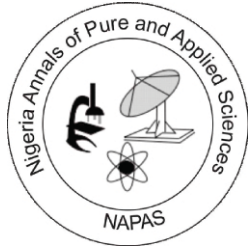


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MOLECULAR SURVEILLANCE OF GENETIC DELETION OF *pfhrp2* IN PLASMODIUM FALCIPARUM POPULATION OF FALSE NEGATIVE MALARIA RDTs IN BENUE STATE, NIGERIA

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Abstract

Malaria case detection and management have been recently improved through the use of rapid diagnostic test (RDT). However, different studies in Nigeria show high prevalence of RDT false negative results. This study was conducted to determine the deletion of *pfhrp2* and their flanking regions among false negative malaria RDTs within different zones of Benue State, Nigeria. 510 Blood samples were collected by finger prick method on filter paper blot between 2018 and 2019. DNA was extracted from filter paper blot using methanol fixation and heat extraction method. *Pfhrp2* genes were amplified by polymerase chain reaction (PCR), resolved by electrophoresis on 2% agarose gel and visualized under UV light. Genotyping of 120 randomly selected false negative isolates using *pfhrp2* genes confirmed that, there was no deletion of *pfhrp2* genes in Benue State, Nigeria. PCR genotyping of 20 randomly selected true negative isolates using *hrp2* genes indicated that, 85% of the true negative isolates were positive for *P. falciparum*. 89% (454/510) of the subjects were identified positive by thick smear microscopy for asexual *P. falciparum* parasites, out of which only 107 (21%) were positive by *PfHRP2* based RDT. Deletion phenomenon of *pfhrp2* was not common in Benue State; hence RDT results could be enhanced through proper maintenance of quality control assurance criteria by avoiding exposure of RDT kits to temperatures above 30°C and relative humidity of above 70% as this is found to degrade the monoclonal antibodies embedded in the RDT kits.

Keywords: Malaria, Diagnosis, *Plasmodium falciparum*, Deletion, *pfhrp2*, False negative.

INTRODUCTION

Malaria in Nigeria is treated blindly without diagnosis in most cases and where diagnostic measures exist, sensitivity of several of them is still in doubt (Adekunle *et al.*, 2014). Microscopy had long been the method of choice for the diagnosis of malaria all over the world. Though, the major drawback of routine microscopy in malaria studies is the requirement of expertise in the parasite identification and power supply (Haditsch, 2014). The challenges of microscopic diagnosis have necessitated the use of rapid diagnostic test (RDT) to ensure prompt and early diagnosis, processing with minimal expertise, very high specificity and sensitivity, and quantitative evaluation of parasitemia to be delivered at a very low cost particularly in highly endemic areas (Wongsrichanalai, 2001).

The diagnosis of *Plasmodium falciparum* malaria using PfHRP2 and PfHRP3 as *P. falciparum* antigen has been widely accepted as a rapid antigen test (Onynekwe *et al.*, 2007; Baker *et al.*, 2005). However, in certain situations, HRP2 based RDT are less sensitive, particularly for parasites that express little or no target antigen, resulting in false negative results (Chenge *et al.*, 2014; Silva *et al.*, 2024). Parasite characteristics such as variable or absent expression of antigens targeted by RDTs can also affect RDT performance (Cheng *et al.*, 2014).

The performance of HRP2 based malaria RDTs in Nigeria for malaria diagnosis has not been consistent and few studies have investigated the use of HRP2 antigen based RDTs with reported sensitivity of 23.4% to 97.7%. Among these there were false negative samples (Adefolahi *et al.*, 2013; Adekunle *et al.*, 2014; Ukpai and Odukaesieme, 2014; Otuu *et al.*, 2015; Oyetunde *et al.*, 2015; Kumari *et al.*, 2021; Silva *et al.*, 2024; Olivera *et al.*, 2025). Therefore, it is hypothesized that these false negative results could be due to deletion of *pfhrp2* gene in these parasites since it has been shown to affect RDT performance in Peru (Gamboa *et al.*, 2010), Senegal (Wurtz *et al.*, 2013), Ghana (Amoah *et al.*, 2016), Mozambique (Gupta *et al.*, 2017), Rwanda (Kozycki *et al.*, 2017), Kenya (Beshir *et al.*, 2017), Congo (Parr *et*

al., 2017), and Eritrea (Berhane *et al.*, 2017). The success of malaria control and elimination is completely dependent on the ability to accurately and rapidly diagnose malaria infection in different settings (Perkins and Bell, 2008). Accurate diagnosis facilitates appropriate and prompt treatment of febrile illness and severe malaria (Gamboa *et al.*, 2010).

False negative RDT results will delay anti-malaria treatment, potentially endangering life and the patient will be a source for ongoing malaria transmission (Cheng *et al.*, 2014). Most Countries are aggressively controlling Malaria, and some are progressing towards elimination, success in this regard relies on appropriate malaria case management based on early diagnosis and prompt treatment with efficacious anti-malaria drugs (WHO, 2001). To improve case management, the World Health Organization (WHO) recommends that parasitological confirmation by microscopy or malaria rapid diagnostic tests (RDTs) is conducted in all patients with suspected cases of malaria prior to commencing treatment (WHO, 2010).

Identifying the prevalence and distribution of *P. falciparum* parasites with deleted *pfhrp2/3* is very important for malaria control. This study seeks to determine the genetic deletion of histidine rich protein-2 in false negative malaria RDT in Benue State, Nigeria.

METHODS

Study Area and Study Design

This study was conducted in Benue State, Nigeria between 2018 and 2019. One hospital was selected each from the three senatorial district of the State, with the adoption of random sampling technique. General hospital Katsina-Ala was selected from Benue North East senatorial district (Zone A), General hospital Makurdi from Benue North-West (Zone B) and General hospital Otukpo from Benue South senatorial district (Zone C).

Informed Consent and Ethical Approval

Informed consent was obtained from the recruited subjects or care givers of the volunteered participants. Ethical approval with reference number MOH/STA/204/VOL.1/79 was obtained

from the Benue State Ministry of Health and Human Services.

Sample Collection and Preparation

Finger prick blood samples were collected from recruited subjects after informed consent from 510 subjects. One drop of blood was used to prepare thick smear, 2-3 drops (50µl) was used for the HRP2 and another 2-3(50µl) were used for filter paper blots in all the three sites. A drop of whole blood was spotted on whatmann No. 1 filter paper before clotting. It was air dried at ambient temperature and kept in a plastic container, sealed with silica gel for deoxyribonucleic acid (DNA) analysis (Koita *et al.*, 2012; Okungbowa and Mordi, 2013).

Microscopic Examination of *Plasmodium falciparum*

Microscopic examination was carried out using methods described in (Norgan *et al.*, 2013; Ojurongbe *et al.*, 2013). Thick blood films were made on pre-cleaned grease-free glass slides. The blood films were fixed by spreading absolute methanol (methyl alcohol) on the microscopic slides. The slides were stained with 10% giemsa stain for 15 minutes, air dried and examined under a microscope with a high-power oil immersion objective lens (X100) for characteristic features of malaria parasites. A total of 100 fields were examined before declaring any sample as positive or negative. Slides were considered positive or negative after two laboratory scientists examined the fields and agreed that they were negative or positive. Slides on which there were disagreements were re-examined by a third time (Koita *et al.*, 2012).

HRP2 based Rapid Diagnostic Test (RDT)

The HRP-2 based RDT (Care Start™ malaria PfHRP-2 Ag RDT) was performed and the result interpreted according to the manufacturer's instructions using a test strip coated with an immobilized immunoglobulin G1 (IgG1) monoclonal antibody against the central histidine-rich repeat region of HRP2. Capillary tubes were used to obtain 50 µl of blood samples from the site of the finger prick and applied to the strip, and

developed using the buffer provided by the manufacturer. Test strips were examined and interpreted after 15-20 minutes. Interpretation of the test results were carried out independently of the microscopic examination and within the time recommended by the manufacturer. Test results were considered invalid if no control band was seen.

Extraction of Parasite DNA from Samples Collected on Filter Paper

Parasite genomic DNA was extracted from blood samples collected on filter paper using the methanol fixation and heat extraction method as described in Lin *et al.* (2005). A 3mm punch was used to punch two 3mm² filter blots of each sample. The punched 3mm filter blots were separately broken in small pieces by separate scalpels. The small pieces of filter blots were packed in Eppendorf tubes and labelled accordingly. 140µl of methanol was pipetted into each of the Eppendorf tubes containing the templates and allowed to stay for 20 minutes. The templates were then aspirated at ambient temperature. 120µl of methanol was pipetted into the Eppendorf tubes containing the DNA templates for the second time after the first was aspirated. It was then incubated in a dry bath incubator at 37°C for 40 minutes and allowed to dry.

The following reagents were pipetted into an Eppendorf tube: 10mM of Hydrogen Chloride (HCl) at a PH of 8.1, 0.5mM of EDTA, 10% w/v of SDS and 20mg/µl of proteinase K. The mixture which was a lysing buffer was mixed using a vortex machine. 150µl of the lysing buffer was dispensed into each of the templates. The buffer (enzyme) was primarily used to lyse the parasite cells and release the DNA. The templates were then incubated in a dry bath incubator for 30 minutes at 60°C. The temperature was increased to 99.9°C for 10 minutes. After the incubation period, the Eppendorf tubes containing the template and the buffer were removed from the dry bath incubator, vortexed and cooled at 4°C in the refrigerator.

PCR Genotyping of Merozoite Surface Protein2 (msp2)

Plasmodium falciparum infections were confirmed by PCR amplification of *P. falciparum* specific gene (pfmsp2). The successful amplification of pfmsp2 gene was an indicative of enough quantity and good quality DNA, which could allow for the amplification of *pfhrp2*, *pfhrp3* and their respective neighbouring genes flanking the central histidine-rich repeat region (Koita *et al.*, 2012). All the samples that amplified pfmsp2 genes were included for final analysis of *pfhrp2* gene deletion. The merozoite surface protein 2 (msp2) genes were amplified using established PCR protocol (Koita *et al.*, 2012; Singh *et al.*, 1999). False negative samples (negative by RDT and positive by microscopy) were randomly selected from the 357 false negatives. Forty (40) of the samples were selected from each of the three sample sites. The samples were genotyped using msp2 genes in order to determine the specific clones that were associated with false negatives in the study area. Briefly, a nested PCR protocol was used. The primary amplification targeted a conserved region of msp2. A secondary PCR reaction was also conducted which amplified a polymorphic region of msp2 (3D7/IC and FC27 strains). The primer sequences as described in (Snounou *et al.*, 2002) were used. The primer sequences for the primary (nest i) reaction for the amplification of msp2 in FC27 and 3D7/IC were: M2-OF ATGAAGGTAATTAACATTGTCTATTATA CTTGTTACCATCGGTACATTCTT M2-OR. For the nested reaction, the primer and the sequences used for FC27 strain were: M2-FCF AATACTAAGAGTGTAGGTGCARATGCTCCA TTTTATTTGGTGCATTGCCAGA ACTTGAAC M2-FCR while for the 3D7/IC was: M2-ICF AGAAGTATGGCAGAAAGTAAKCCTYCTAC TGATTGTAATTCGGGGGATTTCAGTTTGTTTC G M2-ICR. PCR genotyping of msp2 genes were performed in 20µl total reaction volume containing: 1X PCR buffer, 9.2µl of Nucleases free H₂O, 1.5µM MgCl₂, 200µM dNTPs, 0.25µM each of forward and reverse primers (0.5 µl), 0.5 unit of Taq polymerase (New England Bio labs, USA), 5% DMSO and DNA template. The obtained 20µl

of mixture was briefly spun using a micro-centrifuge at 1500 rounds for 30 seconds. The primary amplification of DNA was initially denatured at 95°C for 3 minutes followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 61°C for 1 minute and elongation at 72°C for 1 minute. The final elongation was performed at 72°C for 5 minutes.

For the secondary reaction, the volume per reaction of nuclease free water was increased to 11.2µl while 3µl amplicons of the DNA templates were used. The volumes and concentrations of all the other reagents remaining the same as in nest 1 with a total of 20µl reaction volume. The secondary amplification involved 35 amplification cycles. The annealing temperature was reduced to 58.6°C., while all the other temperatures and periods (time) remaining the same as in nest 1. *P. falciparum* infections were confirmed by PCR amplification of *P. falciparum* specific gene (pfmsp2). The *P. falciparum* msp2 gene was amplified using methods described in (Koita *et al.*, 2012), modified by shortening the number of cycles to 35 and by shortening the annealing temperature to 58.6°C. The initial denaturation time was also reduced to 3 minutes. Four micro litters (4µl) of gel loading dye were dispensed into the PCR tube containing the amplicons. 10µl of DNA ladder was carefully loaded into each of the lane one. Ten µl of PCR amplicons were carefully loaded into the gel, which was ran for 1 hour at 100V. All the digested products and amplicons were resolved on 2.0% agarose gel stained with ethidium bromide. The resolved amplicons were viewed under UV light from a UV transilluminator. Samples were considered positive by PCR genotyping if the msp2 yielded a product following gel electrophoresis.

Detection of *pfhrp2* genes by PCR

Samples that successfully amplified for msp2 genes were considered to have enough quantity and quality DNA and were therefore included for the final analysis of *pfhrp2* gene deletion.

The PCR amplification protocol for exon 2 of *pfhrp2* were adapted from (Baker *et al.*, 2005; Abdallah *et al.*, 2015; Amoah *et al.*, 2016; Parr *et al.*, 2017) with minor modifications. The PCR protocol was performed using the primers and

reaction conditions as described in (Parr *et al.*, 2017) and shown in **Table 1**.

Table 1: Primer Sequences and PCR Reaction Conditions to Amplify *pfhrp2* and Their Flanking Genes

Gene	Primer Name	Primer Sequence	Annealing Temperature
Pfhrp2	HRP2-2F	5' ATTCCGCATTTAATAA TAACTTGTGTAGC 3'	58 ⁰ C
	HRP2-2R	5' ATGGCGTAGGCAATG TGTGG 3'	

The PCR amplification of *pfhrp2* was performed in 20µl total reaction volume. The reagents used were: Nuclease H₂O, 1x PCR Buffer, 200µM dNTPs, 1.5µM MgCl₂, 0.25µM for both forward Primer and Reverse Primer, Taq Polymerase, 2% of Tween 20, 1mg/ml of Bovine Serum Albumin (B.S.A), and DNA Template. The DNA was initially denatured at 95⁰C for 3 minutes followed by 30 cycles of denaturation at 94⁰C for 30 seconds, annealing at 58⁰C for 1 minute and elongation at 72⁰C for 30 seconds. The final elongation was performed at 72⁰C for 5 minutes.

Agarose Gel Electrophoresis of PCR Amplicons

All the digested products and PCR amplicons were analysed by electrophoresis in 2% agarose gel stained with ethidium bromide. Ten micro litres (10µl) of DNA ladder was carefully loaded into each of the lane one. Ten micro litres (10µl) of PCR amplicons were loaded into the gel. The electrophoretic machine was allowed to run for 1 hour at 100v. The resolved amplicons were considered positive by PCR genotyping if the *Pfhrp2* yielded a product following gel electrophoresis.

RESULTS

Specific Clones of *P. falciparum* Associated with the False Negative Phenotype in the Study Area.

The distribution of false negative malaria among the three selected locations as shown in **Table 1** indicated that, false negative (positive by microscopy and negative by RDT) malaria was high (70%, 357/510). The highest false negative prevalence was recorded in Otukpo (72.2%, 135/187) followed by Katsina-Ala (70.6%, 120/170) and Makurdi (66.7%, 102/153), no significant difference ($\chi^2 = 4.95$, $df = 2$, $P > 0.05$) was recorded among the three selected sites.

All the 120 false negative *P. falciparum* isolates yielded products after PCR amplification. This was a confirmation that all the *P. falciparum* isolates used in this study had sufficient quantity and quality DNAs; hence the isolates were included for the final analysis of *pfhrp2* gene deletion. The result of the *P. falciparum* clones associated with the false negative phenotypes in the study area indicated that, 3D7 had the highest distribution by 51% (62/120) followed by FC27 with 33.3% (40/120). Hybrid strains were recorded at 15% (18/120) as shown in **Table 2**. *Msp2* alleles did not amplify at lane 6 but amplified at lanes 2, 3, 4, 5, 7, 8 using 3D7 while lane 12 did not amplify but lanes 9, 10, 11, 13 and 14 amplified using FC27 clones. The *msp2* genes were amplified between 200bp and 480bp by 3D7/IC while FC27 amplified *msp2* between 150bp and 400bp as shown in **Plate 1**.

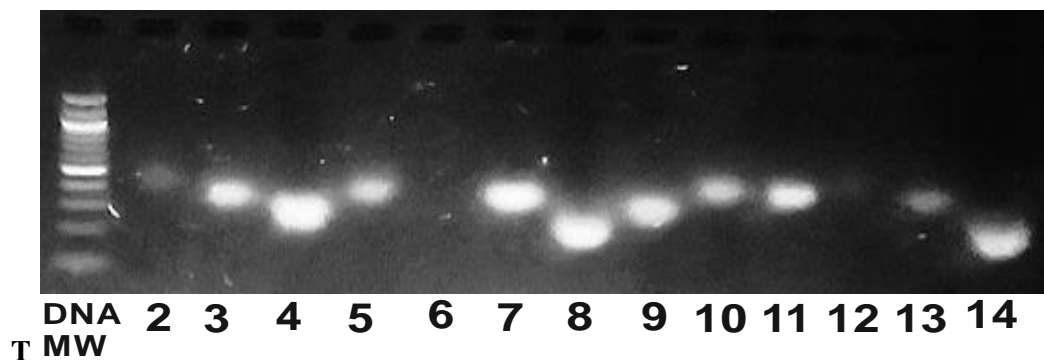
Table 1: Distribution of False Negative Malaria among the three Selected Locations

Location	N0. Examined	False negatives (%)
Makurdi	153	102 (66.7)
Katsina-Ala	170	120 (70.6)
Otukpo	187	135 (72.2)
Total	510	357 (70.0)

$$(\chi^2 = 4.59, df = 2, P > 0.05)$$

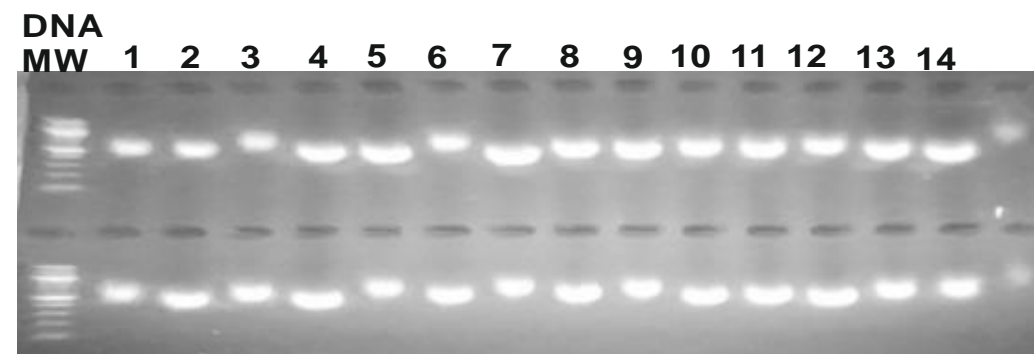
Table 2: Distribution of Specific Clones of *P. falciparum* among False Negative Samples after Genotyping of *msp2* Gene

<i>P. falciparum</i> Clones	Frequency	Percentages %
3D7/IC	62	51.7
FC27	40	33.3
Hybrid	18	15
Total	120	100

**Plate 1: UV Light Image of MSP2 Alleles of 3D7 and FC27 PCR Products during PCR Genotyping of *msp2* Genes****The Presence or Absence of *pfhrp2* Genes among *P. falciparum* False Negative Isolates in Benue State, North Central Nigeria**

A UV. light image showing amplification of *pfhrp2* genes among false negative isolates as shown in **Plate 2**. All the false negative samples employed for the PCR analysis yielded products after PCR

amplification. The *pfhrp2* PCR genotyping result confirmed amplification of *pfhrp2* with 3D7/IC and FC27 strains. The *pfhrp2* exon 1 and 2 assay produced bands between 500bp and 750bp. The samples were accordingly classified as *pfhrp2*+ (positive) indicating that, there were no deletions.

**Plate 2: UV. Light Image of Ethidium Bromide- stained PCR Products After Gel Electrophoresis Showing Amplified *pfhrp2* Genes from RDT False Negative**

Isolates between 500bp and 750bp

Genotyping of histidine rich protein2 (*hrp2*) using true negatives isolates was also carried out by PCR to determine the presents of *P. falciparum*. Twenty (20) true negatives isolates (Negative by both RDT and Microscopy) were randomly selected and genotyped using *hrp2* in order to determine if

P. falciparum were associated with the true negative isolates. Eighty five percent 85% (17/20) of the 20 isolates yielded products after gel electrophoresis (**Fig 1**) between 500bp and 750bp while 15% (3/20) of the isolates on lane K, L, N did not amplify as shown in **Plate3**.

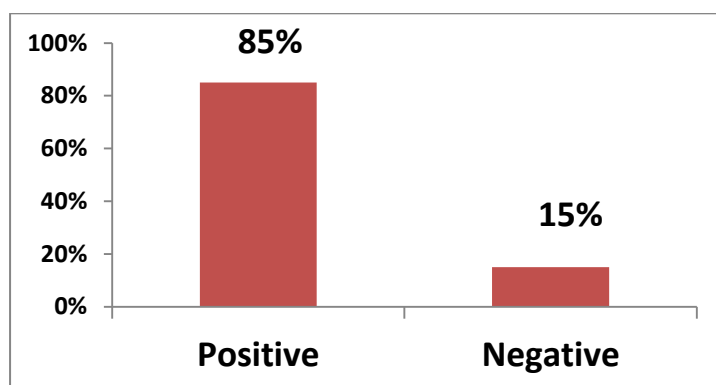


Fig 1: PCR Genotyping of *hrp2* to Identify *P. falciparum* among True Negative Isolates

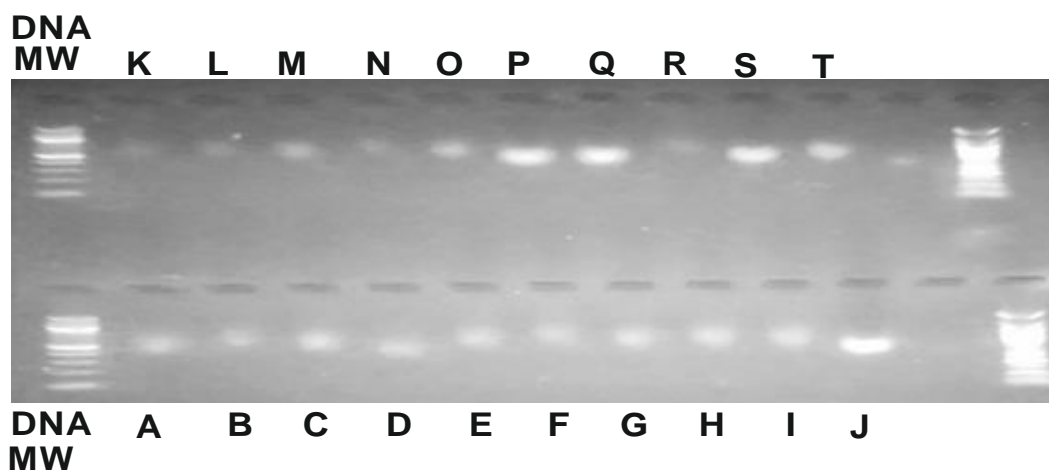


Plate 3: UV. Light Image of Ethidium Bromide- stained PCR Products after Gel Electrophoresis Showing Alleles of *pfhrp2* Genes for RDT True Negative Isolates

DISCUSSION

Distribution of *P. falciparum* by Microscopy and HRP2 Based RDT Kits

The high prevalence (89%) of malaria by microscopy and low (21%) positivity rates of RDT in this study may be due to external factors that may have affected the stability of the RDT such as exposure to extreme temperatures which has been found to be a major contributor to poor performance of RDTs, especially during

transportation as well as storage (Jorgensen *et al.*, 2006; Albertini *et al.*, 2012 ; Chenge *et al.*, 2014). High humidity may have degraded RDTs performance in the study area which was also reported in the United States of America (Abanyie *et al.*, 2010). The RDT poor performance in this study may be as a result of parasite factor or test factors also reported by (Bakere *et al.*, 2005).

The high prevalence of false negative malaria RDT in this study suggested that, false negative

results could be essentially linked to isolates with low parasite densities less than 0.01% (Trouvay *et al.*, 2013). False negative results have also been attributed to an excess of antigen, as well as antibodies that block the detection antibody target site. This prozone phenomenon can be mitigated by sample dilution (Gillet *et al.*, 2009). False negative result in this study could also be as a result of inhibitors in the patients' blood preventing development of the control line as also reported by (Durand *et al.*, 2013). Higher numbers of false negatives have also been identified in Ogun State (Adekunle *et al.*, 2014) and Ghana (Amoah *et al.*, 2016).

Distribution of *P. falciparum* Clones among False Negative Malaria RDT

The genotyping of *P. falciparum* clones using *msp2* indicated that, 3D7, FC27 and hybrid were unevenly distributed in the study area. 3D7 had the highest prevalence (51.7%) while hybrid had the least (15%). The genotyping of *msp2* confirmed the different clones and the sufficient quantity and quality DNAs. All the samples were therefore included for *pfhrp2* gene deletion. All the 120 false negative isolates genotyped for *P. falciparum* clones yielded products after PCR amplification for one or two of the clones. This study shows that, 3D7/IC was the most predominant strain in Benue State, Nigeria.

Presence or Absence of Part or the Entire *pfhrp2* Gene among False Negative Isolates in the Study Area

All the isolates in this study area did not contain any parasites lacking exon1 or exon 2 of *pfhrp2* genes. PCR amplification was performed on 120 false negative samples to estimate the deletion of *pfhrp2* on exon 1, 2 and intron. All the isolates yielded products after gel electrophoresis. This was an indication that, there was no deletion among any of the isolates. The non- deletion of *pfhrp2* in this study was consistent with studies co-conducted in DRC, Gambia, Kenya, Mozambique, and Rwanda (Ramutton *et al.*, 2012), and in French Guiana, France, South America (Trouvay *et al.*, 2013) where none of the isolates contained

parasites lacking the *pfhrp2* genes. However, 2.68% isolates in their study lacked *pfhrp3* exon 2. The low performance of RDT in this study cannot be likened to the deletion of *pfhrp2*, since data from this study revealed that *P. falciparum* isolates in Benue, do not lack *pfhrp2* gene. The low performance of RDT in this study could be attributed to exposure of the RDT kits to temperatures above 30°C or relative humidity above 70%. This low performance could also be as a result of prozone effect or very low parasite density which was also reported in Peru, South America (Baker *et al.*, 2005). The absence of *pfhrp2* gene deletion in Benue State, North Central Nigeria was in variance with previous studies in Mali (Koita *et al.*, 2012), Senegal (Wurtz *et al.*, 2013), Peru (Gamboa *et al.*, 2010; Akinyi *et al.*, 2013; Beldeviano *et al.*, 2015), India (Kumar *et al.*, 2013; Bharti *et al.*, 2016; Kumari *et al.*, 2021), China (Li *et al.*, 2015), Colombia (Murillo *et al.*, 2015; Olivera *et al.*, 2025), Honduras (Abdallah *et al.*, 2015), Ghana (Amoah *et al.*, 2016), Mozambique (Gupta *et al.*, 2017; Silva *et al.*, 2024), Rwanda (Kozycki *et al.*, 2017), Kenya (Beshir *et al.*, 2017), Brazil and Bolivia (Rachid *et al.*, 2017), Congo (Parr *et al.*, 2017), Eritrea (Berhane *et al.*, 2017) which reported various degrees of deletion in either *pfhrp-2* or *pfhrp-3* genes or both. Deletion in one or both *pfhrp2* and *pfhrp3* influences the accuracy of malaria diagnosis. However, in the present study, there was no deletion on either the exon 1 and 2 of *pfhrp2*. Other factors may have been responsible for the poor performance of Care Start™ Ag HRP2 RDT Kits in this study area. Factors in the manufacturing process, transportation, storage, miss-use of RDT kits and even parasite factors may have been responsible for the poor performance of RDT kits in this study, which was also reported in Peru, South America (Baker *et al.*, 2005; Cheng *et al.*, 2014).

Genotyping of True Negative Isolates (Negative by both RDT and Microscopy) Using *msp2* Genes

The present study revealed that, PCR achieved 85% (17/20) prevalence among true negative

isolates (negative by both RDT and microscopy). The 85% prevalence of malaria among true negatives by PCR in this study was consistent with a study conducted in Angola where the study confirmed 125 *P. falciparum* isolates that were both microscopic and RDT negative to be positive by PCR (Fancony *et al.*, 2013). The performance of PCR in this study is consistent with some studies which recently found out that, PCR revealed almost ten times more Plasmodium infection compared to microscopy. This phenomenon takes into account the high number of sub-microscopic infections in a relatively low transmission area (Kaisar *et al.*, 2013). The superiority of PCR over microscopy and RDT in this study is consistent with studies conducted elsewhere (Kumudunayana *et al.*, 2011; Ojuronbe *et al.*, 2013). Contrary to this present study, a study conducted in North – eastern Tanzania by (Minja *et al.*, 2012), indicated that the 351 samples that were both negative by RDT and microscopy were all negative by PCR. Thus, confirming that, both microscopy and RDTs performed equally well in diagnosing true negative cases. The superiority of PCR over microscopy and RDT in this study is also consistent with the findings in Sri Lanka (Kaisar *et al.*, 2013), Angola (Claudia *et al.*, 2013) and Nigeria (Ojuronbe *et al.*, 2013; Chukwuocha *et al.*, 2010). PCR diagnosis of sub-microscopic infection is crucial in order to affect accurate diagnosis, prompt

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- treatment of asymptomatic cases and also to identify and clear potential reservoirs of transmission (Okell *et al.*, 2009; Karl *et al.*, 2011) and reduce malaria related morbidity and mortality.

Conclusion

The genotyping of false negative isolates (negative by RDT and positive by microscopy) using *pfhrp2* genes confirmed that, the deletion of *pfhrp2* was not common in Benue State, North Central Nigeria. The parasite factor (deletion of *pfhrp2* and *pfhrp3* genes) which was previously thought to be the cause of HRP-2 based RDT poor performance in Benue State, Nigeria was actually not the cause of RDT poor performance.

Diagnosis of *P. falciparum* in Nigeria using HRP2 based RDT will surely yield reliable results if the criteria for quality control assurance are strictly observed, such as avoiding exposure of RDT kits to higher temperatures above 30⁰C and higher relative humidity above 70%.

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Conflict of interest

There was no competing of interest during and after the study.

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