The Relevance of Positive Control Wells in Monitoring the Performance of Malaria Rapid Diagnostic Test in the Field for Effective Case Management of Malaria

Okangba, C. C[1], Oyibo W.A.[2], Thompson, E. T.[3], Ejime E.[4], Ajani, T.A.[5], Elikwu, C.J.[6], Okangba, K. K[7]

[1,4,5,6,7] *Department of Medical Microbiology and Parasitology Benjamin Carson (SNR) School of Medicine, Babcock University, Illisan –Remo, Ogun State, Nigeria*

[2]*ANDI Center of Excellence for Malaria Diagnosis/WHO-FIND Malaria Specimen Collection Site, Department of Medical and Parasitology College of Medicine, University of Lagos, Lagos, Nigeria*

[3]*Department of Medical Microbiology and Parasitology, Jos University Teaching Hospitals, Nigeria*

Abstract: **Background: Malaria rapid diagnostic tests (MRDTs) are potential breakthrough in the provision of accurate diagnosis in remote areas, but widescale use is hampered by uncertainty over accuracy under field conditions. Rapid diagnostic tests (RDTs) are central to fulfilling the WHO's recommendation for parasitologic confirmation of all suspected cases of malaria. RDT performance may be compromised when exposed to the high temperature conditions typical of most malaria endemic regions. However, a systematic method to monitor RDT quality and performance in endemic countries is lacking at the present time. Current methods to monitor RDT performance in the field include comparing results from RDTs to diagnoses made by light microscopy and observing health workers perform tests. These methods are not substitutes for direct quality control. Positive Control Wells (PCWs), which contain recombinant malaria parasite antigen, are a novel method for addressing this need for quality assurance. In this study, the suitability of PCWs as quality control kit for malaria RDTs quality assurance testing was evaluated.**

Objective: **Assess the performance of malaria RDTs using Positive Control Wells and compared with highly characterized Quality Control samples in the case management of malaria.**

Method: **A total of thirty-five malaria RDTs compromising of twenty-four Histidine rich protein II, five Plasmodium lactate dehydrogenase and three *Plasmodium falciparum*/ *Plasmodium vivax* Malaria Rapid Diagnostic Tests were monitored for their performance in the field using Positive Control Wells (PCWs) The CTK Positivia Malaria Ag Rapid Test Assay Control kit with Catalog Number C0010 PCWs (Produced by CTK Biotech, Inc. 10110 Mesa Rim road, San-Diego, CA 92121 USA), and then compared with highly characterized Quality Control (QC)samples. Thermal stability was also assessed after one month of storage at elevated temperatures of 35, 40 and 45^{o}C in the incubator and humidity. The QC samples were diluted to different parasitaemia dilutions (2000, 1000, 200, 150, 100 and 50parasites/microliter of blood {µL}) and then tested with the same MRDTs used to evaluate the performance of the PCWs and to determine the antigen concentration of the PCWs. The wells were reconstituted with buffer for both the positive and negative control wells.**

Results: **All the thirty-five MRDTs used to perform this evaluation all gave a positive results, but with different test line intensity ranging from 0.5+ to 3+. The *P. falciparum* specific lines showed more intensity than the pan-specific lines. At elevated temperature, the RDTs also gave positive results, but negative when the PCWs were kept for longer period.**

Conclusion: **This study confirmed that positive control wells have a potential place in routine management of malaria, filling an important gap in ensuring the quality of diagnosis to guide the delivering of potentially life-saving treatment.**

Key words: **Positive Control Wells, Quality Control, Quality Assurance, Malaria Rapid Diagnostic Tests, Malaria, *Plasmodium falciparum***

I. INTRODUCTION

Malaria remains a serious health problem for most of the global population. Over 200 million cases of *Plasmodium falciparum* occur annually worldwide for which prompt treatment is required to prevent death, especially in children under the age of five (WHO, 2015). Poor access to appropriate diagnosis and treatment, and increasing resistance of parasites to drugs, are major contributors to malaria mortality (Sani *et al*., 2013; Okangba *et al*., 2016).The burden of malaria is highest in sub-Saharan Africa. However, travelers from malaria non-endemic countries are also at increased risk of severe disease if exposed to this parasite. In 2018, 228 million cases of malaria occurred worldwide, most of the malaria cases (93%) were from Africa region with Nigeria having the highest burden of malaria (25% of all malaria cases) (WHO, 2019). Nigeria accounts for a quarter of all malaria cases in the WHO African Region. Transmission occurs all-year round in the South, and more seasonal in the North (WHO, 2008). It is one of the principal causes of sickness and death in Nigeria and imposes an enormous socio-economic burden on the country. *Plasmodium falciparum* is

the most prevalent malaria parasite in the African region accounting for 99.7% of the estimated malaria cases in 2018 (WHO, 2019). Approximately, 450,000 death was due to malaria globally, while parasite-based diagnosis is increasing, most suspected cases of malaria are still not properly confirmed, resulting in the overuse of antimalarial drugs or lead to over diagnosis (Oladosu and Oyibo, 2013) and can also lead to poor disease monitoring (WHO, 2018).

Accurate diagnosis of malaria is critical to administering appropriate treatment. In the past, due to the high prevalence of malaria among febrile patients and the availability of cheap anti-malarial drugs, malaria was diagnosed on the basis of clinical symptoms, with only a small proportion of cases confirmed with laboratory tests. However, the World Health Organization (WHO) and Federal Ministry of Health (FMOH) emphasize on parasitological confirmation of all suspected cases of malaria before treatment (WHO, 2015; FMOH, 2015). This decision was made for multiple reasons, including recent reductions in the incidence of malaria in many endemic countries (WHO, 2010; Ceesay *et al*., 2008; Mmbando *et al*., 2008; Steketee *et al.,* 2008) the spread of parasite resistance requiring a switch to more expensive artemisinin based combination therapy (ACT) and the need to reduce drug pressure to prevent development and spread of resistance to ACT.

Malaria diagnosis has relied traditionally, on light microscopic examination of stained blood smears. However, the capacity to conduct quality routine malaria microscopy has been low, resulting in little or no use of the laboratory to confirm suspected cases and a mistrust of laboratory test results by clinicians (McMorrow *et al*., 2008; Nankabirwa *et al.,* 2009). Malaria rapid diagnostic tests (MRDTs) have shown to be comparable or surpass routine microscopy when the use of quality controlled, well performing test kits (Lon *et al*., 2005; Bells *et al*.,2006) is coupled with adequate training and carefully designed bench aids (Harvey *et al*., 2008; D'Acremont *et al*.,2010; Mcmorrow *et al*., 2010;). Malaria RDTs are simple to perform and can be used by non-laboratory staff in health facilities and by community health workers (Thiam *et al*., 2011). The performance of quality controlled RDTs and their ease of use by a wide variety of non-laboratory health workers have also, in part, led to the new WHO recommendation on parasitological confirmation of all suspected cases. Large-scale operational use has raised questions about the accuracy of current RDT technology in tropical conditions. As utilization of RDTs has increased rapidly in the last few years, there is a clear and urgent need to address issues on quality performance and appropriateness of use, particularly in remote endemic areas (Okangba, 2019; WHO, 2019b). The need for Quality Assurance (QA) systems to maintain the quality of microscopy diagnosis of malaria is well established but the extent of implementation varies widely. Quality assurance process must become an integral part of RDT budgets, procurement and implementation plans (WHO, 2019a). Ensuring good manufacturing quality is addressed to an extent by the RDT product evaluation (WHO, 2009; WHO, 2012; WHO, 2013) and pre-procurement lot testing programmes conducted by the WHO, Foundation for Innovative New Diagnostics (FIND) and US Centers for Disease Control and Prevention (CDC). Responsibility for overseeing QA processes, extending from post purchase testing of RDTs to training and supervision of users and control of storage and transport, should be clearly defined and coordinated from a central level. A system of regional and referral laboratories, based on standard operating procedures would test RDTs after purchase and for the duration of shelf-life using Quality Control (QC) panels prepared from wild-type parasites (WHO, 2018; Okangba, 2019).

Positive control wells are composed of recombinant antigen (Lon *et al.,* 2005; Aidoo *et al*., 2012) at concentrations intended to give a positive results that should allow the village health worker or care provider to test an MRDTs from any box before use, ensuring that the kit has not degraded during storage and transport, and boostering confidence that the results of testing may be used to direct therapy. This development promises to greatly improve on the current need to perform comparative microscopy at sentinel sites to monitor MRDT field performance (Perkins and Bells, 2008; Rennie *et al*., 2008). A prototype Positive Control Wells, a point-of-care quality control test for malaria RDTs is developed to monitor the effective of MRDTs in the case management of malaria. These PCWs are small plastic wells coated with a small amount of dried recombinant proteins HRP2, pLDH, and aldolase (the main targets of malaria RDTs), (Lon *et al*., 2005; Tamiru *et al*., 2015). When diluted with a fixed volume of clean (tap) water and applied to an RDT, the recombinant antigen solution produces a positive reaction on the RDT and a band will appear at the test line, indicating that the RDTs is fit for use. In order to select recombinant proteins for prototype PCWs and ensure equivalence to testing against real parasite samples, extensive evaluations of the proteins and PCW prototypes are important (Lon *et al*., 2005; Tamiru *et al*., 2015). These evaluation will not only demonstrate that recombinant proteins are very well detected by RDTs and that PCWs when performed properly, but also demonstrated high stability of the PCW prototypes, essential for effective use in the field (WHO, 2013; WHO, 2019a). The PCWs can therefore be used by health workers to test stocks of RDTs stored at their health facilities and to ensure their validity. The use of PCWs containing recombinant antigens would assist in assuring the performance of RDTs in the field and this would assist in the parasite-base confirmation and in the case management of malaria (Tamiru *et al.,* 2015; WHO, 2018). Quality assurance processes must be transparent, and good communication with manufacturers and end-users during QA development is necessary (Okangba and Oyibo, 2019; WHO, 2008; WHO, 2019).

Hence, the objective of this study is to determine the importance of Positive Control Wells in the performance of

MRDTs in the case management of malaria and also to compare performance of highly characterized quality control samples and PCWs .

Significance of Study

It is paramount that RDT quality can be checked and test quality assured at the level of use, often in remote clinics or in the hands of village volunteers. At present, field users have no method of ensuring that the RDTs they are using are still functioning properly after exposure to heat and humidity during transport and storage.This new technology will provide a simple, low-cost method to test RDTs and ensure quality can be monitored from manufacture to the end-user, addressing several of the current blocks to effective RDT use.

II. STATEMENT OF THE PROBLEM

In the past some RDT have performed badly based on some challenges such as when it is exposed to high temperature, lack of cool chain means of transportation, short shelf life and issue on antigen vs parasitaemia. At present, users have no method of ensuring that RDTs are still functioning properly after exposure to variable transport and storage conditions. The development of Positive Control Wells will address this uncertainty

Rationale

It is vital that the potency of malaria RDTs can be evaluated in the field before it is used for malaria diagnosis especially in remote clinics or in the hands of village volunteers. At present, field users have no method of ensuring that the RDTs they are using are still functioning properly after exposure to heat and humidity during transport and storage. This study is aimed at evaluating the performance of RDTs at the community level employing the use of PCWs and comparing the performance of PCWs and highly characterized quality control samples. This new technology will provide a simple, low-cost method to test RDTs and ensure that quality can be monitored from manufacture to the end-user, addressing several of the current blocks to effective RDT use

Principle of the Positive Control Wells

The positive malaria Ag Rapid Test Assay Control Kit is intended for use with MRDTs as external assay controls to monitor test performance under circumstances such as: a new operator uses the Rapid test, prior to testing specimens, to investigate the cause of repeated invalid results, to verify a higher than expected frequency of positive or negative results, to verify performance of MRDTs when temperature during storage falls outside of 2-30^{o}C , to determine MRDTs performance when a new lot is produced and when new shipment is received and for periodic intervals testing of MRDTs. The positive Malaria Ag Rapid Test Assay Control Kit is comprised of positive controls including Pf-HRP2 Positive Control, Pf-LDH Positive Control and a Negative Control. These are intended for use on the Malaria Pf/Pv, Pf/Pan and Pf Ag Rapid Tests.

III. MATERIALS AND METHODS

The PCW used in this study was the CTK Positivia Malaria Ag Rapid Test Assay Control kit with Catalog Number C0010 PCWs (Produced by CTK Biotech, Inc. 10110 Mesa Rim road, San-Diego, CA 92121 USA). After receipt from the manufacturer via courier, wells were kept in sealed container provided by the manufacturer and stored at approximately 4^{o}C until the time of use. The PCWs were transported in cool ice boxes. Each well was reconstituted with diluents and allowed to stand for 1minute to ensure that the antigen dissolved properly.

Rapid diagnostic test choice

RDTs were chosen based on performance in Round 2 of WHO/FIND/CDC RDT performance evaluations (WHO, 2013) and their availability for purchase (Table one). Performance criteria used for test selection was a panel detection score (PDS) at 200parasite/µl of ≥90 for *Plasmodium falciparum.* PDS for other *Plasmodium species* Based on the above criterion, 35 RDTs were selected for this study (Table one)

Laboratory Procedure

The laboratory procedure was performed at the ANDI centre of Excellence for malaria diagnosis International Malaria Microscopy Training and RDT QA Programme WHO/TDR/FIND Malaria Specimen Bank Site College of Medicine, University of Lagos. Parasitized blood samples were diluted into different parasitaemia levels (50parasites/µl, 100 parasites/µl, 150 parasites/µl, 200 parasites/µl, 2000 parasites/µl and 5000 parasites/µl of blood). Thirty five selected quality assured MRDTs were tested using the positive, negative control well samples and diluted blood QC samples at different parasitaemia levels. This would indicate the dilution / parasitaemia level at which the RDTs reads positive. The PCWs were reconstituted and tested using thirty-five different malaria RDTs purchased from different MRDTs suppliers in Lagos, Nigeria.

How to Reconstitute the Malaria Ag Positive Control stock (Reagent Preparation)

The positive and negative controls were brought to room temperature before opening if stored at 2-8^{o}C. Ten drops of the negative control was added to each of the positive control vials and the stoppers were replaced and then the vials were mixed vigorously for ten times to dissolve the positive control antigen. The solution was left to stand for ten minute at room temperature to ensure that the antigen is completely reconstituted.

Procedure for PCWs Test

- The selected quality assured MRDTs (table one) were opened and allowed to stand at room temperature
- The MRDTs were labeled with the name of the Positive Control or Negative Control samples

- Using the blood transfer device, 5µL of each reconstituted Positive Control and Negative Control was transferred to the sample well of each device respectively
- Two drops of the blood lysis buffer (or Negative Control) to the buffer well of each positive and negative control device
- Results were recorded within 20 minutes.

Standard Protocol for Preparing Quality Control Samples

Five patients were selected for preparation of Quality Control (QC) samples/panels. These patients were based on the following criterias; the patient must have history of malaria symptoms (fever), twelve years or older, no recent intake of anti-malarials in the last two weeks, no clinical anaemia and gave consent. The patients that qualifies were given an identification number and will be asked to complete the case report form and consent form. The patients finger were pricked to collect the required volume blood for preparing the malaria blood smears for malaria microscopy testing and for performing MRDT. Patient with strongly positive MRDTs result (2+ or 3+) and /or parasite density greater or equal to 5,000parasites/µl of blood (treat patient with ACTs) and it must be single species infection. The patient were exposed to HIV counselling. 7mls of venous blood was collected from the patient. 2mls of blood was dispense into the plain bottle for hepatitis B/C and HIV testing while the remaining 5mls of blood was dispense into an EDTA bottle with patient name, age, sex and identification number. The thin and thick blood films were read by two microscopist after staining with giemsa stain and the average parasite count was used to determine the parasite density of the patient. The blood group of the patient were also determined and crossed matched with the parasite free donor blood to prevent agglutination (clump test must be negative)

IV. PROCEDURE FOR QUALITY CONTROL TESTING OF MRDTS

A. Appropriate use of QC sample aliquots for RDT QC testing

The required QC sample aliquots were taken out of the -80^{o}C freezer, placed on a rack and allowed to thaw to room temperature for minimum of 20 minutes and maximum of 30 minutes. The procedure is performed within 12 hours of thawing. The QC samples was used once and left over was discarded

B. Performing an RDT using a QC sample aliquot

The instruction manual studied prior to performing the MRDTs QC test. The QC samples were brought out approximately 30 minutes before test commence. The MRDTs were also brought to room temperature (~25°C) prior to performing the test. The integrity of the MRDTs were also ascertained. Each of the MRDTs were labeled with the QC sample ID, RDT lot number, and date of test (DD/MM/YY), using a marker pen. The QC sample aliquot were vigorously mixed (flick or use micropipette or use vortex) prior to opening and pipetting the blood. The RDTs was performed with the QC sample as per manufacturer instructions, BUT use a micropipette to transfer the specified blood volume to the RDT. A timer was used to to record all steps exactly as per manufacturer instructions. The RDT results was interpreted within the manufacturer recommended time and recorded. The standard color chart provided by WHO/FIND for rating the band intensity from 0 (negative) to 4+.was used to interpret the MRDTs result

Standard Protocol for Quality Assurance Testing of MRDTs in the Laboratory; Using QC Panels

Five QC panels of different parasitaemia levels (50p/µl, 100p/µL, 150p/µL, 200p/µL, 1000p/µL and 2000p/µL) were thawed to room temperature

- Eight locally purchased MRDTs and two quality assured MRDTs (controls) MRDTs were assessed
- The expiring date of the RDTs were checked and manufacturer's leaflet was read so as to correctly and accurately perform the test
- Three test kits each were used for all the low level parasitaemia (50, 100, 150 and 200parasites/µL of blood) for each QC panel, while two test kit each were used for the high level parasitaemia 1000 and 2000p/µL for each QC panel
- All the MRDTs were labeled using a marker or pencil
- 5µL of QC sample was collected with the use of a blood transfer device and dispense into the sample well and the recommended drops of buffer was added into the buffer well
- The result was interpreted within 20 minutes

Procedure for MRDTs Performance Assessment using Positive Control Wells (PCWs)

- The PCWs was brought to room temperature, reconstituted with negative PCWs and then shake vigorously
- Thirty-two MRDTs (27 HRP2 detecting RDTs, 5 pLDH detecting and 3 P.f/ P.v detecting RDTs) were assessed
- Three test kits were used for each RDTs and they were labeled using a marker or pencil
- With the aid of a transfer device 5µL of the reconstituted PCWs was dropped into the sample well and the recommended drops of buffer was added into the buffer well and the results were interpreted within 20minutes

Stability Testing of MRDTs using PCWs

The MRDTs used for performance assessment with the use of PCWs were kept into an incubator set at 45^{o}C, 50 ^{o}C and 60^{o}C for 30days. The MRDTs were brought out of the incubator after the expiration of 30days. The MRDTs were brought to room temperature. The performance of the MRDTs were assessed using the PCWs Three test kits were used for each RDTs and they were labeled using a marker or pencil. With

the aid of a transfer device 5µL of the reconstituted PCWs was dropped into the sample well and the recommended drops of buffer was added into the buffer well. The result was interpreted within 20minutes

Table 1: Detailed Information on Locally Purchased MRDTs in Lagos, Nigeria

S/NO	NAMES OF RDT PRODUCT	Number of Test Kit	PRINCIPLE	STORAGE TEMP	MAN. Date	EXP. Date	Date of Purchase	Locally Purchased PRICE	Lot Number	Date of initial Testing	Supplier Address
1	Global Device	25	Detects P.f antigen only(HRP2)	2°C-30°C	2005	2008	20th May 2008	3,000	MAL 710002	7th July 2008	Lascon Phar.Ltd Idumota Lagos
2	Paracheck Device	25	Detects P.f antigen only(HRP2)	4-45°C	Jan-08	Dec-09	20th May 2008	2,200	31074 31075	7th July 2008	Lascon Phar.Ltd Idumota Lagos
3	SD Bioline(Antibody)	30	Detects Antibody in blood(msp Antigen)	2-30°C	-	Nov 15 2009	20th May 2008	6,500	18090	7th July 2008	C.C.Obi Nig Ltd Yaba,Lagos
4	CTK Biotech(Antigen)	25	Detects PLDH	2-30°C	29-Mar-2007	Sep-08	20th May 2008	6,500	F0327 P2	7th July 2008	Lascon Phar.Ltd Idumota Lagos
5	Wondfo Biotech	50	Detects HRP2 in blood for PF	4-30°C	-	Oct-09	May 28th 2008	4,500	W3771 WIW	7th July 2008	46/48 Iga St. Idumota, Lagos
6	Acon Biotech	40	Detects HRP2 in blood for PF	2-30°C	Sep-08	Sep-10	2nd Feb 2009	4,000	MAL 8090023	4th Feb 2009	Ojax Medics Ltd Iga st. Isale-Eko Lagos
7	Grand time	25	Detects P.f antigen only(HRP2)	2-30°C	-	Oct-10	2nd Feb 2009	4,000	MAL8110031	4th Feb 2009	Global Nig Ltd Isale-Eko, Lagos
8	Embassy	25	Detects P.F ANTIGEN ONLY (H	2-30°C	Sep-08	Sep-11	June,2007	4,000	W3780908W2	4th Feb 2009	Embassy Ltd Isale-Eko, Lagos
9	ICT Combo	25	Detects all plasmodium spps (PLDH)	4-37°C	-	Sep-09	8th Sept2009	7,200	31902	7th July 2008	Directly from Manufacturer in S/Africa
10	CORE(One step)	25	Detects Pf/PV	2-30°C	-	Mar-11	8th Sept2009	3,000	20090426	14th Sept 2009	Coner stone Pharma Ltd Isale-Eko,Lagos.
11	Accu Care	25	Detects PF/PV	4-30°C	Jun-09	May-11	20th April 2009	2,700	MAG 903	14th Sept 2009	Emalis Nig Ltd Isale-Eko, Lagos.
12	Antec	25	DetectsPf (HRP2)	2-30°C	-	Jun-10	NIL	-	20081117	29th April 2009	Chudi Concerns Ltd Isale-Eko,Lagos.
13	BID	30	Detects Pf(HRP2)	4-30°C	Jun-09	Jun-11	NIL	-	73LAB013C	27th Aug 2009	Bundi Int'l Diagnostic Aba,Nigeria.
14	BID	30	Detects all plasmodium spps (PLDH)	4-30°C	Jun-09	Jun-11	NIL	-	110LAB013A	27th Aug 2009	Bundi Int'l Diagnostic Aba,Nigeria.
15	Bioland Malaria of Antigen	30	Detects Pf/pan	2-30°C	Aug-09	Feb-11	NIL	-	MPF010809	1-Oct-09	Codix Pharma Ltd Ajao Est Lagos.
16	Bioland Pf/Pv Cassette	30	Detects Pf/Pv	2-30°C	-	Mar-11	NIL	-	MAL070909	1-Oct-09	Codix Pharma Ltd Ajao Est Lagos.
17	Acon	25	Detects Pf(HRP2)	2-30°C	-	Sep-11	NIL	-	MAL9090012	1-Oct-09	Acon Laboratory Inc.
18	Bioland Pf/pv Strip	25	Detects Pf/Pv	2-30°C	Oct-09	Jan-10	NIL	-	MAL030708	1-Nov-09	Nano sign Inc

19	First Response Malaria test kit	30	Detects Pf(HRP2)	2-30ºC	Apr-11	NIL	NIL	-	56EI709	1-Oct-09	Premier Medical I nc
20	Dr Greg's Malaria test kit	25	Detects Pf/Pv	2-30ºC	NIL	Sep-09	19th Nov 2009	5,200	C90910MA L	1-Nov-09	Olutex Ltd Lagos
21	SD Bioline Pf (Antigen)	30	Detects Pf(HRP2)	4_30°C	Oct-09	Apr-09	NIL	NIL	82018	1-Nov-09	SD Inc
22	SD Bioline PLDH	30	Detects Pf(HRP2)	4_30°C	Oct-09	Apr-09	NIL	NIL	90017	1-Nov-09	SD Inc
23	Bioland Malaria Pf/Pv (strip)	25	Detects Pf/Pv	2-30ºC	Oct-09	Apr-11	NIL	NIL	MAF02100 09	1-Nov-09	Nano sign Inc
24	ICT Pf Malaria test kit	25	Detects Pf (HRP2)	2_37ºC	NIL	Jun-11	NIL	NIL	32412	1-Nov-09	ICT Diagnostic
25	CTK Pf/Pv	25	DetectsPf/Pv	2- 30ºC	Feb 2014						
26	Bioland Combo	25	Detects Pf/pan	2-30ºC	26/10/ 09	Apr-11	Jan-10	NIL	MAL 021009	1-Nov-09	Codix Inc
27	Clearview Pf	25	Detects Pf(HRP2)	4_30°C	NIL	Aug-11	Sep-10	NIL	32556	1-Sep-10	Vision Biotech
28	Clearview Pf	25	Detects all plasmodium spps (PLDH)	4_30°C	NIL	NIL	Sep-10	NIL	NIL	1-Sep-10	Vision Biotech
29	Onsight Pf	25	Detects Pf(HRP2)	4_30°C	Jul-10	Jun-12	Sep-10	NIL	A21008	1-Sep-10	Amgenix Inc
30	Poct Pf	16	Detects Pf(HRP2)	2-30ºC	Feb-12	Mar-12	Mar-10	NIL	201003022 6	1-Mar-10	Intec Producer Inc
31	ParaHit	10	Detects Pf (HRP2)	4_40°C	19/12/ 08	Dec-10	NIL	NIL	400000182 6	1-Mar-10	NIL
32	Cortez	25	Detects Pf (HRP2)	2-30ºC	NIL	Jun-11	NIL	NIL	10020422	1-Apr-10	NIL
33	Tri malaria	30	Detects Pf(HRP2)	4_27°C	Feb-11	Jul-11	Mar-10	NIL	MPF10020 01	April	Trinition Biotech Nig Ltd Abuja
34	Dialab	30	Detects Pf (HRP2)	2-30ºC	NIL	Feb-12	Apr-10	NIL	82040	April	Dialab Austria
35	IDA Paracheck	25	Detects Pf (HRP2)	4_45°C	NIL	Feb-12	Jan-12	NIL	31817	1-Jul-10	Orchid Biomedical System

V. RESULTS

The Performance of Malaria Rapid Diagnostic Tests using Positive Control Wells

A total of thirty-five (35) malaria RDTs were procured from different malaria RDTs suppliers in Lagos state and other selected States in Nigeria (table one). These selected MRDTs were used to evaluate the performance PCWs. From the results recorded from the performance of PCWs using thirty five MRDTs (27 Histidine Rich Protein 2{HRP2} detecting RDTs, 5 Plasmodium Lactate Dehydrogenase {pLDH} and 3 *P.f/P.v* detecting MRDTs) it was observed that all the HRP2 detecting MRDTs tested positive with PCWs having a positive test intensity ranging from 1+ to 3+, the pLDH detecting MRDTs showed a positive test line intensity of 2+ on the P.f band for all the RDTs, but on the Pan band it showed a line intensity of 2+ for only the SD Bioline combo RDT (control RDT), while the P.f/ P.v detecting MRDTs also showed a positive signal on the P.f band and negative signal result on the P.v band for all the three MRDTs evaluated (table two)

To Determine the Antigen Concentration of the PCWs using highly Characterized Quality Control Samples at Different Parasite Dilutions

The prepared highly characterized QC samples were diluted at different parasite dilutions (50p/ul, 100p/ul, 150p/ul, 200p/ul, 1000p/ul and 2000p/ul) and were then used to evaluate the MRDTs used for PCWs evaluation so as to ascertain the antigen concentration of the PCWs since it was not indicated in PCWs kit pack. From the results recorded after performing the test base on the manufacturer's instruction manual and following Good Laboratory Practice that the procured MRDTs which tested positive to PCWs now showed a negative percentage results at 50 parasites/ul (83.8%), 100 parasites/ul (61.3%) and 150 parasites (25.8%). All MRDTs tested positive at 200p/u/, 1000p/ul and 2000p/ul. This results indicates that the parasite concentration of the PCWs will likely be between the range of 150parasites/ul to 200parasites/ul (table three)

Positive Control Wells Stability Test Using Selected MRDTs

Ten selected MRDTs were kept in the incubators at different temperatures for 30 days and tested with reconstituted PCWs. The results recorded signified that all the MRDTs showed a positive test line intensity ranging from 1+ to 3+ for all the ten MRDTs. The control band also showed intensity of 2+ to 3+ for all the MRDTs (table four)

Table 2: Evaluating the Performance of PCWs on different types of MRDTs

Types Of Mrdts		Results		
Hrp2 Detecting Mrdts	*P.F* Band	Control Band		
Sd Bioline Control	2+	2+		
Carestart Control	2+	2+		
Paracheck	2+	3+		
Clearview	1+	3+		
Dr Greg	1+	2+		
Antec	2+	2+		
Parahit	2+	3+		
R And R	1+	2+		
First Response	1+	3+		
Ict	2+	3+		
Grand Medical	1+	2+		
Dialab	3+	2+		
Tri Malaria	2+	3+		
Bioland	3+	2+		
Fmbassy	2+	2+		
Acon	3+	2+		
Abon Cassette	3+	2+		
Poct	1+	2+		
Abon Strip	2+	3+		
Blue Core	1+	2+		
Onsight	1+	2+		
Highgate	1+	2+		
Micropoint	3+	2+		
Global	2+	3+		
P.F/Ldh Detecting Mrdts		*P.F* Band	Pan Band	Control Band
Ict Combo		2+	0	3+
First Response Combo		2+	0	3+
Bioland Combo		2+	0	2+
Sd Bioline Combo Control		2+	2+	3+
R & R Combo		2+	0	2+
P.F/*P.V* Detecting Mrdts		*P.F* Band	*P.V* Band	Control Band
Lifestyle		1+	0	2+
Accurate		2+	0	2+
Ctk		2+	0	2+

Table 3: Results Names Of Mrdts

	Control 1 Carestart					Control 2 Sd Bioline Hrp2					Global Hrp2 Rdt				
Dilutions/Samples	Qc1	Qc2	Qc3	Qc4	Qc5	Qc1	Qc2	Qc3	Qc4	Qc5	Qc1	Qc2	Qc3	Qc4	Qc5
50p/Ul	0.5+	0.5+	0.5+	0.5+	0.5+	1+	1+	1+	1+	1+	0-	0-	0-	0-	0-
50p/Ul	0.5+	0.5+	0.5+	0.5+	0.5+	1+	1+	1+	1+	1+	0-	0-	0-	0-	0-
50p/Ul	0.5+	0.5+	0.5+	0.5+	0.5+	1+	1+	1+	1+	1+	0-	0-	0-	0-	0-
100p/Ul	1+	1+	1+	1+	0.5+	1+	1+	1+	1+	1+	0-	0-	0-	0-	0-
100p/Ul	1+	1+	1+	1+	0.5+	1+	1+	1+	1+	1+	0-	0-	0-	0-	0-
100p/Ul	1+	1+	1+	1+	0.5+	1+	1+	1+	1+	1+	0-	0-	0-	0-	0-
150p/Ul	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+	0.5+	0.5+	0.5+	0.5+	0.5+
150p/Ul	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+	0.5+	0.5+	0.5+	0.5+	0.5+
150p/Ul	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+	0.5+	0.5+	0.5+	0.5+	0.5+
200p/Ul	1+	2+	2+	2+	1+	2+	2+	2+	2+	2+	0.5+	0.5+	0.5+	0.5+	0.5+
200p/Ul	1+	2+	2+	2+	1+	2+	2+	2+	2+	2+	0.5+	0.5+	0.5+	0.5+	0.5+
200p/Ul	1+	2+	2+	2+	2+	2+	2+	2+	2+	2+	0.5+	0.5+	0.5+	0.5+	0.5+
1000p/Ul	2+	2+	2+	2+	2+	3+	3+	3+	3+	3+	1+	1+	1+	1+	1+
1000p/Ul	2+	2+	2+	2+	2+	3+	3+	3+	3+	3+	1+	1+	1+	1+	1+
2000p/Ul	2+	3+	3+	3+	2+	3+	3+	3+	3+	3+	2+	1+	2+	2+	1+
2000p/Ul	2+	3+	3+	3+	2+	3+	3+	3+	3+	3+	2+	2+	2+	2+	2+

QC Samples	LP SD BIOLINE					MICROPOINT					FIRST RESPONSE					CLEARVIEW				
	QC1	QC2	QC3	QC4	QC5	QC1	QC2	QC3	QC4	QC5	QC1	QC2	QC3	QC4	QC5	QC1	QC2	QC3	QC4	QC5
50p/ul	0-	0-	0.5+	0.5+	0.5+	0-	0-	0.5+	0.5+	0.5+	0-	0-	0.5+	0.5+	0.5+	0-	0-	0-	0-	0-
50p/ul	0-	0-	0.5+	0.5+	0.5+	0-	0-	0.5+	0.5+	0.5+	0-	0-	0.5+	0.5+	0.5+	0-	0-	0-	0-	0-
50p/ul	0-	0-	0.5+	0.5+	0.5+	0-	0-	0.5+	0.5+	0.5+	0-	0-	0.5+	0.5+	0.5+	0-	0-	0-	0-	0-
100p/ul	1+	1+	1+	1+	1+	0.5+	0.5+	1+	1+	1+	0.5+	0.5+	0.5+	0.5+	0.5+	0-	0-	0-	0-	0-
100p/ul	1+	1+	1+	1+	1+	0.5+	0.5+	1+	1+	1+	0.5+	0.5+	0.5+	0.5+	0.5+	0-	0-	0-	0-	0-
100p/ul	1+	1+	1+	1+	1+	0.5+	0.5+	1+	1+	1+	0.5+	0.5+	0.5+	0.5+	0.5+	0-	0-	0-	0-	0-
150p/ul	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+	0-	0-	0-	0-	0-
150p/ul	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+	0-	0-	0-	0-	0-
150p/ul	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+	0-	0-	0-	0-	0-
200p/ul	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+	0.5+	0.5+	0.5+	0.5+	0.5+
200p/ul	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+	0.5+	0.5+	0.5+	0.5+	0.5+
200p/ul	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+	0.5+	0.5+	0.5+	0.5+	0.5+
1000p/ul	2+	2+	2+	2+	2+	2+	3+	3+	2+	2+	1+	2+	2+	2+	1+	1+	1+	1+	1+	1+
1000p/ul	2+	2+	2+	2+	2+	2+	3+	3+	2+	2+	1+	2+	2+	2+	2+	1+	1+	1+	1+	1+
2000p/ul	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	2+	2+	2+	2+	3+	1+	1+	1+	1+	1+
2000p/ul	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	2+	2+	2+	2+	3+	1+	1+	1+	1+	1+

QC Samples/ Dilutions	TRIMALARIA					BIOLAND					POCT					EMBASSY				
	QC1	QC2	QC3	QC4	QC5	QC1	QC2	QC3	QC4	QC5	QC1	QC2	QC3	QC4	QC5	QC1	QC2	QC3	QC4	QC5
50p/ul	0-	0-	0-	0-	0-	0.5+	0.5+	0.5+	0.5+	0.5+	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-
50p/ul	0-	0-	0-	0-	0-	0.5+	0.5+	0.5+	0.5+	0.5+	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-
50p/ul	0-	0-	0-	0-	0-	0.5+	0.5+	0.5+	0.5+	0.5+	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-
100p/ul	0-	0-	0-	0-	0-	0.5+	0.5+	1+	1+	1+	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-
100p/ul	0-	0-	0-	0-	0-	0.5+	0.5+	1+	1+	1+	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-
100p/ul	0-	0-	0-	0-	0-	0.5+	0.5+	1+	1+	1+	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-
150p/ul	0.5+	0.5+	0.5+	0.5+	0.5+	1+	1+	1+	1+	1+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+
150p/ul	0.5+	0.5+	0.5+	0.5+	0.5+	1+	1+	1+	1+	1+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+
150p/ul	0.5+	0.5+	0.5+	0.5+	0.5+	1+	1+	1+	1+	1+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+
200p/ul	0.5+	0.5+	0.5+	0.5+	0.5+	1+	1+	1+	1+	1+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+
200p/ul	0.5+	0.5+	0.5+	0.5+	0.5+	1+	1+	1+	1+	1+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+
200p/ul	0.5+	0.5+	0.5+	0.5+	0.5+	1+	1+	1+	1+	1+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+
1000p/ul	1+	1+	1+	1+	1+	2+	3+	3+	2+	2+	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+
1000p/ul	1+	1+	1+	1+	1+	2+	3+	3+	2+	2+	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+
2000p/ul	1+	1+	1+	1+	1+	3+	3+	3+	3+	3+	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+
2000p/ul	1+	1+	1+	1+	1+	3+	3+	3+	3+	3+	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+

	ANTEC					PARAHIT					DR GREG					R AND R				
	QC1	QC2	QC3	QC4	QC5	QC1	QC2	QC3	QC4	QC5	QC1	QC2	QC3	QC4	QC5	QC1	QC2	QC3	QC4	QC5
50p/Ul	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-
50p/Ul	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-
50p/Ul	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-

100p/Ul	0-	0.5+	0-	0-	0.5+	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-
100p/Ul	0-	0.5+	0-	0-	0.5+	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-
100p/Ul	0-	0.5+	0-	0-	0.5+	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-
150p/Ul	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-
150p/Ul	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-
150p/Ul	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-
200p/Ul	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+
200p/Ul	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+
200p/Ul	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+
1000p/Ul	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+
1000p/Ul	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+
2000p/Ul	2+	2+	2+	2+	2+	1+	2+	1+	1+	2+	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+
2000p/Ul	2+	2+	2+	2+	2+	1+	2+	1+	1+	2+	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+

	ICT HRP2					GRAND MEDICAL					WONDFO					HIGH GATE HRP2				
QC Samples/ Dilutions	QC1	QC2	QC3	QC4	QC5	QC1	QC2	QC3	QC4	QC5	QC1	QC2	QC3	QC4	QC5	QC1	QC2	QC3	QC4	QC5
50p/ul	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-
50p/ul	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-
50p/ul	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-
100p/ul	0-	0.5+	0-	0-	0.5+	0-	0-	0-	0-	0-	0-	0.5+	0-	0-	0.5+	0-	0-	0-	0-	0-
100p/ul	0-	0.5+	0-	0-	0.5+	0-	0-	0-	0-	0-	0-	0.5+	0-	0-	0.5+	0-	0-	0-	0-	0-
100p/ul	0-	0.5+	0-	0-	0.5+	0-	0-	0-	0-	0-	0-	0.5+	0-	0-	0.5+	0-	0-	0-	0-	0-
150p/ul	0.5+	0.5+	0.5+	0.5+	0.5+	0-	0-	0-	0-	0-	0.5+	0.5+	0.5+	0.5+	0.5+	0-	0-	0-	0-	0-
150p/ul	0.5+	0.5+	0.5+	0.5+	0.5+	0-	0-	0-	0-	0-	0.5+	0.5+	0.5+	0.5+	0.5+	0-	0-	0-	0-	0-
150p/ul	0.5+	0.5+	0.5+	0.5+	0.5+	0-	0-	0-	0-	0-	0.5+	0.5+	0.5+	0.5+	0.5+	0-	0-	0-	0-	0-
200p/ul	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+
200p/ul	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+
200p/ul	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+
1000p/ul	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+
1000p/ul	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+
2000p/ul	2+	2+	2+	2+	2+	1+	1+	1+	1+	1+	2+	2+	2+	2+	2+	1+	1+	1+	1+	1+
2000p/ul	2+	2+	2+	2+	2+	1+	1+	1+	1+	1+	2+	2+	2+	2+	2+	1+	1+	1+	1+	1+

QC Samples/ Dilutions	ONSIGHT					CTK *P.f/P.v*					ACCURATE *P.f/P.v*					LIFE STYLE *P.f/P.v* KIT				
	QC1	QC2	QC3	QC4	QC5	QC1	QC2	QC3	QC4	QC5	QC1	QC2	QC3	QC4	QC5	QC1	QC2	QC3	QC4	QC5
50p/ul	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-
50p/ul	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-
50p/ul	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-
100p/ul	0-	0-	0-	0-	0-	0-	0.5+	0-	0-	0.5+	0-	0.5+	0-	0-	0.5+	0-	0-	0-	0-	0-
100p/ul	0-	0-	0-	0-	0-	0-	0.5+	0-	0-	0.5+	0-	0.5+	0-	0-	0.5+	0-	0-	0-	0-	0-
100p/ul	0-	0-	0-	0-	0-	0-	0.5+	0-	0-	0.5+	0-	0.5+	0-	0-	0.5+	0-	0-	0-	0-	0-
150p/ul	0-	0-	0-	0-	0-	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0-	0-	0-	0-	0-

150p/ul	0-	0-	0-	0-	0-	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0-	0-	0-	0-	0-
150p/ul	0-	0-	0-	0-	0-	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0-	0-	0-	0-	0-
200p/ul	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+
200p/ul	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+
200p/ul	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+
1000p/ul	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+
1000p/ul	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+
2000p/ul	1+	1+	1+	1+	1+	2+	2+	2+	2+	2+	2+	2+	2+	2+	2+	1+	1+	1+	1+	1+
2000p/ul	1+	1+	1+	1+	1+	2+	2+	2+	2+	2+	2+	2+	2+	2+	2+	1+	1+	1+	1+	1+

QC Samples/ Dilutions	VIKIA					CORE					BID HRP2 KIT					ACCUCARE				
	QC1	QC2	QC3	QC4	QC5	QC1	QC2	QC3	QC4	QC5	QC1	QC2	QC3	QC4	QC5	QC1	QC2	QC3	QC4	QC5
50p/ul	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-
50p/ul	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-
50p/ul	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-	0-
100p/ul	0-	0-	0-	0-	0-	0-	0.5+	0-	0-	0.5+	0-	0.5+	0-	0-	0.5+	0-	0-	0-	0-	0-
100p/ul	0-	0-	0-	0-	0-	0-	0.5+	0-	0-	0.5+	0-	0.5+	0-	0-	0.5+	0-	0-	0-	0-	0-
100p/ul	0-	0-	0-	0-	0-	0-	0.5+	0-	0-	0.5+	0-	0.5+	0-	0-	0.5+	0-	0-	0-	0-	0-
150p/ul	0-	0-	0-	0-	0-	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0-	0-	0-	0-	0-
150p/ul	0-	0-	0-	0-	0-	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0-	0-	0-	0-	0-
150p/ul	0-	0-	0-	0-	0-	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0-	0-	0-	0-	0-
200p/ul	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+
200p/ul	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+
200p/ul	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+
1000p/ul	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+
1000p/ul	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+
2000p/ul	1+	1+	1+	1+	1+	2+	2+	2+	2+	2+	2+	2+	2+	2+	2+	1+	1+	1+	1+	1+
2000p/ul	1+	1+	1+	1+	1+	2+	2+	2+	2+	2+	2+	2+	2+	2+	2+	1+	1+	1+	1+	1+

	Paracheck Hrp2 Rdt					Abon Cassette					Abon Hrp2 Strip					Carestart Hrp2				
	QC1	QC2	QC3	QC4	QC5	QC1	QC2	QC3	QC4	QC5	QC 1	QC 2	QC3	QC4	QC5	QC1	QC2	QC3	QC4	QC5
50p/ul	0.5+	0.5+	0.5+	0.5+	0.5+	0-	0-	0-	0-	0-	0-	0-	0-	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+
50p/ul	0.5+	0.5+	0.5+	0.5+	0.5+	0-	0-	0-	0-	0-	0-	0-	0-	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+
50p/ul	0.5+	0.5+	0.5+	0.5+	0.5+	0-	0-	0-	0-	0-	0-	0-	0-	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+
100p/ ul	1+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+
100p/ ul	1+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+
100p/ ul	1+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	0.5+	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+
150p/ ul	1+	1+	1+	1+	1+	0.5+	0.5+	0.5+	0.5+	0.5+	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+
150p/ ul	1+	1+	1+	1+	1+	0.5+	0.5+	0.5+	0.5+	0.5+	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+
150p/ ul	1+	1+	1+	1+	1+	0.5+	0.5+	0.5+	0.5+	0.5+	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+
200p/ ul	1+	1+	1+	1+	1+	1+	1+	1+	1+	0.5+	1+	1+	1+	1+	1+	2+	2+	2+	2+	2+

200p/ul	1+	1+	1+	1+	1+	1+	1+	1+	1+	0.5+	1+	1+	1+	1+	1+	2+	2+	2+	2+	2+
200p/ul	1+	1+	1+	1+	1+	1+	1+	1+	1+	0.5+	1+	1+	1+	1+	1+	2+	2+	2+	2+	2+
1000p/ul	2+	2+	2+	2+	2+	2+	2+	2+	2+	1+	2+	2+	2+	2+	2+	2+	3+	2+	2+	2+
1000p/ul	2+	2+	2+	2+	2+	2+	2+	2+	2+	1+	2+	2+	2+	2+	2+	3+	3+	2+	2+	2+
2000p/ul	2+	2+	2+	2+	2+	2+	2+	2+	2+	1+	2+	2+	2+	2+	2+	3+	3+	3+	3+	3+
2000p/ul	2+	2+	2+	2+	2+	2+	2+	2+	2+	1+	2+	2+	2+	2+	2+	3+	3+	3+	3+	3+

Table four: Stability testing of MRDTs Using Positive Control Wells

S/No	Types of MRDTs/ Different Storage Temperatures for 30 days	45°C		50°C		60°C	
		P.f band	Control band	*P.f* band	Control band	*P.f* band	Control band
1	SD Bioloine	3+	3+	2+	2+	2+	2+
2	Carestart	3+	3+	2+	2+	1+	2+
3	Paracheck	3+	3+	2+	2+	1+	2+
4	Bioland	2+	3+	2+	2+	1+	2+
5	Clearview	2+	3+	1+	2+	1+	1+
6	First Response	3+	3+	2+	2+	2+	2+
7	Parahit	2+	3+	1+	2+	1+	1+
8	ICT	3+	3+	2+	2+	2+	2+
9	Acon	2+	3+	1+	2+	1+	1+
10	Embassy	2+	3+	1+	2+	1+	1+

VI. DISCUSSION

Malaria rapid diagnostic tests are potential breakthrough in the provision of accurate diagnosis in remote areas, but wide scale use is hampered by uncertainty over accuracy under field conditions. Positive control wells, which contain recombinant malaria parasite antigens, are novel method for address, there is need for QA. The potential of a commercial available positive control well, reconstituted with blood, was assessed for use in routine monitoring of MRDT sensitivity in a remote malaria-endemic region. When maintained at 4^0C, the wells produced a consistent level of pLDH antigen activity, as detected by pLDH – detecting MRDTs, but activity reduced after cumulative exposure to temperatures likely to be encountered over few months in a malaria-endemic area (Lon *et al*., 2005). This limitation was successfully overcome in the field through centralized and controlled storage. Monitoring of MRDT sensitivity was successfully incorporated into routine supervisory visits to remote clinics. However, improved temperature stability of the wells would enhance their potential. The threshold at which the well's signal reduced MRDT sensitivity requires further investigations. The wells show potentials to overcome an important obstacle to the wide implementations of accurate parasite-based diagnosis and appropriate treatment from research data indicate that false-negative results whether due to poor product quality or undetected damage to products after purchase, are likely in widespread operational use of MRDTs (Lon *et al.,* 2005). Together with effects on patient health workers and patients in MRDT use, undermining attempts to introduce parasite-based diagnosis into routine malaria management. This study carried out by Lon *et al*. (2005), indicate that positive control wells have the potentials to fill an important gap in monitoring of MRDT quality. While the product and test lacked sufficient temperature stability for prolonged uncontrolled storage in the tropics, it was sufficiently stable to be deployed in remote area (Chiodini *et al.,* 2007). A positive control well must be stable enough to be stored and used without significant loss of antigen activity. The reconstituted contents must perform similarly to infected human blood at close to the minimum clinically significant parasite density when placed on the RDT, and the wells must be simple and cheap enough to be adopted into routine malaria control programmes (Lon *et al*., 2005; Gersti *et al.,* 2010).

According to manufacturer-provided product specifications, most RDTs require maximum storage temperatures of approximately 30°C above which test performance maybe compromised, especially if tests are stored for prolonged periods at such temperatures. In sub-Saharan Africa, where RDTs are increasingly being used for malaria diagnosis, ambient temperatures are frequently above 30°C and, therefore, in peripheral health facilities where refrigeration is likely to be unavailable for cool storage of RDTs, high temperature exposure is expected to be common (Okangba *et al*., 2016). The length of time a test has to be stored at the higher than the recommended storage temperatures before performance is affected is variable. Data from the WHO/FIND/CDC product evaluation indicate that (Thiam *et al.,* 2011) most RDTs retain performance after prolonged periods above required storage temperatures while some others deteriorate and a minority improve in performance. It is not clear what level of heat exposure and for how long a test needs to be exposed for a definitive adverse effect on performance. Therefore, monitoring RDTs stored under such conditions is critical to an effective QC system. Such test monitoring could be achieved by positive controls are regularly used to test for RDT performance. Unfortunately, because malaria RDTs require fresh or frozen parasitized

blood and recombinantly produced positive control antigens areas yet unavailable, field monitoring has largely been ignored as a component of a comprehensive RDT quality assurance system. This is despite the importance of guaranteeing the quality of RDTs especially in peripheral health facilities where they are more likely to be used.

Most manufacturers recommend that MRDTs be stored between 2 and 30°C (Jorgensen *et al.*, 2007). However the use of RDTs in remote areas entails storage in tropical and subtropical conditions which may be outside the storage temperature of the MRDT. An ideal MRDT should be able to tolerate temperatures of at least 40°C, with peaks of 50°C, under storage for up to 2 years (WHO, 2015; WHO, 2018). There are limited data on the stability of many MRDTs under such conditions at present, and more extreme conditions may occur temporarily during transport. The stability and sensitivity of products may also vary between lots. It is important that users minimize exposure to high temperatures, and to monitor the performance of each lot (WHO, 2004; WHO, 2009; Okangba, 2019). Transportation of MRDTs from the manufacturer, and road transport within the country is very important in ensuring the preservation of the integrity of the MRDTs for better performance. Prolonged exposure to high temperature and humidity will rapidly degrade RDTs, and may occur when exposed to intense heat (above 30^{o}C and after removal from the envelope or if the envelope is damaged (Moody, 2002; Jorgensen *et al*., 2006; Tavrow *et al*., 2010; Okangba and Oyibo 2019).

The result of this study indicated that the Positive Control Wells have the potential to identify faulty MRDTs and to fill an important gap in monitoring RDTs quality. The CTK Positivia Malaria Ag Rapid Test Assay Control kit with Catalog Number C0010 PCWs (Produced by CTK Biotech, Inc. 10110 Mesa Rim road, San-Diego, CA 92121 USA) used for this study, lacked sufficient temperature stability for prolonged uncontrolled storage in the tropics. The consistency of test line intensity indicates a fair degree of consistency in antigen activity of the wells. Some the RDTs kept in the incubators at different temperatures indicated a relatively weaker signal and negative results when tested with the PCWs . This shows that when MRDTs are exposed to high temperature, it deteriorates it's performance. Consistency between manufactured lots must also be assessed. The use of purified or recombinant antigen may enable more consistency between well lots, if more detailed investigation of antigen activity showed this to be a problem. For the PCWs to be useful, it should produce faintly visible line as close to the minimum acceptable RDT sensitivity, so that use in areas without regular re-supply or temperature control would be possible.

The consistency of the test line intensity indicates a fair degree of consistency in antigen activity of the wells. For the well to be consider useful, the wells should produce a faintly visible test line close to the minimum acceptable RDT sensitivity, so that RDTs failing to achieve this sensitivity can be easily distinguished. However, the MRDTs used in this study to evaluate the performance all showed positive result with a test line intensity clearly above the minimum detectable level (>0.5).The CTK PCWs used for the study didn't indicate the antigenmia level of the wells, so therefore, highly characterized QC samples of different parasiteamia levels ranging from 50p/ul to 2000p/ul were used to ascertain the antigen level of the PCWs . it was observed that QC samples at 50p/ul tested on thirty-five different MRDTs showed 74.3% negative result, while at 100p/ul, 60% of MRDTs used gave a negative results and at 150p/ul, showed 25%, negativity. All the thirty-five showed a positive result when QC sample of 200p/ul was used on them. This indicates that antigenmia level of the CTK PCWs is above 150p/ul.

More data is needed on the relationship between antigen concentration and parasite density, and the clinical significance of low parasities densities, to determine whether the PCWs used will detect deterioration below the critical sensitivity required for malaria case management. A parasite density of 100p/ul has been recommended as a minimum acceptable sensitivity for RDTs (WHO, 2013; Tamiru *et al*., 2015; Okangba, 2019). Setting the antigen content of wells to an equivalent to, or close above, this level would be ideal. The RDT test line would then not be visible once the RDTs had deteriorated beyond the critical level.

The use of Positive Control Wells as QC samples appeared to be a feasible method for monitoring RDT performance. However, these results revealed that, for country-specific programmes standardization of the sample on the specific RDT to be monitored is critical to obtaining desired results. The result obtained from this study is similar to observations reported in the WHO/FIND/CDC RDT product evaluation shows HRP2 tests to be more sensitive than pLDH. It was also demonstrated that failing Malaria Rapid Diagnostic Tests could be identified using PCWs. In addition to their utility as QC samples, PCWs can be used in assessing health workers' ability to perform MRDTs and interpret results. Such a use could double as assessing the competency of health workers and test performance at the same time.

VII. CONCLUSION

In conclusion, this study confirms that positive control wells have a potential place in the case management of malaria, quality diagnosis which would help guide appropriate treatment especially in rural areas. Improved stability of the wells would make this easier, and it must be shown that the PCWs have the right antigen content that will clearly distinguish RDTs that are sufficiently sensitive from those that are not sensitive. .

Therefore, to ensure good performance and reliable results from the use of MRDTs for the diagnosis of malaria especially in endemic country like Nigeria, the PCWs should be included inside each MRDTs pack. This will assist in ruling out MRDTs that are sub-standard and ensuring the potency of the MRDTs before it is used on patient for malaria

parasite detection. The use of PCWs should be couraged in an endemic country such as Nigeria where misdiagnosis, over diagnosis and the consequences of failing to treat malaria can be deadly

COMPETING INTERESTS

The authors declare that they have no competing interests

ACKNOWLEDGEMENT

We thank the entire staff of ANDI Center of Excellence for Malaria Diagnosis/WHO-FIND Malaria Specimen Collection Site, Department of Medical and Parasitology College of Medicine, University of Lagos for their invaluable assistance and constructive contribution to carry out this study.

REFERENCE

[1] Aidoo*, Michael Jaymin C Pateland John W Barnwell (2012). Dried *Plasmodium falciparum* infected samples as positive controls for malaria rapid diagnostic tests. *Malaria Journal* 11:239.

[2] Bells, D., Wongsrichanalai, C. and Barnwell, J.W. (2006). Ensuring quality and access for malaria diagnosis; how can it be achieved? *Nature Reviews. Microbiology* 4: 682- 695

[3] Chiodini,P ,L., Katherine B, Pernille J, John W. B, Katherine K. G, Jenny L, Anthony H. Moody, Audie C, and David Bell (2007). The heat stability of Plasmodium lactate dehydrogenase-based and histidine-rich protein 2-based malaria rapid diagnostic tests. *Transaction of the Royal Society of Tropical Medicine and Hygiene* 101: 331-337

[4] D'Acremont, E.K., Narh, B.S., Epokor, M., Akanpiga, S., Quartey, A.A., and Gyapong, J. (2010). Rapid testing for malaria in settings where microscopy is available and peripheral clinics where only presumptive treatment is available: A randomised controlled trial in Ghana. *British Medical Journal*; pp.340: 930.

[5] Federal Ministry of Health (2015): *National Antimalarial Treatment Guidelines* National Malaria. Abuja, Nigeria.

[6] Gersti, G.A. Zhong, K.J, Loregrove, F.E. Graham, C.M., and Jain, K.C. (2010). Evaluation of the Binax NOW ICT Test versus polymerase chain reaction and microscopy for the detection of malaria in returning travelers *Am. J. Trop. Med. Hyg. 69: 598-592.*

[7] Jorgenson, P., Chantap, L., Rebueno, A., Tsuyuoka, R., Bell, D. (2006) Malaria rapid diagnostic tests in tropical climates: the need for cool chain. *American Journal of Tropical Medicine and Hygiene 74: 750-754*

[8] Lon, C. Mark, E.R., Shelley, A.E., (2005). Clinical features cannot predict a diagnosis of malaria or differentiate the infecting species in children living in an area of low transmission. *Trans. R. Soc. Trop. Med. Hyg* 92:45-49

[9] Mayxay M, Newton P.N, Yeung S, Pongvongsa T, white N J, (2004) Short communication: An assessment of the use of malaria rapid test by village health volunteers in rural Laos. *Trop Med Int Health. 9:325-329*

[10] McMorrow D. P., Kawamoto F, Lin K, Laoboonchai A, Wongsrichanalai C. (2008). A comparism of two rapid immunochromatographic tests to expert microscopy in the diagnosis of malaria *Acta Tropics. 82:51-59*

[11] Moody Anthony (2002). Rapid diagnostic tests for malaria parasite. *Clinical Microbiology Review 15(1): 66-78*

[12] Okangba, Chika C., Charles J. Elikwu,, Emmanuel O Shobowale, Opeoluwa Shonekan, Victor Nwadike, Babatunde Tayoa, Azubuike C. Omeonua, Bibitayo Faluyia, Chiamaka Meremikwua, Oyindamola Faladea, Demilade Osobaa, Tolulope Binuyo, Akinboboye Olutosin (2016). Histidine rich protein 2 performance in determining the prevalence of Malaria among patients presenting with clinical symptoms of Malaria. *Scientific Journal of Pure and Applied Sciences*. 5(1) 339-350

[13] Okangba, Chika. C (2019a). Importance of quality assurance testing of malaria rapid diagnostic test in the case management of malaria. *Scientific Journal of Pure and Applied Sciences*. 8(5) 858-875

[14] Okangba, C. C. (2019b). Evaluating the Performance of Locally Purchased Malaria Rapid Diagnostic Test in the Laboratory, Using Highly Characterized Quality Control Samples International Journal of Research and Innovation in Applied Science (IJRIAS) | Volume IV, Issue V, |ISSN 2454-6194

[15] Okangba, Chika C and Oyibo Wellington Aghoghovwia. (2019). A review on performance assessment malaria rapid diagnostic tests in the field. Scientific Journal of Pure and Applied Sciences (2019) 8(7) 876-895

[16] Oladosu, O. O. and Oyibo, W. A. (2013). Overdiagnosis and overtreatment of malaria in children that presented with fever in Lagos, Nigeria. ISRN Infectious Diseases 914675, 6.

[17] Sani, U.M., Jiya, N.M. and Ahmed, H. (2013). Evaluation of malaria rapid diagnostic test among febrile children in Sokoto, Nigeria. *International Journal of Medical Science ISSN* 2167, 3(5), 434-440

[18] Perkins, B.A., Zucker, J.R., Otieno, J., Jafari, H.S., Paxton, L., and Redd, S.C, (1997). Evaluation of an algorithm for Integrated management of childhood illiness in an area of high malaria transmission in Kenya. *Bull World Health Organ* 75:33-42.

[19] Rennie. W., Phetsouvanh, R., Lupisan, S., Vanisaveth, V., Hongvanthong, B., Phompida, S., Alday, P., Fulache, M., Lumagui, R., Jorgensen, P., Bell, D., and Harvey, S. (2007). Minimising human error in malaria rapid diagnosis: clarity of writteninstructions Health worker performance. *Transact Royal Society of Tropical Medicine and Hygiene,* 101:9-18.

[20] Sani, U.M., Jiya, N.M. and Ahmed, H. (2013). Evaluation of malaria rapid diagnostic test among febrile children in Sokoto, Nigeria. *International Journal of Medical Science ISSN* 2167, 3(5), 434-440

[21] Tamiru, Afework., Lucy, Boulanger., Michelle, A. Chang., Joseph, L. Malone., Michael, Aidoo (2015). Field assessment of Dried *P. falciparum* samples for MRDTs quality control and proficiency testing in Ethiopia. *Malaria Journal*. 14:11

[22] Tavrow, P., Knebel, E., and Cogswell, L. (2000). Operations Research Results: Using quality design to improve malaria rapid diagnostic tests in Malawi. Bethesda, MD: *Published for the U.S. Agency for International Development Bethesda USA, by the Quality Assurance Project.* 9-18

[23] Thiam S, Thior M, Faye B, Ndiop M, Diouf ML, Diouf MB, et al. (2011) Major reduction in anti-malarial drug consumption in Senegal after nation-wide introduction of malaria rapid diagnostic tests. PLoS One 6.

[24] Waverly, R., Rattanaxay, P., Socorro, L., Viengsay, V., Bousay, H., Samiane, P., Portia, A., Mila, F., Richard, L., David, B. and Steven, H. (2007). Minimising human error in malaria rapid diagnosis: Clarity of written instructions and health worker performance. *Transaction of Royal Society of Tropical Medicine and Hygiene.* 101:

[25] World Health Organization (2000). New perspective: malaria diagnosis. Report of a joint WHO/USAID informal consultation 25-27 October 1999. World Organization

[26] World Health Organization (2003). Malaria rapid diagnosis, Making it Work. RS/2003/GE/05(PHL)

[27] World Health Organization (2004). Rapid Diagnostic Tests for malaria: Methods Manual for Laboratory Quality Control Testing. Version 2. World Health Organization, Manila

[28] World Health Organization (2008). Methods manual for laboratory Quality Control Testing of Malaria Rapid Diagnostic Tests. UNICEF/UNDP/World Bank/WHO. Special Programme for research and training in tropical disease (TDR). Foundation for Innovative Diagnosis (FIND) 1216 Geneva, Switzerland

[29] World Health Organization: Malaria Rapid Diagnostic Test Performance; Results of WHO product testing of malaria RDTs: Round 2 (2009). 2010 [http://www.finddiagnostics.org/resource-centre/reports_brochures/malariadiagnostic-test-report-round2.html], Accessed 4 May 2010.

[30] World Health Organization, (2010). Malaria Rapid Diagnostic Test Performance: Summary Results of WHO Product Testing of Mmalaria RDTs: Rounds 1 and 2 (2008–2009). WHO, (2011).Management of Severe Malaria, a Practical Handbook. 3rd ed. Geneva: Global Malaria Programme.

[31] WHO, (2012). Malaria Rapid Diagnostic Test Performance. Results of WHO product testing of malaria RDTs: Round 4. Geneva, Switzerland.

[32] World Health Organization, (2013). Geneva, Switzerland WHO, 2013. World Malaria Report 2013. World Health Organization, Geneva, Switzerland

[33] WHO. World malaria report (2014). Geneva: World Health Organization; 2014.

[34] WHO. (2015).Quality Assurance Manual for Malaria Rapid Diagnostic Test evaluation. 3rd edition

[35] World Health Organization (2016). Rapid Diagnostic Tests for malaria: Methods Manual for Laboratory Quality Control Testing. Version 2. World Health Organization, Manila

[36] World Health Organization (2017). Interim notes on selection of types of malaria rapid diagnostic tests in relation to the occurrence of different parasite species. Regional office for Africa and Western Pacific

[37] World Health Organization 2015. The role of RDTs in Malaria Control. Malaria Microscopy / mRDT. World Health Organization, Geneva, Switzerland.
World Health Organization 2019b. False-negative RDT results and implications of new reports of *P. falciparum* histidine-rich protein 2/3 gene deletions. World Health Organization, Geneva, Switzerland.
World Health Organization 2019a. World Malaria Report 2019. World Health Organization, Geneva, Switzerland.