2} \quad (1)$$

Where n = minimum sample size,

Z_{1-a} value of standard normal deviation which is at 95% confidence level has been found to be 1.96.

P=best estimate of the population prevalence obtained from the literature review.

d=proportion of sample error in a given population.

At prevalence rate of *Plasmodium* malaria of 19.2.0% (Yakubu *et al.*, 2019), using 5% precision

at 95% confidence level, the minimum sample size n for this study is calculated as follows:

Where Z=1.96,

P=19.2%, 0.192 d= 5%, 0.05

Therefore, n= (1.96) x 0.192 x (1-0.192)

$$0.05^2$$

n=238

The minimum sample size is 238

Added attrition was = 110% of 238

Total sample size=500 x 3 =1500

Questionnaire

A structured questionnaire was administered for all consenting participants to capture their demographic characteristics which included sex, age, marital status, educational level, whether or not they are on any form of condition they are managing.

Validation of Questionnaire

Content validity was carried out by experts in the subject matter by reviewing the questionnaire to ensure that it covers all relevant topics comprehensively. The questionnaire was also compared with existing validated questionnaire and literature to ensure it covers all aspects. A pilot study was conducted with a small sample of the target population to check for clarity and relevance.

Reliability Test

Cronbach's Alpha test was used to measure the internal consistency of the questionnaire. The reliability was retested by administering the questionnaire to the same group of people at two different points in time and the correlation between the two sets was calculated.

Informed Consent

Written informed consent for participation in the study was sought from the patients in accordance with standards for human experimentation and the Helsinki Declaration.

Ethical Approval

The Ethical approval was sought from the Taraba State Ministry of Health Ethical Committee and presented to the health facilities where the blood samples were collected in compliance with the Declaration on the Right of the Patient (Foster *et al.*, 2001). Before enrolment for the study, the patients/subjects involved signed an informed consent form. Guardian/Parent sign the consent form on behalf of participants below the age of 18 years old.

Collection and preparation of blood specimen

All blood samples were collected by a phlebotomist or Laboratory Technician and screened by a Laboratory Scientist or Technician. With sterile aseptic precaution, about 5mL of venous blood was collected from the cubital vein of adult and/or by pricking the hand or leg in children. Three milliliters (3mL) of the blood samples were transferred into plain bottles to allow for coagulation, whereas the remaining 2mL was transferred into ethylenediaminetetraacetic acid (EDTA) bottles for malaria parasite tests and haematological studies. The coagulated blood samples were centrifuge at 3000 rpm for 10 minutes, the serum transferred into Bijou bottle and stored frozen until required for biochemical analyses (Onyesom *et al.*, 2010). The researcher in Collaborations with the laboratory Technicians or Scientist were responsible for safety issues during sample collection. Participants who reacted negatively or whose skins were damaged during sample collections were referred to a doctor in the facility and the cost implications was handled by the researcher. The researcher did not provide medications for malaria positive participants but were referred to a physician for proper treatment. The participants were made aware and given a typed or written test result regardless whether positive or negative.

Preparation of thick and thin blood films

Thick and thin blood films were prepared on the same slide for each subject and stained using 10% Giemsa stain as described by Cheesebrough (2006). Using a completely clean grease free microscope slide a small drop of blood was added to the centre of the slide and a large drop about 15mm to the one end of the slide. Using a smooth edge slide spreader, the thin blood film was spread immediately without delay, the large drop was spread to make a thick film to cover an area of about 15 x 15mm. the slide was allowed to air dry. The thin film was fixed with absolute methanol for two minutes without touching the thick films.

Giemsa Staining Technique

The giemsa stain was diluted to 10% solution (90ml buffered distilled water pH 7.0 to 10ml Giemsa stain in a measuring cylinder and mixed). The slides were placed on a staining rack. The diluted stain was flooded on the slides and allowed to stain for 45-60 minutes. The slides were washed using buffered distilled water pH 7.0. The back of the slides were wiped off with cotton wool and placed in a clean rack. A drop of oil immersion was applied to an area of the film. An area that was well stained and not too thick was selected and examined for malaria parasites and malaria pigments and was confirmed by examining the thin blood films (WHO, 2020).

Parasite Density Test

Measurement of parasite density of peripheral blood smear was done via Giemsa stained techniques. The films were examined microscopically using x100 objective under oil immersion (Cheesbrough, 2006) as reported by Sumbele *et al.* (2010). Level of parasitaemia was in microliter (µl) of blood thick film preparation (Erhart *et al.*, 2004). According to WHO (2005), level of parasitaemia were graded as low+ (1 to 999/µl), moderate++ (1000 to 9999/µl) and severe++++ (>200,000/µl).

Molecular Genotyping of Parasite

All molecular analysis were performed at the Malaria Genomic Research & Training Center (MGRTC) of the Department of Biochemistry and Nutrition of the Nigerian Institute of Medical Research (NIMR), Yaba, Lagos State, Nigeria.

DNA extraction

Blood sample from patients with uncomplicated malaria was spotted on Whatman 3mm filter paper to make Dried Blood Spot (DBS). The DBS was punctured into a 1.5ml eppendoff tube. Parasite DNA was extracted from the punctured DBS using the phenol/chloroform method (Sambrook *et al.*, 1989). Briefly, the punctured DBS were treated using proteinase K. Then, the phenol extraction/ethanol precipitation method was used to obtain the parasite DNA. This DNA was resuspended in 50 µL of buffer, therefore, 1 µL of the DNA template corresponded to 3 µL of whole blood (Cuhna *et al.*, 2009). The extracted DNA was stored in -20^{o}C until it was used.

DNA amplification

To genotype the parasite, the merozoite surface proteins 1, *msp1* and *msp2* allelic families were targeted for amplification in a nested PCR technique according to Snounou and Beck (1998). Three loci, i.e K1, MAD20 and RO33 were independently amplified from the *msp1* allele while two loci, i.e 3D7 and FC27 were amplified from *msp2*. The *msp1* gene was amplified in a primary PCR reaction, while the K1, MAD20 and RO33 were amplified in a secondary PCR step. Likewise, the *msp2* allele was amplified in a primary PCR step, while the 3D7 and FC27 were amplified in a secondary PCR reaction. The primer sequences are shown in the table below.

Statistical analysis

The data obtained was analysed using SPSS V28. Chi-square was used to compare the prevalence of malaria infection between age, sex, occupation and educational status of the subjects. The Odd Ratio (OR) was determined using logistic regression to find the relation between the malaria infection and the haematological parameters. The general linear model was used for differentiation of haematological parameters of patients examined likewise biochemical parameters. T-test was also used to determine relationship between haematological and biochemical parameters of malaria parasitized and non-parasitized subjects. Statistical significance was set at 0.05.

RESULTS

Comparative Diagnostic Performance of PCR, RDT and Microscopy in the three study sites

Nested PCR, RDT and Microscopy methods were used to screen for *P. falciparum* in the study populations. From site Jalingo, 85(75.9%), 70(62.5%) and 97(86.6%) samples were positive using nested PCR, RDT and Microscopy, while 27(24.1%), 42(37.5%) and 15(13.4%) were negative respectively. For site Zing, 85(73.3%) were positive for PCR, while 31(26.7%) were negative; 81(69.8) were positive for RDT and 35(30.1) were negative, while 104(89.7%) were positive with Microscopy and 12(10.3%) negative. Also, 82(75.2%), 50(45.9%) and 103(94.5%) were positive respectively for nested PCR, RDT and Microscopy, while 27(24.8%), 59(54.9%) and 6(5.5%) were negative respectively for the

methods from site Lau. In all, microscopy method record the highest number of positive samples.

Table 1: Comparative Diagnostic Performance of PCR, RDT and Microscopy in the three study sites

Study Sites (n)	PCR		RDT		MICROSCOPY	
	Pos. (%)	Neg. (%)	Pos. (%)	Neg. (%)	Pos. (%)	Neg. (%)
Jalingo (112)	85(75.9)	27(24.1)	70(62.5)	42(37.5)	97(86.6)	15(13.4)
Zing (116)	85(73.3)	31(26.7)	81(69.8)	35(30.1)	104(89.7)	12(10.3)
Lau (109)	82(75.2)	27(24.8)	50(45.9)	59(54.9)	103(94.5)	6(5.5)
Total	252 (74.8)	85(25.2)	201 (59.6)	136 (40.4)	304(90.2)	33(9.8)

Comparing the sensitivity and specificity of RDT and Microscopy

The number of True Positive (TP) and True Negative (TN) recorded are 65 and 22 for RDT and 75 and 5 for Microscopy in Jalingo, 73 and 23 for RDT and 77 and 4 for Microscopy in Zing and 48 and 25 for RDT and 82 and 6 for Microscopy in Lau. The specificity and sensitivity of RDT from Jalingo, Zing and Lau are respectively 81.5% & 76.5%, 72.2% & 85.9% and 92.6% & 58.5%. Also, the specificity and sensitivity of Microscopy are 18.55% & 88.2%, 12.9% & 90.6% and 22.2% and 100% respectively**.** Owing to the higher sensitivity of the PCR method compared to Microscopy and RDT, *P. falciparum* detection by PCR was used as the reference method.

Comparing the sensitivity and specificity of PCR and Microscopy

The number of True Positive (TP) and True Negative (TN) recorded are 85 and 11 for PCR and 75 and 5 for Microscopy in Jalingo, 85 and 14 for PCR and 77 and 4 for Microscopy in Zing and 82 and 9 for PCR and 82 and 6 for Microscopy in Lau. The specificity and sensitivity of RDT from Jalingo, Zing and Lau are respectively 47.8% & 95.5%, 51.8% & 95.5% and 50.0% & 96.9%. Also, the specificity and sensitivity of Microscopy are 18.55% & 88.2%, 12.9% & 90.6% and 22.2% and 100% respectively**.** Owing to the higher sensitivity of the PCR method compared to Microscopy and RDT, *P. falciparum* detection by PCR was used as the reference method. From study site Jalingo, 22 (19.6%) and 5 (4.5%) false positive results Microscopy and RDT were negative for PCR and 10 (4.5%) and 20 (9.0%) false negatives Microscopy and RDT. This shows that

Microscopy has a four-fold false positive detection error rate than RDT, while RDT has a two-fold false negative detection error rate. 56 (50%) were positive for the three methods. From study site Zing, the number of false positive for Microscopy and RDT are respectively 27 (23.3%) and 8 (6.9%), while PCR corrected that were false negatives were 8 (6.9%) and 12 (10.3%) respectively. Also from site Lau, 21 (19.3%) false positives were recorded for Microscopy, while only 2 (1.8%) were recorded for RDT. Microscopy had 0 (0%) false negatives while RDT has 34 (31.2%) that were nested PCR corrected (Table 26). The same trend in the number of false positives and false negatives was observed in all the three sites, while Microscopy generally has higher false positive rate, RDT has a higher false negative rate.

Table 2: Comparing the Sensitivity, Specificity and Predictive values of RDT and Microscopy

Sites (n)	RDT						MICROSCOPY					
	TP	FP	TN	FN	Specificity (%)	Sensitivity (%)	TP	FP	TN	FN	Specificity (%)	Sensitivity (%)
Jalingo (112)	65	5	22	20	81.5	76.5	75	22	5	10	18.5	88.2
Zing (116)	73	8	23	12	74.2	85.9	77	27	4	8	12.9	90.6
Lau (109)	48	2	25	34	92.6	58.5	82	21	6	0	22.2	100
Total (337)	186	15	70	66	82.4	73.8	234	70	15	18	82.4	92.9

TP: True Positives; FP: False Positives; TN: True Negatives; FN: False Negatives.

Specificity = TN/(TN + FP)

Sensitivity = TP/(TP + FN)

Positive Predictive Value (PPV) = PPV = True Positives / (True Positives + False Positives)

Negative Predictive Value (NPV) = True Negatives / (True Negatives + False Negatives)

PPV of RDT = 92.5%

NPV of RDT = 51.5%

PPV of Microscopy = 76.9%

NPV of Microscopy = 45.5%

Table 3: Comparing the Sensitivity, Specificity and Predictive values of PCR and Microscopy

Sites (n)	PCR						MICROSCOPY					
	TP	FP	TN	FN	Specificity (%)	Sensitivity (%)	TP	FP	TN	FN	Specificity (%)	Sensitivity (%)
Jalingo (112)	85	12	11	4	47.8	95.5	75	22	5	10	18.5	88.2
Zing (116)	85	13	14	4	51.8	95.5	77	27	4	8	12.9	90.6
Lau (109)	82	9	9	2	50.0	97.6	82	21	6	0	22.2	100
Total (337)	252	34	34	8	50.0	96.9	234	70	15	18	82.4	92.9

TP: True Positives; FP: False Positives; TN: True Negatives; FN: False Negatives; n: number

Specificity = TN/(TN + FP)

Sensitivity = TP/(TP + FN)

Positive Predictive Value (PPV) = PPV = True Positives / (True Positives + False Positives)

Negative Predictive Value (NPV) = True Negatives / (True Negatives + False Negatives)

PPV of PCR = 88.1%

NPV of PCR = 50.0%

PPV of Microscopy = 76.9%

NPV of Microscopy = 45.5%

DISCUSSION

Comparative sensitivity and specificity of Rapid Diagnostic Test, Microscopy and Polymerase Chain Reaction (PCR)

In malaria control, efficient diagnosis of *P. falciparum* infection is as important as the treatment of the disease, as a false positive diagnosis could result in the administration of drug to an uninfected person, a practice which has been attributed to contribute to antimalarial drug treatment failure (Bloland, 2001), or a false negative diagnosis, which can leave an infected person untreated, resulting in an increased risk of morbi-mortality as well as continued transmission of the disease in the population (Berzosa *et al.*, 2018)

Microscopy method had a higher number of positive samples detected than nested PCR and RDT methods from the three study sites. This observed higher detection rate of microscopy across the sites when compared to PCR could be as a result of human error in slide reading. This is because all the false negatives of Microscopy across the three study sites were all a "+" positive detection, which is almost next to a negative slide reading. However, the microscopist could have read artifacts as a "+" parasite detection. This claim is supported by the report of McKenzie *et al.* (2003).

The True Positive (TP) and True Negative (TN) values are important parameters to validate the detection capacity of diagnosis methods and have been used to calculate their Sensitivity and Specificity (Berzosa *et al.*, 2018). From the three sites, RDT has the highest false negative (FN) values with a total of 66 while microscopy recorded a total 18 fasle negative, which corroborates the study of Ogunfowokan *et al.* (2020) in Southern Nigeria where he found RDT to have higher FN values when compared to Microscopy (Ogunfowokan *et al.*, 2020). On the other hand, microscopy has the highest values of TP with a total of 234 versus 186 of RDT. The result from this study shows that microscopy is a better diagnostic technique than RDT. This claim is further supported by the sensitivity and specificity of both methods. Microscopy has a higher sensitivity from the three sites than RDT and a total of 92.6% which is closer to the WHO recommendation of 95% sensitivity than RDT which has a total sensitivity of 73.8% across the three sites. Although RDT has a higher specificity from the three sites compared to Microscopy, both methods have the same total specificity value of 82.4%. The observed relative low performance of RDT when compared to Microscopy is due to the higher FN value of RDT when compared to microscopy and PCR. As HRP-based RDT kit was used in this study, this

may be due to presence of *hrp2* gene deletions in the study sites, which tend to limit the efficiency of RDT as a diagnostic technique as observed by Luchavez *et al.* (2011). This therefore calls for *hrp2* gene deletion prevalence study in the three study sites and Taraba state as a whole.

CONCLUSION

These data provide valuable information for surveillance of *P. falciparum* infection and assessing the appropriateness of the current malaria control strategies in the endemic area. Results of this study suggest that the conventional diagnostic methods for the detection of malaria parasite (microscopy and RDT) are not adequate when accurate epidemiological data are needed to monitor, control and develop malaria elimination interventions. PCR permitted for the accurate diagnosis, confirming it as a valuable tool for epidemiological surveys, mass screening, and assessment of interventions for malaria elimination. This research will be helpful for future treatment strategies that would be tailored to the specific needs of Taraba and Nigeria's malaria- endemic populations.

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