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Assessment of Accuracy and Effectiveness of Rapid Diagnostic Test for Malaria Diagnosis at Primary Health Centres in Abeokuta Nigeria

A. Idowu Olufunmilayo[1*], M. Odeyemi Oluwatosin[1] and S. Babalola Ayodele[1]

[1]Department of Pure and Applied Zoology, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

Malaria is a deadly disease that needs proper and prompt diagnosis in order to treat its symptoms as early as possible. Rapid diagnosis test is a pre-requisite for the effective treatment of malaria in other to reduce the mortality and morbidity of the disease especially at the Primary Health Centre (PHC) facilities. This study compares RDTs test results from PHCs with malaria Quantitative Buffy Coat (QBC) and microscopy test results. A total of 113 subjects with clinical signs of malaria were enrolled after obtaining consent of patients at the Primary Health Centres and questionnaires administered to assess awareness and use of RDTs kit. Storage and compliance to standards of usage of the Kits were observed. The results were analyzed using SPSS version 16.0. There was a significant difference ($p<0.05$) in sensitivity to malaria parasite between the three diagnostic methods as QBC was more sensitive compared with other diagnostic methods, while Microscopy was more sensitive compared with RDT kits. A total number of 86(76.1%), 28(24.8%) and 39(34.5%) malaria positive cases were detected by QBC, RDT and Microscopy respectively. Out of the 86(76.1%) blood samples confirmed positive by QBC, 27(31.4%) and 38(44.2%) positive cases were detectable by RDT and Microscopy respectively. Furthermore, Microcopy detected 15(53.6%)

**Corresponding author: Email: tomiwo2@gmail.com;*

of the total positive cases detected by RDT, while RDT was able to detect 15(34.5%) of the total positive cases detected by microscopy. When compared with QBC, RDT shown a sensitivity and specificity of 32.56% (95% CI= 22.84 – 43.52%) and 31.76% (95% CI= 22.09% - 42.76%) respectively. On the other hand, 71.8% (95% CI=55.12% - 84.98%) sensitivity and 87.1% (95% CI= 78.02 – 93.35%) specificity was shown by RDT when microscopy was used as gold standard. Compliance to manufacturer's instruction on RDT usage was poor as some of the health workers collected the blood sample directly from the pricked finger into the sample well rather than the designated capillary pipette method, while others did not comply with time before reading the results of the kits. The result from this study showed that the sensitivity and accuracy of RDTs kit is low and there is need for proper training of the health workers to avoid misuse of the kit.

Keywords: Malaria; microscopic; RDT; QBC; sensitivity; specificity; diagnosis.

1. INTRODUCTION

Malaria is a major cause of death in tropical and sub-tropical countries. According to the World Malaria Report 2013, the mortality rate of malaria in WHO African Region is 36% [1].

Malaria is transmitted to humans by mosquitoes of the genus *Anopheles*. Malaria is known to be caused by four plasmodia species, namely *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale* and *Plasmodium malariae*, with *P. falciparum* being the most lethal [2]. Malaria is a potential medical emergency and should be treated accordingly. In most malaria endemic countries of sub-Saharan Africa, the current standard for laboratory confirmation of a clinical malaria diagnosis is a peripheral blood film, examined microscopically. However, microscopic based diagnosis of malaria is labour-intensive requiring trained staff and quality equipment attributes that are scarce in resource-poor settings [3-5].

Delays in diagnosis and treatment are leading causes of death in many countries. The quest for newer and easier diagnostic methods for malaria has picked up momentum in the last 10 years; and the development of the rapid diagnostic test (RDT) has opened a new avenue, calling for a review of the existing method [6].

RDT is important because early diagnosis and treatment are essential for reducing the morbidity and mortality and it also contribute for a fast and right treatment. Since there is urgent need for new, simple, quick, accurate, and cost-effective diagnostic tests to determine the presence of malaria parasites, to overcome the deficiencies of light microscopy, numerous new malaria-diagnostic techniques have been developed [6].

Unlike convectional microscopic diagnosis by staining thin and thick peripheral blood smears, and QBC technique, RDTs are less expensive and faster. RDT do not require laboratory equipment. For efficient treatment and management of malaria, rapid and accurate diagnostic testing is imperative. The lack of proper diagnostics results in a waste of already scarce resources and impacts negatively on the prompt treatment of malaria [2].

RDT have been developed in different test formats like the dipstick, strip, card, pad, well or cassette; and the latter has provided a more satisfactory device for safety and manipulation. The RDT provide quick result, require less skilled persons as compared to microscopic diagnosis, do not require electricity or any equipment and develops patient's confidence as well as heath services. Like other diagnostic tests, various conditions for manufacture, transport, storage, and the method of RDT. RDT offers the potential to extend accurate malaria diagnosis to areas where microscopy services are not available such as in remote locations or after regular laboratory hours.

There are several controversies about the use of RDTs such as recent treatment of malaria can affect the result of the kit [7]. Also the case of false positive which is as a result of cross reactivity with rheumatoid factors and false negative resulted due to deletion or mutation of the hrp-2 gene [8-9] is major controversies.

Although RDT is useful, this study is to find out if malaria parasite tests carried out using RDT_S kits is really reliable, efficient, and accurate in the context of its usage primarily in health care centers in Abeokuta and its environments. The objective of the study is to investigate the reliability, accuracy and efficiency of histidine-rich protein II (HRP-II) antigen of malaria *Plasmodium falciparum* kit. The aim of this study is to determine the level of awareness of RDTs kit

among patients, know if the patients and health workers trust the result of the kit, find out if health workers supports continuous usage and observe the procedures involving the use of RDT's kit.

2. MATERIALS AND METHODS

2.1 Study Location

This study was carried out in Abeokuta, South-West Nigeria. Abeokuta is the capital of Ogun State and is located in the rainforest belt. Three Health Centers at Abeokuta South Local Government (Oke-Ilewo), Abeokuta North Local Government (Iberekodo and Sabo), and Odeda Local Government (Alogi and Osiele) were used as the study centers.

2.1.1 Questionnaire administration

Questionnaires were administered to patients to know if they were aware of the kit and its usefulness and to the health workers, to find out their perception of the efficacy and accuracy of the test results from RDTs kit. Questionnaires were not administered to 32 (28.3%) of the patients as a result of weak condition shown by the patients during the course of the study.

2.1.2 Inclusion criterion

The participants are patients who experienced the symptoms of malaria which include fever, vomiting, and headache. Their temperature was taken using thermometer.

2.1.3 Laboratory investigations

Blood sample (2ml) were also collected and examined in the laboratory for malaria parasite. All the 113 patients presented clinical symptoms of malaria before the collection of the blood.

2.1.4 Microscopy

Thick and thin blood films were made with smear from the blood from EDTA bottle on a clean, grease free slide, then spread, air dried(thick film) and fixed in 70% methanol(thin film) then stained with 10% of Giemsa stain for 30 minutes and rinsed with water, air-dried and then viewed under the microscope [10].

2.2 Rapid Diagnostic Test (RDT)

The RDT kit used in this study is SD BIOLINE Malaria Antigen P. f. kit which is used for rapid, qualitative test for the detection of histidine-rich protein II (HRP-II) antigen of malaria *Plasmodium falciparum* in human whole blood.

2.2.1 SD BIOLINE malaria antigen P.f rapid test procedure

The patient's finger was cleaned with alcohol swab and then allow to dry before pricking the finger. The patients' fingers were pricked using lancet to get blood. Capillary pipette was used to draw 5 µl blood. The 5 µl blood was dropped in the round sample well then 4 drops of assay diluent was added into the square assay diluent well. The result can be read after 15 minutes to 30 minutes. If there is a line on the area marked "C" in the result window, the result is negative, two lines "C" and "T" in the result window indicate positive. Invalid result occurs when there is no "C" line in the result window.

2.3 Quantitative Buffy Coat (QBC)

Approximately 55-65 µl of blood was taken into a capillary orange, potassium oxalate and fitted with a cap. A plastic float was inserted inside the QBC microhaematocrit centrifuge at 12,000 rpm for 5 minutes. The tube was then mounted and examined through a paralens advance fluorescence microscopy.

The principle of QBC technique is based on the fact that on centrifugation at a high speed, the whole blood separates into plasma, buffy coat and packed red cell layer. Due to acridine orange dye, the malaria parasite nucleus stains green and the cytoplasm orange in the region between the red blood cells and granulocytes and within the granulocytes and where parasites are most abundant.

2.4 Data Analysis

The data was analyzed using SPSS and chi-square to test for significant difference in the results from the diagnostic tests. Also Venn diagrams were used to present the relationships between the three diagnostic tests.

3. RESULTS

3.1 Demographic Information and Perception of Patients about RDT Kits

Majority of the respondents 46(56.8%) were within the age range of 15-34 years followed by age range 35-54 years (25.9%) while only 1(1.2%) of the respondents was greater than 75 years of age. A high proportion 42(51.9%) of the respondents were married, while 36(44.4%) of

the respondents were single. Majorly, the respondents were females with a frequency of 45(55.6%) while 36(44.4%) of the respondents were males. A high proportion 32(39.5%) of the respondents were students followed by traders, 20(24.7%) while civil servants and artisans recorded a frequency of 10(12.3%) and 11(13.6%) of the total respondents respectively (Table 1).

Majority of the respondents 41(50.6%) had tertiary education, which is then followed by 27(33.3%) who had secondary education, while 7(8.6%) of the respondents had primary education. Only 6(7.4%) of the respondents had no formal education (Table 1).

Table 1. Demographic information of patients at the health facilities

Variables	Frequency (%) N=81
Age (Years)	
< 15	4(4.9)
15-34	46(56.8)
35-54	21(25.9)
55-74	9(4.9)
>75	1(1.2)
Marital status	
Married	42(51.9)
Single	36(44.4)
Widow	3(3.7)
Sex	
Male	36(44.4)
Female	45(55.6)
Occupation	
Artisans	10 (12.3)
Civil servant	11(13.6)
Student	32(39.5)
Trader	20(24.7)
Others	8(9.9)
Education	
None	6(7.4)
Primary	7(8.6)
Secondary	27(33.3)
Tertiary	41(50.6)

All the respondents showed malaria symptoms and 60(70.1%) of the respondents claimed to have used either anti-malaria drugs or herbs before coming to the health center.

Majority of the respondents 66(81.5%) claimed not to have heard about RDT kit before. However, 10(15.2%) of the patients that claimed not to be aware of RDT knew other tests that can be used for the diagnosis of malaria parasite. Furthermore, 15(18.5%) of the respondents claimed to be aware of RDT kit in which only 6(40.0%) claimed to trust the result of RDT kits.

3.2 Prevalence of Malaria Cases Using Different Diagnostic Methods

There was a significant difference ($p<0.05$) in sensitivity to malaria parasite between the three diagnostic methods as QBC was more sensitive compared with other diagnostic methods, while Microscopy was more sensitive compared with RDT kits.

A total number of 86(76.1%), 28(24.8%) and 39(34.5%) malaria positive cases were detected by QBC, RDT and Microscopy respectively. Out of the 86(76.1%) blood samples declared positive by QBC, 27(31.4%) and 38(44.2%) positive cases were detectable by RDT and Microscopy respectively (Fig. 1). On the other hand, QBC was able to detect 27(96.4%) and 38(97.4%) of the total positive case detected by RDT and Microscopy respectively (Fig.1). Furthermore, Microcopy detected 15(53.6%) of the total positive cases detected by RDT, while RDT was able to detect 15(34.5%) of the total positive cases detected by Microscopy (Fig. 1).

Out of the 88 positive cases discovered in the universal set, 15(17.0%) blood samples were positive to the three diagnostic tests (Fig. 1). Only 1(1.1%) blood sample was declared positive by RDTs alone, while Microscopy and QBC declared 1(1.1%) and 36(41.0%) blood samples positive alone respectively. Furthermore, 12(14.0%) blood samples were declared positive by QBC and RDTs alone, 23 (26.1%) blood samples were declared positive to QBC and microscopy alone while none of the blood samples were declared positive by microscopy and RDTs alone (Fig. 1).

In this study, 26(96.3%) of the total number of negative cases detected by QBC also tested negative to Microscopy and RDT respectively. Out of the universal set of 98 negative cases revealed by the Venn diagram, 25(25.5%) were declared negative by the three diagnostic tests, while 23(23.5%) and 12(12.3%) negative samples were detected by RDTs and microscopy alone respectively. Furthermore, none of the samples tested negative to QBC alone (Fig. 2).

Only 1(1.0%) blood sample was negative to QBC and RDTs alone, while 36(36.7%) blood samples were declared negative by RDTs and microscopy alone (Fig. 2). Furthermore, 1(1.0%) of the blood

samples was declared negative by QBC and microscopy alone (Fig. 2).

3.3 Sensitivity and Specificity of RDT Using Microcopy and QBC as Control

Using QBC as control, RDT shown a sensitivity and specificity of 32.56% (95% CI= 22.84 – 43.52%) and 31.76% (95% CI= 22.09% - 42.76%) respectively. On the other hand, a 71.8% (95% CI=55.12% - 84.98%) sensitivity and 87.1% (95% CI= 78.02 – 93.35%) specificity was shown by RDT when microscopy was used as gold standard.

3.4 Perception of Health Workers on Efficacy of RDT Kit for Malaria Diagnosis

Respondents health workers that participated in this study works at different departments in the various health facilities. A high proportion of the health workers 22(44.0%) were Nurses by profession, followed by 9(18.0%) that were Community Health Extension Workers. 8(16.0%) of the health workers were laboratory technologist while the least proportion 3(6.0%) of the respondents were pharmacist (Table 2).

Table 2. Demographic information of health workers

Variables	Frequencies N(%) N= 50
Sex	
Male	14(28.0%)
Female	36(72.0%)
Education	
Polytechnics	13(26.0%)
School of nursing	17(34.0%)
School of health technology	3(6.0%)
University	17(34.0%)
Occupation	
Community health extension workers	9(18.0%)
Nurse	22(44.0%)
Doctor	4(8.0%)
Laboratory technologist	8(16.0%)
Public health officer	4(8.0%)
Pharmacist	3(6.0%)

Majority 48 (96.0%) of respondent health workers claimed to be aware of RDTs kit, in which 24 (50.0%) of them claimed to use the kit frequently, while the remaining 24 (50.0%) claimed to seldom use it. Out of the 50 health workers that participated in this study, 47(94.0%) claimed to know other tests used for diagnosis of malaria parasite apart from RDT kits.

Furthermore, 47(94.0%) of the health workers believed the RDT kits are good, while the remaining health workers 3(6.0%) rated it to be fair. 42(84.0%) of the respondent health workers opined that the results of RDTs kit are accurate and efficient while 27(54.0%) believed that the kit is more accurate and efficient than other diagnostics tests.

3.5 Observation Made Involving the Procedure of RDTs Kit by the Health Workers

The use of designated capillary pipette for blood collection for RDT test were jettisoned by some of the health workers because they considered the use of the designated capillary pipette to be cumbersome and they were clumsy while using RDT kit, instead blood drops were collected directly into the blood sample hole thus without taking cognizance of the expected volume of blood to be used which is 5 µl.

It was also observed that health workers were not paying attention to the drops of buffer solution because they sometime drop more than five drops even flooding the assay buffer well. Invalid test results (when the control did not appear/show in the result window) were misinterpreted as positive result especially when the patients show symptoms of malaria. In some cases there is premature reading and discarding the kit without waiting for the recommended 15-20 minutes.

3.6 Storage and Challenges Associated with the Use of RDTs Kit in Health Facilities Visited

Some of the respondent health workers stored the kit in cool and dry place while the rest stored it on their tables. Most of the health workers claimed that they did not experience any difficulty in using RDTs kit while others identified collecting blood sample with micropipette as a challenge. Some said waiting for the result is as well as difficult because it takes time. Some said needle pricking of the patients for blood sample is demanding while some considered reading the result is demanding as well.

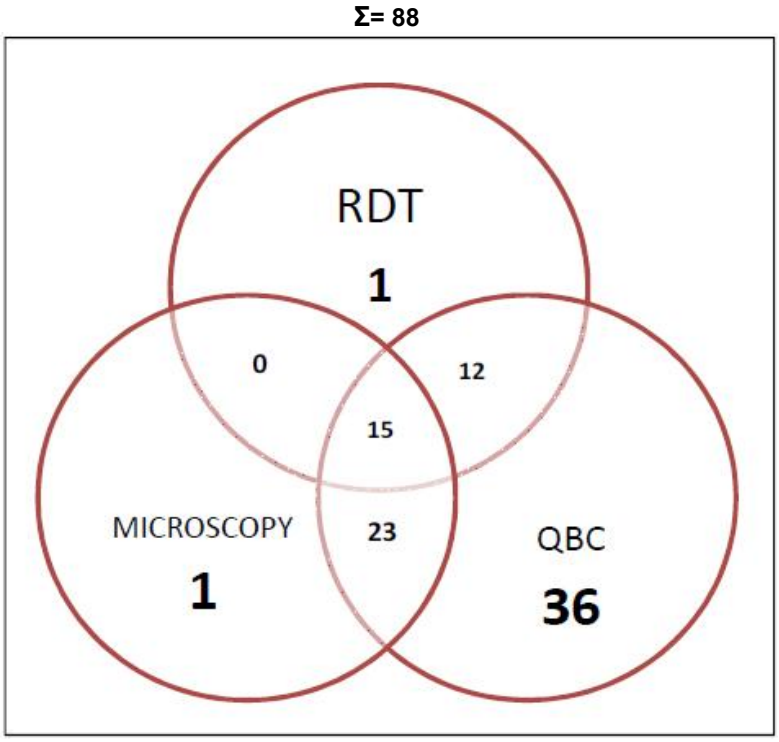

Fig. 1. Relationship between the three diagnostic tests using positive cases

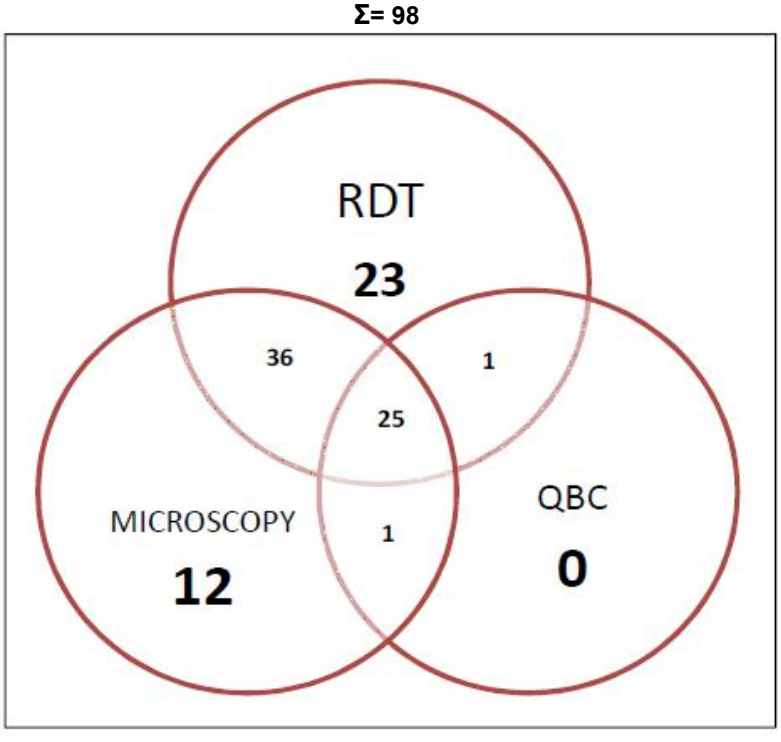

Fig. 2. Relationship between the three diagnostic tests using negative cases

4. DISCUSSION

Malaria is a deadly disease that needs proper and prompt diagnosis in order to treat its symptoms as early as possible. In this study, QBC was more sensitive when compared to other diagnostic methods, while RDT kits showed the least sensitivity of all the three diagnostic methods employed in this study. RDTs were able to detect only 31.4% of the total number of positive cases detected by QBC. Specimen sampling and preparation is a major factors that can affect the performance of RDTs kit. RDT kits are designed primarily for testing capillary blood obtained through a finger-prick [11] and should be collected by a graduated capillary pipette provided with the kit. However, we observed that some of the health workers collected the blood sample directly from the pricked finger into the sample well rather than the designated capillary pipette method. Studies have shown that the accuracy of RDTs can be affected by wrong blood and reagent (buffer) volume [12].

Another factor that could be responsible for the low sensitivity of the RDTs kit was recent treatment with anti-malarial therapy by the patients, as about 60% of the respondents claimed to have used one anti malaria drug or the other. Studies have shown that recent treatment with anti-malarial therapy can have a variable effect on the specificity and accuracy of RDTs result [12].

Appropriate storage of RDTs kit is very imperative, as its affects its performance [12]. Our observatory study revealed that some of the health workers did not comply with the standard environment within which the kit should be stored. Studies have shown that RDTs kit deteriorate more quickly on exposure to moisture (humidity) and high temperature because it rely on antibody-antigen interactions [8,12].

About 96.3% of the total number of negative cases detected by QBC was also declared negative by RDTs and Microscopy respectively. However, only 31.4% and 44.2% of the negative cases detected by RDTs and microscopy respectively were declared negative by QBC. This implies that RDTs and Microscopy reported 68.6% and 55.8% false negative samples respectively when measured against the effectiveness of QBC. Several reasons could be responsible for the large number of false negative results reported by RDTs in this study.

One possible factor is the level of parasitaemia, which might have been influenced by the recent treatment with anti-malarial therapy. Studies have shown that false positive results shown by RDTs are associated with low level of parasitaemia in the specimen [10].

Other factors that could be responsible includes; possible genetic heterogeneity of PfHRP2 expression, deletion of HRP-2 gene, presence of blocking antibodies for PfHRP2 antigen or immune-complex formation and prozone phenomenon at high antigenemia [8,10].

In this study, the sensitivity and specificity of RDT increased when Microscopy was used as gold standard compared to that of QBC as gold standard. RDTs was able to detect 71.8% of every positive cases and 87.1% of every negative cases detected by microscopy. The implication of this is that QBC may be very sensitive even at low parasitaemia, thus detecting more positive cases.

5. CONCLUSION

The rate of false negative results of RDTs kit is high; health workers should be trained on the proper and accurate use of RDT kit. Good and proper storage environments should be made available at the health centers so as to increase the performance of the kits.

QBC method can easily be carried out by trained personnel; it provides a dependable method for diagnosis of malaria. It is handy in laboratories that screens large number of samples and in malaria endemic areas where parasite level is low [13]. However, in situations where adequate laboratory back up is not available, rapid diagnosis test can be employed if its appropriately used.

ETHICAL CLEARANCE AND INFORMED CONSENT

Ethical approval was obtained at the Ogun state Ministry of Health and the consent form was given to the patient to seek their approval before recruiting them into the study.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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