

1 **Factors affecting the validity of PfHRP2-based rapid diagnostic test kit in the diagnosis of**  
2 **malaria in Afikpo, Ebonyi State: a pilot study.**

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6 **ABSTRACT**

7 **Aim:** To assess the factors affecting the validity of histidine-rich protein II (HRP2)-based rapid  
8 diagnostic test (RDT) kit for diagnosis of malaria in Afikpo North Local Government Area, Ebonyi  
9 State.

10 **Study design:** Quantitative methods using both structured questionnaires and serological tests  
11 were employed to collect data on factors affecting the validity of HRP2-based RDT kit results for  
12 malaria diagnosis.

13 **Place and duration of study sample:** Four different health facilities including Akanu Ibiam  
14 Federal Polytechnic Medical Centre, Unwana, between November 2022 and March 2023.

15 **Methodology:** We interviewed eleven (11) health workers (HWs) (5 males and six females) on  
16 factors affecting the validity of RDT kits. We also included 50 patients (19 males and 31  
17 females; aged 1-40 years) with symptoms of malaria to validate some of the factors mentioned  
18 by the health workers. Replicates of CareStart<sup>®</sup> TM malaria RDT kits were used to test some of  
19 the factors on blood samples collected from the patients. Data collected was analyzed using the  
20 Chi-square test.

21 **Result:** The majority of the HWs use microscopy (54.5%) while 45.5% use RDT for the  
22 diagnosis of malaria. The majority of the HWs claimed that the quantity of buffer reagent (50%)  
23 and wait time (14.3%) affects the performance of RDT kits. However, there was no statistically  
24 significant difference ( $p>0.05$ ) in the prevalence of malaria (36%) among the different buffer  
25 quantities and wait times tested. Hence, the tested buffer reagent volumes and wait times do  
26 not affect the performance of rapid diagnostic tests. It is important to validate these findings in  
27 future studies with a larger sample size.

28  
29 **Keywords:** malaria, rapid diagnostic test kit, buffer reagent, wait time.

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31 **INTRODUCTION**

32 Nigeria accounts for the highest proportion (27%) of global malaria cases [1]. Pregnant women  
33 and children under five years old are most affected. Most malaria-related deaths happen within  
34 a few days from the time an individual starts showing symptoms, and when early diagnosis and  
35 prompt treatment with an effective antimalarial drug are delayed. Hence, prompt, and accurate  
36 laboratory diagnosis is important for both rapid and effective management and surveillance of  
37 malaria [2]. Diagnosis of malaria using light microscopy and polymerase chain reaction (PCR)  
38 methods are highly recommended due to their high sensitivity and specificity, but specific  
39 challenges have limited their use in malaria-endemic countries like Nigeria. Fortunately, the  
40 introduction of rapid diagnostic test (RDT) devices has made these challenges surmountable [3-  
41 6].

42 A malaria rapid diagnostic test (mRDT) kit is a device used to confirm malaria infection in  
43 patients presenting symptoms. It detects specific *Plasmodium* antigens in a small quantity of  
44 fresh blood using lateral flow immunochromatography [7]. The types of mRDT in use can detect

45 one or combination of the following antigens: (1) Histidine-rich protein 2 (HRP2)- a water-soluble  
46 protein -produced by trophozoites and gametocytes of *P. falciparum* only; (2) *Plasmodium*  
47 lactate dehydrogenase (pLDH) enzyme produced by all *Plasmodium* species infective to  
48 human; and (3) *Plasmodium* aldolase enzyme produced by all species of human *Plasmodium*  
49 parasites [8].

50 Although, mRDT is recommended for malaria diagnosis in resource-limited areas that are  
51 endemic for malaria, studies have reported varying levels of its usage and adherence to test  
52 results among health workers (HWs) [9]. Reports had shown that RDT was not used by health  
53 workers in Enugu state because of the pervasive notion that RDT results were inaccurate [10]. A  
54 previous study also showed that 37.6% of health workers in Ebonyi State had a poor perception  
55 of malaria RDT kits [9]. Low uptake and confidence in rapid diagnostic test kits may result in  
56 inappropriate prescription of antimalarial drugs, economic waste and delayed detection of the  
57 primary cause of malaria-like symptoms.

58 Hence, this pilot study is aimed at assessing factors affecting the validity of RDT kit results in  
59 the diagnosis of malaria in the study area.

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## 61 MATERIAL AND METHODS

### 62 Study Area

63 The study was conducted in Afikpo under the Afikpo North Local Government Area (L.G.A.) of  
64 Ebonyi State, Nigeria. Ebonyi state has the highest prevalence of malaria in southeast Nigeria  
65 [11] and malaria is the most prevalent medical condition treated in healthcare facilities across  
66 the state [12, 9].

67 Afikpo is located between latitudes 5° 4' N and 6° 3' N and longitudes 7° 5' E and 7° 55'E. There  
68 are predominantly two main seasons: the rainy season between April – October and the dry  
69 season between November – March. The annual rainfall is about 160mm – 220mm with  
70 maximum precipitation occurring between July and September. The atmospheric temperature is  
71 between 23.4°C and 29.9°C and relative humidity is between 60 – 80%. Afikpo is bounded in the  
72 North by Ohaozara L.G.A., Ebonyi State, in the East by Afikpo South L.G.A., in the South by  
73 Cross River State, and the West by Abia State. The population of people in Afikpo is about  
74 672,000 persons [13-15].

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### 76 Administration Of Questionnaires

77 Well-structured questionnaires were administered to medical laboratory scientists (MLS)  
78 working in hospitals and primary health centres within three communities (Afikpo, Enohia, and  
79 Unwana) within Afikpo North L.G.A. The questionnaires contained questions for collecting data  
80 on sex, age, level of education, marital status, knowledge of causes of malaria, mode of malaria  
81 transmission, diagnosis of malaria, and perception of factors affecting the validity of RDT kits.

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### 83 Sampling of malaria symptomatic patients

84 Blood samples were collected from 50 malaria patients at the Polytechnic Medical Centre. The  
85 blood samples were those for malaria by the medical lab scientist at the Polytechnic Medical  
86 Centre.

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## 88 Procedure for malaria rapid diagnostic test

89 The expiry date of the packet was checked, and the gloves were worn. The packet was opened.  
90 Three cassettes were placed on a clean flat surface and labelled clearly with the patient's ID  
91 number. The loop was used to put the drop of blood into the square hole marked "S" and the  
92 loop was discarded. Two drops of buffer were put into the round hole marked "A", for low buffer  
93 volume 1 drop of buffer was put, and for high buffer volume 4 drops of buffer was put. After the  
94 buffer was added, we waited for 20 minutes to read the test results then the results were  
95 recorded.

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## 97 How the RDT result was interpreted

- 98 i. One red line in the window "C" and one red line in the window "T" means that the patient  
99 was positive for malaria.
- 100 ii. One red line in window "C" and no line in window "I" means that the patient was negative  
101 for malaria.
- 102 iii. No line in the window "C" means the test was invalid.
- 103 iv. A window line "T" and no line in the window "C" also means the test was damaged. The  
104 results were invalid. Another test was conducted if the result was invalid.

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## 106 Testing the effect of varying buffer volume and wait time on the accuracy of malaria RDT 107 tests.

108 Carestart™ RDT was used for this study.

109 3 replicates of RDT were used to conduct tests on each blood sample as shown below.

110 1 RDT for low buffer volume with 1 drop

111 1 RDT for high buffer volume with 4 drops

112 1 RDT for recommended buffer volume (2 drops) by the manufacturer (control).

113 The same procedure was followed for the various wait times tested: low (10 minutes), high (40  
114 minutes), and recommended (20 minutes).

115 The results of the tests were recorded and compared to the microscopy test result.

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## 117 Data analysis

118 Data collected was entered in SPSS software version 16.0 for analysis. The chi-square test and  
119 Fisher's test were used to determine the effect of buffer volume on the accuracy of malaria test  
120 results. The result was presented using percentages in standard tables.

121

## 122 RESULTS AND DISCUSSION

123 **Demographic characteristics of health workers (HWs) interviewed.**

124 The data presented in Table 1 indicates that 45.5% of the medical laboratory scientists (MLSs)  
125 who were interviewed were male while 54.5% were female. Most of the MLSs (72.7%) fall within  
126 the age range of 31-40 years and all of them have completed tertiary education (100%). A  
127 majority of them (63.3%) have work experience of 6-10 years and work in hospitals (45.5%).

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129 **Demographic characteristics of malaria symptomatic patients.**

130 A higher proportion (62%) of patients diagnosed for malaria were female while 38% were male.  
131 In terms of their age, most of the patients (64%) are within the age bracket of 21-30 years (Table  
132 2).

133

134 **Usage and factors affecting the validity of RDT according to the health workers (HWs).**

135 Results in Table 3 indicate that 54.5% of MLS use microscopy while 45.5% use RDT to  
136 diagnose malaria in their laboratories. Concerning whether RDT is more accurate than  
137 microscopy, the majority (81.8%) responded "no" while 18.2% said "yes". Concerning the factors  
138 that may affect RDT results, 54.5% believed that buffer quantity can affect RDT results while  
139 others mentioned wait time (9.1%), wrong placement of buffer and blood (9.1%), climatic  
140 conditions (7.1%), and expired RDT kit (7.1%). However, 18.2% have no idea of any factor that  
141 can affect RDT results.

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**Table 1: Demographic characteristics of health workers  
(HWs) interviewed**

	n(%)
<b>Sex</b>	
Male	5 (45.5)
Female	6 (54.5)
<b>Age (years)</b>	
20-30	1 (9.1)
31-40	8 (72.7)
41-50	1 (9.1)
>50	1 (9.1)

**Level of education**

Secondary	0 (0)
Tertiary	11 (100)

**Years of work experience**

1-5	1 (9.1)
6-10	7 (63.6)
11-15	1 (9.1)
16-20	0 (0)
>20	2 (18.2)

**Type of healthcare**

Primary Health Centre	4 (36.4)
Medical Centre	1 (9.1)
Hospital	5 (45.5)
Private Medical Lab.	1 (9.1)

**Total** **11**

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**Table 2: Demographic characteristics of patients diagnosed for malaria.**

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	n(%)
<b>Sex</b>	
Male	19 (38)
Female	31 (62)
<b>Total</b>	<b>50 (100)</b>
<b>Age (years)</b>	

0-10	5 (10)
11-20	8 (16)
21-30	32 (64)
31-40	5 (10)
<b>Total</b>	<b>50 (100)</b>

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**Table 3: Health workers' usage and factors affecting the validity of RDT kits.**

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	n(%)
<b>How MLS diagnose malaria in their lab.</b>	
Use RDT	5 (45.5)
Use microscopy	6 (54.5)
<b>Total</b>	<b>11</b>
<b>Is RDT more accurate than microscopy?</b>	
Yes	2 (18.2)
No	9 (81.8)

<b>Total</b>	<b>11</b>
<b>Perceived factors that affect RDT result</b>	
Wait time	2 (14.3)
Buffer quantity	7 (50.0)
Wrong placement of buffer and blood	1 (7.1)
Climatic conditions	1(7.1)
Expired RDT	1(7.1)
No idea	2 (14.3)
<b>Total</b>	<b>14</b>

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**Effect of buffer quantity and wait time on RDT result.**

There was no significant difference ( $p=1$ ) in the number of samples that tested positive and negative within the various test groups. Out of the 50 samples analyzed, 36.0% were positive to *Plasmodium falciparum* while 64.0% were negative. Concerning quantities of buffer volumes tested, the prevalence of malaria (36%) was the same at low, normal, and high buffer volumes respectively. Concerning the various wait times tested, the prevalence of malaria (36%) was also the same at low, normal, and high wait times respectively.

**Table 4: Effect of buffer quantity and wait time on validity of RDT kits used for diagnosis of malaria in symptomatic patients.**

Test groups	RDT result		Total	Fisher's test
	n(%)			
	Positive	Negative		

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**Quantity of buffer**

Low	18 (36.0)	32 (64.0)	50 (100)	
				Z=0
Normal (recommended)	18 (36.0)	32 (64.0)	50 (100)	
				P=1
High	18 (36.0)	32 (64.0)	50 (100)	

**Wait time**

Low	18 (36.0)	32 (64.0)	50 (100)
Normal (recommended)	18 (36.0)	32 (64.0)	50 (100)
High	18 (36.0)	32 (64.0)	50 (100)

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**165 Discussion**

166 The accuracy and validity of malaria rapid diagnostic test (RDT) kits can be influenced by  
167 various factors, and temperature, humidity and RDT preparation are among such factors [16].  
168 The findings from this study showed that the majority of the health workers had a wrong  
169 perception of factors that can affect the validity of RDT kits in the diagnosis of malaria. There is  
170 no valid report that reagent (buffer) volume and wait time affected the performance of RDT kits.  
171 Going a step further in this study, we reported that the various volumes of buffer solution and  
172 wait time assessed did not affect the validity of RDT kit performance.

173 It is interesting to note that the use of inappropriate buffer reagents can affect the performance  
174 of RDT kits. Buffer reagents play a crucial role in ensuring the proper functioning of RDTs by  
175 facilitating the reaction between the sample and the test components. In the case of malaria  
176 RDTs, the buffer solution is designed to optimize the conditions for the interaction between the  
177 target antigens in the patient's blood and the antibodies or other detection molecules on the test  
178 strip. Substitution of buffer reagents can affect the performance of RDT kits in several ways  
179 including its sensitivity and specificity, reaction rate on the test strip, interference with test  
180 components, and storage stability [17, 18]. However, in the present study, substitution of buffer  
181 reagent was not investigated.

182 Hence, the use of buffer reagents recommended by the RDT kit manufacturer is encouraged to  
183 ensure optimal performance. The present does not in any way encourage RDT kit users to  
184 deviate from manufacturers' specific instructions on the preparation and use of the buffer  
185 solution. It is recommended that users of RDT kits refer to the specific product documentation

186 and guidelines provided by the RDT kit manufacturer for accurate information on buffer reagents  
187 and test procedures.

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## 189 **Conclusions**

190 The various buffer reagent volumes and wait times tested in the diagnosis of malaria using an  
191 RDT device did not affect the validity of the RDT kits.

192

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198 self-funded; hence, we acknowledge the co-authors for providing funds to conduct the study.

199

## 200 **Competing interests**

201 The authors declare that no competing interests exist.

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## 203 **Authors' contributions**

204 OTS designed the study, wrote the protocol, performed the statistical analysis, and wrote the  
205 first draft of the manuscript. ENU and IRK collected the data and managed the literature  
206 searches. All authors read and approved the final manuscript.

207

## 208 **Consent**

209 All authors declare that oral consent was obtained from the patients that participated in the  
210 study before sample collection.

211

212 **Ethical Approval:** the study proposal was reviewed by the biology research unit in the Science  
213 Laboratory Technology department, A.I.F.P.U., Ebonyi State. Approval to conduct the study was  
214 also obtained at the Medical Centre of the host institution. Oral consent was sought from study  
215 participants before blood sample collection.

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