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ORIGINAL ARTICLE

Impact of Storage Conditions on the Efficacy of Malaria Rapid Diagnostic Tests in Tropical Settings

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ABSTRACT

Background and Objectives. Malaria remains a significant global health challenge, with Nigeria bearing the highest burden. This study evaluated the impact of storage conditions on the performance of commercially available malaria rapid diagnostic tests (RDTs) in Uyo Local Government Area, Akwa Ibom State, Nigeria. **Materials and Methods.** Malaria prevalence was assessed across five health stations using CareStart™ pLDH/HRP-2 and HRP-2 RDTs. Blood samples were collected from patients under informed consent. The RDTs were stored under low (14.5°C–23.5°C) and moderate (24.6°C–34.5°C) temperatures, with varying humidity levels. Sensitivity, specificity, positive predictive values (PPVs), and negative predictive values (NPVs) of the RDTs were compared against microscopy. Chi-square analysis was used to assess the relationship between variables, with significance set at $p < 0.05$. **Results.** Temperature significantly affected HRP-2 RDT performance at some stations, while pLDH/HRP-2 RDTs were less impacted. High humidity negatively influenced RDT performance, particularly under elevated humidity conditions. Sensitivity varied across storage conditions, ranging from 44.0% to 85.0% for HRP-2 and from 61.3% to 90.0% for pLDH/HRP-2. A total of 26/53, 85/172, and 128/165 pLDH/HRP-2 RDTs were stored under low, medium, and high humidity, respectively. The overall correlation between RDT performance and humidity was statistically significant ($p < 0.05$). For HRP-2 RDTs, 155/336 and 34/54 were stored at low and moderate temperatures, respectively. The association between HRP-2 RDT performance and temperature was also statistically significant ($\chi^2 = 5.277$, df = 1, $p = 0.022$). **Conclusion.** Exposure to high temperatures and humidity can degrade RDT components and reduce diagnostic accuracy. These findings underscore the need for regular quality control and proper storage conditions to maintain RDT efficacy and support malaria control efforts in Nigeria and other endemic regions.

Keywords: Humidity, Malaria, Malaria Rapid Diagnostic Tests (mRDTs), Temperature

1. INTRODUCTION

A report from a globally recognized public health organization shows that there are 241 million estimated cases of malaria in the world, this global record spread across eighty-five endemic countries, of which Nigeria takes the largest share [1]. This current data showed that there was an increase of 14 million cases of malaria in the world compared to the previously estimated value of 227 million cases. This sharp increase could have been attributed to the outbreak experienced with the global pandemic of COVID-19 in the world.

Meanwhile, between 2000 and 2015, there was a reduction in the incidence of malaria in the world; however, the number of incidences also witnessed an increase between 2019 and 2020, and this increase has also been linked to the global shutdown that the world experience during the period of increase in the incidence of malaria cases [2]. A total of 562,000 people died of malaria in 2015 while the total death due to malaria was 558,000 individuals in 2019. Besides, the estimated death rate increased by 12% in the year 2020 to 627,000 individuals. This increase has also been attributed to the effect of the COVID-19 pandemic that ravaged the whole world during this period. The estimated number of people at risk of death per one hundred thousand populations dropped to 13 individuals in 2019 from 15 people per one hundred thousand risks in 2015. Currently, the number of an individual at risk of death per one hundred thousand has increased to 15 individuals.

Almost all countries with the highest malaria burden are in the African region. Nigeria accounts for the highest burden at 27%, followed by the Democratic Republic of the Congo at 12%, and Uganda at 5% [3]. Nigeria remains the epicenter of malaria infection in Africa. The infection is one of the major causes of morbidity and mortality in Nigeria, with many children and adults reporting for malaria diagnosis and treatment in tertiary, secondary, primary, and private health facilities in the country [4]. Severe malaria in children, adolescent, and some cases among adults often present with extreme weakness, impaired consciousness in some cases, anaemia, respiratory distress, convulsions in many children, hypoglycemia, and other symptoms that are associated with severe cases of malaria infection [4]. World Health Organization recommended that the gold standard for malaria diagnosis is the use of microscopy; however, some factors are potentially affecting the use of microscopy in the diagnosis of malaria infection in endemic areas, such factors include: well trained and adequately expertise microscopists, labour intensive [5], well-maintained quality reagent, high-quality binocular microscopes, and above all, it's time consuming [2].

Other laboratory methods for diagnosing malaria, including polymerase chain reaction (PCR) and enzyme-linked immunosorbent assay (ELISA), are costly and necessitate well-equipped lab facilities with highly trained personnel [6]. In Nigeria, these diagnostic techniques are limited to hospitals with substantial financial resources. However, immunochromatographic rapid diagnostic tests (RDTs) offer a viable alternative for diagnostic services in settings where the financial power is low. Malaria rapid diagnostic test measures the level of parasite-derived proteins in the bloodstream, the method is often recommended when there is no reliable microscopy method/expertise, or when the number of health care seeking individuals is high [7]. RDT method is relatively less time-consuming compared to microscopy and it could be carried out by personnel with limited training [8, 9]. When RDTs were designed, their function is often parasite species-specific or genus-specific, by detecting the expressed antigens by the different species of the malaria parasite [10]. The antigens detected by various malaria RDTs include *P. falciparum* histidine-rich protein 2 (*Pf*HRP2), *P. falciparum*-specific lactate dehydrogenase (LDH), *P. vivax*-specific LDH, pan-LDH, and pan-aldolase [11, 12].

RDTs are sensitive to environmental conditions, especially temperature and humidity [13]. Antibodies and antigens can get denatured when exposed to high temperatures, which reduces their specificity and sensitivity. Over time, test components may deteriorate as a result of high humidity [14]. However, extended exposure to light, particularly in the form of intense sunshine, can cause the chemicals and materials employed in RDTs to degrade. This may lead to poorer test findings and, in rare instances, false-positive outcomes. Unlike flow-through tests (involve movement through the membrane), lateral flow test involves the movement of the samples along the nitrocellulose strip [15]. As a result of this design, malaria RDTs are heat-stable, however, if the temperature exceeds certain threshold, they tend to underperform. Furthermore, their operation is also impaired by cold environment, as the gold conjugate gets damaged by temperature below 0^{o}C. Meanwhile, these RDTs can stay for as long as more than 18 months and remain active in their operation. RDTs have an expiration date that often serves as a reminder of their shelf life. Because the components of expired RDTs may deteriorate with time, using them can result in lower specificity and sensitivity. Consequently, regular quality control procedures are crucial. Based on the aforementioned, this study was designed to understand the effect of storage condition likely to be encountered during operational use in endemic areas on the commercially available malaria RDTs in Uyo Local Government Area.

2. MATERIAL AND METHODS

Akwa Ibom State is situated in the South-South geopolitical zone of Nigeria. It is bordered by Cross River State to the east, Abia State to the north, Rivers State to the west, and the Atlantic Ocean to the south. The state enjoys a tropical rainforest climate, characterized by high humidity and heavy rainfall. The state experiences two main seasons: the rainy season (March to November) and the dry season (December to February). The temperature is between 22.2^{0}C and 40.1^{0}C [16]. According to the 2006 census, Akwa Ibom had a population of approximately 3.9 million people. Current estimates indicate that the population has grown significantly. The state is predominantly inhabited by the Ibibio, Annang, Oron, and Eket ethnic groups, each with its own language, culture, and traditions. The state is one of Nigeria's leading oil-producing states, contributing significantly to the nation's oil and gas output. The state has numerous oil fields and is home to several major oil companies. The state has a growing industrial sector, with industries ranging from food processing to petrochemicals.

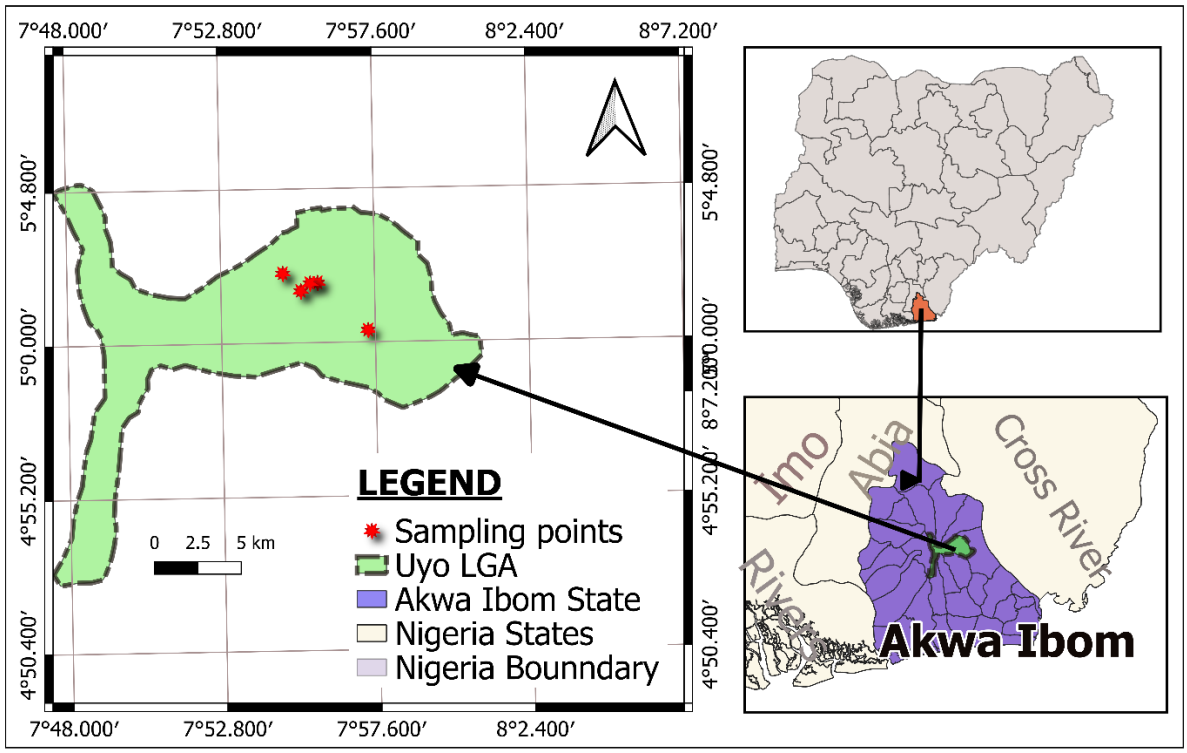

Figure 1. Map of Uyo LGA.

Study Population

The study subjects were all patients who visited health centres with a request form for a malaria diagnosis at the relevant stations. Children under 10 years old who had parental or guardian permission and who came with them to the registration/sample collection location were taken into consideration.

Blood sample collection and storage condition

All blood samples used were derived from a single patient who presented for a malaria infection test at each station. The samples were collected after obtaining informed consent and with the approval of the institutional ethical review committee. Two commercially available RDTs (CareStart^{TM} and SD Bionline) were studied: one designed to detect pLDH, while the other designed to detect pLDH/HRP-2. Upon delivery to the health stations, malaria RDTs were stored in accordance with the manufacturer's guidelines in non-air-conditioned environments, then allocated to separate groups for storage under the following temperatures: moderate (14.5°C- 23.5°C) and high (24.6°C-34.5°C) for up to three weeks. The range of temperature used in this study was same as those found on various shelves where the RDTs are procured for normal laboratory procedures. Prior to the start of the study, the incubators were stabilized at the required temperature for three days before placing the RDTs to be tested inside. At scheduled time intervals, RDTs were taken out of storage and allowed to reach room temperature for 1 to 2 hours before testing. They were then used according to the manufacturer's instructions. To minimize inter-operator variability, the same personnel conducted the test readings at each station. Due to the different formats of the products, blinding the technicians to the results of other products was not feasible. However, each station was kept blinded to the results from other stations until the study was completed Afterward, according to Williams et al. [17], RDTs were subjected to one of the following conditions: air-dried for 2 hours and stored with the desiccant provided in their original packaging (low humidity); air-dried and stored without desiccant (moderate humidity); or stored without air-drying with wet tissue paper (high humidity). In addition, thick blood films were prepared, stained, and examined under the microscope by two experienced medical laboratory scientists.

Data analysis

Data entry and cleaning were performed in a spreadsheet,then exported to R for analysis. The dataset included participant demographics, parasite types, and RDT results. Sensitivity, specificity, positive predictive values (PPVs), and negative predictive values (NPVs) of the RDT compared to microscopy were calculated with 95% confidence intervals (CI). According to WHO (2000) guidelines,

RDTs must have at least 95% sensitivity and 90% specificity. Malaria prevalence was analyzed by stations. Data were presented in tables and graphs, with statistical significance determined using the Pearson Chi-square test and a p-value threshold of 0.05.

Ethical considerations: Ethical approval for this study was obtained from the Research Review Committee of the University of Port Harcourt, Port Harcourt, Rivers State, Nigeria. The study was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki. Patient anonymity was strictly respected throughout the research process.

3. RESULTS

The ratios of total malaria-positive cases were 134/390, 189/390, 239/390, and 278/390 by clinical, HRP-2, pLDH/HRP-2, and microscopy methods. The prevalence of malaria infection across all age groups of respondents was less than half by clinical method while the prevalence was more than half across all ages by pLDH/HRP-2 and microscopy methods (Figure 2).

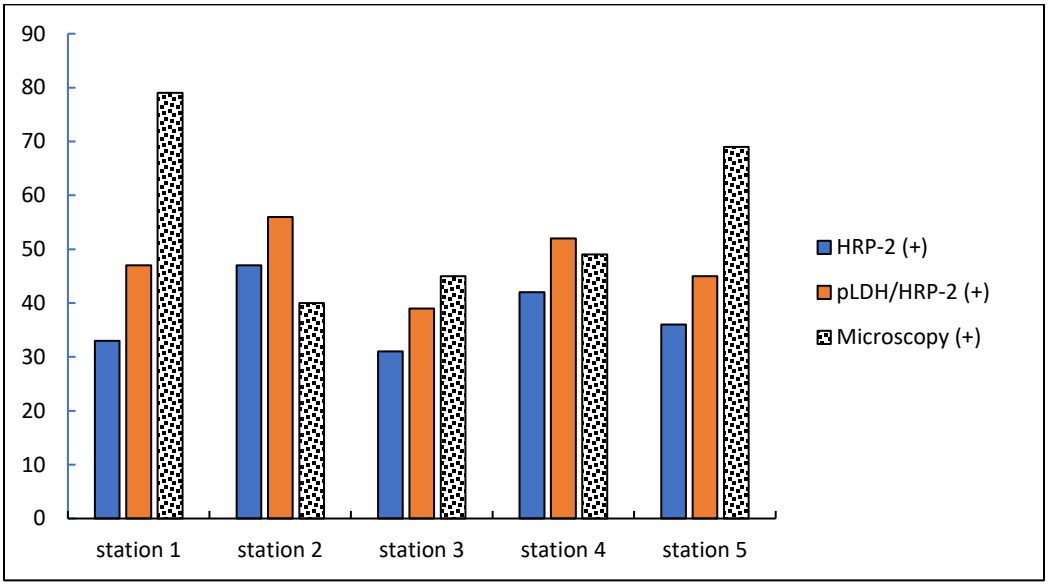

Figure 2. Prevalence of Malaria Parasite Infection based on sampling stations.

Temperature was categorized as low (14.5°C- 23.5°C) and moderate (24.6°C-34.5°C) during the study. In Station 1 hospital, all the RDTs (CareStart™ pLDH/HRP-2 and HRP-2) stored under moderate temperature tested positive for malaria parasite, while more than half of the CareStart™ pLDH/HRP-2 stored under low temperature tested positive to malaria infection. A significant difference occurred between temperature and HRP-2 RDT (χ^2 = 7.44, p = 0.006) (Table 1), but the difference between temperature and CareStart™ pLDH/HRP-2 was not significant (χ^2 = 3.634, p = 0.057). In station 2, a large proportion of the HRP-2 (43/74) and pLDH/HRP-2 (53/62) stored under low temperature tested positive for malaria parasites. The observed differences in the means of pLDH/HRP-2 and temperature were statistically significant (χ^2 = 4.740, p = 0.029) but the mean difference between temperature and HRP-2 was not significant (p > 0.05). A large percentage of both single and pLDH/HRP-2 RDTs stored under moderate temperature , tested positive for malaria parasites while the proportion of HRP-2 stored under low temperature was below average (42.4%). The mean difference of both single and combo RDTs in relation to storage temperature was not significant in station 3. A low proportion (20.0%) of CareStart™ pLDH/HRP-2 stored under moderate temperature tested positive for malaria parasites while no HRP-2 RDT stored under moderate temperature tested positive for malaria parasites in station 4 (Table 2). Meanwhile, more than half of both single and combo RDTs stored under low temperature tested positive for malaria parasites in station 4. On the other hand, more than half of HRP-2 and pLDH/HRP-2 RDTs stored under moderate temperature, tested positive for malaria parasites in station 5. In stations 4 and 5, the mean difference for both HRP-2 and pLDH/HRP-2 RDTs in relation to temperature was statistically significant (p<0.05). The overall prevalence of malaria parasites by HRP-2 and pLDH/HRP-2 RDTs in relation to the storage temperature is represented in Table 2. A total proportion, 155/336 and 34/54, of HRP-2 stored under low and moderate temperatures was reported in this study. The overall mean

between HRP-2 and temperature was statistically significant (χ^2 = 5.277, p = 0.022) but pLDH/HRP-2 RDTs and temperature did not show any significant difference.

Table 1. Prevalence of Malaria Parasite Infection Based on Temperature.

	Station 1		Station 2		Station 3		Station 4		Station 5	
Temperature	HRP-2 (%)	pLDH/HRP-2 (Combo) (%)	HRP-2 (%)	pLDH/HRP-2 (Combo) (%)	HRP-2 (%)	pLDH/HRP-2 (Combo) (%)	HRP-2 (%)	pLDH/HRP-2 (Combo) (%)	HRP-2 (%)	pLDH/HRP-2 (Combo) (%)
Low	28 (37.8)	42 (56.8)	43 (58.1)	53 (71.6)	28 (37.8)	36 (48.6)	42 (56.8)	51 (68.9)	32 (43.2)	40 (54.1)
Moderate	5 (100)	5 (100)	4 (66.7)	3 (50.0)	3 (60.0)	3 (60.0)	0 (0.0)	1 (20.0)	4 (80.0)	5 (100)
Total	33 (41.8)	47 (59.5)	47 (69.1)	56 (82.4)	31 (43.7)	39 (54.9)	42 (53.2)	52 (65.8)	36 (38.7)	45 (48.4)
Chi-square	7.441	3.634	0.018	4.740	0.584	0.056	6.059	4.982	16.851	22.891
P-value	0.006	0.057	0.605	0.029	0.445	0.813	0.014	0.026	0.001	0.001

Humidity was divided into low, medium, and high during the study. In station 1, CareStart™ pLDH/HRP-2 RDT stored under low, medium, and high humidity tested positive in between 50% and 68.3% malaria cases, while HRP-2 RDT tested positive in 41.8% of the overall malaria cases (Table 2). There was no significant difference between humidity and RDTs (HRP-2 and CareStart™ pLDH/HRP-2). In station 2, a large proportion of pLDH/HRP-2 stored under low (3/5), medium (28/34), and high (25/29) humidity tested positive for malaria parasites. The observed differences in the means of RDTs (HRP-2 and pLDH/HRP-2) and humidity were not statistically significant.($p > 0.05$). For HRP-2 stored under low, medium, and high humidity in station 3, less than half tested positive for malaria parasite under low and medium humidity; however, pLDH/HRP-2 stored under low and high humidity tested positive for a high proportion of malaria parasite. The mean difference of both single and combo RDTs in relation to humidity was not significant in station 3. A low proportion (38.2%) of CareStart™ pLDH/HRP-2 stored under medium humidity tested positive for malaria parasites while no HRP-2 RDT stored under same humidity tested positive to low (32.4%) proportion of malaria parasite in station 4 (Table 2). Meanwhile, more than half of CareStart™ pLDH/HRP-2 RDTs stored in high humidity tested positive to malaria parasites in station 5. In stations 4 and 5, the means difference for both HRP-2 and pLDH/HRP-2 RDTs in relation to humidity were statistically significant ($p < 0.05$). The overall prevalence of malaria parasites by HRP-2 and pLDH/HRP-2 RDTs in relation to the humidity is shown in Table 2. A total proportion, 26/53, 85/172, and 128/165 of pLDH/HRP-2 stored in low, medium, and high humidity were recorded in this study. The overall mean between RDTs (HRP-2 and pLDH/HRP-2) and humidity was statistically significant ($p < 0.05$).

Table 2. Prevalence of Malaria Parasite Infection Based on Humidity.

		Station 1		Station 2		Station 3		Station 4		Station 5	
Humidity	No. Examined	HRP-2 (%)	pLDH/HRP-2 (Combo) (%)	HRP-2 (%)	pLDH/HRP-2 (Combo) (%)	HRP-2 (%)	pLDH/HRP-2 (Combo) (%)	HRP-2 (%)	pLDH/HRP-2 (Combo) (%)	HRP-2 (%)	pLDH/HRP-2 (Combo) (%)
Low	8	3 (37.5)	4 (50.0)	2 (40.0)	3 (60.0)	3 (30.0)	6 (60.0)	5 (62.5)	7 (87.5)	6 (27.3)	6 (27.3)
Medium	30	8 (26.7)	15 (50.0)	25 (73.5)	28 (82.4)	15 (41.7)	17 (47.2)	11 (32.4)	13 (38.2)	8 (21.1)	12 (31.6)
High	41	22 (53.7)	28 (68.3)	20 (69.0)	25 (86.2)	13 (52.0)	16 (64.0)	26 (70.3)	32 (86.5)	22 (66.7)	27 (81.8)
		5.256	2.738	2.117	1.689	1.524	1.798	10.776	20.196	17.078	22.994
P-value		0.072	0.254	0.347	0.430	0.467	0.407	0.005	0.001	0.001	0.001

On comparing the performance of HRP-2 test with the malaria diagnostic method, the sensitivity of HRP-2 for station 1, station 2, station 3, station 4, and station 5 were 44.0%, 85.0%, 51.1%, 69.4%, and 52.2 % respectively (Table 3). The specificity for station 1 hospital, station 2, station 3, station 4, and station 5 were 100%, 53.6%, 69.2%, 73.3%, and 100% respectively. The highest Positive Predictive Value (PPV) of 100% each was recorded for station 1 hospital and station 5; however, the lowest Negative Predictive Value (NPV) of 8.7% was recorded for station 1. The highest Positive Likelihood Ratio (PLR) of 2.6 (95% CI: 1.4-4.85) was recorded in station 4 (Table 3).

Table 3. Sensitivity, Specificity, and Predictive Values of HRP-2 Rapid Diagnostic Test using Microscopy as the Standard.

Health facility	Positive (%)	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	Positive Likelihood Ratio (95% CI)	Negative Likelihood Ratio (95% CI)	Accuracy (95% CI)
Station 1	33 (41.8)	44 (32.6-55.9)	100 (39.8-100)	100 (89.4-100)	8.7 (2.4-20.7)	-	0.56 (0.46-0.68)	46.8 (35.5-58.4
Station 2	47 (69.1)	85 (70.2-94.3)	53.6 (33.9-72.5)	72.3 (57.4-84.4)	71.4 (47.8-88.7)	1.83 (1.2-2.8)	0.28 (0.1-0.6)	72.1 (59.9-82.3)
Station 3	31 (43.7)	51.1 (35.8-66.3)	69.2 (48.2-85.7)	74.2 (55.4-88.1)	45.0 (29.3-61.5)	1.7 (0.9-3.2)	0.71 (0.5-1.1)	57.6 (45.4-69.4)
Station 4	42 (53.2)	69.4 (54.6-81.8)	73.3 (54.1-87.7)	81.0 (65.9-91.4)	59.5 (42.1-75.3)	2.6 (1.4-4.85)	0.4 (0.3-0.7)	70.9 (59.6-80.6)
Station 5	36 (38.7)	52.2 (39.8)	100 (85.8-100)	100 (90.3-100)	42.1 (29.1-55.9)	-	0.5 (0.4-0.6)	64.5 (53.9-74.2)

The performance of pLDH/HRP-2 test when compared with the malaria diagnostic method, the sensitivity of pLDH/HRP-2 for station 1, station 2, station 3, station 4, and station were 61.3%, 90.0%, 66.7%, 77.6%, and 62.3 % respectively (Table 4). The highest specificity value of 91.7% was recorded for station 5 while the lowest value of 28.6% occurred in station 2. The highest Positive Predictive Value (PPV) of 97.9% was recorded for station 1; however, the lowest Negative Predictive Value (NPV) of 45.8% was recorded for station 5. The highest positive likelihood ratio (PLR) of 7.5 (95% CI: 2.0-28.6) was recorded in station 5. The highest pLDH/HRP-2 test accuracy of 69.9% was recorded for Station 5.

Table 4. Sensitivity, Specificity, and Predictive values of pLDH/HRP-2 Rapid Diagnostic Test using microscopy as the standard.

Health facility	Positive (%)	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	Positive Likelihood Ratio (95% CI)	Negative Likelihood Ratio (95% CI)	Accuracy (95% CI)
Station 1	47 (59.5)	61.3 (49.4-72.4)	75.0 (19.4-99.4)	97.9 (88.7-100)	9.4 (2.0-25.0)	2.5 (0.5-13.5)	0.5 (0.3-1.0)	62.0 (50.4-72.3)
Station 2	56 (82.4)	90.0 (76.3-97.2)	28.6 (13.2-48.7)	64.3 (50.4-76.6)	66.7 (34.6-90.1)	1.3 (1.0-1.6)	0.4 (0.1-1.1)	64.7 (52.2-75.9)
Station 3	39 (54.9)	66.7 (51.1-80.0)	65.4 (44.3-82.9)	76.9 (60.7-88.9)	53.1 (34.7-70.9)	1.9 (1.1-3.4)	0.5 (0.3-0.8)	66.2 (54.0-77.0)
Station 4	52 (65.8)	77.6 (63.4-88.2)	53.3 (34.3-71.7)	73.1 (59.0-84.4)	59.3 (38.8-77.6)	1.7 (1.1-2.5)	0.4 (0.2-0.8)	68.4 (56.9-78.4)
Station 5	45 (48.4)	62.3 (49.8-73.7)	91.7 (73.0-99.0)	95.6 (84.9-99.5)	45.8 (31.4-60.8)	7.5 (2.0-28.6)	0.4 (0.3-0.6)	69.9 (59.5-79.0)

4. DISCUSSION

As per the ASSURED criteria, the ideal rapid test should be: A = affordable, S = sensitive, S = specific, U = user-friendly (easy to perform with minimal steps and training), R = robust and rapid, E = equipment-free or requiring minimal equipment, and D = deliverable to those in need [18]. We evaluated the temperature and humidity robustness of a malaria RDT brand and found that, despite the varying incubator temperatures, three stations showed no significant changes in test results, while two stations experienced a significant effect. Exposure to heat, humidity, or other adverse conditions can lead to decreased test accuracy [13]. A study by Chiodini et al. [14] demonstrated that tests based on *P. falciparum* histidine-rich protein-2 (HRP-2) maintained their sensitivity after exposure to high temperatures, whereas those based on *Plasmodium* lactate dehydrogenase (pLDH) exhibited reduced sensitivity under the same conditions.

Factors such as epidemiological characteristics, the season of data collection, and reference standards were considered to play a vital role in the final result of the performance of the CareStart™ pLDH/HRP-2 RDT [19]. The present research found low sensitivity for CareStart™ pLDH/HRP-2 and HRP-2 RDT, which agrees with previous study [19]. The low performance of RDT could be due to a low density of malaria parasites and a reduced population of positive participants [19]. The long-term trends of anti-malarial use, as well as the use of counterfeit, less valuable, and unlawful medications, could also be attributed to the progression of the selective pressure on malaria parasites in the blood stream and the consequent low parasitaemia below the kit detection threshold. Furthermore, low performance of the RDT could be a result of the manufacturing process or environmental conditions [10]. Maintaining the quality and assurance of malaria RDTs requires keeping perishable medical materials at the appropriate storage temperature and humidity [20] because abnormal storage conditions could degrade immunochromatographic RDTs [21]. Research has demonstrated that some RDTs' performance during their shelf-life is significantly impacted by simulated high temperatures, and prolonged exposure to significant temperatures can cause RDTs to lose acceptable sensitivity or malfunction [22]. A study has shown that this constraint can be eliminated for malaria case management by using easy, inexpensive, and locally accessible technology [20]. During this study, the low and moderate temperatures in the health facilities seemed to be within a tolerance range for the RDTs, as there was no significant difference between the temperature and the RDTs used in most of the stations. The variability in RDT performance after heat exposure

can stem from multiple factors. Although high temperatures are known to denature proteins, but the degree of resistance varies based on protein sequence and structure, which may explain the observed differences in stability [23]. Additionally, the quality of materials used in the strip itself could contribute to degradation [24]. In an analysis done on commercially available RDTs (50) by the world health organization, they found that the stability of 37 was between 2°C – 30°C while the remaining 13 had stability up to 40°C [25]. In another study by the same organization, forty-eight malaria RDTs were exposed to heat (45°C) and humidity of 75% for sixty days; the RDTs were used to test malaria. They found that 57.8% had viability under these conditions with *P. falciparum*, while 10% remained viable for *P. vivax* or other malaria parasites [18]. In Nigeria and many other African countries, the issue of high temperature is common, as a result, all measures needed to be considered before storing or transporting RDTs from one point to another or within a health care facility. A study in Brazil which is considered a temperate region, RDTs were stored in a place that is higher that the recommended for fifteen months, the result shows that the sensitivity for *P. falciparum* and *P. vivax* (79.7% and 85.7% respectively) was below the WHO recommended value. Similar results were also reported from Senegal and Ethiopia [25], [26]. In order to reduce the impact of degradation on RDTs, provision of cold storage facilities in health care facilities and during transportation should be considered at all times. Storage of RDTs in evaporative cooling boxes in Cambodia has resulted in about 97% reduction in the thermal stress and the performance of the RDTs were almost within the desired result; the storage period was also extended from 210 days to close to one year [20]. RDTs stored in temperature, less that 8°C were compared with RDTs stored in an ambient temperature in Afghanistan. They result showed that invalid results were higher in RDTs stored in an ambient temperature compared to the other storage method [27].

The links between the biomarkers used at the control and test lines of the RDT nitrocellulose strip become weaker in the presence of high humidity [21]. Condensation of atmospheric water vapour causes humidity, which is crucial in conditions where high humidity and high temperatures coexist. Meanwhile, condensation will be avoided by using a desiccant to lower the relative humidity inside the device package, as recommended by the WHO. More so, the RDT device should not be utilized if it is saturated; instead, it should be discarded [28]. The significant difference between different levels of relative humidity and the RDTs shows the negative effect of increased relative humidity on the performance of the RDTs used during this study. This agrees with previous studies where high relative humidity reduced the sensitivity, specificity, and accuracy of malaria test kits in Bamako, Mali [29]. The effect of humidity on RDTs cannot be relegated because abnormal humidity could impair the performance of several commercially available RDTs [30]. A study done by Barbé et al. [21] assessed the effect of desiccant (protect against degradation due to humidity) on RDTs; with package as recommended by WHO and European Community (EC). Their result shows that of the 50 malaria RDT assessed, 22 self-indicating or 8 partial-indicating silica gel contained the toxic cobalt dichloride as humidity indicator. Also, colour change indicating humidity saturation was observed for 8 RDT products of sachets inspected. In addition, they also found that all RDTs with partial-indicating silica gel, sachets with no colour indicating beads were observed, less than half (47%) instruction for use of RDT products with indicating desiccants failed to state that humidity saturation should be checked before using the product, indicating the inability of the RDTs products sampled to meet-up with the international standard required as regard humidity.

The RDTs had a low NPV, making it less reliable for excluding malaria. On the other hand, a greater PPV in this study indicates that patients would receive an accurate malaria diagnosis and avoid needless medication. Likelihood ratio (LR) is a crucial parameter for assessing the precision of a diagnosis and is dependent on the prevalence of the disease condition. It is the proportion of test subjects with a particular disease compared to the expected test result. LR links the likelihood of an illness in a particular patient between the pretest and the posttest [31]. Positive Likelihood Ratio (PLR) is one of the markers for confirming a diagnosis, but negative Likelihood Ratio (NLR) is a reliable indicator for disconfirming a diagnosis. The higher accuracy of CareStart™ pLDH/HRP-2 RDT over HRP-2 showed better performance of CareStart™ pLDH/HRP-2. However, a higher positive likelihood ratio and negative likelihood ratio of HRP-2 were recorded during this study. Our result is in consonance with similar results in Calabar, South-south Nigeria [31].

Inadequate storage and shelf-life management of RDTs can have significant implications. Sensitivity and accuracy may be compromised, leading to false-positive or false-negative results, which impact patient care and malaria prevention efforts [32]. Inaccurate RDT data can result in misdiagnosis, with false positives causing unnecessary treatments and potential side effects, while false negatives could lead to undertreatment. Improper storage and management can also result in the loss of valuable diagnostic tools, necessitating the disposal of unusable tests and causing direct financial losses for the healthcare system [33]. This inefficient allocation of resources diverts funds from other critical aspects of malaria control, such as treatment, prevention, or surveillance, to replace unusable RDTs. Additionally, repeated issues with unreliable RDTs due to poor storage can erode healthcare workers' trust in these diagnostic tools, negatively affecting patient care. Poor storage practices can lead to erroneous surveillance data and misdiagnosis, undermining efforts to control and eradicate malaria and exacerbating the disease's spread.

5. CONCLUSION

The performance of malaria rapid diagnostic tests (RDTs) is significantly influenced by environmental factors such as temperature and humidity, which can compromise their sensitivity, specificity, and overall diagnostic accuracy. The degradation of RDTs due to heat and humidity showed the need for proper storage, transport, and quality assurance measures to preserve their diagnostic integrity. To mitigate this challenge, healthcare facilities and malaria control programs should prioritize the provision of cold storage infrastructure, improved supply chain management, and training for healthcare workers on RDT handling. Adoption of cost-effective storage solutions, such as evaporative cooling. Furthermore, stringent regulatory oversight is needed to ensure that RDTs meet international quality standards before deployment in malaria-endemic regions.

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