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# STUDIES ON DIAGNOSTIC AND PREVENTIVE METHODS OF *Plasmodium falciparum* INFECTION IN BENUE STATE, NIGERIA.

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## ABSTRACT

Malaria is a febrile illness caused by parasites of the genus Plasmodium and transmitted by female *Anopheles* mosquitoes. This study was aimed at evaluating the diagnostic and preventive methods of *Plasmodium falciparum* among patients attending Government hospitals in Benue State, Nigeria. Blood samples of 1200 consenting patients were collected through finger prick and observed for *P. falciparum* using rapid diagnostic test kit (*Pf*RDTs). Thick and thin blood films were prepared for microscopic examination. Transmission and control factors in relation to the disease among the patients were obtained using questionnaire. The prevalence of the infection using microscopy and *Pf*RDTs were 37.1% and 34.4% respectively. There was significant differences between the diagnostic tools used ($\chi^2$=757.859, df= 1 P< 0.001). The detection of the infection in relation to diagnostic parameters using microscopy as reference test showed a sensitivity, specificity, positive predictive values and negative predictive value at 95% confidence interval (CI) recorded at 83.6% , 94.6%, 90.1% and 90.7% for RDTs. The males were more infected with the prevalence of 237(53.3%) across the age groups than their female counterparts who had prevalence of 208(46.7%). The infection was statistically significant ($\chi^2$= 52.348, df =3, P< 0.001). Patients that did not use malaria preventive methods had higher prevalence of 68.1% compared to patients that used combined methods of prevention (15.2%). A significant difference was observed between patients that did not use malaria preventive methods and those that did ($\chi^2$126.223, df =3, P<0.001). These results suggest that CareStartTM (Pf) malaria RDTs are useful in malaria diagnosis. However, false-negative RDT results were detected and may be of great concern in malaria management.

## INTRODUCTION

Malaria is an acute febrile illness caused by parasites of the Plasmodium family and transmitted by female *Anopheles* mosquitoes (WHO, 2017). It is estimated that malarial infects more than 228 million people globally. In Nigeria, (25%) cases and almost 24% of all global malaria deaths were recorded in 2018 (WHO, 2019).

Malaria diagnosis is one of the most neglected areas of malaria research. Improving diagnostic accuracy in malaria control systems can be challenging in terms of technical and finance (Wongsrichanalai *et al.*, 2007). However, the common malaria diagnostic methods are clinical diagnosis and routine parasitological- based tests (FMoH, 2014). The clinical diagnosis is not exact, but remains the basis of malaria treatment for the majority of febrile patients in developing countries; where majority do not have access to medical laboratory most especially in the rural areas. This method is not expensive and most widely practiced (Wongsrichanalai *et al.*, 2007).It is based on the patients' signs and symptoms, and on physical findings at examination. The earliest symptoms of malaria are very non-specific and variable, and include fever, headache, weakness, chills, dizziness, abdominal pain, diarrhea, nausea, vomiting, anorexia, and pruritus (Tangpukdee *et al*., 2009).

Routine parasitological- based tests available to confirm a clinical diagnosis of malaria (FMoH, 2014). These are microscopy, Rapid diagnostic Test (RDTs) and molecular methods. Microscopic diagnosis using blood smears plays an important role in malaria diagnosis because of its ability to diagnose and differentiate each species of malaria, and so it is used as the gold standard for any new detection technique (Kasetsirikul *et al*., 2016). It is the most economic, preferred, and reliable diagnosis of malaria. (Nwoke, 2018; Otubanjo, 2013). However, the limitations of microscopy such as few technical expertise, inadequate reagents, electricity supply, require time and other accessory have posed difficulties to routine use (Adebisi *et al.,* 2016).While, Rapid Diagnostic Test (RDTs) is a device that detects malaria antigen in a small amount of blood, usually 5–15 µl by immunochromatographic assay with monoclonal antibodies directed against the target parasite antigen and

impregnated on a test strip. It is simple and do not require laboratory facilities (WHO,2009; Wongsrichanalai *et al.,* 2007;Tangpukdee *et al*.,2009; Maltha *et al*., 2013). According to FMoH, (2014), histidine rich protein 2 (HRP-2) based RDTs antigen remains in the blood stream for at least two weeks after all viable parasites have been killed by treatment. This test therefore cannot be used to retest an individual patient who returns with symptoms within two – three weeks of treatment. Microscopy should be used for such patient. Molecular methods have proven to be one of the most specific and sensitive diagnostic methods, particularly for malaria cases with low parasitemia or mixed infection. They are mostly used for research. Despite the awareness of non malaria causes of febrile illness, presumptive treatment of fevers, with antimalarial medicines continues in the developing nation (Adebisi *et al*., 2018: Mac *et al*., 2019). Irrational use of antimalarial drugs in the past has led to the emergence of resistant malaria parasites (Amir *et al*., 2018). In order to reduced the development of resistance to drugs and lessen the consequences of failure of therapy, the World Health Organization advocate for test-based management of malaria and restricting artemisinin-based combination therapy (ACT) to only parasitologically confirmed cases (WHO, 2015).

Despite government interventions such as the distribution of insecticide-treated bed nets (ITN), indoor residual spraying (IRS), improved diagnostic testing, treatment by artemisinin combination therapy (ACT) and campaigns on preventive and control methods ,malaria still remains endemic in Benue State ( NMFS, 2011 and NMCP/RBM, 2009). Therefore, this study focused on prevalence of *P. falciparum*, diagnostic and preventive methods used among patients in Benue State, Nigeria.

## MATERIAL AND METHOD

### Study Area

The study was conducted in six selected General Hospitals in Benue State. The General Hospitals located in Adikpo, Gboko, Naka, Okpoga, Otukpo and Sankera . Benue State is located in the north central of Nigeria, with its capital at Makurdi. Its geographic coordinates are longitude 7° 47' and 10° 0' East. Latitude 6° 25' and 8° 8' North. The State has a total population of 4,253,641 in 2006 census , with an average population density of 99 persons per $km^2$. Made up of 2,144,043 males and 2,109,598 females the state has a sex ratio of 1.02, a literacy rate of 44.7% among the population aged 6 years and above, and a population density of about 130 persons per square kilometer Agriculture is the mainstay of the economy, engaging over 75% of the state farming population. Benue State is the nation's food basket because of its rich agricultural produce. The State also boasts of one of the longest stretches of river systems in the country with great potential for a viable fishing industry, dry season farming through irrigation and for an inland water highway. Most of the people are farmers while the inhabitants of the river areas engage in fishing as their primary or important secondary occupation. Malaria is transmitted through the bites of infected female *Anopheles* mosquitoes. These *Anopheles* species are widely distributed in Benue State. Malaria is present throughout the year with a marked increase during the wet/rainy season due to suitable climatic factors most especially temperature as reported by Manyi *et al.*(2014).

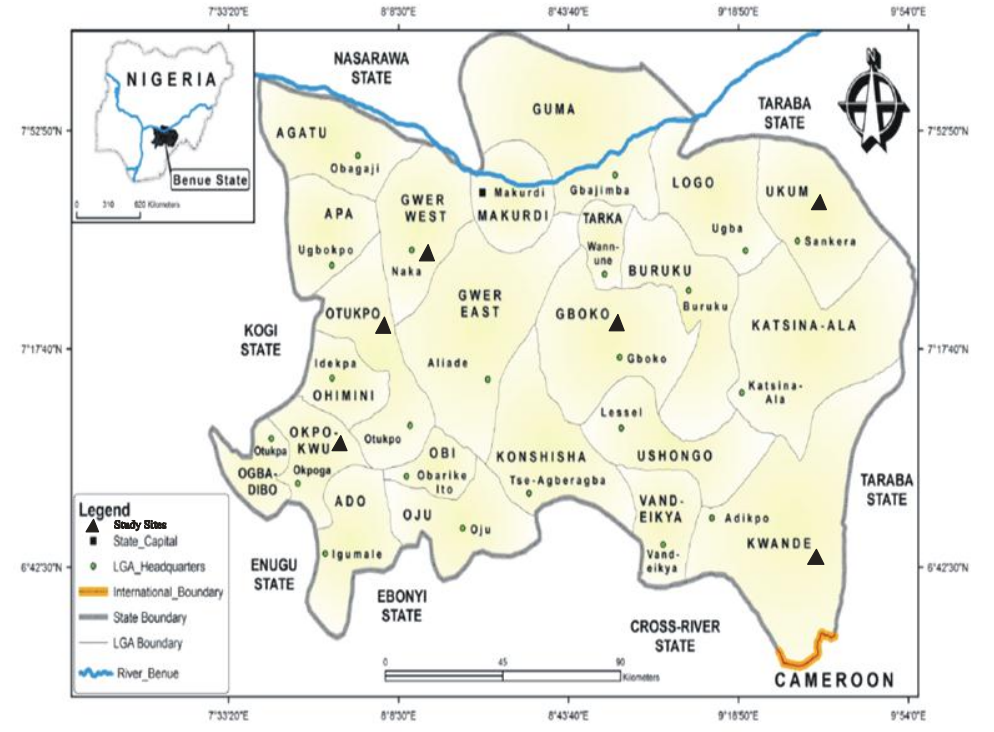


**Figure 1: Map of Benue State, Nigeria (Ministry of Lands and Survey, Makurdi 2015).**

**Study Design/Study population**
The study was conducted between September, 2018 and August, 2019. It was hospital based and it considered only outpatient subjects who were referred to the Laboratory Department for medical diagnosis. The population consisted of 1200 subjects, including both children and adults are screen for *P. falciparum* Infection. Patients' epidemiological data were obtained using questionnaires

**Sample collection and Parasitological Technique**
The left thumb of participants was thoroughly cleaned with methylated spirit and a sterile lancet was used to prick the finger to obtain blood sample. Thick and thin blood films were made on clean slides and a drop of the blood was used with an RDT kit, the "CareStart Malaria HRP2 from Access Bio, Inc." and labeled accordingly . The RDTs were used according to manufacturer's instructions. (WHO, 2009).Thick and thin blood films were prepared on microscope slides collected by finger prick blood samples. After proper fixation, the prepared slides were then stained with 10% Giemsa (v/v). Blood films were examined microscopically using X 100 (oil immersion) objectives as described by Cheesbrough (2010).

**Questionnaire administration:** A structured questionnaire was used to collect data on the sex, age and malaria preventive measures.

**Statistical Analysis:** Simple percentage and Chi-squared test were used for data presentation. Statistical analysis was performed using Statistical Product and service solution (SPSS) software version 18 (IBM., USA). Chi-squared test was used to compare prevalence of malaria infection between age, sex and preventive methods used by the subjects. The significance level was considered at $P \leq 0.05$.The Sensitivity, Specificity, Positive predictive value and Negative Positive predictive were determine by using the formula below:

$$\text{Sensitivity} = \frac{\text{No. of True Positive}}{\text{No. of True Positive} + \text{No. of False Negative}}$$

$$\text{Specificity} = \frac{\text{No. of True Negative}}{\text{No. of True Negative} + \text{No. of False Positive}}$$

$$\text{PPV} = \frac{\text{No. of True Positive}}{\text{No. of True Positive} + \text{No. of False Positive}}$$

$$\text{NPV} = \frac{\text{No. of True Negative}}{\text{No. of False Negative} + \text{No. of False Negative}}$$

**ETHICAL APPROVAL**
Permission was sought for and obtained from the Ethical Committee of the Hospitals Management Board, Makurdi. Patients presenting themselves for Laboratory test in the selected General hospitals were duly informed on the significance of the study. Informed consent of adults and parents/ guardians of the children were obtained before blood sample collection for the tests and administration of questionnaire.

**RESULTS**

A total of 1200 subjects were screened for *P. falciparum* infections among patients attending General Hospitals, in Benue Sate. This gave a total prevalence of 445(37.1%) by microscopy. The *falciparum* malaria prevalence was higher in General Hospital Naka 103(51.3%) and least in General Hospital Gboko 44(22.0%). There was statistically significant ($\chi^2$ =50.464, df=5 and p<0.001) (Table 1). Also, Prevalence of *falciparum* malaria of 413(34.4%) was recorded in this study using rapid diagnostic test (RDTs) (table 2). The malaria Prevalence was higher in Naka 94 (47.0%) and least in Gboko 43(21.0%). There was statistical difference between the study areas in Benue State ($\chi^2$=42.594, df= 5 and P< .001). The diagnostic parameters of RDTs when compared microscopy as reference test for sensitivity, specificity, Positive predictive value (PPV) and Negative predictive value (NPP )were 83.6%, 94.6%, 90.1% and 90.7% respectively (Table 3).

The prevalence of *P. falciparum* infections in relations to age class and sex, age group < 5 years recorded the highest prevalence of 147 (49.7%) while the age group 15 years and above recorded the lowest prevalence of 81 (22.0%). *P. falciparum* infections in relation to sex, the males recorded the highest infection of 237(53.3%), while the females recorded the lowest infection rate of 208(46.7%). However, there was significant difference ($\chi^2$=52.348,df =3 and P < 0.05) between the of infection and the age of the subjects (Table 4).

Malarial infection in relation to preventive measures used among subjects in the study area showed that those that did not use any of the protective measure recorded highest infection of 66(68.1%) and the least prevalence rate was observed among those that used combined method with 53(15.2%). A significant difference was observed in malarial infections rate between subjects that did not use any malaria preventive methods and those that used ($\chi^2$=126.223, df=3 and P<0.5) (Table 5).

**Table 1: Prevalence of *Plasmodium falciparum* infections in relation to locations using microscopy.**

| Location | No positive percentage (%) | No negative percentage (%) | Total(%) |
|---|---|---|---|
| Adikpo | 80 (40.0) | 120 (60.0) | 200 (100) |
| Gboko | 44 (22.0) | 154 (78.0) | 200 (100) |
| Naka | 103 (51.3) | 97 (48.5) | 200 (100) |
| Okpoga | 87 (43.5) | 113 (56.5) | 200 (100) |
| Otukpo | 54 (27.0) | 146 (73.0) | 200 (100) |
| Sankera | 77 (38.5) | 123 (61.5) | 200 (100) |
| Total | 445 (37.1) | 755 (62.9) | 1200 (100) |

$\chi^2$= 50.464 df=5 p=<0.05

**Table 2: Prevalence of *Plasmodium falciparum* infections in relation to locations using RDTs.**

| Location | positive percentage (%) | Negative percentage (%) | Total(%) |
|---|---|---|---|
| Adikpo | 80 (40.0) | 120 (60.0) | 200 (100) |
| Gboko | 43 (21.0) | 157 (78.50) | 200 (100) |
| Naka | 94 (47.0) | 106 (53.0) | 200 (100) |
| Okpoga | 79 (39.5) | 121 (60.5) | 200 (100) |
| Otukpo | 49 (24.5) | 151 (75.5) | 200 (100) |
| Sankera | 68 (34.0) | 132(66.0) | 200 (100) |
| Total | 413(34.4) | 787(65.6) | 200(100.0) |

$\chi^2$= 42.594, df=5 p=<0.05

**Table 3: Diagnostic parameters of RDTs with microscopy test as reference test.**

| Parameters | Estimated value (%) | 95% confidence interval | |
|---|---|---|---|
| | | Lower limit (%) | Upper limit (%) |
| | | | |
| Sensitivity | 83.6 | 79.7 | 86.8 |
| Specificity | 94.6 | 92.6 | 96.0 |
| Positive predictive value (PPV) | 90.1 | 86.7 | 92.7 |
| Negative predictive value (NPV) | 90.7 | 88.4 | 92.6 |

**Table 4: Prevalence of *P. falciparum* Infections in Relation to Age and Sex.**

| | No. Examined | | | No. Positive | | | % Positive | | |
|---|---|---|---|---|---|---|---|---|---|
| Age Group | Male (%) | Female (%) | Total | Male | Female | Total (%) | Male | Female | Total % |
| < 5 | 107 (36.1) | 189 (63.9) | 296 | 84 | 63 | 147(49.7) | 57.1 | 42.9 | 100 |
| 5 – 9 | 154 (50.8) | 149 (49.2) | 303 | 71 | 67 | 138(45.5) | 51.4 | 48.6 | 100 |
| 10 – 14 | 86 (36.9) | 147 (63.1) | 233 | 39 | 40 | 79(33.9) | 49.4 | 50.6 | 100 |
| 15 and above | 138 (37.5) | 230 (62.5) | 368 | 43 | 38 | 81(22.0) | 53.1 | 46.9 | 100 |
| **Total** | **485 (40.4)** | **715(59.6)** | **1200** | **237** | **208** | **445(37.1)** | **53.3** | **46.7** | **100** |

$\chi^2$= 52. 348 df=3 P <0.05

**Table 5: Prevalence of *Pasmodium falciparum* infection relation to protective mechnanism using microscopy test.**

| Protective mechanism | Positive | Negative | Total |
|---|---|---|---|
| Insecticide spray | 196 (45.9) | 231 (54.1) | 427 (100) |
| Use of ITNS | 130 (39.6) | 198 (60.4) | 328 (100) |
| Combination | 53 (15.2) | 295 (84.8) | 348 (100) |
| Non user | 66 (68.1) | 31 (31.9) | 97 (100) |
| Total | 445 (37.1) | 755 (62.9) | 1200 (100) |

$\chi^2$= 126.223, df=3, p=<0.05

## DISCUSSION

Malaria is endemic and stable; being a major cause of morbidity and mortality in Benue State, Nigeria. The results of this study shows the prevalence of 37.1% and 34.4% recorded by microscopy and CareStart$^{TM}$*Pf* malaria RDTs kit respectively. This is lower than the overall malaria prevalence of 59% and 64% recorded by

Azikiwe *et al*. (2012). Also, Mac *et al*. (2019) reported 55.1% and 37.2% prevalence of malaria in Abuja and Nasarawa among healthy individual employing Microscopy and HPR-2 diagnostic test (RDT).While, Egbuche *et al.*(2019) reported 57.1% and 22.0% for Microscopy and diagnostic test (RDTs) respectively. However, the malaria prevalence in this study was higher than 15.1% and 21.6% in Ibadan, Nigeria by Adebisi *et al*. (2018) and 23.2% and 25.9% in Kastina-Alas, Benue State Nigeria, by Ikpa *et al*. (2017). Both studies used light microscopy and CareStart$^{TM}$*Pf* malaria RDTs respectively.

The study reveals sensitivity, specificity, positive predictive value and negative predictive value for RDT in this study. The result in this study is comparable with an earlier report in Kastina-Ala Benue State, Nigeria, with Sensitivity of 86.8% and 95.7%, while specificity of 91.5% and 97.0% in asymptomatic and symptomatic malaria respectively by Ikpa *et al*.(2017) which stated an agreement between the sensitivity of microscopy and RDTs in malaria diagnosis. Similar results were reported by Sheyin and Bigwan (2013), using Carestart$^{TM}$ - Histidine Rich Protein (HRP)-2, recorded sensitivity and specificity values of 78.4% and 97.6%, positive predictive value of 93.3% and negative predictive value of 80.1 in Jos, Nigeria. The finding was also similar to sensitivity and specificity of 84.5% and 98.2%, negative predictive value (93.1%) and positive predictive value 95.6% as observed by Mac *et al.* (2019) in Abuja and Nasarawa state Nigeria. The finding in this study is higher compared to a similar study conducted at Akure by Obimakinde *et al*. (2018) which showed a sensitivity of (65%) RDT compared to Microscopy (71.43%).

The slight different in the results, could be due to false positive or false negative reading .Wongsrichanalai *et al*. (2007) observed that the different may be due to microscopist experience or several factors in the manufacturing process as well as environmental conditions may affect RDT performance. Also, false negative results may be caused by deletion or mutation of the *hrp-2* gene. It could be that participating subjects had recently been treated with antimalarial drugs before presenting in the secondary health facilities. Ikpa *et al*. (2017) noted that, the consequence of a false negative diagnosis is a threat to life of the patient being tested, as the patient may be denied appropriate treatment with antimalarial drugs despite being infected with *P. falciparum* parasites. The RDT kit employed in the investigation of the prevalence, sensitivity and specificity of malaria parasite in this study showed effective and reliable results for detecting *P. falciparum*, although, microscopy remains as gold standard.

In this study, the highest malaria prevalence was observed in patients of the age groups < 5years and 5-9 years. The least prevalence was recorded in the age groups of 15 years and above. This contrasts the reports of highest malaria prevalence in older age groups in Benue, Ibadan and Osogbo by Houmsou *et al*. (2017); Okonko *et al.* (2010) and Nassar *et al*. (2019). Children between ≤ 5 years of age were the most vulnerable group. This is in agreement with WHO report that children under 5 years of age constituted 69% out of estimated malaria deaths around the world (WHO, 2016). Malaria prevalence was statistically significant in different age groups( $p < 0.05$).The high infection observed among these age groups could be due to low immune system to malaria infection and inadequate protection against mosquito bites. This age groups plays around and they often expose their body thereby prone to mosquito bites. This finding is consistent with the reports of previous study in South-Eastern Nigeria by Umeanaeto *et al.* (2019) who reported that high prevalence infection among children (≤ 15 years) of age and further stated that children born to immune mothers are protected against the disease during the first half year of life by maternal antibodies. As they grow older, after continued exposure from multiple infections with malaria parasites over time, they build up an acquired immunity and become relatively protected against the disease and blood stage parasites.

This study has revealed high malaria infection among male than in female counterparts. This finding is similar to the work of Nassar *et al*. (2019) who reported that malaria infection is higher in male than female. Also, Mac *et al*. (2019) reported that female subjects showed slightly low prevalence compared to male counterparts. This could be due to exposure of body parts by male than female or refusal to sleep under insecticide-treated nets. The males were more infected with malaria across the age groups than their female counterparts except females in age group of 10-14 years that recorded slightly higher than male counterparts. This could be due to ignorance on the part of young females concerning the use of protective measures or may be due to differences in gender related activities of females who frequently assist their mothers in house chores such as sweeping the compound, washing of dishes outside at dawn and dusk, as well as fetching of water which make the females more prone to infective mosquito bites as compared to their male counterparts.

However, Yohanna *et al.*(2019) opined that gender did not affect the prevalence of malaria among patients. The reason for this differences in prevalence between females and males cannot be scientifically traced to any reason in particular, it may have occurred by chance as reported by Manyi *et al.* (2018) and Mac *et al*.(2019).

The preventive methods are effective in various ways. But the combination of ITNs and Insecticide spray seemed to be more effective than the use of single protective measures. The present study reveals higher prevalence of malaria among subjects that did not use any malaria preventive methods, followed by those that use insecticide spray alone and insecticide treated bed nets. The least prevalence was recorded among those that

use combination of ITNs and Insecticide. The finding is similar to previous report by Amuta *et al*. (2014) who observed that preventive methods are variously effective and non- users recorded highest infection of malaria than those that use preventive measures. The observed difference in malarial infection between subjects that did not use any malaria preventive methods, those that used single and combined methods of prevention, could due to location, ignorance or poverty. However, the use of insecticide was one of the most preferred methods of mosquito control by many participants; it is possible that insecticide were applied at least once a week. This could be the cause of high malarial infection observed among patients that used insecticide sprays alone, or could be due to mosquitoes resistance to the chemical or to the low quality of the insecticides and the short duration of the insecticide sprays which lasts for few hours only could be another factor. The use of insecticide treated bed nets/ Long Lasting Insecticidal Nets (LLINS) has been shown to reduce malaria infections. The prevalence recorded in this study could be due to unavailability of treated nets by those who desired to procure them, or too expensive or refusal to sleep under insecticide treated nets or discomfort primarily due to heat and perceived low mosquito density were the most widely identified for non use of ITNS. The finding is similar to previous report by Manyi *et al.,* (2018) who observed that the high prevalence could be by chance; child's refusal to sleep under insecticide-treated nets or because of ignorance on the part of their parents concerning the use of insecticide-treated nets which make them more prone to infective mosquito bites. Amuta *et al*. (2014) observed that good result of this combination of preventive methods could just reflect a synergy between the chemical components against mosquitoes. The present study reveals that malaria remains moderately high among patients attending General Hospitals in Benue State, Nigeria, despite the efforts by Government to reduce the burden of malaria in Nigeria. Combined method of prevention (ITNs and Insecticide spray) showed good results in preventing malaria among patients as observed by Hile *et al*. (2012).

## CONCLUSION /RECOMMENDATIONS

Malaria is endemic and stable; being a major cause of morbidity and mortality in Nigeria. This study has revealed that the malaria infection is considered moderately high compare to other studies. It has also revealed that all age groups are prone to malaria infections, but children (≤ 15 years) are highly vulnerable to malaria attack. There is need to intensify campaigns on preventive and control methods. The masses should be encouraged to use combination of preventive methods like Insecticide spray and insecticide-treated bed-nets (ITNs) / Long Lasting Insecticidal Nets (LLINS) so as to reduce the risk of Anopheles mosquitoes' bites. Microscopy as gold standard revealed a slightly high sensitivity. However, this result suggest that CareStartTM (*Pf*) malaria RDT is suitable for the diagnosis of *P. falciparum* malaria . Therefore, clinical findings should always be confirmed by a laboratory test for malaria to avoid irrational use of antimalarial drugs.

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