

# A Comparative Analysis of Different Diagnostic Techniques for Malaria

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**Abstract:** The ability to detect malaria parasite with two immunochromatographic Rapid Diagnostic Test (RDT) kits namely: NOVA malaria kit and SD-Bioline were evaluated by comparison with stained blood film microscopy using thick and thin stained blood films. The work was done between July and August 2010. Blood samples of 202 patients were examined for malaria parasites. The Rapid diagnostic test using SD-Bioline and NOVA malaria kit was also performed using colour bands at lines 1 and 2 to interpret positive and negative test results. The observed prevalence rates were 158(78.2%), 131(64.9%) and 59(29.2%) by microscopy, SD-Bioline and NOVA malaria kit respectively. Analysis of results showed that the sensitivity and specificity rates were 77.7% and 80% for SD-Bioline and 36.3% and 97.6% for NOVA malaria kit respectively. Test Accuracy was 78.2% and 49% for SD-Bioline and NOVA malaria kit respectively. The positive predictive values were 93.1% and 98.3% respectively while the Negative predictive values were 50.7% and 28.7% respectively. Statistical test of the techniques effectiveness at 5% and 1% alpha levels showed that there is significant difference between the performance of RDT kits and the film microscopy technique. Comparison of infection rates using microscopy, SD-Bioline and NOVA malaria kit between the sexes and among four age groups (0-15years), 16-30years, 31-45years and >45 years) showed no significant difference at 1% and 5% alpha levels. Rapid diagnostic tests are recommended for emergency cases to commence treatment and also in epidemiological studies; however, it must be verified with microscopy which remains the gold standard.

**Keywords:** immunochromatography, kit, microscopy, malaria, NOVA, SD-Bioline, RDT.

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## I. INTRODUCTION

Malaria is a major public health problem in over 90 countries worldwide especially in Africa, where it is the leading cause of mortality in children under the age of five years. It accounts for 40% of public health expenditure, 30-50% inpatients admission and up to 50% of outpatients visits [1]. Lately, [2] noted that there were an estimated 438, 000 malaria deaths worldwide. Most of these deaths occurred in the African Region (90%), followed by the South-East Asia Region (7%) and the Eastern Mediterranean Region (2%). The direct financial and indirect cost such as loss of productivity, earning and absenteeism from school has major impacts on both social and economic development of people in malaria endemic areas [3]. [4] Noted that suffering mosquito-borne diseases such as malaria can affects the academic activities of students for a reasonable period of time. Therefore, since malaria is an entirely preventable and treatable mosquito-borne illness [5, 6], prompt and early diagnosis is the key to effective management and control of the disease [7].

Microscopy is one of the established reliable methods of diagnosis of malaria and it is a valuable technique when correctly carried out. Unfortunately, technical requirement and trained personnel as well as the need for precision in microscopy often hamper the reliability of the technique, especially in areas where trained personnel and equipment are grossly inadequate [8]. Due to the technicalities involved in the diagnosis of malaria by the microscopy method, the interest of scientists was raised in the development of more rapid diagnostic methods for malaria, which also would require less expertise training.

The clinical diagnosis, otherwise called prognosis of malaria which is more widely used based on the symptoms of malaria, is rather non-satisfactory. This is because the symptoms of malaria are very "non-specific" and overlaps with those of other febrile (feverish) illnesses [9].

The advantage of the chromatographic diagnosis is their ability to give rapid results which are similar to that of microscopy [8]. Another major advantage is that they do not require high level man power training. The need for a rapid diagnosis of malaria requiring less comprehensive technical training led to the manufacture of various types of chromatographic kits for malaria diagnosis. Although the immunochromatographic tests are exceptionally easy to perform, there are some inherent problems of sensitivity and specificity which measure accuracy of the diagnosis; hence the aim of this research centres on ascertaining the diagnostic accuracy and performance characteristics of the two immunochromatographic test strips (SD-Bioline and NOVA malaria kit) in relation to microscopy in malaria diagnosis.

## II. MATERIALS AND METHODS

### Study Area:

The study was done at VIVA medical Laboratory and Uchenna Hospital, both in Nnewi. Nnewi is a semi-urban area whose inhabitants are mainly businessmen and women with some civil servants and students.

### Study population:

Patients attending Viva Medical laboratory and Uchenna hospital laboratory were used for the study. The patients gave their consent prior to blood collection. Blood samples (2ml) were collected from each of the 202 patients via venipuncture into a blood collection container containing ethylene diamine tetra-acetic acid (EDTA) anticoagulant. The blood samples in the container were mixed well by inversion. Thick and thin blood films were made, stained and examined for malaria parasites according to [10].

### Rapid Diagnostic Test:

The remaining blood samples were used for Rapid Diagnostic Test (RDT) of SD-Bioline malaria Pf/Pv and NOVA malaria kit Pf/Pv. These were done following the manufacturer's instructions. Presence of colour band(s) in the test and control regions was read as positive while the presence of only one colour band at the control line (C) was read as negative. Whenever the control (C) failed to react and show no colour band, it was considered invalid test.

### Data Analysis and Performance calculations:

Performance characteristics were calculated using the standard format of World Health Organization [9, 11]. Key variables were:

$$\text{Sensitivity \%} = \frac{\text{No of true positive (TP)}}{\text{TP} + \text{No of false Negative (FN)}} \times 100$$

$$\text{Specificity \%} = \frac{\text{No of true Negative (TN)}}{\text{TN} + \text{No of false positive (FP)}} \times 100$$

$$\text{Negative predictive value (NPV)\%} = \frac{\text{TN}}{\text{FN} + \text{TN}} \times 100$$

$$\text{Positive predictive value (PPV)\%} = \frac{\text{TP}}{\text{TP} + \text{FP}} \times 100$$

$$\text{Test accuracy (TA)\%} = \frac{\text{TP} + \text{TN}}{\text{All test (n)}} \times 100$$

$$\text{False positive rate (FPR)\%} = \frac{\text{FP}}{\text{FP} + \text{TN}} \times 100$$

$$\text{False Negative rate (FNR)\%} = \frac{\text{FN}}{\text{FN} + \text{TN}} \times 100$$

### III. RESULT

Of the 202 blood samples examined for malaria parasites, 158(78.2%) were positive using microscopy technique, 131(64.9%) using SD Bioline and 59(29.2) using NOVA kit. The prevalence values recorded by microscopy were higher, followed by SD Bioline while NOVA malaria Kit gave the least prevalence measurement. Females were more infected than the males in all the categories (Table 1). Age groups 16-30 years were infected with malaria parasite more than others in all the categories. The difference among the different age and gender groups was not statistically significant ( $X^2_{\text{obs}} < X^2_{\text{tab}}$  at 5% and 1% alpha levels) [Table 2]. Among the three diagnostic methods, Microscopy gave the highest number of positive results and less number of negative results. It gave either positive or negative results. SD Bioline gave the highest number of true positive results 122(60.4%) and false positive results 9(4.5%) while NOVA Kit gave the highest true negative 41(20.3%) and false negative results 102(50.5%) [Table 3]. The performance characteristics of the two RDT kits calculated showed that the sensitivity of SD Bioline was 77.7% while the specificity was 80.0%. Again, the sensitivity of NOVA kit was 36.3% while the specificity was 97.6% (Table 4).

**TABLE I: Prevalence of malaria by sex of the patients using different diagnostic techniques**

Gender	Number examined	Number positive (%)		
		Microscopy	SD Bioline	NOVA kit
Male	94	67(71.3)	59(62.8)	25(26.6)
Female	108	91(84.3)	72(66.7)	34(31.5)
Total	202	158(78.2)	131(64.9)	59(29.2)

**TABLE II: Prevalence of malaria by age of the patients using different diagnostic techniques**

Age group (years)	Number examined	Number positive (%)		
		Microscopy	SD Bioline	NOVA kit
0-15	45	34(75.6)	24(53.3)	8(17.8)
16-30	61	52(85.2)	45(73.8)	21(34.4)
31-45	54	44(81.5)	38(70.4)	18(33.3)
>45	42	28(66.7)	24(57.1)	12(28.6)
Total	202	158(78.2)	131(64.9)	59(29.2)

**TABLE III: Performance Characteristics of the RDTs in comparison to Microscopy**

Performance Characteristics	Total blood samples examined	Total positive	Total negative	True positive	False positive	True negative	False negative
Microscopy	202	158(78.2)	44(21.8)	0	0	0	0
SD Bioline	202	131(64.9)	71(35.1)	122(60.4)	9(4.5)	35(17.3)	36(17.8)
NOVA Kit	202	59(29.2)	143(70.8)	58(28.7)	1(0.5)	41(20.3)	102(50.5)

**TABLE IV: Sensitivity and specificity of the RDTs kits**

RDT kits (%)	Sensitivity (%)	Specificity (%)	Test Accuracy	Positive Predictive Value (%)	Negative Predictive Value (%)	False Positive Rate (%)	False Negative Rate (%)
SD Bioline	77.7	80.0	78.2	93.1	50.7	20.0	49.3
Nova kit	36.3	97.6	49.0	98.3	28.7	2.4	71.3

#### IV. DISCUSSION

In this study, 158(78.2%) out 202 blood samples that were positive using microscopy technique was higher than 40(20.0%) positive malaria samples observed among 200 individuals in Ekwulumili, Anambra State using microscopy [12]. It was also higher than 150(50%) malaria positive observed with microscopy among 300 blood samples in Ihiala, Anambra State [13]. The variations in the percentage of the malaria positive samples in the different location may not be as a result of the microscopy diagnostic method used. This may be attributed to the epidemiological factors that aid malaria transmission in the different study areas [14].

In the study, both male and female were infected with malaria parasites although more females than males were infected in the microscopy. The age group 16-30 years recorded the highest prevalence rate while 45 years and above recorded the least using microscopy. This may be because the age groups of 45 years and above are knowledgeable enough to protect themselves more from mosquito bite knowing the consequences of exposing themselves. These agree with [15, 16] who also observed that both sexes are equally susceptible to malaria infection.

SD-Bioline kit also showed that more females than males were infected with malaria parasite. The age group 16-30 years also recorded the highest percentage with SD-Bioline. [1] Also observed that the younger age groups are more infected with malaria.

The observations with NOVA malaria kit is also in line with the findings of microscopy and SD-Bioline kit. The performance characteristic of the RDTs in relation to microscopy shows that SD-Bioline kit and NOVA malaria kit showed false positive and false negative results. Microscopy recorded no false positive nor false negative result. This agrees with the findings of [8] who observed that 5 patients tested positive by RDT kits and then tested negative with microscopy. [17,18,19] also observed false positive and false negative using RDT kits in the diagnosis of malaria. Also, the observations of [8] agrees with the findings of this work where a few patients that tested positive with SD-Bioline and NOVA kit, but then tested negative with microscopy. Statistical analysis on the comparison of the RDT with the microscopy showed that there is significant difference among the techniques using chi-square analysis and Randomized block design statistical analysis.

The general performance of the two rapid diagnostic test kits showed that SD-Bioline has higher sensitivity than NOVA kit. NOVA kit was higher in specificity than SD-Bioline kit. The results agree with [11] who evaluated the performance of a RDT kit. [20] Assessed the diagnostic capacity of three rapid diagnostic kits against microscopy. The performance of SD-Bioline is moderately well and much better than that of NOVA kit judging with their sensitivity and test accuracy.

## V. CONCLUSION

The rapid diagnostic tests are rapid, easy to perform and interpret without much training and expertise, but comparing with the microscopy showed significant differences. The evaluation of the two RDT kits showed that the SD-Bioline kit is quite reliable than the NOVA kit. This nevertheless does not rule out their usefulness in rapid and quick diagnosis of malaria in critical clinical cases and epidemiological researches but confirmatory test must be carried out using microscopy especially where information on the parasitic load is needed. Therefore, microscopy diagnosis remains the most reliable method of malaria infection diagnosis. Further, researches should be done on other available RDT kits so as to determine their accuracy in malaria diagnosis. Manufacturers of these RDT kits should improve the quality, sensitivity and specificity of these kits so as to avoid false positive and false negative results.

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