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User Experience of Diagnostic Skill of SD Bioline Malaria Ag P.f Rapid Diagnostic Test

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ABSTRACT

To achieve success in the war against malaria, quality diagnosis must be made a top priority. With the introduction of malaria rapid diagnostic test (mRDT), malaria testing has witnessed rapid increase especially in non laboratory settings such as homes, consulting rooms and patient's bedside. Despite the increase in access to malaria testing, clinical outcomes, and in many occasions, malaria rapid diagnostic test results are rarely in tandem with microscopy assessment of the same patient. These discrepancies necessitated this study to assess the diagnostic skill of mRDT results obtained using SD Bioline Malaria Ag P.f an antigen based mRDT kit. Using giemsa stained thick blood smear microscopy method as the reference standard, a total of 110 hospital patients were tested, out of these numbers, giemsa stained thick blood smear identified 83 (75.5%) positive while SD Bioline Malaria Ag P.f identified only 26 (23.6%) positive having a sensitivity of 27.7%, accuracy of 42.7%, PPV 88.5% and NPV 28.6%. From these findings, it was observe that there are many malaria infected patients that the SD Bioline Malaria Ag P.f failed to identify. Yet among these numbers, there are also those that SD Bioline Malaria Ag P.f wrongly identified as positive i.e, false positive 3 (2.7%) and those that were actually positive but could not be detected by SD Bioline Malaria Ag P.f i.e, false negative 60 (54.5%). The accuracy of SD Bioline Malaria Ag P.f from the study therefore stood at 42.7% when compared with the microscopy method, implying that it has poor diagnostic performance.

INTRODUCTION

Malaria diagnosis has not been very successful as desired. As deadly as the disease is, it has no definite diagnostic method which is devoid of one shortcoming or another. The world is waiting for that method which apart from being accurate is also quantitative and specific. Of all the available methods, microscopy is the most reliable hence referred to as the gold standard; but it is fraught with so many shortcomings which include not being rapid with reduced sensitivity at low parasite density, affected by poor optical resolution of the microscope and the microscopist, shortage of adequately trained malaria microscopists and poor quality of staining reagents. The malaria real time qualitative polymerase chain reaction (Rt qPCR) is also not sensitive at low parasitaemia where it can yield false negative result. It is expensive and not readily available in the rural areas and it is also susceptible to false positive results with contaminants, persistent antigenemia after successful treatment and primer errors. The malaria Rt qPCR can also yield false negative results in situations where gene mutation occurred. Following these anomalies, the burden of accurate diagnosis now lies in clinical diagnosis where a combination of factors such as disease endemicity level, malaria season and the age group under consideration contribute to proffering near accurate prediction of malaria infection on patients [1, 2]. The RDT method is not different. The gains in the RDT method include the speed, and interpretability of its result, however, issues of accuracy, robustness, goodness of use and scalability still desire a lot of consideration. The introduction of RDT is to offer prompt treatment following accurate early detection of the parasite for eradication of the disease.

In this study, we determined the quality and reliability of results generated from the use of a malaria rapid test kit incorporated with monoclonal antibodies to *Plasmodium falciparum* histidine-rich protein II (*Pf*HRP II) antigen[3] such as SD Bioline Ag P.f kit. The choice for this product is because it is one of the most extensively used malaria rapid diagnostic test (mRDT) kit in Plateau State Specialist Hospital (PSSH) Jos, Nigeria and in many public and private outlets for rapid diagnosis of malaria in children and adults who presented in the hospital and are suspected of having malaria in other facilities. We compared the malaria RDT results with those of giemsa stained thick blood smear microscopy.

As a rule in PSSH, all samples tested using mRDT must also be subjected to giemsa stained thick blood smear microscopy for confirmation. Now, if the malaria rapid diagnostic test can be used for testing before treatment, why is microscopy, which is more technical, time consuming and subjected to many other limitations necessary as confirmatory test? One may want to ask the question: what is actually wrong with the mRDTs that they cannot form final confirmatory results? To this end, a more reliable rapid diagnostic method, or improved diagnostic skill of current malaria RDT is still needed in our health system to address the shortcomings seen in the antigen-based RDT.

In this study, we analyze the diagnostic performance of SD Bioline Malaria Ag P.f in order to discover valid, novel, potentially useful and understandable patterns in data generated from malaria diagnosis using this diagnostic model; with giemsa stained thick blood smear microscopy as the standard.

SD Bioline Malaria Ag P.f is a rapid test kit that utilizes monoclonal antibody to the parasite antigen histidine-rich protein II (*Pf*HRP II) to detect the presence of malaria parasite in patient's samples. This parasite antigen is specific for *Plasmodium falciparum*[4, 5] and can persist for up to 5 weeks in a patient even after successful treatment[6, 7, 8]. This persistent antigenaemia and specificity to *Plasmodium falciparum* constitute significant limiting factors to the credibility of diagnostic accuracy of the kits utilizing *Pf*HRP II as diagnostic marker.
The malaria rapid diagnostic test kit is a qualitative assay which can be likened to a binary classification predictor that produces a correct or wrong prediction, in other words, true positive or not (false positive) and true negative or not (false negative) results.

MATERIALS AND METHODS

Research Design

This study was conducted to generate adequate information about current practices as related to the quality and integrity of results gotten from the most popular and widely used mRDT kit in Nigeria, in other to guide policy making in the aspect of malaria diagnosis if the war against malaria is something serious. To accomplish this, SD Bioline Malaria Ag P.f the was used to test each patient in the hospital and at the same time, a thick blood smear was made on microscope slide for giemsa stained microscopy which was used as the standard for quality assessment of the RDT results.

Study Setting

The study was undertaken between June and September, 2019 in the children outpatient department of Plateau State Specialist Hospital (PSSH), Jos, Plateau state Nigeria. A total of 110 hospital patients were attended to using SD Bioline Malaria Ag P.f and giemsa stained thick blood smear microscopy. Samples were collected only from

patients whose provisional diagnosis requested for malaria test which were exclusively children under the age of fifteen years.

Ethical clearance: Ethical clearance was obtained from Jos University Teaching Hospital (JUTH) Ethical Committee.

Materials

RDT kit comes with all materials that are required. For the microscopy we used microscope slide, giemsa stain, oil immersion and light microscope.

Sampling Process

Microscopy: Smears for thick blood film were made on clean, grease-free microscope slides from fresh blood samples smeared directly on the slide. Smears were allowed to dry at room temperature and stained using giemsa stain. The smears were examined with x100 magnification under oil immersion objective. At least a total of 100 fields were examined before declaring the slide as positive or negative (9).

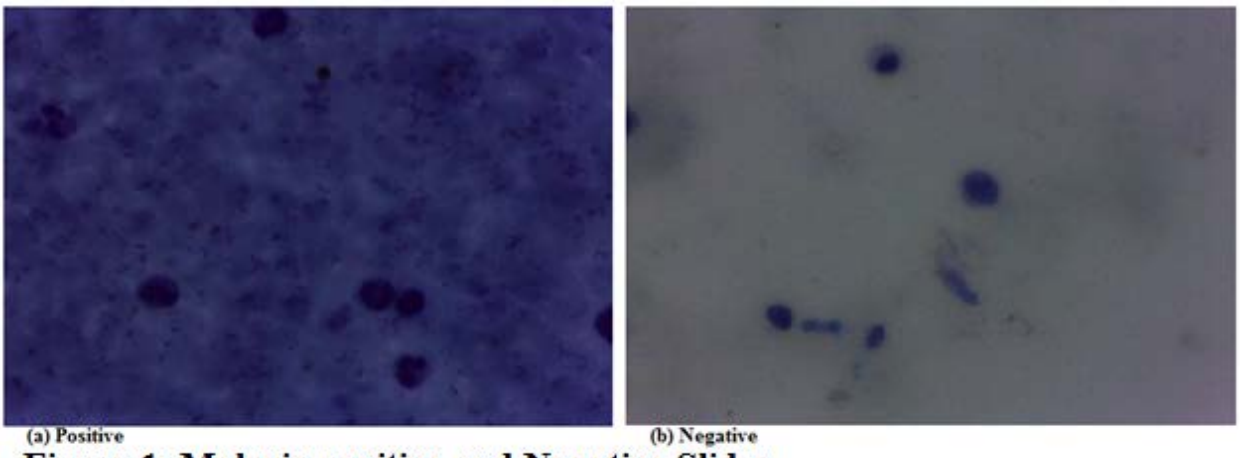

Figure 1: Malaria positive and Negative Slides

Rapid Diagnostic Testing using SD Bioline Malaria Ag P.f

Specimen was collected using a sterile lancet**.** The area to be lanced was cleaned with an alcohol swab. The end of the finger tip was squeezed and pierced with a sterile lancet provided. The first drop of blood was whipped away with sterile gauze or cotton. With a capillary pipette (5µl) provided while gently squeezing the tube, the open end was immersed in the blood drop and then the pressure was gently released to allow blood flow into the capillary pipette to the black line mark. The blood in the capillary pipette was transferred into the round specimen well. Four drops of assay diluent was dropped vertically into the square assay diluent well. The test was allowed to run for a minimum of 15 minutes and a maximum of 30 minutes before the result was read [10].

Interpretation of RDT Results

Negative: Negative result is indicated by presence of one coloured band (control line 'C') within the result window.

Positive: The presence of two coloured bands (test line 'P.f' and control line 'C') within the result window indicates positive result.

Invalid result: If the coloured line (control line 'C') is not visible within the result window after performing the test, the result is considered invalid. All the invalid results were retested using a new test kit [10].

RESULTS

A total of 110 study participants were enrolled into the study. 83 (75.5%) of the patients tested malaria positive for giemsa stained thick blood smear microscopy while the RDT kit – SD Bioline Malaria Ag P.f had 26 (23.67%) (Table 1, figure 2).

Table 1: Summary of Results.

	Microscopy N (%)	SD Bioline Malaria Ag P.f N (%)
Positive	83 (75.5)	26 (23.6)
Negative	27 (24.5)	84 (76.4)
Total	110	110

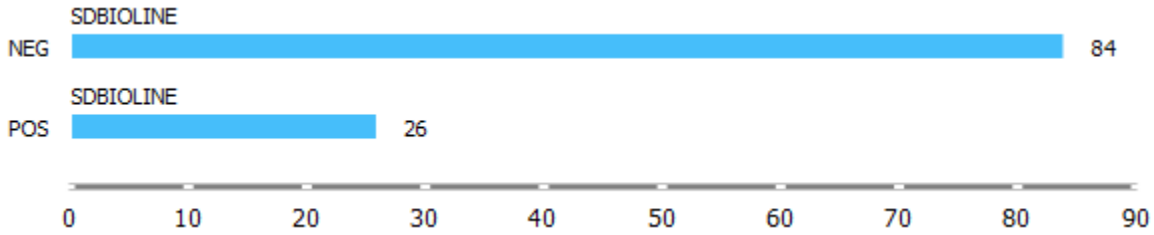

Figure 2: Box plot for attribute 'RDT_USED' grouped by 'RDT_RESULT'.

To recognize the performance characteristics of our model (RDT), the number of true positive (TP) i.e, the number of models testing positive when microscopy is positive, true negative (TN) i.e, the number of models testing negative when microscopy is testing negative, false positive (FP) i.e, number of models testing positive when microscopy is testing negative and false negative (FN) i.e, the number of models testing negative when microscopy is testing positive for SD Bioline Malaria Ag P.f were given as follows: 23 (20.9%), 24 (21.8%), 3 (2.7%) and 60 (54.5%) respectively (table 2). Figure 3 illustrates further.

Table 2: Diagnostic Performance Parameters

Justification	SD Bioline Malaria Ag P.f N (%)
True positive (TP)	23 (20.9)
True negative (TN)	24 (21.8)
False positive (FP)	3 (2.7)
False negative (FN)	60 (54.5)

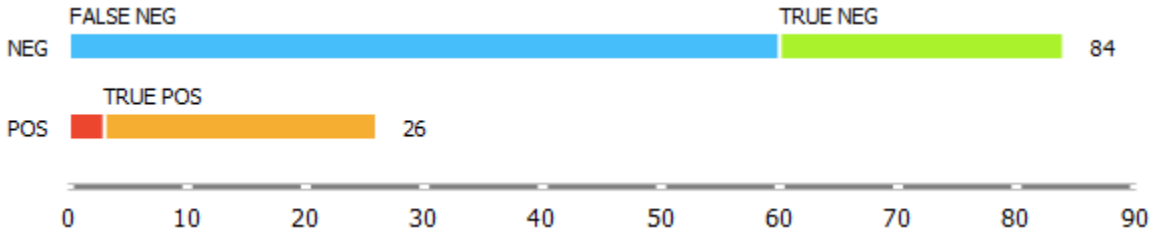

Figure 3: Box plot showing performance characteristics of RDT - SD Bioline Malaria Ag P.f.

From our findings, the performance characteristics of the RDT kit - SD Bioline Malaria Ag P.f is 27.71% sensitivity and 88.89% specificity. Positive predictive value of SD Bioline Malaria Ag P.f is 88.46% while its negative predictive value is 28.57%. The diagnostic accuracy from the study gave SD Bioline Malaria Ag P.f as 42.73% (table 3).

Table 3: Summary of Diagnostic Performance of SD Bioline Malaria Ag P.f.

Diagnostic measure	**Specificity value**
Sensitivity / Recall	0.2771
Specificity	0.8889
Positive Predictive Value	0.8846
Negative Predictive Value	0.2857
Accuracy	0.4273
Precision	0.8846
Error Rate	0.5727

Classification rate/accuracy is given by the equation:$(TP + TN)/(TP + TN + FP + FN)$

Recall: $(TP/TP + FN)$

Precision:$(TP/TP + FP)$

To assess the diagnostic skill of SD Bioline Malaria Ag P.f, the receiver operating characteristic (ROC) curve was generated and the area under the curve (AUC) was used as summarized in figure 4.

Table 4: Comparison of the RDT with microscopy using 2 x 2 contingency table also known as confusion matrix.

	Microscopy		
	Positive	Negative	Total
RDT			
SD Bioline	N (%)	N (%)	N (%)
Positive	23 (27.7)	3 (11.1)	26 (23.6)
Negative	60 (72.3)	24 (88.9)	84 (76.4)
Total	83 (100)	27 (100)	110 (100)
Recognition	27.71 (Sensitivity)	88.89 (Specificity)	42.73 (Accuracy)
Precision	23/26 = 88.46%		
Recall	23/83 = 27.71%		
Error rate	0.5727		
F - score	0.422 = 42%		

Note: Sensitivity is calculated as: true positive / true positive + false negative.
Specificity is calculated as: true negative / true negative + false positive, F-measure (2*Recall*Precision)/(Recall+Precision).

Table 4 gives the summary of malaria diagnosis results as produced by SD Bioline Malaria Ag P.f and giemsa stained thick smear microscopy. Given equal number of datasets for both microscopy and RDT, the receiver Operating Characteristics (ROC) curve was preferred for investigating the observations. By plotting the false positive rate in the x-axis versus the true positive rate in the y-axis using a number of candidate threshold values between 0.0 and 1.0, we can deduce the diagnostic skill of the RDT from area under the curve (figure 4). Tables 5a and b are displays of confusion matrixes constructed from the results of evaluations of SD Bioline Malaria Ag P.f as seen in the Orange data mining data visualization software algorithms corroborating information in table 4.

Table 5a: Confusion matrix for Logistic Regression (showing number of instances)

		Predicted				
		FALSE NEG	**FALSE POS**	**TRUE NEG**	**TRUE POS**	**∑**
Actual	**FALSE NEG**	60	0	0	0	**60**
	FALSE POS	0	0	2	1	**3**
	TRUE NEG	0	0	24	0	**24**
	TRUE POS	0	0	0	23	**23**
	∑	**60**		**26**	**24**	**110**

Table 5b: Confusion matrix for Logistic Regression (showing proportion of predicted)

		Predicted				
		FALSE NEG	**FALSE POS**	**TRUE NEG**	**TRUE POS**	**∑**
Actual	**FALSE NEG**	100.0 %	NA	0.0 %	0.0 %	**60**
	FALSE POS	0.0 %	NA	7.7 %	4.2 %	**3**
	TRUE NEG	0.0 %	NA	92.3 %	0.0 %	**24**
	TRUE POS	0.0 %	NA	0.0 %	95.8 %	**23**
	∑	**60**		**26**	**24**	**110**

Key: Actual = Microscopy observations, Predicted = mRDT observations.

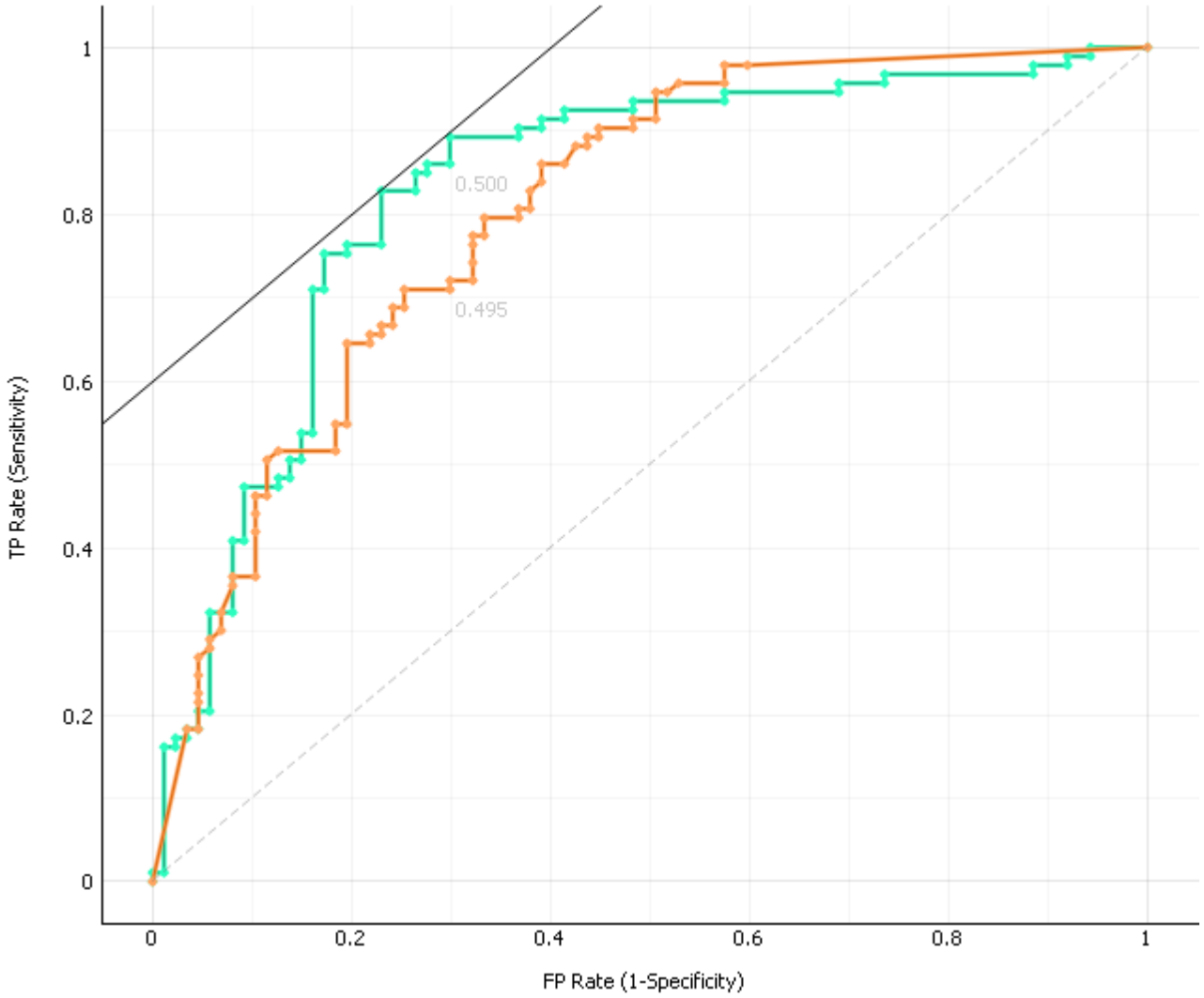

Figure 4: ROC Analysis of SD Bioline.

The true positive rate is calculated as the number of true positives (TP) divided by the sum of the number of true positives (TP) and the number of false negatives (FN), TP rate is also referred to as sensitivity. This describes how good SD Bioline Malaria Ag P.f is at producing the positive results. The false positive rate is calculated as the number of false positives (FP) divided by the sum of the number of false positives (FP) and the number of true negatives (TN). It tells us how often a positive result is produced when the actual result is negative. Large values on the y-axis of the ROC plot (figure 4) indicate higher true positives and lower; false negatives [11].

From the curve, the summary of diagnostic skill of SD Bioline Malaria Ag P.f can be deduced from the Area under the Curve (AUC). The lower the values on the x-axis of the plot the lower the false positive and higher true negatives. The larger values on the y-axis indicate higher true positives and lower false negatives.

The size of different results produced by SD Bioline Malaria Ag P.f is presented in a bar chart (figures 2, 3 and 5). The RDT recorded a high negative result rate. The measure of true positive, false positive, true negative and false negative results can be seen in figure 3.

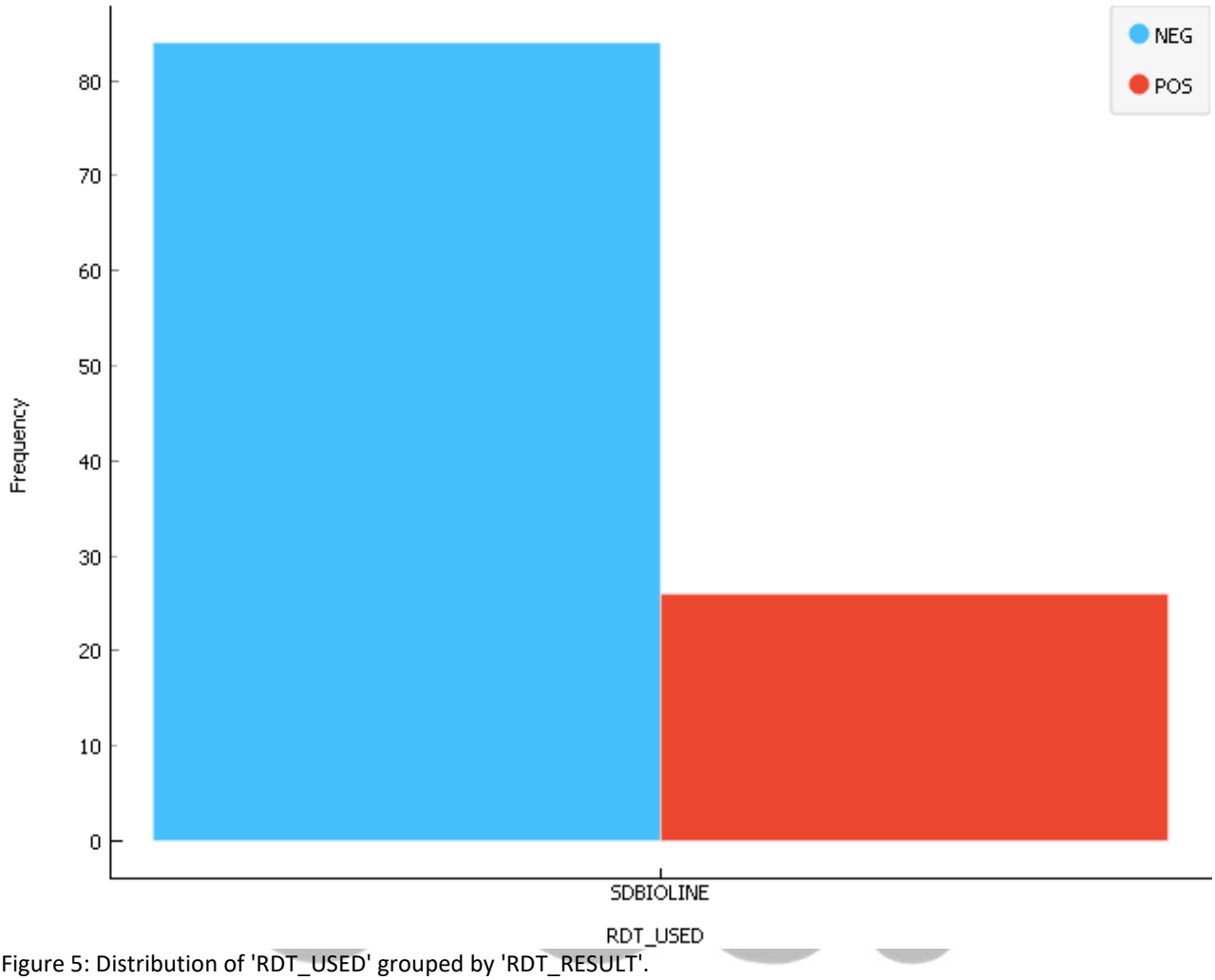

Figure 5: Distribution of 'RDT_USED' grouped by 'RDT_RESULT'.

Generally speaking, from the microscopy, findings indicate that malaria infestation was high among the study population as seen when values in false negative and true positive are put together (figures 3 and 6). This may be a result of collective contributions of season and geographic location.

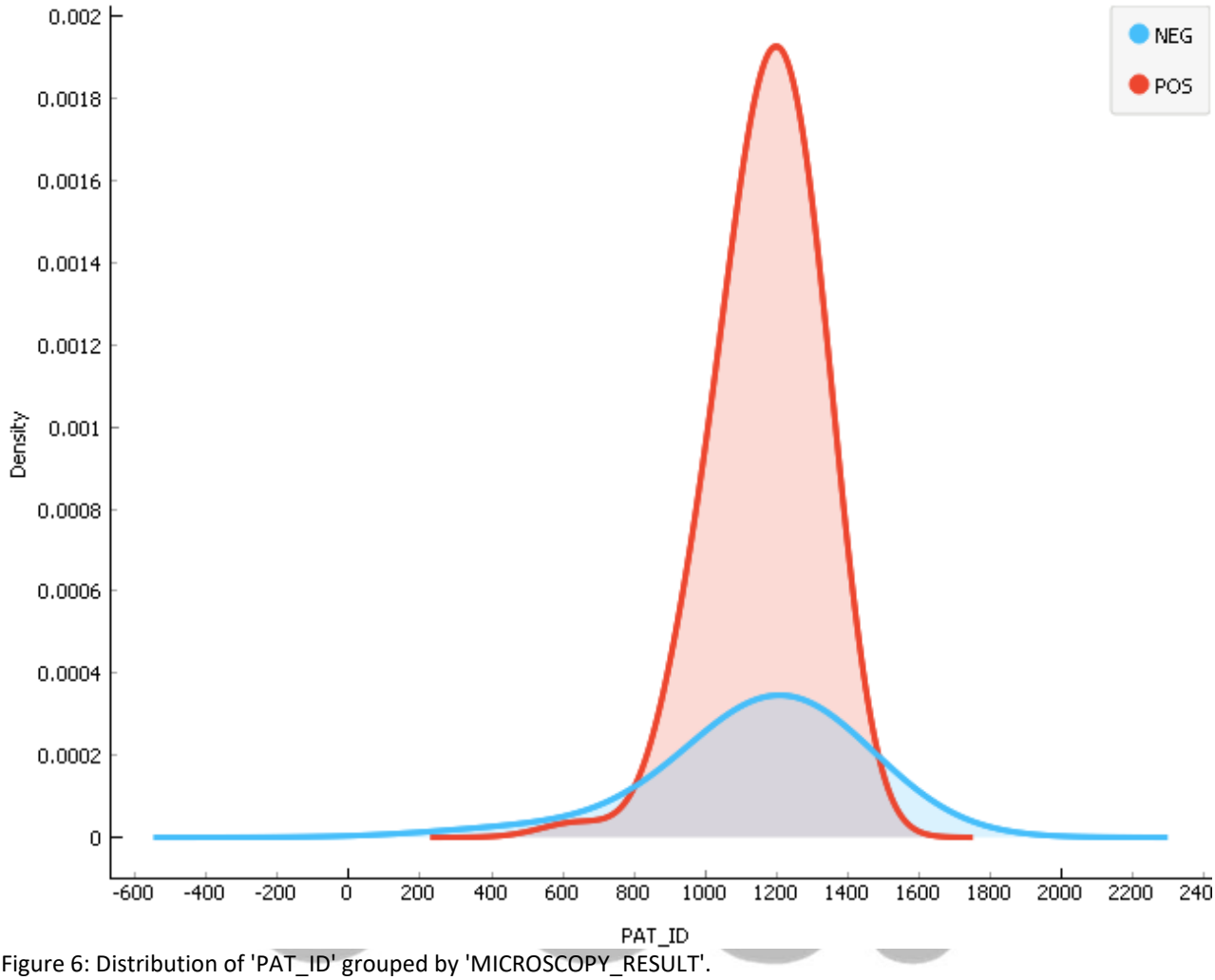

Figure 6: Distribution of 'PAT_ID' grouped by 'MICROSCOPY_RESULT'.

Settings

Sampling type: Stratified 10-fold Cross validation

Target class: Average over classes

Scores

Model	AUC	CA	F1	Precision	Recall
Logistic Regression	0.559	0.427	0.422	0.885	0.277

Discussion

In this study, the skill of the RDT SD Bioline Malaria Ag P.f in malaria diagnosis has been investigated**.** The observed 75.5% positive rate for microscopy is consistent with observations from previous studies by Dozie & Chukwuocha, 2016 in Owerri (microscopy 72% and HRP II RDT 72% - positive rates); James et al, 2012 in Sokoto (microscopy 70.6%, HRP II RDT 54.6% - positive rates, sensitivity 54.2%, specificity 80.4%, NPV 57.5% and accuracy 76.8%); and Oyetunde et al. 2015 in Ijebu Ode Ogun state (microscopy 66.8%, RDT 36.8% positive rates, sensitivity 42.5%, specificity 87.1%, PPV 86.6 and NPV 43.5%) (table 7). Our observation of 23.6% positive rate, sensitivity 27.7%, specificity 88.9% and 42.7% accuracy on the RDT SD Bioline Malaria Ag P.f is however not consistent with any of the findings in the past studies in different locations in Nigeria and it is significantly (p = 0.005) short of acceptable standard expected of a diagnostic tool. Our test model has over 50% error rate. On a flip side of this, it could be possible that the most prevalent species in this part of the world is non *falciparum Plasmodium* species (eg: *vivax, malariae, ovale*, etc). These species do not respond to *Pf*HRP II-based mRDT.

Table 7: Observations from Some Studies on Malaria RDTs in Nigeria.

	Microscopy	*Pf*HRP II RDT					
Author and year	Positive Rate	Positive Rate	Sensitivity	Specificity	Accuracy	PPV	NPV
Dozie & Chukwuocha, 2016 Owerri.	72%	72%	_	_	_	_	_
James et al, 2012 Sokoto.	70.6%	54.6%	54.2%	80.4%	76.8%	_	57.5%
Oyetunde et al. 2015 Ijebu Ode Ogun state.	66.8%,	36.8%	42.5%,	87.1%,	_	86.6%	43.5%)
Ifeanyichukwu Jos****	75.5%	23.6%	27.7%	88.9%	42.7%	88.5%	28.6%

Rose, Oluwasogo & Ayodele, 2017 Kwara state	57.1%	49.5%	86.3%	99.6%	_	_	_

From tables 5a and 5b we are able to see how SD Bioline Malaria Ag P.f is confused when it produces its results and the type of errors it makes. To ensure diagnostic reliability of SD Bioline Malaria Ag P.f manufacturers and their partners need to re-evaluate its diagnostic response based on the following classifier attributes:

Accuracy

Its accuracy predicting class label. The classification model (SD Bioline Malaria Ag P.f) has low classification accuracy (42.7%) so it can not be a model of choice.

Speed

SD Bioline Malaria Ag P.f has quick turnaround time but the speed is not enough if it is short in other classifier attributes.

Robustness

SD Bioline Malaria Ag P.f does not produce extraneous results when used within its ambit of specification, rather invalid (missing) results are occasionally seen arising from uncertain causes. It was observed that with the use of normal saline, table or distilled water as buffer, or running the test with patient's serum as conditions may prompt from time to time SD Bioline Malaria Ag P.f often yields positive results with or without blood sample. It was also observed that the recommended volume of buffer solution often fail to drive the sample up to the result window, while by increasing the volume of the buffer, it tends to over dilute the antigen especially in low parasitemia, making the antigen undetectable thereby leading to false negative result or invalid result.

Scalability

SD Bioline Malaria Ag P.f is manual procedure which result is visually observed and it remains on the test kit for relatively a long period. It is quite scalable however certain situations do impact on its results. For instance, faint coloured bands which are not tracked early enough may disappear before reading time.

Interpretability

The understanding and insight provided by the model. SD Bioline Malaria Ag P.f displays clear unambiguous results when there is sufficient and satisfactory reaction.

Goodness of rules

SD Bioline Malaria Ag P.f lack good compactness of classification rules from loss of robustness giving it somewhat distorted decision tree size and pattern (data not indicated).

Sustained accuracy, robustness, goodness of rules and improved scalability are key issues to be considered in any effort to improve diagnostic quality of SD Bioline Malaria Ag P.f**.**

For the avoidance of doubts, this study was conducted with SD Bioline Malaria Ag P.f kits of lot numbers: 05CDD122A, 05CDD142A and 05CDD161A in ascending order of quantity used (figure 7). Records of the RDT results are verifiable from the children outpatient department of PSSH Jos, Plateau State, Nigeria starting from 17/06/2019 to 20/09/2019.

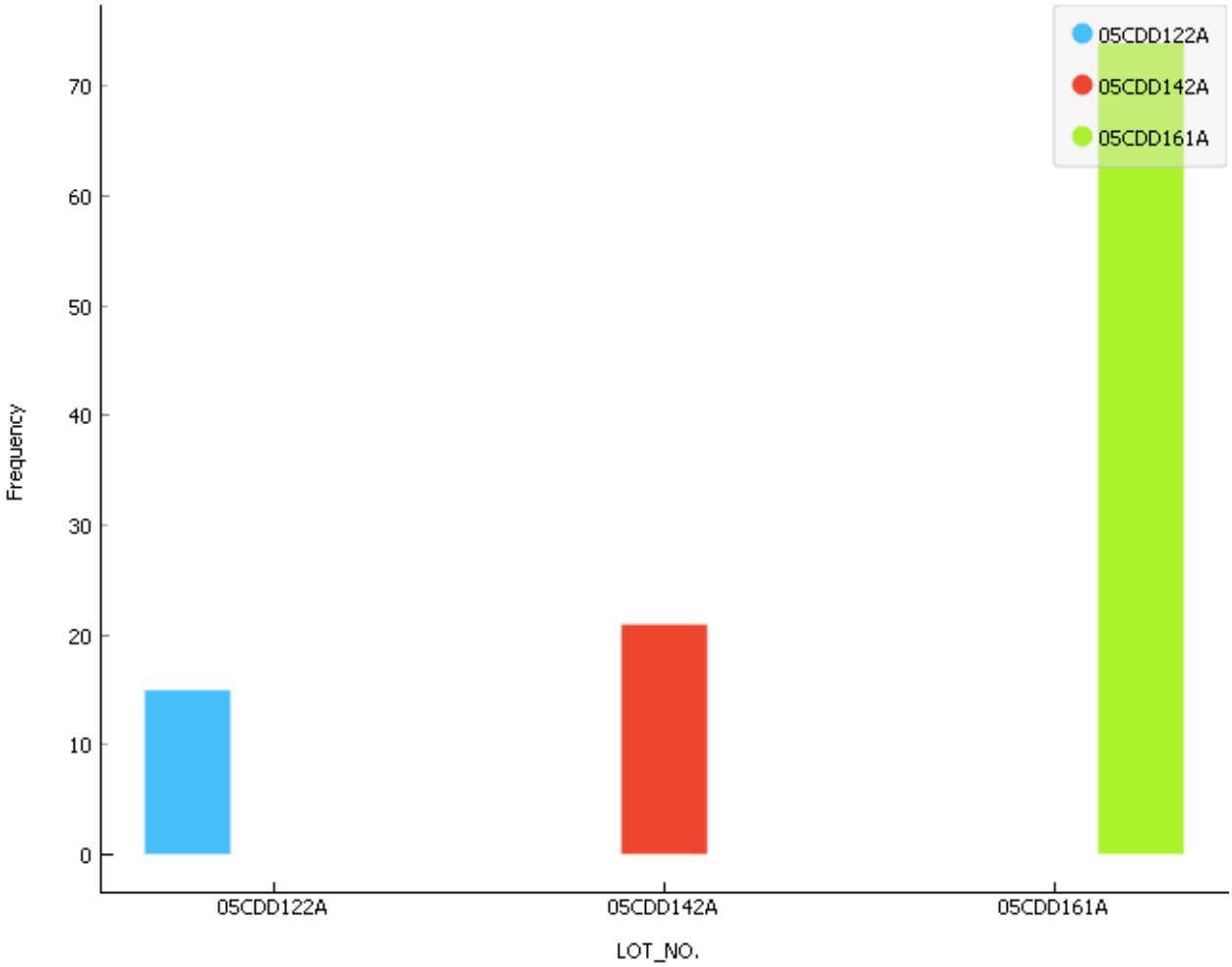

Figure 7: Lot number distribution of SD Bioline Malaria Ag P.f kits used.

SUMMARY OF FINDINGS, CONCLUSION, AND RECOMMENDATIONS

Summary of Findings

The diagnostic proficiency of SD Bioline Malaria Ag P.f has been evaluated in this study. The findings are exactly the field experience using the product. On a serious note malaria has enjoyed so much attention over the years yet its impact has remained unabated. At various times and places, governments have set up several agencies to tackle malaria. Nongovernmental organizations are also not left behind. Donor agencies have continued to fund institutions and individuals in malaria research programmes. Despite all these efforts, it still appears as if the world is not making progress in winning the war against malaria. Certainly we must be getting it wrong somewhere. For this reason, we conducted a study to investigate the quality of results obtained from the use of one of the most

popular mRDT brands in Nigeria - SD Bioline Malaria Ag P.f. In our study, a total of 110 hospital patients were tested using this brand. Out of these numbers, giemsa stained thick blood smear identified 83 (75.5%) positive while SD Bioline Malaria Ag P.f identified only 26 (23.6%) positive with sensitivity of 27.7%, accuracy of 42.7%, PPV 88.5% and NPV 28.6%. From these findings, one would observe that there are many malaria infected patients that the SD Bioline Malaria Ag P.f failed to identify. Yet among these numbers, there are also those that SD Bioline Malaria Ag P.f wrongly identified as positive i.e, false positive 3 (2.7%) and those that were actually positive but could not be detected by SD Bioline Malaria Ag P.f i.e, false negative 60 (54.5). The accuracy of SD Bioline Malaria Ag P.f from the study therefore stood at 42.7% which is not favourable as a diagnostic tool of choice especially for a menace like malaria. It is therefore not doubtful that discrepancies like these are responsible for slow progress in malaria elimination interventions apart from emergency of resistant strains of the parasite and its vector.

Conclusions

Diagnosing *Plasmodial* disease – malaria, has not been as successful as desired especially in settings such as where this study was undertaken. As deadly as the disease is, it has no definite diagnostic method which is devoid of one shortcoming or another. The world waits for that method which apart from being accurate is also quantitative and specific. Of all the available methods, microscopy is the most reliable; but it is fraught with so many shortcomings which include not being rapid, reduced sensitivity at low parasitaemia, affected by poor optical resolution of the microscope and the microscopist, shortage of adequately trained malaria microscopists and poor quality staining reagents. The malaria real time qualitative polymerase chain reaction (Rt qPCR) also has its own shortcomings that limit its application.

Findings from this study indicate that the mRDT examined is not measuring up to expectation despite its popularity. No wonder the disease burden is rising in spite of efforts already put place. For instance, the world health organization (WHO) reported in 2016 that 91 countries reported a total of 216 million cases of malaria, an increase of 5 million cases over the previous year. The global tally of death reached 445,000 deaths, about the same number reported in 2015. WHO Africa region continues to account for about 90% of malaria cases and deaths worldwide. Fifteen countries all but one in sub-Saharan Africa – carry 80% of the malaria burden, (WHO world malaria report, (2017)). The same report indicated that 15 countries but one in sub Saharan Africa carrying 80% of the malaria burden. This staggering statistics is coming at the heels of the introduction and use of mRDT. Something must therefore be wrong with the system that of all coordinated effort already put in place overtime virtually no progress is being made.

In this study, the RDT of choice in PSSH and in most other facilities in Nigeria was being investigated; it was observed from the study that the RDT is actually not helping out. Little wonder most renowned health facilities in Plateau State such as Jos University Teaching Hospital, Bingham University Teaching Hospital and Our Lady of Apostles Hospital have long jettisoned the use of RDTs for malaria testing. PSSH where it is still being used in the

state is only in the children outpatient department; where they use it before initiating emergency treatment of children with febrile illnesses when malaria is suspected to be the cause. It is noteworthy here that clinicians often do not rely on the results from these malaria RDTs when they look doubtful following clinical findings.

Recommendations

The World Health Organization (WHO) recommended RDT standards include a sensitivity of 95% for the detection of 100 /µl parasites and 95% specificity. It is obvious that the model evaluated fell below expected standard. In the study, the diagnostic performance of SD Bioline Malaria Ag P.f was poor with classification accuracy of 42.7% and recall (sensitivity) of 27.7% (tables 4 and 5).

With the accuracy of 42.7% and sensitivity of 27.7, our model is clearly underperforming and therefore, should no longer be recommended until issues of quality are adequately addressed about it. This is important as none of the past studies in Nigeria had attained up to 95% WHO recommendation for sensitivity. Discontinuation of the use of the malaria RDTs in Nigeria is important as nobody would like to be a victim of wrong diagnosis. The consequence of wrong diagnosis can lead to wrong treatment decision, distorted epidemiologic record and poor clinical outcome. Considering the information so far gathered from this study, the following recommendations should be considered for the use of malaria rapid diagnostic tests:

1. Malaria rapid diagnostic tests should be discontinued forthwith;
2. Research should be conducted to ascertain factors responsible for poor performance of the RDT kits used in Nigeria so as to come up with solutions that will improve the accuracy, robustness, goodness of rules and scalability of the products.
3. Malaria specialist centers manned by adequately trained malaria microscopists and funded properly with adequate instruments, where residents can obtain free quality malaria diagnosis should be established in every electoral ward or attached to all the primary health centers throughout the country to encourage early detection and treatment for total elimination of the parasite. This is because an uninfected mosquito does not transmit malaria parasite. Government and development partners should endevour to provide every necessary logistics for effective functioning of the centers; and
4. Research should be sponsored for the development of more reliable qualitative malaria rapid test kits based on clime peculiarities to enhance early and accurate diagnosis and treatment.

Limitations of the Study

The study witnessed quite a few limitations ranging from taking up measured volume of blood samples from restive children to poor capillary migration of recommended volume of buffer up to the result window. When this happens we will have no option than to add buffer and in some cases add more sample to the test which alter manufacturer's recommendation and these do impact the outcome of the study.

Contribution to Knowledge

The problem that prompted this study was the problem of inconsistent result by malaria rapid diagnostic test kits used in Nigeria. The problem with the mRDTs is something that involves the participation of several stakeholders to solve. This study has therefore contributed to knowledge by providing relevant information and guidance to individuals, health care service providers, test kit manufactures as well as health corporate organizations on the need to be alive to a prominent factor militating against winning the effort to eliminate malaria.

Suggestions for Further Studies

In order to advance the course of effective diagnosis and treatment of mostly febrile illnesses of protozoan blood parasites, I suggest further studies in the following areas:

1. Scientific study of studies of the effects of malaria misdiagnosis on the treatment decision, epidemiologic records, or clinical outcomes.
2. Molecular basis for reactive widal tests in the absence of *Salmonella* antibody.
3. A Broad spectrum diagnostic solution for parasitic blood diseases.

Acknowledgement

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REFERENCES

[1] A. Dicko, C. Mantel, B. Kouriba, I. Sagara and M.A. Thera *et al*., (2005). *Season, fever prevalence and pyrogenic threshold for malaria disease definition in an endemic area of Mali*. Trop. Med. Int. Health, 10: 550-556.

[2] Y.W. Mwangi, M. Mohammed, H. Dayo, R.W. Snow and K. Marsh, (2005). *Clinical algorithms for malaria diagnosis lack utility among people of different age groups*. Trop. Med. Int. Health,10: 530-536.

[3] C. Wongsrichanalai, M.J. Barcus, S. Muth, A. Sutamihardja and W.H. Wernsdorfer, (2007). *A review of malaria diagnostic tools: Microscopy and rapid diagnostic test (RDT).anthones inhibitors of α-glucosidase and glycation from Garcinia nobilis*. Am. J. Trop. Med. Hygiene, 77: 119-127.

[4] A. Moody, (2002) *Rapid diagnostic tests for malaria parasites*. Clin Microbiol Rev 15: 66-78.

[5] D. Bell, C. Wongsrichanalai, J.W. Barnwell, (2006) *Ensuring quality and access for malaria diagnosis: how can it be achieved?* Nat Rev Microbiol 4: 682-695.

[6] K. Abba, J..J. Deeks, P. Olliaro, C.M. Naing, S.M. Jackson, et al. (2011) *Rapid diagnostic tests for diagnosing uncomplicated P. falciparum malaria in endemic countries*. Cochrane Database Syst Rev : CD008122.

[7] L.B. Ochola, P. Vounatsou, T. Smith, M.L. Mabaso, C.R. Newton, (2006). *The reliability of diagnostic techniques in the diagnosis and management of malaria in the absence of a gold standard*. Lancet Infect Dis 6: 582-588.

[8] M.L. Wilson, (2012) Malaria rapid diagnostic tests. Clin Infect Dis 54: 1637-1641.

[9] B.I. Garba, A.S. Muhammad, A.Musa, B. Edem, Yusuf, N.K. Bello, A.D. Adeniji, T. Kolawole, (2016). *Diagnosis of Malaria: A Comparison between Microscopy and Rapid Diagnostic Test among Under-Five Children at Gusau, Nigeria.* Sub-Saharan Afr J. Med 2016, 3:96-101.

[10] SD Bio Malaria AgPf (05FK50): Standard Diagnostics, Inc. Yongin – si, Gyeonggi – do, Repoblic of Korea http://www.standard dia.com).

[11] J. Brownlee, (2019). Machine Learning Mastery: How to use ROC Curves and Precision – Recall Curves for Classification in Python.