

Comparative Assessment of Some Rapid Diagnostic Test (RDT) Kits for Malaria Diagnosis in Ibadan, Nigeria

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Abstract

Background: Deployment of sound diagnostic deliverables remains a crucial component of malaria control and prevention programme in Africa. This study aims to make a comparative assessment of the efficacy of three famous brands of rapid diagnostic kits (RDT) available in Nigerian market, with the traditional Giemsa staining (microscopic) method, in testing for malaria in endemic zones, also to provide relevant information and guidance to individuals, health care service providers, test kit manufacturers as well as health corporate organizations.

Methods: Giemsa microscopy along with RDT kits (*Acon*, *Paracheck* and *SD Bioline*) were carried out on 525 patient samples presumed to present with acute uncomplicated malaria by clinical diagnosis. In addition, the total WBC count and haematocrit were conducted on the blood samples.

Results: Out of the 525 samples recruited, three hundred (300) 57.1% were found positive by Giemsa microscopy. SD Bioline had a positivity rate of 260 (49.5%) while Acon and Paracheck trailed behind with 200 (38.1%) and 150 (28.6%) samples respectively. The sensitivity, specificity and efficiency of the three RDT kits were as follows: SD Bioline (86.3%, 99.6%, 92%); Paracheck (50%, 97.7%, 70.4%) and Acon (66.7%, 100%, 80.9%) respectively. Children within the age bracket 0-10 years had the highest malaria positivity rate ($F=5.29$; $p < 0.05$). 115 (38.3%) of children in this age group were positive for malaria with Mean PCV of 30.65 ± 0.52 compare to non-malaria control. The dominant malaria species was *P. falciparum* with 280 (93.3%) cases. However, an appreciable cases of *P. falciparum* & *P. vivax* 15 (5.0%) along with *P. falciparum* & *P. malariae* 5 (1.7%) co-infections were confirmed. It was also observed that the haematocrit value for individuals correlated inversely with the parasite density ($r = -0.78$; $p < 0.05$).

Conclusion and Recommendation: Giemsa microscopy method still remains the gold standard for malaria diagnosis in limited resources endemic zones and recommends that imported RDT kits for malaria should be validated before use in developing countries.

Keywords: Giemsa Microscopy; Rapid Diagnostic Kits; Parasite Density; Co-Infection; Malaria Control

INTRODUCTION

Malaria is a protozoan infection of red blood cells transmitted through bites of blood-feeding female *Anopheles* mosquitoes. It has been declared one of the most dreaded diseases of mankind as it accounts for 0.584 million deaths annually (WHO, 2014). Most of the deaths occur among children under the age of 5 years, with some childhood deaths resulting mainly from cerebral malaria and anaemia (Yusuf *et al.*, 2010). Recent statistics show that while it is declining in other regions of the world due to deliberate interventions, Africa seems to be an exception. Although recent estimates suggest that malaria mortality rates decreased by an impressive 47% between 2000 and 2013 globally, and by 54% in World Health Organization's (WHO) African Region, malaria still remain a major public health problem in a number of countries (Abdoulaye *et al.*, 2016). Eighty percent of malaria cases occur in Africa and about 40% of malaria deaths occurred in just two countries – Nigeria and the Democratic Republic of Congo (WHO, 2013).

Nigeria, with a population density of 134,031,164, records more than 100 million cases of malaria and a mortality of 300,000 annually (USA malaria fact sheet, 2015). Efforts aimed at malaria control through the use of insecticide treated bed-nets seem not to be yielding the desired result, possibly due to wrong use or abuse of the material, low rate of ownership and coverage, poor or low community compliance and commitment to follow / practice and implement training expertise received from past malaria control programmes and low literacy level in these populations. Although malaria is curable, its endemicity and re-occurrences, coupled with its high mortality in children (WHO, 2013) and pregnant women, pose a major challenge in Africa especially in Nigeria. Four major species of *Plasmodium* cause malaria - namely *Plasmodium falciparum*, *Plasmodium malariae*, *Plasmodium ovale* and *Plasmodium vivax*. A fifth specie, *Plasmodium knowlesi* was first known to infect humans in 1965, but its current prevalence rate is comparatively insignificant. One of the challenges in malaria management, particularly among children, is inaccurate diagnosis of the condition (Olukosi *et al.*, 2015). Clinical diagnosis of malaria in a region with many tropical infectious diseases has limited reliability since signs and symptoms are similar for many of these diseases. Clinical diagnosis of malaria without laboratory support may lead to malaria misdiagnosis and maltreatment (Oladosu and Oyibo, 2013). The situation is compounded by

drug resistance to existing anti-malarial drugs. One of the control strategies to combat malaria is to improve diagnosis and treatment (Wilson, 2012). Though, examination of a thick blood smear by Giemsa staining technique remains the preferred method for malaria diagnosis, it is however labour-intensive and time consuming. As reliable as this technique is, a few cases may still be missed. Nonetheless, Giemsa microscopy offers a lot of advantages over other diagnostic techniques. Microscopic examination of blood films for malaria is only feasible in standard laboratory settings which are often unavailable in many rural areas. Consequently, the use of rapid diagnostic technique (RDT) kits for malaria diagnosis is becoming widespread in recent times. For instance, Ikwuobe *et al.*, 2011, advocated the use of RDT at community pharmacies in Nigeria. Continuous influx of imported RDT kits into the Nigerian market with little or no regulation is a cause for concern and has subtle tendency to compromise malaria management and control on a nation-wide scale. The assumption that all RDT kits work pretty alike and exhibit high efficacy is misplaced and has a tendency to worsen efforts aimed at malaria control in Nigeria.

The aim of this study is to make a comparative assessment of the efficacy of three famous brands of rapid diagnostic kits found in market along with the Giemsa staining microscopy method in testing for malaria. The goal is to determine the efficacy and relative strength of each product in detecting malaria parasite so as to enable end users make informed choices, unlike a situation in which all kits are assumed to be of equal quality and standard which might lead to coercive, poor informed and indiscriminate purchase. This in turn should aid or enhance better quality assurance and control of laboratory diagnostics for malaria and ultimately promoting better health care delivery.

MATERIALS AND METHODS

Study Site

A total of 525 patients provisionally recruited and diagnosed for acute uncomplicated malaria at Glory Diagnostica Ojoo, Ibadan. Patient samples were obtained from Mount Zion, Fajimi Memorial, Mosolape, Olufunmilayo and Olushola Hospitals, all in Ojoo area of Ibadan, Nigeria

Sample collection and analysis

Before sampling, temperature of each patient was measured with the aid of a mercury-type clinical thermometer. Biometric data *i.e.* the age and sex of patients were recorded. Five (5) mls of venous blood from each patient was collected into a clean plastic EDTA container for haematocrit value determination and total white blood cell (WBC) count. Thin and thick blood films were stained by Giemsa for malaria microscopy. At the beginning of each test procedure, individual patient blood samples were thoroughly mixed and tested for malaria parasitemia separately with *Acon*, *SD Bioline* and *Paracheck* kits respectively. Known positive and negative controls for malaria parasite were run along with patients' test samples. Haematocrit or packed red cell volume (PCV) were performed by filling a plain capillary tube to $\frac{3}{4}$ levels with blood from an EDTA container by capillary flow. One end of the capillary tube is sealed with plasticine and placed in a haematocrit centrifuge and spun for 5 mins at 5,000 revolutions per minute. The spun capillary tube is read with a haematocrit reader. Normal PCV values ranged from 40-55% and 36-45% for male and female individual respectively.

Total white blood cell (WBC) count

0.02 ml of each blood sample was added to 0.38 ml of Turk's solution and gently mixed. After leaving on the bench for five minutes, the mixture was charged into a Neubauer counting chamber, allowed to settle for another two minutes before counting was done under x10 objective of the microscope.

Giemsa technique for staining thick and thin films

Dried thin films on staining rack were fixed for 1 minute with methyl alcohol. 5% aqueous Giemsa stain was applied and allowed to stain for 20 minutes. The stain was rinsed off with buffered distilled water (pH 7.2). Stained slides were allowed to dry and examined under x100 objective. Similarly, thick films were allowed to dry, flooded with 5% aqueous Giemsa stain without pre-staining fixing and left for 30 minutes. Buffered distilled water was also applied to rinse off the stain. On drying, slides were examined under the x100 objective.

Parasite density estimation (number/ μ l) by counting parasites against white blood cells

A portion of the thick film where white cells are evenly distributed and parasites well stained is selected for examination. 200 white blood cells (WBCs) were systematically counted. Asexual forms of the parasites in the field covered are estimated concurrently. The number of parasites per μ l of blood is calculated using the W.H.O formula:

$$\frac{\text{No of parasites counted} \times \text{Individual WBCs}}{200(\text{WBCs})}$$

A slide is declared negative when there is no parasite detected / counted within 200 WBCs.

Testing with different RDT kits

STANDARD DIAGNOSTIC (SD) BIOLINE METHOD: Test device is removed from the foil pouch and brought to room temperature. 20 μ l of whole blood is added to the sample well(s) of the test device. 3 drops of assay diluent is added into the sample well(s). The timer is set. A pink colour developing within 10 minutes

indicates a positive result.

PARACHECK (Pf) TEST METHOD (Orchid Biomedical Systems): Paracheck (Pf) kit components are brought to room temperature before testing. 5µl of anticoagulated blood is delivered into sample well A. Six (6) drops of the clearing buffer is delivered into well B. The mixtures are allowed to react. The timer is set. A pink colour within 15 minutes denotes a positive result.

ACON: Test strip is removed from the foil pouch and used immediately. 10µl of whole blood is transferred into a specimen tube. 3 drops of buffer is added into the same specimen tube. Test strip is immersed into the contents of the test tube, taking caution not to exceed the maximum line on the test strip. The timer is set. A pink colour within 10 minutes indicates a positive result.

Interpretation of result

SD / Bioline: The presence of two or three colour bands ('1', '2' and c within the result window, no matter which appears first, indicates a positive result for Pf (*P. falciparum*) or Pv (*P. vivax*) and positive control respectively for SD Bioline.

Paracheck and Acon: Two distinct coloured lines appear. One in the control (c) and another in the test region. This indicates a positive result for Paracheck / Acon. Negative is indicated by no coloured line appearing in the test region but only in the control region (c).

Ethical considerations

All aspects of the study were approved by Olabisi Onabanjo and Kwara State Universities Ethical Review Boards. Verbal and written Informed consent was obtained from all individuals before blood sample collection. They were assured of voluntary participation, confidentiality of their roles and the opportunity to withdraw at any time without prejudice in line with Helsinki declaration was guaranteed.

RESULTS

Table I: Malaria positivity and mean parasite density according to sex

Sex	Total Population	No Positive	% Positive	% Relative Positivity	Mean and SEM of Parasite Density
Males	286	161	53.7	56.3	3297.36 ± 539.83
Females	239	139	46.3	58.2	2866.44 ± 474.57
Total	525	300	100.0

Table I showed that there is no significant difference as regards malaria positivity according to sex. $t = 0.73, P \text{ value} > 0.05$

Key: N = Number positive, % = Percentage

Table II: Parasite density of sample population by age group

Age (Years)	No Positive	% Positive	Mean and SEM of Parasite Density
0-10	115	38.3	5164.05 ± 1183.96
11-20	44	14.6	4530.75 ± 698.63
21-30	63	21.0	851.02 ± 279.48
31-40	40	13.3	1839.08 ± 874.28
41-50	24	8.0	1341.23 ± 663.73
>50	14	4.7	520.85 ± 141.94

Table II showed that there is statistical difference across the age groups. Highest parasite density was found between age (0 – 10) years.

Table III: Relative malaria positivity by RDT kit used

	Giemsa Thick Smear (Microscopy)		Acon RDT		SD Bioline RDT		Paracheck RDT	
	N	%	N	%	N	%	N	%
Positive	300	(57.1)	200	(38.1)	260	(49.5)	150	(28.6)
Negative	225	(42.9)	325	(61.9)	265	(50.5)	375	(71.4)
Total	525	(100.0)	525	(100.0)	525	(100.0)	525	(100.0)

Table III revealed that Giemsa thick smear have the highest malaria positivity of 300 (57.1%) of total samples examined. This is followed by SD Bioline RDT kit with a malaria positivity of 260 (49.5%) of total samples examined. The Giemsa gold standard is seen to be a better diagnostic tool for malaria diagnosis than the RDT.

Table IV: Malaria positivity by *Plasmodium species* type

<i>Plasmodium Species</i>	No Positive (%)	Mean Parasite Density (SEM)
<i>P. falciparum</i>	280 (93.3)	2,244.86 (± 327.94)
<i>P. falciparum</i> & <i>P. vivax</i>	15 (5.0)	15,916.67 (± 812.67)
<i>P. falciparum</i> & <i>P. malariae</i>	5 (1.7)	13,520.00 (± 780.64)
Total	300 (100.0)	...

Table IV showed *P. falciparum* to be the most prevalent species. A significantly high parasite density was found in *Plasmodium* co-infection than in single infection ($F = 51.21, P < 0.05$).

Table V: Sensitivity, Specificity and Efficiency of RDT kit

RDT	Acon	SD Bioline	Paracheck
Sensitivity	66.7%	86.3%	50.0%
Specificity	100.0%	99.6%	97.7%
Efficiency	80.9%	92.0%	70.4%

Table V showed SD Bioline to have the highest efficacy of the RDT kits; it has a sensitivity, specificity and efficiency of 86.3%, 99.6% and 92.0% respectively.

Table VI Comparison of the haematocrit value in malaria and non-malaria subjects

Subject	No Positive	Mean PCV ($\pm SEM$)
With Malaria	300	30.65 \pm 0.52
Without Malaria	225	37.74 \pm 0.34

$r = -0.78; t = 11.33, Pvalue < 0.05$

Table VII: Frequency of subjects with fever among malaria positive patients

Malaria	YES (%)	NO (%)	TOTAL
Positive	242 (89.0)	58 (22.9)	300
Negative	30 (11.0)	195 (77.1)	225
Total	272 (100.0)	254 (100.0)	525

$X^2 = 233.63, P < 0.05$

Key

Fever = axillary temperature > 37.5°C

Discussion

Early diagnosis of malaria most especially in rural settlement or Primary Health Care (PHC) level is vital to combating the disease in all endemic regions. In addition, Nigeria had set for itself the year, 2015 to achieve one of the millennium development goals (MDGs), which is to combat the trio of deadly infectious diseases – malaria, tuberculosis and the human immunodeficiency-virus (HIV) infection. One of the ways to control any infectious disease is to cure the infection in affected patients, in order to limit its spread (WHO, 2010). To achieve this effectively, patients require to be diagnosed promptly and appropriately, combining both clinical and laboratory tools. In recent times, efforts at achieving this goal has led to the increased use of rapid diagnostic test (RDT) kits for malaria diagnosis, particularly in the field and rural communities where laboratory facilities are non-existent (Ikwoobe *et al.*, 2011).

This study compared the efficacy of three different but commonly used rapid diagnostic test (RDT) kits, namely Acon, Paracheck and SD Bioline along with Giemsa microscopy as the gold standard. When malaria positivity for 525 specimens were analysed using different RDTs alongside the Giemsa microscopy method, the latter was able to detect highest number of malaria positivity, constituting 300 (57.1%) of total samples examined. The best of the RDT kit (SD Bioline) had a malaria positivity of 260 (49.5%) of total samples diagnosed. Its positivity in relative percentage to the Giemsa method was 86.7%. Results obtained from this study confirm that the microscopy method remains the reference standard and a better diagnostic tool for malaria diagnosis in the laboratory than the RDTs. This view is supported by Wilson, 2013 who posited that the sensitivity of microscopy is not 100% but varies from region to region and depends to some extent on the skill of the microscopist (Olukosi *et al.*, 2015) and the degree of parasitaemia in a given specimen.

The sensitivity and specificity obtained in this study with SD Bioline is relatively similar to the observation made in the study of Awortu *et al.*, 2007 in whose report the SD Bioline sensitivity was (47%) while specificity was (100%) for malaria parasite. Also the sensitivity and specificity obtained in this study with Paracheck were dissimilar to the observation of Mendiratta *et al.*, 2007 who found Paracheck to be 92.6% sensitive and 98.6% specific. However, Awortu *et al.*, 2007 and Mendiratta *et al.*, 2007 worked on a single type kit only. Observed disparity in the result obtained with Paracheck in this study and that of Mendiratta *et al.*, 2007 is still not clear and calls for further investigations.

SD Bioline is seen as the best RDT kit of the three kits analysed in this study, being able to detect low level parasitaemia. This makes it more sensitive than the others. Besides, it has a sensitivity and specificity of 86.3% and 99.6% respectively. Moreover, it was generally observed that the higher the parasite density, the heavier the colour intensity. In other words, the degree of parasitaemia may be estimated by the extent of colour produced on the test strip, implying that in field work, the intensity of strip colour during / after testing with this kit may be used to give a rough assessment of parasite density in the patient.

From the results obtained, there was no significant difference by gender. ($t = 0.73$, $p > 0.05$) i.e., malaria infection is not influenced by sex of an individual. Amoo *et al.*, 2008 obtained a similar result, implying that each sex is exposed and affected equally by the bite of mosquitoes. In addition, malaria infection and development in the host is not gender-biased (Table 1).

In considering malaria infection according to patients' age, the highest level of parasitaemia was observed in the age group (0-10) years. This could be as a result of the naïve nature of the immune status within that age bracket, making children more susceptible to malaria infection. It is pertinent to note that Awortu *et al.*, 2007 also recorded similar results, with the highest level of parasitaemia in their own study considering age group (1-5years), while assessing malaria frequency, using only the SD Bioline kit.

Furthermore, *Plasmodium falciparum* was found to be the most predominant species among the infecting parasite species, constituting 93.3% of total malaria cases identified in this study. This is in consonance with the submission of Bouree and Taugordeau, 1993 that in many tropical countries, *P. falciparum* is the predominant specie. *Plasmodium falciparum* infections are known to be highly pathogenic and since it is the most prevalent in this study, it may account for why malaria disease takes a great toll on the Nigerian population. Co-infection by other malaria parasite strain seen together with *P. falciparum* accounted for the remaining (6.7%). Hamer *et al.*, 2009 recorded 9.3% for other malaria strains. In addition, a higher parasite density was observed in *Plasmodium* co-infection than in single-specie infections in this study. Significantly, a lower haematocrit value ($t = 11.33$, $p < 0.05$) was found more commonly in subjects with malaria than in subjects without malaria i.e., a strong negative correlation was observed between parasite density and haematocrit value ($r = -0.78$, $p < 0.05$). Biswas *et al.*, (1999) had similar results of low haematocrit values with malaria positive subjects. Also, the level of anaemia in this study was significant in malaria species co-infection than in single-specie infections. A high parasite density implies that a greater number of red blood cells are parasitized, with a consequence of higher red cell destruction that eventually culminates into severe anaemia. This connotes that haematocrit value determination is very paramount and should be conducted along with malaria parasite test, especially among children in whom severe anaemia may quickly result in fatality. Aregawi *et al.*, (2011) observed a reduction in anaemia cases after a scale-up of malaria control programmes in Zanzibar region of Tanzania.

With a temperature of $>37.5^{\circ}\text{C}$ taken as the benchmark for fever, two hundred and forty-two (242) subjects, representing 80.7% had fever symptoms out of 300 found positive for malaria parasite ($p < 0.05$). This suggests that among people with pyrexia in the tropics, malaria parasite screening should be one of the key tests to be conducted while not excluding other probable causes. Muller *et al.*, (2003) recorded 894 (55%) positive cases for malaria parasite out of 1640 subjects having fever symptoms.

Finally, it is pertinent to mention one important diagnostic advantage in microscopy which RDTs lack - the ability of the analyst to observe the morphological features of the parasite under the microscope. This makes it possible for the microscopist to identify the different parasite forms and stages commonly seen under the microscope, a feature which has a lot of implications for the diagnosis of critical parasite forms like schizonts and gametocytes. The inability of RDTs to detect such parasite forms may scale down the gravity of detection of infection especially during severity, thereby contributing directly or indirectly to morbidity and mortality. Wilson *et al.*, (2012) listed several other pitfalls in using RDTs for the diagnosis of malaria to include: possibility of cross reactions of the antigens in the immunochromatographic strip with rheumatoid factor, autoantibodies and certain other non-malarial infections; false positive results for *Plasmodium* species that are absent in blood when *P. falciparum* is in high concentration; the continued presence of pHRP-2 antigens in blood several weeks after treatment, even when parasite is already cleared from the blood and inability to fairly assess the degree of parasitaemia, among others.

CONCLUSION

Giemsa staining technique has been re-established in this study to be the best routine method for malaria diagnosis. Assessment of results from this study has shown SD Bioline as the best RDT kit among the three kits assessed, being the kit with highest sensitivity, specificity and efficiency. It may thus be used fairly reliably where facilities for microscopy does not exist.

Though *Plasmodium falciparum* was the prevalent malaria agent among the subjects, higher parasite density was more common in co-infections involving *Plasmodium falciparum* and other *Plasmodium* species. Demonstration of higher parasite density has shown that children within the age 0-10 years are at high risk of

malaria infection.

Results from this study indicate that the degree of malaria parasitaemia in a patient correlates inversely with the patient's haematocrit level. Low haematocrit values found among malaria infected subjects demonstrated that anaemia remains one of the major symptoms of *Plasmodial* infection in Ibadan.

Recommendations

Although SD Bioline has shown a proven efficacy in the diagnosis of malaria in this study, it can only be recommended as a preliminary adjunct to Giemsa staining technique in malaria diagnosis. Laboratorians in developing countries should continue to employ the Giemsa quantitative analysis for routine purposes so as to achieve effective diagnosis, disease control and efficacious treatment of malaria. It is imperative that malaria microscopy, being a reliable, affordable and accessible technique be assessed regularly against emerging technologies for malaria diagnosis. Anaemia, one of the consequences of malaria parasitaemia in children should be screened routinely to prevent fatality, when malaria is suspected in children.

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Conflict of Interest

We declare no competing interest.

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