# DIAGNOSTIC EVALUATION OF RAPID DIAGNOSTIC TESTING IN MALARIA DIAGNOSIS IN A PRIMARY HEALTH CARE FACILITY IN SOUTH-SOUTH NIGERIA

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## ABSTRACT

**Introduction:** Malaria is a public health burden with high morbidity and mortality. Sub-Saharan Africa experiences the highest prevalence, morbidity and mortality of malaria, with Nigeria accounting for 80%. Malaria parasitological diagnosis is critical to effective malaria management. The study evaluated the diagnostic performance of rapid diagnostic testing (RDT) in diagnosing malaria in a remote primary health care facility in south-south Nigeria.

**Methods:** This retrospective study evaluated 386 malaria cases detected over 11 months in a primary health care facility. The RDT in the study contains *the Plasmodium histidine-rich protein 2 (HRP-2) and Plasmodium lactate dehydrogenase (pLDH)*, branded as SD Bio line Malaria Ag P.*f/Pan.* The study used the statistic software 'MEDCALC (https://www.medcalc.org) and VassarStats (www.vassarstats.net)' to evaluate the sensitivity, specificity, predictive values, false and positive rates, likelihood ratios, malaria prevalence and diagnosis accuracy of the RDT against Giemsa-stained microscopy.

**Results:** The rapid diagnostic test (RDT) performed relative well compared to microscopy (the gold standard) at the sensitivity of 86.96% (95% CI: 76.18 - 93.49) verse 100% (95% CI: 93.43 - 100) and the specificity of 97.16% (95% CI: 94.49 - 98.61) verse 100% (95% CI: 98.53 - 100), respectively. Of the 386 samples, malaria prevalence was respectively 17.88% (95% CI: 14.26 - 22.15) by the RDT and 17.69% (95% CI: 14.11 - 21.93) by microscopy. The diagnostic accuracy of both the RDT and microscopy was 95.34% (95% CI: 92.73 - 97.21) and 100% (95% CI: 99.06 - 100), respectively.

**Conclusion:** The inter-test agreement between the rapid diagnostic test (RDT) and microscopy in the study is excellent at the Kappa value of 0.95 (0.93 - 0.97). The rapid diagnostic test (RDT) performed relatively lower than the Giemsa-stained microscopy in the study. The study findings are however consistent with other relative studies.

**Key words:** Malaria diagnosis, microscopy, rapid diagnostic testing.

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## INTRODUCTION

Malaria is a serious public systemic febrile disease that has very high morbidity and mortality rates. In 2017, the Wealth Health Organization reported annual global malaria death toll at 456, 000 [1]. Africa and Asia bear the most global burden of malaria with highest rates of malaria morbidity and mortality. Africa bears 90% of global malaria prevalence, 80% of which is borne by the sub-Saharans. Of the 91% malaria deaths occurring in Africa, children die from malaria more with worst under-5 children malaria mortality[1-3]. In Nigeria, malaria is endemic (holoendemic), affecting the vulnerable population groups such as the children, pregnant women, and low-socioeconomic groups[1, 4]. Malaria prevalence differs across Nigeria, depending on geographic regions, age groups, periods and rural/urban settlements. Several studies have reported prevalence rates between 14.7% and 85.7% in Nigeria in different periods

and across the six geopolitical zones and age groups[5]. Malaria burden is largely due to persistent transmission; along with misdiagnosis, over diagnosis, overtreatment, drug resistance, and poor malaria-vector control[6-8]. Though malaria symptoms are non-specific, fever is the most predominant symptom with headache, joint pains, muscle pains and poor appetite common as well[8].

A worrisome trend in clinical practice in many health facilities is poor malaria parasitological testing even with suspicious nonspecific symptoms and travel history that should prompt malaria screening and diagnosis. The factor promoting clinical malaria management without parasitological testing is either lack of or limited laboratory facilities. Health care providers' attitude is another challenge in managing malaria. Assumption that every fever is caused by malaria without proper diagnostic work up to differentiate the etiological agents is not evidence-based practice. Malaria symptoms are non-specific, bacterial and viral infections can produce symptoms commonly associated with malaria. Even other parasitic and fungal infections are not left out in the differentials of malaria[9-19]. Essentially, parasitological testing or parasite-based diagnosis of malaria as part of evidence-based malaria management helps in reducing malaria parasite resistance and transmission.

Parasite-based diagnosis (parasitological testing) of malaria is central to effective management of malaria. Malaria diagnostics are expected to be at least 95% sensitivity and specificity[8, 15, 18]. Though malaria microscopy is the 'gold standard' for malaria parasites diagnosis, other non-microscopic diagnostics are in practice now with reassuring results[8]. Alternative malaria diagnosis methods (detection of *Plasmodium* species like *falciparum, vivax, ovale, knowlesi and malariae*) which are technically less complex to operate, with short turn-around time, are important for timely management of malaria (especially in emergency settings). The clinical significance of such test methods (such as rapid diagnostic test, RDT) is the fact that malaria can be managed promptly, which potentially helps in preventing malaria complications and mortality that would result from delayed treatment or misdiagnosis. Many countries have adopted the practice of combining rapid diagnostic tests with microscopy in combating malaria burden for effective management and have reported improved malaria care[2,19-20]. Microscopy detects *Plasmodium spp*. and quantifies the parasites, but it can be time consuming and it requires an experienced microscopist to make accurate diagnosis. Hence, rapid diagnostic testing (RDT) with high sensitivity and specificity rates is necessary for accurate and timely diagnosis of malaria. Rapid diagnostic testing is useful in all levels of healthcare institutions for the diagnosis of malaria, especially in poor-resource settings like remote primary health care with limited laboratory facilities[21].

For clinical use of rapid diagnostic tests (RDTs) for malaria diagnosis, the sensitivity is expected to be greater than 95% as recommended by World Health Organization[8]. Many RDTs have demonstrated higher sensitivities with others performing below the bench mark sensitivity rate (95%). In a study Partec rapid diagnostic test® (PT) was sensitive at 100% and specific at 97.4%, while Binax Now® (BN RDT) performed at 97.2% sensitivity and 93.3% specificity[22]. Factors such as level of parasitemia, storage conditions and operator's incompetency can influence the performance of RDTs[1, 8, 14-15,19]. Elsewhere, some commercially available RDTs (First Response, SD Bio line and CareStart) performed at varied sensitivity and specificity rates[20].Performance consistency and reliability of RDTs are confronted with some factors such as cross-reactivity, level of parasitemia and storage conditions. Hence, a rapid diagnostic test that performs very well in one circumstance may report low in another scenario depending on interference of factors. However, in favourable operating conditions, a rapid diagnostic test should be consistent and reliable in diagnosing malaria.

To address the prevalence burden and clinical/presumptive diagnosis of malaria (which has largely contributed to malaria drug resistance) effective malaria management based on parasitological testing cannot be overemphasized [4].Therefore, the purpose of this study was to evaluate the 'diagnostic performance of Rapid

Diagnostic Testing (RDT)' in diagnosing malaria in a 'remote primary health care facility in South-South Nigeria.

## MATERIALS AND METHODS

This retrospective study retrieved primary data (raw data from in-clinic laboratory records)on malaria cases diagnosed by rapid diagnostic and microscopy. The study evaluated the primary data on previous malaria cases of 11 months retrieved from the records from April 2017 to February 2018. The study site is a 'remote primary health care facility' (LafargeHolcim site clinic, Mfamosing) in an industrial setting which sees several clinical cases among industrial employees in Cross Rivers State, south-south Nigeria. The study region is a rainforest zone with high malaria transmission prevalence.

The two diagnostic tests in use in the clinic are Giemsa-stained microscopy and rapid diagnostic tests (RDTs), SD Bio line being the predominant RDT in use. The sample size was estimated using the formula:

Samples size, n = (Distribution of 50%) / (Margin error% / confidence level score)$^2$.

- $n = Z^2pq / e^2$, where e is the desired level of precision (i.e. the margin of error) at 5%
- p is the (estimated) proportion of the population with the desired attribute at 50%
- q is 1- p
- z-value is 1.96 at 95% confidence interval

The sample size (n) was calculated as $1.96^2 \times 0.5(1-0.5) / 0.05^2 = 3.8416 \times 0.5(0.5) / 0.0025 = 0.9604 / 0.0025 = 384.16$, giving a minimum sample size of 385.

The SD Bio line used in the study contains the *Plasmodium histidine-rich protein 2 (HRP-2) and Plasmodium lactate dehydrogenase (pLDH)*, labelled SD Bio line Malaria Ag P.*f/Pan (other species),* which is a three-band lateral-flow immunochromatographic antigen detection test in a cassette format. The Rapid Diagnostic Test (RDT) detects *Plasmodiumfalciparum and non-falciparum (as pan)* species.

The in-clinic laboratory that provided the primary malaria data for the study is maintained according to the World Health Organization (WHO) laboratory standards. The study centre has adequate and competent laboratory scientists/microscopists who handle the two malaria testing methods considered in the study.

The rapid diagnostic test (RDT), SD Bio line Malaria Ag *Pf/Pan*, in use in the in-clinic laboratory is maintained at the manufacturer's recommended operating conditions and it uses capillary blood to detect malaria parasites. The procedure or technique involved follows:

- collecting capillary blood with a picker following finger prick with safety blood lancet;
- dropping the blood (about 5µl) into the immune-chromatographic film well;
- adding four drops of an appropriate diluent in the dilution well; and reading off results after about 15 - 20 minutes.

The RDT strip was read valid if the control line/band appeared, and was positive for either falciparum or pan or both with colour lines/bands appearance in addition to the control line or band.

For statistical analysis, the author used the statistic software 'MEDCALC (https://www.medcalc.org) and VassarStats (www.vassarstats.net)' to evaluate the sensitivity, specificity, predictive values, false and positive rates, likelihood ratios, malaria prevalence and diagnosis accuracy of the RDT in malaria diagnosing malaria against Giemsa-stained microscopy. Both the RDT (SD Bio line Malaria Ag *Pf/Pan*) and microscopy detected *Plasmodium spp., P. falciparum mono infection, and P. falciparum*co-infection. The rapid diagnostic test [RDT], SD Bio line Malaria Ag P.f/Pan, used in the study contained the Plasmodium histidine-rich protein 2 [HRP-2] and Plasmodium lactate dehydrogenase [pLDH]; a three-band lateral-flow immunochromatographic antigen detection test in a cassette format which detects P. falciparum and non-falciparum species designated as Pf/Pan). The microscopy results were used to determine the accuracy of the rapid diagnostic test in terms of true positive (TP), true negative (TN), false positive (FP) and false negative (FN) RDT results. The sensitivity was

calculated as TP/TP+FN and the specificity as TN/TN+FP. Others performance metrics/parameters calculated also include positive predictive value (PPV, the proportion of positive smears among the total positive RDT results), negative predictive value (NPV, the proportion of positive smears among the total number of negative RDT results), false positive rate (1–sensitivity), false negative rate (1–specificity), likelihood ratios (LR+ = sensitivity/1-specificity; LR- = 1-specificity/sensitivity). Youden index which is calculated by the formula (sensitivity + specificity) – 1 was considered in the calculation.

Appropriate approval was received from the management of LafargeHolcim to access the malaria data. But there was no need for obtaining consents because it is a retrospective study.

**RESULTS**

The results were evaluated in tables 1 to 5as displayed in the last five pages after the references, which captured the detection of *Plasmodiumfalciparum and non-falciparum species.*

Table 1 is a 2 x 2 (two-way) contingency table evaluating the accuracy of the rapid diagnostic test (RDT) in detecting malaria parasites against Giemsa-stained microscopy.

In table 2, the overall *Plasmodium spp*. positivity rate by both RDT and microscopy of the 386 samples investigated was 17.86% (95% CI: 14.26 - 22.15). And the overall negativity rate was 82.12% (95% CI: 77.85 - 85.74).

There was no statistical significant difference in detecting *Plasmodium spp.* (that is, all species without discriminating) by both test methods in assessing the disease prevalence, diagnosis accuracy and the negativity rates in both tables 1 and 2.

**Table 1:** Malaria diagnosed by rapid diagnostic test (SD Bio line Malaria Ag *Pf/Pan*) compared to microscopy.

| Rapid Diagnostic Test | Microscopy | | |
|---|---|---|---|
| (RDT) | Positive | Negative | *Total* |
| Positive | 60 | 9 | *69* |
| Negative | 9 | 308 | *317* |
| *Total* | *69* | *317* | *386* |

*Chi-squared = 0.000; P=1.000 (not statistically significant)*

**Table 2:** Malaria diagnosed (*Plasmodium spp.*) by Giemsa-stained Microscopy and Rapid Diagnostic Test (RDT) with their comparisons.

| Plasmodium spp. | Number of samples | Negative | Microscopy | RDT | Microscopy & RDT | Microscopy positives but RDT negatives | Microscopy negatives but RDT positives |
|---|---|---|---|---|---|---|---|
| All species | 386 | 317 | 69 (17.88%) | 69 (17.86%) | 60 (15.54%) | 9 (2.33%) | 9 (2.33%) |
| P.*falciparum* | | | 65 (16.84%) | 57 (14.77%) | 52 (13.47%) | 13 (3.37%) | 5(1.29%) |
| P.*falciparum/ vivax/ovale* | | | 4 (1.04%) | 12 (3.11%) | 3 (0.78%) | 1 (0.26%) | 9 (2.33%) |
| **P-value** | **<0.0001** | | | | | | |

Table 3 shows 86.96% sensitivity and 97.16% specificity of the SD Bio line combination RDT. The accuracy for diagnosing malaria by the RDT was 95.34%, having excellent inter-test agreement with microscopy at 0.95 (table 3).

**Table 3:** Performance accuracy of RDT (SD Bio line Malaria Ag *Pf/Pan*) compared to microscopy for the diagnosis of malaria (*Plasmodium spp.*).

| ***Parameter*** | **Rapid Diagnostic Test** |
|---|---|
| Sensitivity (95% CI) | 86.96% (76.18 - 93.49) |
| Specificity (95% CI) | 97.16% (94.49 - 98.61) |
| Positive (95% CI) | 17.88% (14.26 - 22.15) |
| Negative (95% CI) | 82.12% (77.85 - 85.74) |
| Positive predictive value (95% CI) | 86.96% (76.18 - 93.49) |
| Negative predictive value (95% CI) | 97.16% (94.49 - 98.61) |
| False positive rate (95% CI) | 13.04% (6.50 - 23.83) |
| False negative rate (95% CI) | 2.84% (1.39 - 5.51) |
| LR+ [C] (95% CI) | 30.23 (15.98 – 58.69) |
| LR+ [W] (95% CI) | 6.67 (3.60 - 12.34) |
| LR - [C] (95% CI) | 0.13 (0.07 - 0.25) |
| LR - [W] (95% CI) | 0.03 (0.02 - 0.06) |
| Disease (malaria) prevalence | 17.88% (14.26 – 22.15) |
| Accuracy | 95.34% (92.73 - 97.21) |
| Kappa value | 0.95 (0.93 - 0.97) |
| Youden index | 0.84 |

*95% CI=confidence interval; LR+ = positive likelihood ratio; LR - =negative likelihood ratio; [C] = conventional;[W] = weighted by prevalence*

The two-way contingency tables 4 and 5 are about the discriminatory detection of *Plasmodium species* by the RDT at 80% and 75% sensitivity rates for *P.falciparum and non-falciparum* infections, respectively.

**Table 4:*P. falciparum*** diagnosis by rapid diagnostic test (SD Bio line Malaria Ag *Pf/Pan*) compared to microscopy.

| Rapid Diagnostic Test (RDT) | Microscopy | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Positive | Negative | *Total* | *Sensitivity 95% CI* | *Specificity 95% CI* | *PPV 95%CI* | *NPV 95% CI* | *Accuracy 95%CI* | *Youd* |
| Positive | 52 | 5 | *57* | *80.00% (68.23-88.90)* | *98.44% ( 96.40-99.49)* | *91.23% ( 81.21-96.16)* | *96.05% ( 93.73-97.53)* | *95.34% ( 92.73-97.21)* | *0.78* |
| Negative | 13 | 316 | *329* | | | | | | |
| *Total* | *65* | *321* | *386* | | | | | | |

**Table 5:** *P. falciparum/vivax/ovale* co-infection diagnosis by rapid diagnostic test (SD Bio line Malaria Ag *Pf/Pan*) compared to microscopy.
*Chi-squared = 16.761; P<0.0001 (statistically significant)*

| Rapid Diagnostic Test | Microscopy | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Positive | Negative | *Total* | *Sensitivity 95% CI* | *Specificity 95% CI* | *PPV 95%CI* | *NPV 95% CI* | *Accuracy 95%CI* | *Youd* |
| Positive | 3 | 9 | *12* | *75.00% (19.41 - 99.37)* | *97.64% (95.57 - 98.92)* | *25.00% (12.38 - 44.02)* | *99.73% ( 98.56 - 99.95)* | *97.41% (95.29 - 98.75)* | *0.73* |
| Negative | 1 | 373 | *374* | | | | | | |
| *Total* | *4* | *382* | *386* | | | | | | |

## DISCUSSION

Overall, the superiority of microscopy over the combination SD Bio line Malaria Ag *Pf/Pan* RDT in diagnosing malaria observed in this study was not remarkable; the two malaria testing methods were excellently correlated at κ = 0.95. In the study, the Giemsa-stained microscopy was superior to the combination RDT SD Bio line Malaria Ag *Pf/Pan* by 2.07% for detecting *P.falciparum.* This performance superiority by microscopy is not significant enough to play down on the application of rapid diagnostic testing in detecting Plasmodium species, especially in poor-resource healthcare settings where it is highly needed. Malaria prevalence and diagnosis accuracy by microscopy were 17.69% (95% CI: 14.11 - 21.93) and 100% (99.06 - 100), respectively.

The test accuracy of the RDT was impressive at 95.34% (95% CI: 92.73 - 97.21) which implies malaria can be diagnosed or detected adequately and accurately using rapid diagnostic testing with all precautions, favourable conditions and diagnostic factors observed. The study rapid diagnostic test (RDT) falsely detected malaria parasites at 13.04% which is over 3% more than the benchmark (less than 10%) by the World Health Organization (WHO)[17]. A study elsewhere reported false positive rate at 11.5% by SD Bio line *Pf/Pan*[17]. The clinical significance of false positivity is exposing the body systems to anti-malaria medications when there is no malaria. This can lead to exposure to drug adverse effects and

development of malaria parasite resistance to anti-malarial drugs, potentially compounding malaria burden. Another observation that is capable of contributing to increased malaria transmission is that the RDT SD Bio line Malaria Ag *Pf/Pan* in the present study missed 2.84% (table 3) of the malaria cases (false negativity).

The World Health Organization (WHO) set out a test sensitivity bench score at 95% at parasite density of 100/µl for relevant and reliable routine diagnostic test use[8]. Although the RDT in this study was sensitive at 86.96% overall, elsewhere combination SD Bio line Malaria Ag *Pf/Pan* and several other commercially available rapid diagnostic tests (RDTs) have performed at higher sensitivity rates to include 97.4% by SD Bio line Malaria Ag *Pf/Pan*[23]; 96.16% by Malaria *Pf/Pan* One Step Rapid Test[3]; 97.2% by BN RDT[22]; and >98% by SD Bio line *Pf*, CareStart Malaria HRP2 or HRP2/pLDH[2], respectively. A possible explanation for the relative low or poor sensitivity of the RDT reported in this study might have resulted from low parasitemia which was not detected. However, in some other studies the sensitivity of SD Bio line RDT was lower than the finding of this study[14,19-20,24].

A further research is required to establish RDT sensitivity at various parasite density levels in a prospective cross-sectional study using larger sample sizes in the same region or other regions in Nigeria. This is to gain more evidence regarding the reliability and accuracy of RDTs in detecting malaria parasites, which can enhance effective malaria management, especially in remote health care facilities with limited laboratory services.

## CONCLUSION

In the index study, the RDT used performed relatively comparative with Giemsa-stained microscopy with excellent inter-test agreement, κ = 0.95. The study justifies the clinical use of *'rapid diagnostic testing'* for parasite-based diagnosis of malaria. Additionally, rapid diagnostic testing is a point-of-care diagnostic procedure that requires less expertise and that has short turn-around time with results in few minutes.

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**Competing interests**

This study is void of competing interests for any interest institution, company, persons or group.

**Authors' Contributions**

Though Author[1] extracted and processed the primary data, both authors contributed equally to the study format and writing.

**Consent**

This is a retrospective study in which there was no direct participant or patient contact and obtaining consents was not required.

**Ethical Approval**

Approval to access the clinic records was received from the relevant authority of the primary health care facility where the study was conducted retrospectively.

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