

# A Comparison of the Rapid Diagnostic Test and Microscopy for Malaria Diagnosis in Children Under five in Abia State, Nigeria

Ugo U. Enebeli<sup>1\*</sup>, Agwu N. Amadi<sup>1</sup>, Okechukwu K. Iro<sup>2</sup>, Evangeline T. Oparaocha<sup>1</sup>, Eunice A. Nwoke<sup>1</sup>, Nnenna N. Oti<sup>3</sup>, Sally N. O. Ibe<sup>1</sup>, Uchekchukwu M. Chukwuocha<sup>1</sup>, Chinyere R. Nwufu<sup>1</sup>, Chinasa O. Amadi<sup>1</sup>, Iheanyichukwu Esomonu<sup>4</sup>

<sup>1</sup>Department of Public Health, Federal University of Technology, Owerri, Imo State, Nigeria.

<sup>2</sup>Department of Environmental Health Sciences, Abia State University, Abia State, Nigeria.

<sup>3</sup>Post Graduate School, Federal University of Technology, Owerri, Imo State, Nigeria.

<sup>4</sup>Department of Environmental Sciences, Federal University of Technology, Owerri, Imo State, Nigeria.

\*Corresponding author

**Abstract** – Children under five years bear the largest burden of malaria in Nigeria, and accurate diagnoses are important in malaria control and eventual eradication efforts. However, some reports on the accuracy of the most common malaria tests – microscopy and rapid diagnostic tests (RDTs) – are presently not satisfactory. Thus, the aim of this study is to determine the accuracy of malaria RDTs using microscopy as a gold standard, among children in Abia State, Nigeria. Between June 2015 and June 2018, blood samples were collected from 1,209 children in house to house surveys and tested for malaria using RDT and microscopy in Abia State. These data were used to assess the discriminatory accuracy of the RDT against the microscopy as a gold standard by analysis of its sensitivity and specificity. The predictive accuracy of the RDT was assessed by the positive predictive value (PPV) and negative predictive value (NPV) of the RDT against the microscopy at  $p=0.05$ . There was a statistically significant agreement between the results of the RDT and microscopy ( $p=0.001$ ). The prevalence of childhood malaria by RDT and microscopy were 58.5% and 46.5% respectively. And the sensitivity, specificity, PPV and NPV of RDT were 79.36%, 56.26%, 63.08% and 72.51% respectively. This study concludes that there is need for accurate and reliable malaria diagnostic methods to be on track for achieving Sustainable Development Goal 3, and recommends that community diagnoses of childhood malaria should be combined with more specific diagnoses to improve the overall child health in Abia State, Nigeria.

**Keywords** – malaria; diagnosis; microscopy; rapid diagnostic test; sensitivity; specificity; children

## I. INTRODUCTION

In most countries where malaria is endemic, malaria is commonly diagnosed by clinical features, rapid diagnostic tests (RDT) or microscopy (Mfuh *et al.* 2019). Unfortunately, imprecise clinical diagnosis is the main basis of therapeutic care for many febrile children in developing countries due to limited primary health services and hard to reach laboratory diagnostics (Mukry *et al.*, 2017). Because clinical diagnosis alone has very low specificity and results in overtreatment, the

World Health Organization (WHO) recommends that prompt malaria diagnosis, either by microscopy or malaria RTD, should be routinely carried out in all patients with suspected malaria before treatment is administered (WHO, 2015).

Microscopy is considered the gold standard for malaria diagnosis (Mukry *et al.*, 2017). Although specific, microscopy needs expertise in microscopy and is unreliable at low parasitaemias. Thus RDTs were developed for ease of use as well as its capacity to diagnose malaria in low-grade parasitaemia (below the detection limit of microscopy) (Mfuh *et al.*, 2019). In Nigeria, quality-assured Histidine-Rich Protein II (HRP 2) – based RDT is recommended for the diagnosis of malaria in all age groups (FMOH, 2011).

According to Fagbamigbe (2019) however, reports on the accuracy of malaria tests are presently not satisfactory. This is evidenced by the widened discrepancy in malaria prevalence in the Nigeria Malaria Indicator Surveys of 2010 (with a 10% discrepancy between prevalences of 52% by RDT and 42% by microscopy) and of 2015 (18% discrepancy between prevalences of 45% by RDT and 27% by microscopy) (Fagbamigbe, 2019).

Since children under five are currently the most vulnerable group affected by malaria in Nigeria (WHO, 2019), it is important to study the accuracy of the tests in children in order to provide information on its ability to “discriminate between and/or predict disease and health” (Eusebi, 2013). This study thus sets out to determine the accuracy of malaria rapid diagnostic tests (RDTs) among a representative sample of children in Abia State, using microscopy as the reference standard.

## II. MATERIALS AND METHODS

This study was conducted between June 2015 and June 2018 using house to house surveys in Abia State in the South East of Nigeria. Abia State is located within latitudes

4°40' and 6°14' north and longitudes 7°10' and 8° east and spans 5,834km<sup>2</sup> of land and forest vegetation.

A cross sectional design was used for this study. The State was subdivided into the 3 senatorial zones and a multi-stage technique was used. First, 3 LGAs were randomly selected from each of the 3 senatorial zones, giving a total of 9 LGAs. In the second stage, 3 communities were randomly selected from each of the 9 LGAs, giving 27 communities with an estimated under-five population of 11,349. Using 10% of the entire under-five population, a sample size of 1,135 was derived; and an additional 5% was added for non-response totalling 1.190. Thus, in the third stage, 44-45 households with under-fives were randomly selected in each of the 27 communities, giving a total of 1209 households.

All 1,209 under-five children in the selected households were tested for malaria using Carestart<sup>TM</sup>RDT (Access Bio Inc., NJ, USA) and microscopy (using 2-5mL of venous blood) by trained field laboratory scientists. The caregivers were orally told of the results of the RDT and children that had positive RDT results were offered antimalarial treatment according to the Nigeria malaria treatment protocol. The venous samples were labelled and transported in EDTA tubes in cold boxes to research laboratory at Abia State University Teaching Hospital, Aba for thick blood film microscopy.

Data analysis for this study was done with Statistical Package for Social Sciences (SPSS) version 22 (International Business Machine, 2015). The methods of data analysis were: descriptive statistics with frequency tables and graphs; and inferential statistics using chi square to determine associations between variables.

### III. RESULTS

#### 3.1 Age and Sex Characteristics of the Under-Five Children

Table 3.1 shows that 22.4% of the study children were in the age group of 0-11 months and comprised of 8.1% males and 14.3% females; 15.0% were in the age group of 12-23 months and comprised of 11.8% males and 3.2% females; 9.6% were in the age group of 24-35 months and comprised of 4.1% males and 5.5% females; 32.6% were in the age group of 36-47 months and comprised of 13.6% males and 19.0% females; while 20.4% were in the age group of 48-59 months and comprised of 13.9% males and 6.5% females.

Table 3.1: Age and Sex Characteristics of the Under-Five Children

Age Group (Months)	Male n (%)	Female n (%)	Total N (%)
0-11	98 (8.1)	173 (14.3)	271 (22.4)
12-23	143 (11.8)	38 (3.2)	181 (15.0)
24-35	50 (4.1)	66 (5.5)	116 (9.6)
36-47	164 (13.6)	230 (19.0)	394 (32.6)
48-59	168 (13.9)	79 (6.5)	247 (20.4)
Total	623 (51.5%)	586 (48.5%)	1,209 (100.0%)

#### 3.2 Prevalence of Childhood Malaria using RDT and Microscopy

Table 3.2 shows that the six-month childhood malaria prevalence using RDT and Malaria are 58.5% and 46.5% respectively.

Table3.2: Prevalence of Childhood Malaria using RDT and Microscopy

Malaria Diagnostic Test	Malaria Presence	Frequency	Percent
Malaria RDT	Malaria	707	58.5
	No Malaria	502	41.5
Total		1209	100.0
Malaria Microscopy	Malaria	562	46.5
	No Malaria	647	53.5
Total		1209	100.0

#### 3.3 Comparison of the Rapid Diagnostic Test and Microscopy for malaria diagnosis

The Chi-square ( $\chi^2$ ) test was used to establish any association between the RDT tests and microscopy tests. A  $p=0.000$  showed that there is a statistically significant relationship between malaria screening based on rapid diagnostic tests (RDT) and parasitological diagnosis of malaria. Table 3.3 shows that the true positives (TP) are 446 (36.9%) and the true negatives (TN) are 364 (30.1%). As well, the false positives (FP) are 283 (23.4%) and the false negatives (FN) are 116 (9.6%).

Table 3.3: Comparison of the Rapid Diagnostic Test and Microscopy for malaria diagnosis

Rapid Diagnostic Test for Malaria	Microscopy		Total, n (%)
	Positive (+) n (%)	Negative (-) n (%)	
Positive (+)	446 (36.9)	283 (23.4)	707 (58.5)
Negative (-)	116 (9.6)	364 (30.1)	502 (41.5)
Total	562 (46.5)	647 (53.5)	1209 (100.0)

The discriminatory accuracy of the RDTs was measured by their sensitivity and specificity in this study. The Sensitivity of RDTs compared to Microscopy, that is the probability that a truly infected child will test positive is 79.36%. That is, sensitivity is TP divided by TP + FN = 446 divided by 446+116, expressed as a percentage. The Specificity of RTDs compared to Microscopy, that is the probability that an uninfected child will test negative is 56.26%. That is, specificity is TN divided by TN+FP, expressed as a percentage.

The Predictive accuracy of the RDTs was measured by their positive predictive values and negative predictive values. The Positive Predictive Value (PPV) is 63.08% and this is the TP divided by TP+FP expressed as a percentage. That is, PPV is 446 divided by 446+283, expressed as a

percentage. And the Negative Predictive Value (NPV) is 72.52%, and is the TN divided by TN+FN in percentage. That is, NPV is 364 divided by 364+116, expressed as a percentage.

#### IV. DISCUSSION

Among the 1209 under-five children in the study area, there were more males (52.1%) than females (47.9%). These findings are similar to recent reports on under-five male-female population distribution in Nigeria (NPopC, 2018; NBS & UNICEF, 2017; NDHS, 2013, and NPopC & ICF, 2014). The most common age group in this study were the 36 – 47 month olds at 32.6%, followed by the 0 – 11 month olds at 27.1, the 48 – 59 month olds, the 12 – 23 month olds and the 24 – 35 month olds. This pattern is similar to the NDHS of 2013 with the most common age group as the 36 to 47 month olds (20.8%), followed by the 0 – 11 month olds (20.5%), the 48 – 59 month olds (20.0%), the 12 – 23 month olds (19.6%) and the 24 – 35 month olds (19.1%)(NPopC & ICF, 2014). Thus, the age structure of the under-fives in this study is a close reflection of the demographic structure of Abia State and Nigeria. This shows that there was minimal age bias within the study participants.

The result of the childhood malaria prevalence in the study areas showed a higher prevalence with RDT than microscopy at 58.5% and 46.5% respectively. These findings are in agreement with the results of recent studies comparing RDT and microscopy as malaria diagnostic tools:

the 2015 Nigeria Malaria Indicator Survey got 21.1% with RDT and 8.2% with microscopy (NMEP, NPopC, NBS & ICF International, 2016); Falade *et al.*, (2016) got 77.4% with RDT and 54.1% with microscopy; and Mfuh *et al.*, (2019) got 45% with RDT and 31% with microscopy.

The discriminatory accuracy of the RDTs compared to microscopy was measured by their sensitivity and specificity in this study. The sensitivity of RDT in this study was 79.36%, and this is comparable to several studies carried out recently (Mfuh *et al.*, 2019; Ayogu *et al.*, 2016; Fagbamigbe, 2019; Umegbolu & Madukwe, 2018).

The Specificity of RDTs compared to Microscopy in this study is 56.26%. Lower specificities of 40.7% and 46.7% were reported by Falade *et al.* (2016) and Murungi *et al.* (2017) respectively. Generally, the specificity of RDTs are lower than their sensitivities, and this could be explained by the continued presence of Histidine-Rich Protein 2 (HRP2) antigens in the blood after successful treatment of a malaria episode, leading to false positives with the RDT (WHO, 2013).

The Predictive accuracy of the RDT, using the microscopy as the gold standard, was measured by their positive predictive values and negative predictive values. The Positive Predictive Value (PPV) of the RDT in this study is 63.08%, which is in agreement with the outcomes of studies by Fagbamigbe (2019), Falade *et al.* (2016) and Ojurongbe *et al.* (2013) at 57.5%, 65.0%, and 67.7% respectively.

The Negative Predictive Value (NPV) of RDT is 72.52% in this study. This is close to the findings of Ojurongbe *et al.* (2013), Mfuh *et al.* (2019) and Falade *et al.* (2016) at NPVs of 67.7%, 78.0% and 82.7% respectively. The low PPV (63.08%) and higher NPV (72.52%) suggest that the chances of having malaria when RDT test is positive is only 63% while the chances of being disease-free when RDT test is negative is higher at 72%.

#### V. CONCLUSION

The result of this study showed a statistically significant relationship between malaria screening based on rapid diagnostic tests and parasitological diagnosis of malaria. Generally, there was a higher prevalence of childhood malaria in Abia State with RDT than microscopy at 58.5% and 46.5% respectively. The RDT was more sensitive than specific at 79.36% and 56.26% respectively.

Malaria diagnosis is invaluable in the control of malaria, as well as in its eventual eradication. Microscopy remains the current gold standard for malaria diagnosis, however RDTs are convenient and easy to use, although its accuracy is challenged by several issues. This study has highlighted the discriminatory and predictive accuracies with the use of RDT in under-fives in Abia State, Nigeria and recommends that as much as possible, RDTs should be combined with microscopy in order to improve the diagnosis of malaria among the under-fives in Abia State, Nigeria.

There is also need for regular quality and performance checking of RDTs in a malaria-endemic country like Nigeria following the recommendation of WHO (2005) that RDT should be implemented with a comprehensive quality control strategy. There is also need for health workers to read and easily understand the instruction manual in the packaging of the kit. Additionally, positive controls are also required to be available in field settings. Total adherence to these recommendations is expected to improve the discriminatory and predictive accuracies of results from malaria RDTs.

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#### CONFLICT OF INTEREST STATEMENT

The authors have declared there is no conflict of interest.

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