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# Performance Characteristics of Two Immunochromatography Kits in the diagnosis of Malaria in Port Harcourt, Southern Nigeria.

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***Abstract:*** *Malariais a major cause of morbidity and mortality in Nigeria and Africa as a whole and a menace to humanity in the tropical regions of the world, yetproperdiagnosisof thisdeadlydiseaseposes a great challenge to mostendemicnations of the world. In view of this, astudywasdone to determine the performance characteristics of two commercially available Rapid Diagnostic Test (RDT) kits; CTK Onsite and SD Bioline relative to Giemsa-stained blood film microscopy within Port Harcourt, Southern Nigeria. A total of 400(226 males and 174 females) subjects between the ages of 11 and 60years were recruited for this study at the Out Patients Department (OPD) of the University of Port Harcourt Teaching Hospital(UPTH). The studyshowed a sensitivity,specificity, Positive Predictive Value, Negative Predictive Value and Accuracy of 78.7%, 100%, 100%, 73% and 86.5% respectively for CTK Onsite comparedto 36.2%, 100%, 100%, 47.4% and 59.5% recorded for SD Bioline. Hence this study showed that CTK Onsite is a better choice kit than SD Bioline for effective diagnosis of falciparum malariabut microscopy remain the gold standard as it detected more malaria cases than both kits.*

***Keywords –****Blood film, Malaria, Microscopy, Performance Characteristics, RDT.*

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## I. Introduction

Malaria, an avoidable, treatable nevertheless a deleterious disease caused by the *Plasmodium* parasite is spread by the inoculative blood meal bite of an infected female Anopheles mosquito[1]. There are five human plasmodia species transmitted from persons to persons, this include the lethal species; *P. falciparum*, low mortality species; *P. vivax, P. ovale, P. malariae,* and latelydocumented *P. knowlesi,* reported in the forested regions of South-East Asia and the Island of Borneo [2,3]. Malaria is the principal cause of death and morbidity aside HIV/AIDS in the tropical regions of the world, with unquantifiable direct and indirect burdens like malnutrition, anaemia, Low birth weight and numerous Disability Adjusted Life Years (DALYs) in these endemic zones as well [4]. It is a crucial public health menace in developing countries causing considerable morbidity and mortality especially in Sub-Saharan Africa[5,6] and almost half of the world's population is endangered to this deadly disease [7].

WHO, in the year 2016 estimated 216 million cases of malaria globally and 445,000 mortalities thereof with 70% of these mortalities occurring among children under the age of 5. It is a huge drain to the economy of many nations of the world, as a whooping US$2.7 billion was spent on malaria control and elimination by governments of malaria endemic countries and international partners in the year 2016 alone. The situation is not different in Nigeria, the most populous black nation as 50% of the population has at least one episode of malaria yearly while children under 5 have between 2 – 4 average attacks in a year [8]. Mortality from this disease is very high in Nigeria and DR Congo as both countries account for 40% of the estimated global death [9]. Despite these global commitments to this deadly disease, proper diagnosis is still a great challenge to Nigeria and most sub-saharan nations. Clinical diagnosis, an imprecise diagnostic method still remains the basis of therapeutic care for the majority of febrile patients in malaria endemic areas, which had most times led into mis-diagnosis or under-diagnosis, hence indiscriminate use of malaria drug which had contributed to the steady rise reported in malaria drug resistance seen lately[10]. Stained blood smear microscopy remains the gold standard for malaria diagnosis, as it is sensitive and allow the quantification of parasitemia but it requires trained microscopist, potent reagents as well as constant power supply which are mostly unavailable in these endemic zones, hence the advent of immune-chromatographic Rapid Diagnostic Test (RDT) kits which abridge the shortcomings of microscopic diagnosis [11]. The use of these kits had consistently risen within years as about 1.66billion kits were sold between 2010 and 2016, but their performances had been questioned by several researchers due to

some inhibiting factors like cross reactivity with antibodies, extreme temperatures and high humidity [12] which could affect their performances, due to paucity of this data in this region, this study seek to assess the performance characteristics (Sensitivity, Specificity, Positive Predictive Value, Negative Predictive Value and Accuracy) of two RDT kits; CTK Onsite™Ab-*Pf/Pv*(R01111C) and SD Bioline® Ag-*Pf*(05FK50)sold within Port Harcourt, Southern Nigeria.

## II. Materials and Methods

1.1 Study area

This study was carried out at the Out-Patients Department (OPD) of the University of Port Harcourt Teaching Hospital (UPTH), Port Harcourt, Rivers State of Nigeria. Port Harcourt, the capital of Rivers State is located in the South-South part of Nigeria. It lies in the tropical wet climatic zone, characterized by abundant rainfall with little dry season. The monsoon season occurs between April and October, resulting in heavy rainfall of between 2000 and 2500mm with temperature of up to $25^0$C and a relatively fairly stable and constant humidity. UPTH is a tertiary health institution which serves as a referral centre for the local health facilities within the state as well as neighbouring states. It located at latitude $4^0$53'59"N and longitude $6^0$55'45"E with an elevation of 15m above sea level.

1.2 Ethical Consideration

Ethical clearance to undertake this research was gotten from Centre for Research Management and Development, University of Port Harcourt as well as University of Port Harcourt Teaching Hospital Research Ethics Committeewhile informed consent form was obtained from patients above 18years and parents or guardians of those below 18years for approval.

1.3 Study Population

The study population is made up of 400 (226 males and 174 females) random subjects between the ages of 11 and 60 at the Out Patient Department (OPD) of UPTH who reported for medical check-up between November 2017 and April 2018. Blood samples were collected following standard procedures and practices.

1.4 Collection of Blood Samples

Venipuncture technique was used to collect two milliliters of blood into an ethylene-diamine tetra-acetic acid (EDTA) bottles which were labelledappropriately.All collected samples were transported to the parasitology laboratory of the Department of Animal and Environmental Biology of the University of Port Harcourt within 1 -2hours of collection for examination. Demographic Information was collected from every participant in the study using standardized questionnaires given to the adult and to the guardians of subject below 18years.

1.5 Laboratory Analysis

Examinations of blood samples for malaria parasites were done using the thin and thick blood films on separate slides, which were stained with 10% Giemsa stain. The slides were then examined under oil immersion lens (×100) of a light microscope as described by Cheesbrough[13].For quality assurance in microscopy examination of slides, two trained microscopists viewed each blood film before declaring the slide positive or negative but when conflicting results arose, a third senior microscopist was consulted. Detection of malaria parasites using RDT was done using the SD® Bioline Ag-*Pf* and CTK Onsite™Ag-*Pf/PV* kits with strict adherence to the manufacturer's instructions. The SD® Bioline Ag-*Pf* is designed for the detection of *falciparum* malaria antigen while the CTK Onsite™Ab-*Pf/Pv*is designed for detection of both *falciparum and vivax* malaria antibodies.

1.6 Statistical Analysis

The data was analyzed using descriptive statistics such as frequency and percentages. Chi-square level of significance set at P <0.05. Sensitivity (Sn), Specificity (Sp), Positive Predictive Value (PPV), Negative Predictive Value (NPV) and Accuracy (Acc) for each kit was determined against light microscopy. True Positive (TP), True Negative (TN), False Positive (FP) and False Negative (FN) were used in determination of the characteristics of these kits. Sensitivity, specificity, PPV, NPV and Accuracy were calculated using equation (1), (2), (3), (4) and (5) respectively.Results were interpreted at 95% confidence interval (CI).

$$\text{Sensitivity} = \frac{TP}{TP+FN} \times 100 \quad (1)$$

$$\text{Specificity} = \frac{TN}{FP+TN} \times 100 \quad (2)$$

$$\text{Positive Predictive Value} = \frac{TP}{TP+FP} \times 100 \quad (3)$$

$$\text{Negative Predictive Value} = \frac{TN}{TN+FN} \times 100 \quad (4)$$

$$\text{Accuracy} = \frac{TP+TN}{TP+FN+FP+TN} \quad (5)$$

## III. Results

The results showed that 216(54.0%) out of the 400 participants tested positive for malaria with the CTK Onsite™Ab-*Pf/Pv*kit, 92(23.0%) with the SD Bioline® Ag-*Pf* Kit while 254(63.5%) was recorded with microscopic examination($P<0.05$). *P. falciparum* was the only species found during the study. The prevalence of falciparum malaria based on age and sex is shown Table I, while the variables used in determining the performance characteristics of both kits are shown in Table II. The overall sensitivity for detection of *P. falciparum* by CTK Onsite™Ab-*Pf/Pv*was 78.74% (95% CI: 73.2 – 83.6) while that of SD Bioline® Ag-*Pf*was 36.22% (95% CI: 30.3 – 42.5). Specificity for CTK Onsite™Ab-*Pf/Pv* and SD Bioline® Ag-*Pf* was 100% (95% CI: 97.5 – 100.0) and 100% (95% CI: 97.5 - 100) respectively as shown in Table III. The study indicated that the sensitivity of CTK Onsite™Ab-*Pf/Pv* was higher than that of SD Bioline® Ag-*Pf*, though they have the same specificity. The positive predictive value for detection of *P. falciparum* malaria by both kits was 100% while the negative predictive value (NPV) for CTK Onsite™Ab-*Pf/Pv* and SD Bioline® Ag-*Pf* kits was 73.0% (95% CI: 68.1–77.0) and 47.4% (95% CI: 45.1-49.7), respectively. The accuracies for CTK Onsite™Ab-*Pf/Pv*and SD Bioline® Ag-*Pf* was 86.5% (95% CI: 82.8 - 89.7) and 59.5% (95% CI: 54.5 – 64.4) respectively as shown in Table III.

**Table I:**Malaria prevalence in relation to diagnostic techniques

| | | Microscopy | | CTK Onsite™ | | SD Bioline® | |
|---|---|---|---|---|---|---|---|
| Criteria | No Examined | Non-infected (%) | Infected (%) | Non-infected (%) | Infected (%) | Non-infected (%) | Infected (%) |
| Total | 400 | 146(36.5) | 254(63.5) | 184(46.0) | 216(54.0) | 308(77.0) | 92(23.0) |
| Male | 226(100%) | 86(38.1) | 140(61.9) | 116 (51.3) | 110 (48.7) | 188 (83.2) | 38 (16.8) |
| Female | 174(100%) | 60(34.5) | 114(65.5) | 68 (39.1) | 106 (60.9) | 120 (69.0) | 54 (31.0) |
| 11-20years | 70(100%) | 24(34.3) | 46(65.7) | 28 (40.0) | 42 (60.0) | 52 (74.3) | 18 (25.7) |
| 21-30years | 120(100%) | 38(31.7) | 82(68.3) | 52 (43.3) | 68 (56.7) | 86 (71.7) | 34 (28.3) |
| 31-40years | 88(100%) | 46(52.3) | 42(47.7) | 56 (63.6) | 32 (36.4) | 72 (81.8) | 16 (18.2) |
| 41-50years | 70(100%) | 22(31.4) | 48(68.6) | 28 (40.0) | 42 (60.0) | 52 (74.3) | 18 (25.7) |
| 51-60years | 52(100%) | 16(30.8) | 36 (69.2) | 20 (38.5) | 32 (61.5) | 46 (88.5) | 6 (11.5) |

**Table II**: Variables used in determining the Performance Characteristics ofRDT Kits in Relation toMicroscopy

| Variables | CTK Onsite™ | SD Bioline® |
|---|---|---|
| True Positive | 200 | 92 |
| False Positive | 0 | 0 |
| True Negative | 146 | 146 |
| False Negative | 54 | 162 |

**Table III**: Performance Characteristics of the RDT Kits relative to Microscopy

| RDT Kit | SN (95% CI) | SP (95% CI) | PPV | NPV (95% CI) | ACC (95% CI) |
|---|---|---|---|---|---|
| CTK Onsite™ | 78.7% (73.2-83.6) | 100% (97.5-100) | 100% | 73.0% (68.1-77.0) | 86.5% (82.8-89.69) |
| SD Bioline® | 36.2% (30.3-42.5) | 100% (97.5-100) | 100% | 47.4% (45.1-49.7) | 59.5% (54.5-64.4) |

Key: CI= Confidence Interval, SN=Sensitivity, SP= Specificity, PPV=Positive predictive value, NPV=Negative Predictive Value, ACC= Accuracy

## IV. Discussion

Using Giemsa Microscopy as the Gold Standard, the malaria prevalence recorded in this study was 63.5%. This is comparable to 60.6% and 68.4% reported in Kano [14] and GombeState[15] respectively, higher than 48.1% in Borno State [16] and 23.3% in Anambra State[17] but lower than 72% in Osun State[18] and 85.7% in Enugu State [19] of Nigeria. The significantly high prevalence recorded in this study could be attributed favourable environmental conditions (Temperature 26-30$^0$C) and fairly stable humidity (90-100%) which supports the optimum breeding of Anopheles species, the vector of malaria. *P. falciparum* was the only species recorded in this study which is similar to the findings of previous studies[6,7]. Majority of the the study population were within the 21-30years age group, likewise, this age group carried the larger portion of the infection rate with 82(68.3%) out of the 120(100%) in this age group infected. This is similar to findings of Sanji*et al.,* [20], this could be attributed to the fact that this age group represent the larger portion of the active working group, who spend more time working, setting out before dawn and retiring late after dusk which

predisposes them to mosquitoes, some people in this age group are also involed in nocturnal jobs like security guards whichmakes them vulnerable to mosquitoes.

The availability of sensitive, accurate and affordable RDT kits for the diagnosis of malaria is highly desirable. The sensitivity, specificity and diagnostic accuracy recorded for the two RDT kits was lower than that of microscopy, the diagnostic standard. The general performance characteristic of the kits showed that CTK Onsite™Ab-*Pf/Pv* kit is more sensitive and accurate than the SD Bioline® Ag-*Pf* although it recorded 54 false negative results, this could be as a result of low parasitemia, as sensitivity of RDT kits decreases with low parasitemia[21]. This could also be due to the fact that CTK Onsite™Ab-*Pf/Pv*detect malaria antibodies unlike SD Bioline® Ag-*Pf* which detect antigen. SD Bioline® Ag-*Pf*also showed a very low sensitivity (36.2%) which contradicts the work of Pembele*et al.*,[22] who recorded a sensitivity 87.7%, but it is worthy of note that the specificity of SD Bioline® Ag-*Pf*recorded in this study is higher(100%) than 79.9% reported in the same work [21].

## V. Conclusion

In this study, we demonstrated that there is high malaria prevalence in Port Harcourt and that CTK Onsite™Ab-*Pf/Pv*kit is more sensitive and accurate than the SD Bioline® Ag-*Pf*kit although, microscopy supersedes both kits. This buttress the fact that microscopy still remains the 'gold standard' in malaria diagnosis and should be opted for in areas with the technical know-how. We recommend the use of Insecticide Treated Nets (ITNs) as there is active malaria transmission in Port Harcourt. Environmental sanitation and dislodgement of mosquitoes breeding sites should be regularly carried out to reduce breeding and emergence of mosquitoes.

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**Conflict of Interest**: We declare that there is no conflict of interest.

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