

A Comparative Study on Malaria Rapid Diagnostic Kit and Conventional Giemsa Stained Blood Film Microscopy

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Abstract: ***Aim:** This study was aimed at comparing the rapid diagnostic kit with that of conventional Giemsa stained thick and thin blood film microscopy. **Background:** Accurate diagnosis of malaria cases in rural areas is a very difficult task due to lack of steady power supply and lack of well trained scientists to handle microscopy. Since rapid diagnostic test produces result within short period of time there is high preference to the rapid diagnostic kit without recourse to sensitivity, specificity, accuracy and reliability of the result obtained. **Methods:** Blood samples were collected from 144 children and adults with fever/history of fever and 20 control specimens at BMSH, Port Harcourt. Rapid diagnostic test was performed using CareStart Plasmodium falciparum malaria (HRP 2) test kit by access Bio while Giemsa stained thick and thin blood films was used for conventional microscopic method. **Result:** Results were analyzed and compared using conventional microscopy as reference standard. The blood films indicated that 73.6% of the patients were positive for P. falciparum while rapid test recorded 30.6% for P. falciparum. Rapid test failed to diagnose malaria at concentration less than 100 per microliter of blood. Its sensitivity and specificity were 37.7% and 89% respectively. Reliability of the result was below average (40.3%) and its accuracy was 51%. The Giemsa method was significantly sensitive than the RDT ($P = 9.51 \times 10^{-15}$). **Conclusion:** The advantages of RDT requiring little training and produces result in short period of time considered the use as alternative diagnostic tool in malaria endemic area but should not be rely upon as complete diagnostic tool for malaria.*

Keywords: malaria, rapid test, giemsa, diagnostics, microscopy

1. Introduction

Malaria is a mosquito-borne disease of humans and other animals caused by eukaryotic protist of the genus plasmodium. It is widespread in the tropical and subtropical regions due to significant amount of rain fall, consistent high temperatures, and warm humidity along into stagnant water. Malaria is responsible for over 90% of reported cases of tropical disease in Nigeria [1].

Laboratory confirmation of malaria infection requires the availability of a rapid, sensitive and specific, test at an affordable cost. Conventional methods of laboratory diagnosis use microscopic examination of stained thick blood films which requires technical expertise and availability of a good quality microscope and also time-consuming. On the other hand, rapid diagnostic tests (RDTs) can potentially provide accurate diagnosis to all at risk populations, reaching those unable to access good quality microscopy services in endemic areas. It works by detecting specific malaria antigens or emerges [2].

Epidemiology of malaria is dependent on environment, vectors, parasite and host. Their interplay determines the two polar epidemiological extremes: stable and unstable malaria. However, the transmission pattern of malaria does vary within the same country sometimes within short distances [3]. According to the World Malaria Report 2011, malaria is prevalent in 106 countries of the tropical and semi-tropical world with 35 countries in Central Africa bearing the highest burden of cases and death.

Malaria diagnosis involves identifying malaria parasites or antigens/products in patient blood. There are four principal methods for diagnosis of malaria: clinical, microscopy, antigen test and molecular methods. Some advantages and shortcomings of these methods have been described, related to sensitivity, specificity, accuracy, precision, time consumed, cost-effectiveness, labour intensiveness, the need for skilled microscopists and the problem of inexperienced technicians.

Conventional light microscopy is the established method for the laboratory confirmation of malaria. It is sensitive when used by skilled and careful technician and can detect densities as low as 5 – 10 parasites per microlitre of blood. When parasites are found, they can be characterized by terms of their species and circulating stage. Though it is relatively expensive, it provides permanent record (the smear) of the diagnostic findings.

Rapid diagnostic tests (RDTs) for the diagnosis of malaria are based in the detection of antigens (Histidine rich protein 2), Aldolase, and parasite lactate dehydrogenase) derive from malaria parasites lysed blood using immunochromatographic methods. The result is usually a coloured test line, obtained in 5 – 20 minutes. It requires no capital investment on electricity, simple to perform and easy to interpret [4]. Today several commercial test kits are available with most frequently employing dipsticks bearing monoclonal antibodies directed against the parasite target antigens. The products come in various formats like cassettes, dipsticks, card-flaps.

2. Methodology

2.1 Research Design

A total of 144 blood samples were collected from patients attending Braithwaite Memorial Specialist Hospital, Port Harcourt. The patients (children and adult) had history of malaria symptoms like fever, malaise, headache, vomiting etc but without previous anti-malaria treatment within two weeks of attending the hospital. 20 control samples were also collected from students of Rivers State University of Science and Technology who had no malarial symptoms were also included in the study.

2.2 Sample Processing

Sample collection was done using lancet. The area was clean with no alcohol swab, and then the fingertip was pierced with a sterile lancet. The first drop of blood was wiped and pipette provided was used to collect the blood enough for thick, thin film and RDT method for each patient. These samples were examined immediately (RDT) and films prepared for microscopy.

2.3 Rapid Diagnostic Test Device: RDT used in this study is the careStart malaria HRP 2 test kit by Access Bio Inc. The test was performed according to the manufacturer's instructed 0.05ml of whole blood was dropped into sample well. 2 drops of assay buffer was added into Assay buffer well immediately. The result was read after 20 minutes.

2.4 Giemsa Staining Technique

Giemsa stain dilution of 1 in 30 (3%) was made by mixing 1ml of stain and 29ml of buffered water. The films were covered with diluted Giemsa for 30 minutes and then washed in buffered water pH 7.2. The films were dried in a vertical position. At least 100 high power (100 x objectives) microscope fields were examined for parasites using oil immersion.

2.5 Statistical Analysis

Descriptive statistics of percentage and bar chart were used to summarize the data collected and inferential statistics of T-test and Russell's used to compare the two techniques of malaria detection.

3. Results

3.1 Study Population

A total of 144 patients were tested for malaria parasite, 44 patients representing 30.6% were positive using rapid diagnostic technique and 106 patients representing 73.6% were positive using Giemsa stained thick film technique. The control subjects when tested similarly recorded 1 positive RDT case out of 20 and 4 positive cases by thick film technique. This is shown in Table 4.1

The comparative performance of the rapid diagnostic test and standard thick film microscopy showed 40 positive cases and 34 negative cases of RDT that corresponded with that of

Giemsa stained thick film microscopy result, 4 false positive (positive RDT/ negative Microscopy) and 66 false negative (negative RDT/positive Microscopy) results were obtained, in which microscopic examination showed 58 cases with parasite density/ul less than 100, and 8 cases with parasite density per microliter of more than 100.

The positive and negative predictive index values of CareStart malaria test kit compared with thick blood films were 91% and 34% respectively. The rapid test showed 40 true positive results, which corresponded with the sensitivity value of 37.7% when compared with microscopy result. The specificity of the kit is 89.47% corresponding with true negative result of 34, while the accuracy and reliability values of the kit is 51% and 40.3% respectively. The microscopy method was significantly sensitive than the RDT ($p=0.0001$).

4. Discussion

This study compared the diagnosis of malaria by a new rapid test, (CareStart rapid test by Access Bio Inc) with the traditional microscopy of Giemsa stained thick blood film. The result showed significant difference between the two methods. The performance characteristic of the CareStart malaria test kit has been compared with laboratory microscopy result. Positive slides of *falciparum* malaria parasites were very high 106 (73.6%) in contrast to rapid diagnostic test device. The lower percentage of malaria with rapid test may be attributed to low parasitemia, deterioration of the kit due to storage condition [5] and inability of the test kit to detect other species of *Plasmodium*. Again false positive results recorded may be attributed to detection of antigens produced by gametocytes in infection where only gametocytes were present or it is possible that some of the patients might have treated themselves of malaria before test. Such individuals may have residual malarial antigens, which can test positive with rapid test kit. The false positive results according to [6] may reflect the non-specific binding of the antibody to the capture lines. Marked difference was also observed by [7] where 89% positive thick film microscopy cases was recorded against 7% positive cases by RDT though kits are of different manufacturing companies.

The rapid diagnostic test even though it uses small quantity of blood, had a threefold of detection to the range of 100 parasites per microlitre of blood compared to 5 by thick film microscopy. The test also claims that since it is antigen specific, can distinguish between all species of human malaria parasites because of antigenic differences between their parasite isoenzymes [8]. The findings could be faulted due to the low recovery rate in the study [7].

Therefore, the rapid test technique was not reliable enough since there is the possibility of low parasite density cases which will escape detection. This observation is consistent with [9]. It is suggested that under the condition of low parasite density and prevalence, conducting active malaria surveillance using rapid malaria test is not justifiable. The sensitivity of this RDT kit is low (37.7%) indicating that it is more likely to fail in diagnosing a positive patient as having the disease especially in cases of low parasitemia. Its relatively high specificity shows that a patient is not

incorrectly diagnosed as having a malaria parasite. Though the accuracy of test results obtained with RDTs is of average, its reliability based on [6] calculation is below average and T-test data analysis showed very high significant result of RDT against Microscopy. Thus RDT should not be depended only upon in diagnosis of malaria.

There are compelling reasons to justify the use of rapid malaria diagnostic test especially in developing countries like Nigeria. Definitive diagnosis of infection in malaria control activities must therefore require the use of laboratory.

This we believe is an invaluable malaria diagnostic tool of the future if all the necessary control measures are in place. It would do away the need for slides, their transportation to centralized laboratories and the required infrastructure and trained staff and in situation where the electricity supply is very erratic like Nigeria, the rapid test is a perfectly suitable alternative. This will certainly improve the diagnostic accuracy of malaria at the primary health care level and by community health workers.

The major setback of the rapid test is that the cost per unit diagnosis may be currently high and its present use in malaria control programmes may only be cost effective in areas of low transmission where the cost of setting up a microscopic laboratory and equipment exceeds the cost of the rapid test. The rapid test can be modified by using finger-prick samples instead of venepuncture. This further simplifies the test both in terms of cost and trained personnel and therefore this method of collecting blood sample for the rapid test is advocated.

5. Future Prospects of this Study

Future prospects of this study are hinged on the fact that malaria is grossly under diagnosed using the conventional methods, any rapid technique should be complimentary and not counterproductive. The need for further comparative study on other rapid diagnostic kits is imperative.

References

[1] Alaba, A. O. (2005). Malaria and Rural Household productivity in Oyo State. A PhD thesis submitted in the Department of Economics, University of Ibadan

[2] UNICEF (2011). Malaria diagnostic: a guide for selecting rapid diagnostic test (RDT) kits. 1st edition

[3] World Health organization (2009). Impact of malaria control. In: World malaria Report, 2009 2744.

[4] Moody, A. (2002). Rapid diagnostic tests for malaria parasites. *Clinical Microbiology Review*, 15, 66 – 78

[5] Nyenke, C. U. (2013). Basics of specimen collection and analysis for health workers. 2nd edition. Jaenoc International Company, Nigeria.

[6] Russelle, E. C., Nongnu, I. M., Alongrot, P., Chalermpon, K., Nattawan, R., Roberts, S. M. and Jetsumon, S. (2002). Short Report: Failure of the optimal rapid Malaria test as a tool for the detection of asymptomatic malaria in an area of Thailand endemic for *Plasmodium falciparum* and *P. vivax*. *American Journal of Tropical Medicine and Hygiene*, 67(6), 563 – 565.

[7] Ukaji, D. D., Ezeiruaka, F. C., Eze, E. M. and Nyenke, C. U. (2011). Comparison of rapid diagnostic test and peripheral blood film for detection of malaria parasite from immuned adults in Elele, Rivers State. *Nigeria Biomedical Science Journal*, 8(3), 29-31.

[8] Mason, D. P., Kawamoto, F., Lin, K., Laoboonuchai, A. and Wongsrichanalai, C. (2002). A comparison of two rapid field immunochromatographic tests to expert microscopy in the diagnosis of malaria. *Acta Tropical Journal*, 82, 51 – 59.

[9] Tidi, S. K. and Akogun, O. B. (2005). Effectiveness of the rapid test technique (RTT) in malaria diagnosis and chloroquine treatment failure in Yola, Nigeria. *Nigeria Journal of Parasitology*, 26, 49 – 54.

Table 4.1: Malaria parasite detection using two different methods

Technique used	Total no. examined	No. positive	% positive	No. negative	% negative
Giemsa thick film	144	106	73.6	38	26.4
RDT kit	144	44	30.6	100	69.4
Controls	20	Thick film 4	20	16	80
		RDT 1	5	19	95

Table 4.1C: Comparative performance characteristics of rapid diagnostic test and microscopy

Raid Test Kit	Microscopy			
		Positive	Negative	
	Positive	40	4	44
	Negative	66	34	100
	Total	106	38	144

Author Profile



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