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EVALUATION OF MICROSCOPY AND RAPID DIAGNOSTIC TEST KIT FOR MALARIA INFECTION DETECTION

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Abstract

This study was aimed at comparatively evaluating the performance of Microscopy and Rapid Diagnostic Test (RDT) kit as diagnostic tools for detecting malaria infectivity among Western Delta University Students (WDU). A total of 210 students of WDU, Oghara made up of 50(23.8%) and 160(76.2%) males and females respectively were enrolled for the study. Out of the 50(23.8%) male students screened by Microscopy method, 35(70.0%) and 15(30.0%) participants tested positive and negative for *Plasmodium falciparum* Malaria Parasite respectively. Out of the same number of male subjects, 21(42.0%) and 29(58.0%) tested positive and negative respectively for malaria parasitaemia by RDT technique. Out of the 160 female students screened, 95(59.4%) and 65(40.6%) were positive and negative respectively for malaria infection by Microscopy. By the RDT method, 38(23.5%) and 122(76.5%) females were positive and negative respectively for malaria infection. On the whole, malaria infection prevalence rates by Microscopy and RDT were 61.9% and 28.1% respectively. Malaria infection had no correlation with sex. A paired T-test of both techniques at 95% confidence interval showed that there was no significant difference (**P > 0.05**) for positive results and there was a significant difference (**P < 0.05**) for negative results. Performance characteristics of Bioline RDT kit used included sensitivity (71.4%), specificity (87.5%) PPV (50.0%) and NPV (77.3%) as against 100% each for Microscopy. Paired T-test evaluation of performance of both methods indicated a significant difference in performance of Microscopy over RDT (**P < 0.05**). The 61.9% and 28.1% prevalence rates of malaria infection obtained in this study showed that malaria is endemic in WDU and indeed Oghara. Despite the lower performance of RDT over Microscopy in this study, RDT is still an alternative to Microscopy particularly in rural healthcare facilities where there is no trained experienced Microscopist to read stained thick films for detection of malaria infection. However, either of the two methods should not be used singly but a combination of both wherever possible.

Keywords: Evaluation, Microscopy, RDT, Malaria, Infection, Detection

INTRODUCTION

Malaria is one of the highest killer diseases affecting most tropical countries especially Africa (Baboo *et al*., 2008). Malaria is a disease caused by the bite of an infected anopheles mosquito which harbors the plasmodium parasite (WHO, 2005). It is a life threatening disease caused by different species of Plasmodium parasites which are found in humans and non-humans (Bashir *et al.,* 2019). Whereas *Plasmodium vivax, Plasmodium falciparum, Plasmodium malariae* and *Plasmodium ovale* are found in humans, *Plasmodium knowlesi, Plasmodium berghei* and *Plasmodium vinkei* are found in non-humans (Mayxa *et al.,* 2004). Of all the species, *Plasmodium falciparum* and *Plasmodium vivax* are widely spread and are mostly found to be associated with cases of mixed-species of malaria or co-infection or genotype of *Plasmodium* parasites (Mayxa *et al.,* 2004).

Malaria presents itself with different symptoms ranging from fever to chills, headache, excessive sweating, muscle pains and shivering. These symptoms interface with symptoms of other disease conditions and therefore, treatment cannot be based on symptoms but on actual diagnosis of the plasmodium species (Berkley *et al*., 2005;

Chandrainohan *et al*., 2002; Hamer *et al*., 2007)

The World Health Organization (WHO) estimated that 3.2 billion people are at risk of being infected with malaria and according to the latest estimates, 219 million cases of malaria occurred globally in 2017 and the disease led to 407,000 deaths (WHO, 2018). Millions of people are affected in Africa, Asia and South America and according to WHO (2015), malaria is endemic in over 100 countries with about 3million people at risk of infection. The disease has remained a major public health concern in Nigeria because the disease is responsible for 13% child and 11% maternal mortality (WHO, 2005). It also accounts for 30,000 deaths each year and about 60% of outpatient visits to healthcare facilities in Nigeria (WHO, 2005).

Late and inefficient diagnosis of malaria largely caused by *Plasmodium falciparum* in tropical Africa are factors that account for the increasing morbidity, mortality, drug resistance and the associated economic losses accompanying malaria infection and there is therefore need for an express and accurate diagnostic method for its detection (Acheampong *et al*., 2011). In developing countries like Nigeria, Microscopy is the method that is mostly employed in the diagnosis of malaria infection and hence, it is referred to as the "gold standard" method for the laboratory diagnosis of malaria (Mayxa *et al.,* 2004). Microscopy is still considered as the "gold standard" for malaria diagnosis in endemic countries because it has a sensitivity of 50-500 parasites/µl (Moody, 2002), is inexpensive and allows the identification of species as well as parasite density count (Feleke *et al.,* 2017; WHO, 2009). The drawbacks however, in the use of Microscopy especially in remote areas include, the fact that it is cumbersome to operate, requires trained personnel, is unaffordable due to high cost and lack of constant electricity (WHO, 2016).

As a result of the need therefore, for rapid and accurate detection of malaria parasites in the effective treatment and management of malaria, Malaria Rapid Test kits have been developed for the parasitological diagnosis or confirmation of malaria (Kozycki *et al*., 2017). The most widely used RDTs for malaria are based on the detection of parasite histidine-rich protein II (HRP2) and *Plasmodium* lactate dehydrogenase (Ugah *et al.,* 2017). The major constraints of RDTs however, are false positive results because HRP2 persists in the blood for several days after infection clearance (Humar *et al*., 1997) and false negatives due to gene deletions (Kozycki *et al*., 2017). Despite the stated constraints, Microscopy is less advantageous to rapid diagnostic test because RDTs provide quick and reliable results and less skilled persons are needed to read the results. Moreover, RDTs do not require electricity or any form of equipment (Mayxa *et al*., 2004).

Despite the availability of both techniques for malaria parasitaemia detection, some health professionals in endemic countries have somewhat lost confidence in test results and resolved to treat all fever cases systematically as malaria (WHO, 2016; Mangham *et al.,* 2011). This may result in over-diagnosis or under-diagnosis of malaria with the accompanying excessive use of antimalarial drugs or negligent

(blind) treatment all of which invariably contribute to malaria morbidity and development of antimalarial drug resistance (Ugah *et al*., 2017; Metoh *et al.,* 2010). More studies are needed therefore, to test and compare the performance of both diagnostic methods to ascertain their accuracy and reliance in malaria infection detection. This study therefore, is aimed at assessing the performance of microscopy and rapid diagnostic test kit for malaria infection detection among Western Delta University undergraduate students with the following objectives:

- To screen for malaria parasites in the peripheral venous blood of students using **Microscopy** method.
- To screen for malaria parasites in the peripheral venous blood of students using **Rapid Diagnostic Test (RDT)** method.
- To Statistically Compare the performance of both methods for malaria infection detection.

MATERIALS AND METHODS

Ethical Clearance

Participating students were all adults aged between 18 – 37 years (average 22years) who gave their oral and written informed consent to take part in the study.

Study Design/Study Area

This study was a cross-sectional type of which samples were collected or obtained from randomly selected consenting students of Western Delta University, Oghara, Delta state, Nigeria. This work was carried out consistently from March to June, 2020 in the Microbiology Department of the institution.

The University, established in 2008, is situated in the Western end of Delta State, close to Koko junction, along Benin-Sapele Road, Nigeria. The University has three Colleges and a student population of about one thousand.

Sample Size Determination/Sampling

Sample size for this study was computed in line with a scheme provided by Charan and Biswan (2013) of which the calculated minimum sample size "N" was 186. This was arrived at with a 95% confidence interval, a P value of 0.1407, a malaria prevalence rate (based on report of Udijih *et al*, 2017) of 14.07% and a margin error set at 0.05. For convenience however, a total of 210 subjects were enrolled for the study.

Using 5ml sterile needles and syringes, about 3mllllitres (3ml) of venous blood was collected from 210 randomly selected Western Delta University, Oghara students made up of 50(23.8%) male and 160 (76.2%) female students. Whole blood samples were collected by venipuncture (by tying a tourniquet around the upper arm and surface-sterilizing the arm with 70% ethanol to sterilize and stimulate increased blood pressure in the veins) and dispensed into sequestrinized (also known as ethylene diamine – tetra-acetic acid or EDTA) anti-coagulated blood containers properly mixed by standard method and labeled appropriately. Both symptomatic and asymptomatic students were recruited for the study because almost 90% of participants did not show or feel any visible signs/symptoms of plasmodiasis or malaria fever.

All collected specimens were processed within 2-24hours of collection. Where there

was inevitable delay in screening, affected samples were refrigerated (at 4^0 C) in a functional refrigerator with steady voltage.

Malaria Diagnosis by Microscopy

A drop of whole blood of each sample was placed on the centre of a grease-free slide (after appropriate labeling). Using the corner of a spreader, a circular thickness was made with the drop of the blood to obtain a thick film. The films were allowed to air-dry on a laboratory bench while being protected from flies, ant and dust particles (Bashir *et al*., 2019). All labeled thick films slides were placed on a staining rack and flooded with 10% V/V Giemsa stain solution for 15minutes (Brooks *et al*., 2004) and excess stain removed with drops of water while wiping the back of the slide with tissue paper. All well-labeled stained slides were air-dried by putting them in an upright position in a partitioned wooden block. Oil immersion drops were put on the dry thick films and were examined for sexual stage parasites (ring forms or trophozoites) under the microscope using X100 oil immersion objective lens and results were recorded. Slides were examined by a trained Medical Laboratory Scientist and Microscopist. A thick film was declared negative after five minutes of not observing asexual parasites over about 100 microscopic fields (Hopkins *et al*., 2008). The presence of malaria parasites was determined and reported using the plus (+) sign scheme (Achempong *et al.*, 2011).

Malaria Diagnosis Using RDT

The blood samples used for microscopy were also used for RDT screening. The test is a one-step Malaria Antigen *P. falcipraum* (HRP-II) Rapid Test Kit which is an Immunochromatographic test coated with monoclonal antibody that recognizes the specific Histidine Rich Protein – 2 (HRP - 2) associated with the presence of *Plasmodium falciparum.*

Procedure

The RDT kit used was Bioline SD Malaria Ag P.F Kit (Standard Diagnostics, Korea). The test device, buffer and specimen were allowed to equilibrate at room temperature (10 – 30^0 C) prior to testing. The test cassette was removed from the foil pouch by tearing at the notch and then placed on level surface laboratory bench. With a capillary pipette, 5ml (0.05ml) of whole blood was slowly added into the sample well and 4 drops of clearing buffer (or assay diluent) were vertically added to the buffer well. All cassettes were well labeled before use. The blood-buffer mixture was allowed to run across the test control window. Results were read within 15-20minutes (Moody, 2002).

Performance Characteristics

The performance characteristics of the Bioline SD Malaria Ag P.F kit are 99.7% sensitivity and 99.5 specificity.

INTERPRETATION OF RESULTS

Positive Result

The presence of two coloured band lines (test line and control line) within the result window, regardless of which band appears first, indicates a positive result.

Negative Result

The presence of one coloured band (control line C) within the result window indicates a negative result.

Invalid Result

If the one coloured band (control line C) is not visible within the result window after performing the test, the result is considered invalid. Instructions may not have been followed correctly or the test may have deteriorated beyond the expiration date.

Determination of Performance Characteristics of RDT Used

The performance characteristics include sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV).

Sensitivity

The sensitivity of the RDT used was calculated by taking the percentage of positive malaria test by RDT from the total of positive malaria by microscopy (Moody, 2002).

$$\text{Sensitivity} = \frac{true\ positive}{True\ positive + false\ negative} \text{ X } 100$$

Sensitivity was defined as the probability that a truly infected individual will test positive.

Specificity

The specificity of the RDT was determined by taking the percentage of Malaria negative test by RDT from the total number of malaria negative samples by microscopy (Baker *et al*., 2010).

$$\text{Specificity} = \frac{True\ Negative}{True\ Negative + False\ positive} \text{ X } 100$$

Specificity was defined as the probability that truly un-infected individuals will be tested negative (Lee *et al.,* 2006).

Positive Predictable Value (PPV)

This is the probability that those individuals testing positive by rapid diagnostic test (RDT) were truly infected.

$$\text{PPV} = \frac{True\ positive}{True\ Positive + False\ positive} \text{ X } 100$$

Negative Predictive Value (NPV)

This is the probability that those individuals testing negative by rapid diagnostic test (RDT) were truly uninfected.

$$\text{NPV} = \frac{True\ Negative}{True\ Negative + False\ Negative} \text{ X } 100$$

Data Analysis

Sensitivity, specificity, PPV and NPV were calculated (in percentages) and computed values compared with the standard (microscopy) of 100% hypothetical sensitivity, specificity, PPV and NPV (Standard Diagnostics, Korea).

Results

A Total of 210 students consisting of 50(23.8%) males and 160(76.2%) females were screened for malaria parasitaemia using the standard Microscopy and Rapid Diagnostic Test (RDT) methods. Out of the 50(23.8%) male students screened by microscopy method, 35(70.0%) and 15(30.0%) tested positive and negative respectively for trophozoites or ring forms of *Plasmodium falciparum* malaria parasite. Comparatively, out of the same number of male students screened by RDT method, 21(42.0%) and 29(58.0%) were positive and negative respectively for malaria parasitaemia (**Table 1**).

On the other hand, 160(76.2%) female students were screened using both methods. Ninety-five 95(59.4%) and 65(40.6%) tested female students were positive and negative respectively for malaria parasitaemia by microscopy method whereas, rapid diagnostic test method recorded 38(23.5%) and 122(76.5%) malaria parasite positive and negative cases (**Table1**).

Table 1: Sex Distribution of Malaria Parasitaemia by Microscopy and RDT Methods

Table 1: Sex Distribution of Malaria Parasitaemia by Microscopy and RDT Methods

Sex	Total Samples Screened	Microscopy		RDT		P-value
		Number positive	Number Negative	Number Positive	Number Negative	
Males	50 (23.8%)	35(70.0%)	15(30.0%)	21(42.0%)	29(58.0%)	**12.706**
Females	160(76.2%)	95(59.4%)	65(40.6%)	38(23.5%)	122(76.5%)	**6.314**
Total	**210(100.0%)**	**130(61.9)**	**80(38.1)**	**59(28.1%)**	**151(71.9%)**	

On the whole, 130(61.9%) and 80(38.1%) participants tested positive and negative respectively for malaria parasite by microscopy method while 59(28.1%) and 151(71.9%) participants tested positive and negative respectively for malaria parasite using rapid diagnostic testing methods.

Critical $t_{0.05\ (1),\ 1}$ = **1.651** (Paired T test Comparing **POSITIVE** Results of Both Methods)
P value = **12.706** at 95 C.I
P > 0.05

Critical $t_{0.05\ (1),\ 1}$ = **6.768** (Paired T test Comparing **NEGATIVE** Results of Both Methods)
P value = **6.314** at 95 C.I
P < 0.05

The data on subjects with true/false negative and true/false positive Microscopy and RDT results are presented in **Table 2.**

True positives are people with malaria disease who tested positive. False negatives are people with the disease but tested negative while true negatives are people without the disease who tested negative and false positive are participants without the disease but tested positive. In the Table also, the comparison of RDT results with the results of microscopy using the 2 x 2 contingency table is shown. For microscopy, 110 samples tested positive while 100 samples tested negative for malaria parasitaemia.

Table 2: Comparison of Microscopy with RDT Method

Method	Positive	Negative	True positive	False positive	True negative	False negative
Microscopy	110	100	110	0	100	0
RDT	56	154	39	17	137	0

In the case of RDT, 56 and 154 samples tested positive and negative respectively for malaria parasitaemia. In Microscopy method more participants who truly had malaria tested positive for malaria compared to RDT (true positive). Microscopy method had no record of participants who truly were not suffering from malaria but tested positive (i.e. false positive) compared to RDT method.

Table 3 shows the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of both malaria diagnostic methods used. Rapid Diagnostic test method has 71.4% sensitivity, 87.5% specificity, 50% PPV and 77.3% NPV. Therefore, RDT detects 71.4 % of individuals suffering from malaria, 87.5% of individuals free (not having) malaria, 50.0% of participants that tested positive and actually have malaria and 77.3% of participants that tested negative and did not actually have malaria.

Microscopy conversely, recorded sensitivity, specificity, PPV and NPV of 100% each and this is significantly higher than the sensitivity, PPV and NPU obtained for RDT method ($P < 0.05$).

Table 3: Sensitivity, Specificity and Other Parameters of Microscopy and RDT Methods Used

Parameters	Microscopy	RDT	P-value
Sensitivity (%)	100	71.4	**3.182**
Specificity (%)	100	71.4	
PPV (%)	100	71.4	
NPV (%)	100	71.4	

Critical $t_{0.05\,(1),\,3}$ = **3.5942** (Paired T test Comparing Results of Both Methods)
P value = 3.182 at 95 C.I
P < 0.05

DISCUSSION

In this work, the performance of the microscopy over rapid diagnostic test kits (as malaria diagnostic tools) is evaluated. Microscopy is the most widely used tool for the diagnosis of malaria and when in capable hands, it is very sensitive for detecting parasitaemia (Nandwani *et al.*, 2005). Microscopy (often described as gold standard for malaria detection) can also give important information to the clinician (Medical Doctor) about the species, parasite stages and parasite density. However, good quality of microscopy is difficult to implement and maintain because it is labour intensive and requires highly skilled and regular quality control (Azikiwe *et al.*, 2012). On the other hand, the use of malaria RDTs which appears to be the most rapid method to detect the presence of malaria parasite and requires minimum or no training at all, is recommended by WHO when reliable microscopy is not available.

Routine malaria diagnosis is focused on detection of asexual parasite stages in the stained thick blood film using microscopy or detection of parasite antigen using RDT. Findings in this study showed malaria prevalence rates of 61.9% and 28.1% by microscopy and RDT respectively suggesting that prevalence rate obtained by microscopy (61.9%) was much higher than that obtained by RDT method (28.1%). This finding is not in agreement with the report of Tekola *et al.* (2008) which stated a high malaria prevalence rate of 80% by RDT and low rate of 48.9% by microscopy. Findings in this study are however consistent with 66.8% and 36.8% malaria positivity by microscopy and RDT respectively (Oyeyemi *et al.*, 2015). Going by microscopy alone, high malaria prevalence rates of 76.8% and 93.4% have been reported in Okada, Edo State and Odoakpu, Onitsha by Otajevwo (2013) and Ilozumba and Uzozie (2009) respectively. These rates are higher compared to findings in present study. Male malaria parasitaemia reports of 70.0% by microscopy and 42.0% by RDT are obviously higher compared to female malaria prevalence rates of 59.4%

by microscopy and 23.5% by RDT in this study which suggest that the male participants in this study seem to be more vulnerable to malaria attack than their female counterparts. This suggestion (position) is inconsistent with the report of Bashir *et al.* (2019) which stated that the rate of malaria positivity by microscopy is not connected with gender and also that malaria positivity by RDT was not connected with gender. This is similar to the findings of Garba *et al.* (2006) which stated that the positivity of microscopy and RDT is not connected with gender of the individual. Performance evaluation of both techniques showed there was no significant difference of Microscopy over RDT for positive malaria parasite results ((**P > 0.05**) and a significant difference in performance of both methods (**P < 0.05**). Performance characteristics of both methods showed that Microscopy significantly performed better than RDT.

The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of the Bioline RDT kit used in this work are 71.4%, 87.5%, 50.0% and 77.3% respectively. The sensitivity rate or level obtained in this work is a little low compared to 96%, 97%, 79%, 78.4%, 91.1%, 90.9%, 87.1% and 93% sensitivity rates obtained by some previous authors (Msellem *et al*., 2009; Mahende *et al*., 2016; Garba *et al.*, 2006; Oyeyemi *et al.*, 2015; Bouchachart *et al.*, 2004). On the other hand, the 71.4% sensitivity obtained in this study is high when compared to low values of 42.5%, 40.3% and 37.7% reported by previous authors (Osei – Yaboah *et al*., 2016; Abdul-Kadir *et al.*, 2015; Brown and Azikiwe, 2014).

Similarly, 87.5% specificity level obtained in this work is consistent with 89.6%, 89.0%, 97.8%, 99.6% and 100% sensitivity rates obtained by some previous authors in similar studies (Abdul-Kadir *et al.*, 2015; Abdul-Kadir *et al.*, 2015; Brown and Azikiwe, 2014).

In this study, the positive predictive value (PPV) and negative predictive value (NPV) obtained were 50.0% and 73.3% respectively. This was inconsistent with the NPV (98.5%) and PPV (84.3%) results obtained by Mahende *et al*. (2016). Findings in this work were however consistent with PPV (50.0%) and NPV (53.7%) reported by some authors (Garba *et al*., 2006).

The sensitivity reported in this work is yet to attain the 95% recommended by WHO (WHO, 2000). This low sensitivity is disadvantageous as it will impair control intervention. However, the higher specificity (87.5%) will improve the cost effectiveness of malaria diagnosis since the RDT is unlikely to miss out the non-infected individuals.

CONCLUSION

This study has shown that malaria (positive) parasitaemia detection occurred more with the microscopy than with RDT tool despite the not too high performance characteristics of RDT kit used (in view of WHO set standard). However, despite the lower performance of RDT compared to microscopy in this study, RDT is still an alternative to microscopy particularly in rural health facilities where there is no trained experienced microscopist to read stained thick films for detection of malaria infection. Indeed, based on the fact that

both methods have peculiar advantages and disadvantages, accurate diagnosis should not be based on one method only but a combination of both.

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