

# Evaluating the use of microscopic examination and rapid diagnostic tests to diagnose malaria in North Central Nigeria

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## Abstract

The global impact of malaria has spurred interest in developing prompt and accurate diagnostic strategies to provide an effective management of the disease. The aim of this study was to compare rapid diagnostic tests (RDTs) for malaria with routine microscopy. Samples were collected randomly from 364 febrile out-patients with clinical suspicion of malaria from four hospitals in North Central Nigeria. Results from the rapid diagnostic kits were analysed and compared to those obtained by general microscopy. Of the 364 out-patients involved in the study, 218 (59.89%) tested positive for *Plasmodium falciparum* by RDTs, whereas 263 (72.256%) tested positive by microscopy. There are significant differences ( $P < 0.05$ ) in infection rates between RDT and microscopy. The sensitivity, specificity and negative predictive values of RDTs compared to microscopy are low, while the positive predictive value is high. Evaluation of RDTs against the parasite-positive panel with parasite densities of  $< 1000$  parasites/ $\mu\text{L}$ , between 1000-5000 parasites/ $\mu\text{L}$  and above 5000 parasites/ $\mu\text{L}$  was 11.73, 30.61, 57.65% for RDTs compared to 6.11, 27.95 and 65.94% for microscopy, respectively. Test line intensity increases with increase in parasite densities for both methods.

## Introduction

Malaria is the most common single diagnosis made in most countries in Africa.<sup>1</sup> The

accuracy of clinical diagnosis is limited by the low specificity of symptoms and signs of malaria.<sup>2</sup> Presumptive anti-malaria treatment for any fever without scientific investigation of possible alternative causes is widely practiced, and studies suggest that this leads to significant over-use of anti-malarial medicines throughout Africa.<sup>3,4</sup> With growth of resistance to older anti-malarial drugs, newer but more expensive drugs have been developed as first line drugs, like the artemisinin combination therapy which has largely replaced the older drugs like Chloroquine and Sulphadoxine/Pyrimethamine in most African countries.<sup>5</sup> The widespread availability and affordability of these drugs especially in rural areas depend on subsidy.<sup>6</sup> This may become unsustainable if most anti-malarial drugs continue to be given to patients who do not have malaria. If patients with bacterial disease, an important cause of avoidable death in children in Africa<sup>7,8</sup> are treated as malaria cases, they may not receive appropriate treatment.<sup>9</sup>

The World Health Organization has recommended that management of all malaria cases should be confirmed by quality-assured, parasite-based diagnosis before treatment is started.<sup>10,11</sup> Improving the diagnosis of acute febrile illness so that anti-malaria drugs are targeted to patients who need them and alternative diagnosis sought in others is therefore a matter of public health priority in Africa.

In Nigeria one of the four key interventions towards malaria control is the introduction of parasitological confirmation of malaria cases by rapid diagnostic tests (RDTs) and scaling up of diagnosis by microscopy.<sup>12</sup> The general objective being to achieve timely and equitable access to malaria diagnosis and treatment by all sections of the population and as close to the home as possible. The use of RDTs as a definitive diagnostic tool where microscopy is not readily available to confirm suspected malaria cases have been proposed; however, its performance must be evaluated to avoid over or under diagnosis of malaria cases before it can replace a more tedious microscopy. The specific objective of the study is to examine performance of RDTs compared to microscopy which is the gold standard in North Central Nigeria.

## Materials and Methods

### Study area

The study was carried out in hospital settings. Four hospitals with large attendance and standard diagnostic laboratories were selected, namely Adonai Hospital Mararaba, Nasarawa State, Blessed Trinity Hospital in New Karu Local Government, Jikwoyi Medical Centre in

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Abuja Municipal Area Council and Asokoro District Hospital, with Hospital Attendance cutting across the Abuja, Kogi and Niger and Nasarawa States as well as other parts of the North Central Zone. The study was conducted over a period of 5 months from February to June, part of which fell in the rainy season when the prevalence of malaria is normally at its peak.

### Study population

From among febrile hospital attendees [patients with fever ( $\geq 37.5^\circ\text{C}$ )], 364 patients (91 per hospital) were selected. Test outcomes were recorded for each of the centres. Positive cases were further examined to ascertain parasite density/ $\mu\text{L}$  of blood.

### Parasitological analysis

Thick and thin smears were made on the same slide for each patient from finger prick using a sterile lancet. Two slides were made for each patient. The first slide was the read (R) slide (that is the slide that was read), while the other slide was archived (A) slide. The thin films were fixed with methanol and left to dry. The prepared smears were stained with 10% Giemsa at a pH of 7.2 for 35 min. The staining process was quality controlled to ensure that the morphology of the malaria parasites in positive slides were distinct and clear. The parasite den-

sity was computed as the number of parasites per 500 leucocytes on a thick film and reported as parasites per microlitre of blood assuming an average white blood-cell count of 8000 per  $\mu\text{L}$ .<sup>13</sup> Stained slides were examined under the light microscope using  $\times 100$  objective lens (immersion oil). A slide was considered negative after 100 high power fields have been examined. Another Microscopist, the second reader, was made to re-read each slide. Parasite counts of >20% discordance between two readers were re-read by a third reader, who served as the tie breaker.

### Rapid diagnostic tests

This device detected malaria antigen in a small amount of blood, usually 5-15  $\mu\text{L}$ , by immune-chromatographic assay with monoclonal antibodies directed against the target parasite agent (*Plasmodium falciparum* and *Plasmodium vivax*) and impregnated on a test strip. The results usually a coloured test line was obtained after 5-20 min. RDT requires no capital investment or electricity, are simple to perform and easy to interpret. Sensitivity and specificity tests were performed on 2 RDTs namely First Response (Premier Medical Corporation, Watchung, NJ, USA) and CTK (Biotech Inc., Oklahoma City, OK, USA). CTK

was used in this study because it was more sensitive and specific.

### Ethical approval

Ethical approval was obtained from the management of each of the participating hospitals including the ethics committees of Federal Capital of the Territory of Abuja, Nigeria.

### Data analysis

The collected data were analysed using SPSS version. Proportions were compared by Chi-square tests.

## Results

RDT showed detection of *P. falciparum* in 218 (59.89%) out of 364 out-patients with males 103 (47.25%) and females 115 (52.75%) tested from the four hospitals; whereas microscopic results showed detection of *P. falciparum* in 263 (72.25%) out of 364 patients with male 118 (44.87%) and females 145 (55.13%) tested from the four hospitals (Table 1). Chi-square analysis showed that the increase in difference between the RDT and the microscopy results was significant ( $P < 0.05$ ).

The sensitivity, specificity, positive predictive value and negative predictive values of RDTs against blood slide microscopy for all species of malaria combined are shown in Table 2. The overall sensitivity was 45.32% (95% CI 3.92-5.84). Specificity of RDTs was 40.89% (3.42-5.01) overall. The overall positive predictive value was 59.89%, while negative predictive value was very low (27.75%).

Test performance showed that evaluation of RDTs against the parasite-positive panel with parasite densities of <1000 parasites/ $\mu\text{L}$  revealed 11.73%, between 1000-5000 parasites/ $\mu\text{L}$  30.61% and above 5000 parasites/ $\mu\text{L}$  57.65% compared to 6.11, 27.95 and 65.94% for microscopy, respectively (Table 3). Analysis showed that RDT is also sensitive ( $P > 0.05$ ) compared to microscopic examination.

## Discussion

Recently in Nigeria the use of RDT as a fast and easier method of malaria diagnosis has been sponsored to replace the tedious and time-consuming microscopic methods. Still, this study was initiated to compare the sensitivity of RDTs in accurately determining malar-

**Table 1. Detection of *Plasmodium falciparum* in peripheral blood of out-patients according to gender using rapid diagnostic test and microscopy.**

Categories	Overall infected patients		Male infected patients		Female infected patients	
	n	%	n	%	n	%
RDT	218	59.89	103	47.25	115	52.75
Microscopy	263	72.25	118	44.87	145	55.13

RDT, rapid diagnostic test.

**Table 2. Performance of different rapid diagnostic methods compared to Giemsa stain microscopy.**

	Microscopy		Sensitivity (%) (95% CI)	Specificity (%) (95% CI)	PPV (%)	NPV (%)
	Positive	Negative				
RDTs						
Positive	218	146	45.32 (3.92-5.84)	40.89 (3.42-5.01)	59.8	27.75
Negative	263	101	-	-	-	-

RDTs, rapid diagnostic tests; PPV, positive predictive value; NPV, negative predictive value; CI, confidence interval.

**Table 3. Estimation of parasite density of the thick film positive results vs test line intensity of rapid diagnostic test positive results from four selected hospitals in North Central Nigeria.**

Hospital	Microscopy, parasite density ( $\mu\text{L}$ )			RDT, test line intensity		
	<1000/UL	1000-5000/UL	>5000/UL	Faint	Visible	Very visible
Adonai Hospital Mararaba	30	27	5	8	12	32
Blessed Trinity Hospital	47	14	2	6	13	29
Jikwoyi Medical Centre	45	11	3	4	17	25
Asokoro District Hospital	29	12	4	5	18	27
Total						
n	151	64	14	23	60	113
%	65.94	27.95	6.11	11.73	30.61	57.65

ia infections. Results showed that prevalence of malaria is generally high in North Central Nigeria. Despite the difference between the overall prevalence of malaria detected by the two tests in our study, the overall sensitivity observed was low (45.32%). This indicates that there was a substantial non-overlap between the positives detected by each test. The positive predictive value was generally high (60%). Other reports indicate that RDTs have shown a comparable level of accuracy to microscopy in clinical settings.<sup>14,15</sup> Even though the clinical history of the participants was not recorded in our study, evidence from other studies showed that RDT positive cases missed by microscopy might be individuals who had been treated but in whom antigenemia persists.<sup>15,16</sup>

Jamshaid and others in a similar study compared two commercial assays, RDTs with microscopy for confirmation of symptomatically diagnosed malaria where a total of 750 patients were examined. The ICT malaria Pf/Pv test kit failed to detect malaria infection in 93 (34%) of 271 malaria patients and optimal test failed to detect malaria infection in 41 (15%) of 271 malaria patients. The sensitivities of ICT malaria Pf/Pv and OptiMAL tests for detection of *P. falciparum* infection were 81 and 87% respectively and their sensitivities decreased significantly to 23 and 44% respectively at parasite densities of <500/μL which is similar to the result obtained in this study.<sup>17</sup>

In another work on RDTs and microscopy for guiding outpatient treatment of febrile illness in Tanzania: a randomized trial, 49.22% tested positive by microscopy while 46.16% tested positive by RDT. The factor of parasite density in the sensitivity of RDT in detecting malaria parasite in peripheral blood played a vital role here<sup>9</sup> and other studies have shown that test line intensity increases with increase in parasite densities.<sup>18</sup> The lack of sensitivity of RDTs at low parasitaemia compared to microscopy is one of the shortcomings noted here which is also reflected in our findings.

Moody and others pointed out that RDTs can be useful in screening febrile returnees from endemic areas<sup>19</sup> and according to World Health Organization, on the use of RDTs, they are recommended in situations exceeding microscopy capability such as in an outbreak or in occupationally exposed groups.<sup>20</sup> As RDTs improve, including in sensitivity and in ability to measure parasitaemia levels, at least semi-quantitatively, the scope of RDT application will expand. Current RDTs cannot replace microscopy; other factors such as the quality of the products, storage temperature and humidity, and end users' performance can affect the diagnostic accuracy.

## Conclusions

In conclusion, the results of this study adds to the evidence that non-microscopic rapid tests for the detection of plasmodial antigens may develop into important diagnostic tools and can prove to be a valuable adjunct to clinical assessment of the patient's blood film microscopy under certain circumstances. The study design was such as to make a direct comparison of the performance of microscopy and RDTs in four different hospitals. These tests are rapid and simpler to perform and to interpret, however, their sensitivity indicates that they should not yet be regarded as first line diagnostic tests, but with time, further improvement in technology of production and better storage and handling, they hold the promise of becoming the first line diagnostic tools for malaria taking over from microscopy which remains the gold standard for now.

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