Failed Initial Quality Control Testing of Procured Malaria Rapid Diagnostic Tests in Lagos State, Nigeria

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Abstract: **Deployment of sound diagnostic tests remains a crucial component of malaria management, prevention and control in Africa. Rapid diagnostic tests (RDTs) address the need for accurate diagnosis of malaria particularly in resource limited settings. Misdiagnosis which remains too frequent resulting in antimalarial treatment (Oladosu and Oyibo, 2013). Malaria rapid diagnostic tests (MRDTs) have the potential of significantly improving the diagnosis of malaria in developing countries, especially where there is no adequate microscopy service for the diagnosis of malaria or act as a back-up to microscopy for inexperience personnel. However, the absolute reliance of these tests remains a problem due to uncertainty of the quality of the test and lack of confidence since there is no regulation and lack of proper quality control measures on ground (Okangba, 2019a). There are different commercially available MRDTs in the market these include SD Bioline®, which detects the antibodies of *Plasmodium falciparum* and *Plasmodium vivax* using recombinant antigen of merozoite surface protein (MSP), Global Device®, Paracheck *Pf*® and Wondfo Biotech® detects Pf histidine-rich proteind (HRP2) and CTK Biotech® detects parasite lactate dehydrogenase (pLDH) in all the species of *Plasmodium spp*. The quality assessment and heat stability of five commercially procured RDTs (SD Bioline®, Global device®, Paracheck®, Wondfo Biotech® and CTK Biotech®). The Quality Assurance (QA) was carried by using prepared Quality Control (QC) samples at different parasitaemia dilutions. The MRDTs failed the initial quality control testing (QC), since none had up to 100% positive as recommended by WHO. SD Bioline® had percentage positive of 83.3% while Global device®, Paracheck®, Wondfo®, and CTK Biotech® had percentage positive of 33.3%, 16.7%, 33.3% and 8.3% respectively. For heat stability testing, the RDTs were stored at different temperatures for 50days: Global device®, CTK biotech® and Wondfo® were kept at 40^{0}C, and 45^{0}C, Paracheck® at 45^{0}C and 50^{0}C, while SD Bioline® was kept at 40^{0}C. They all showed a substantial fall in percentage test line positivity. All the MRDTs gave a 0% positivity expect for SD Bioline, which gave percentage positive of 25% at 40^{0}C temperatures respectively. The marked decline in the performance of the MRDTs can be adversely affected by the high temperatures to which they were exposed to in a tropical country, manufacturer's defects, poor storage facility, mishandling in the course of transportation and use of sub- standard materials in production .There is need for proper regulatory body to regulate the manufacturing and importation of RDTs against any unwholesome practice. Also, there is need to consider the importance of stability of diagnostic test during procurement.**

Key words: **Quality Control, Quality Assurance. Malaria Rapid Diagnostic Test, Failed initial testing, Stability, Temperature, Procure.**

I. INTRODUCTION

Malaria Rapid Diagnostic Tests (RDTs) have a major role in malaria management, particularly in providing blood-based diagnosis in remote locations where microscopy-based diagnosis is unavailable (Okangba *et al.,* 2016). Like other diagnostic pathology tests, various conditions of manufacture, transport, storage and use may impair their accuracy. Malaria RDTs have frequently performed well indiagnostic trials, but unexplained poor sensitivity has also been recorded in field and laboratory trials(Jelinek *et al*., 1999. Iqbal *et al*., 1999, Mankhambo *et al*., 2002, Huong *et al*., 2002, Okangba *et al.,* 2016, Okangba, 2019a). Malaria RDTs, as referred to as immunochromatographic lateral flow devices thatdetect parasite antigen. Capture of dye-labelled „signal" antibody-antigen complex by a fixed „capture"antibody produces a visible line on a nitrocellulose strip, signifying a positive test result. Differentproducts target various antigens specific to plasmodia. Blood, product reagent and labelled antibody antigen complex are drawn along the nitrocellulose-fibre strip by capillary action and flushing with a solution reagent buffer solution.. Performance of malaria RDTs is therefore dependent on several factors, including the rate of flow of blood up the nitrocellulose strip, the adherence of capture antibody (Ab) to the strip, ability of the Ab to bind antigen (Ag), and the integrity of the signal Ab-dye conjugate (Baker *et al*., 2005; Sani *et al.,* 2013). All these are subject to

deterioration in adverse transport and storage conditions, and rates of deterioration and their effect on outcomes can vary between products (Moody, 2002, Murray *et al.*, 2008). .

The relationship between antigen concentration and parasite density can vary with the degree ofsequestration of parasites, the stage of parasite growth, and the persistence of antigen after reduction or elimination of the parasite population. (Baker *et al.*, 2005) The antigen concentration of QC samples with a given parasite density may therefore vary within certain limits, and the parameters used for preparing QC samples must take this into account. Wild parasites rather than cultured parasites are used for preparing the QC samples for the same reason, as the relationship between cultured parasite densityand antigen concentration of the culture medium is likely to vary significantly from that expected invivo. Variation in the structure of some parasite antigens affects binding to antibody (Baker *et al*., 2005). This variation should be taken into account when interpreting failure of tests against samples with low parasite density, and in the choice of QC samples to verify these results.

The QC samples are derived from fresh blood and prepared and stored in a manner designed to minimize loss of antigen or other changes that may affect RDT performance (WHO, 2012).

Malaria rapid diagnostic tests, and rapid tests for other diseases including HIV and Hepatitis B and C,are biological tests that deteriorate on exposure to high temperatures, and deteriorate rapidly onexposure to high humidity. They may also deteriorate through freeze-thawing. To maintain sensitivity,it is important to store in as close as possible to the conditions specified by the manufacturer. Most manufacturers recommend that RDTs be stored between 2 and 30°C. However the use of RDTs in remote areas entails storage of RDTs in remote areas entails storage in tropical/subtropical conditions which may be outside the design parameters of the RDT. To be used in these areas, an ideal RDT should be able to tolerate temperatures of at least 40°C, with peaks of 50°C, under storage for up to 2 years (WHO, 1999). There are limited data on the stability of many RDTs 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 2012, WHO, 2014, Okangba, 2019b)

Reliance on RDTs to guide malaria case management is expected to increase. Therefore a quality assurance system for RDTs is needed to ensure there are good practices related to manufacturing, purchase, transport, storage, and technical use by health workers. A method of monitoring these practices is to implement quality control (QC) procedures at a number of different stages: Prior deploy ment to the field (lot testing) and by health workers prior to use in the field. An integral component of the lot testing is the development and use of quality control samples to test the threshold sensitivity of RDTs to determine if deterioration has occurred To ensure that each lot of a product has the high standards specified for the product by the manufacturer, RDTs should be tested on receipt from a manufacturer prior to use in the field (initial testing) and further testing be performed at a later date on withheld RDTs (long-term testing) (WHO, 2014).This initial lot testing of RDTs will provide some confidence about the quality of RDTs used (WHO, 2012; WHO, 2016).

World Health Organization recommends that RDTs be implemented with a comprehensive quality control strategy. Firstly, RDTs should be purchased from a manufacturer that follows good manufacturing practices (GMP). Secondly, each lot of RDTs should be tested on arrival in the country of use to ensure that the tests were not exposed to extreme temperatures or other conditions that may affect RDT performance. RDT performance is measured by testing known dilutions of parasites (typically 200 and 2,000 parasites/μL) and ten negative quality control samples. WHO also recommends post deployment testing at the health facility level, but these recommendations are less developed. Suggested mechanisms include sentinel site monitoring, increased training and supervision, and teaching healthcare workers problem-solving skills when RDTs are not performing well (Bells *et al.*, 2006; WHO, 2016). Once all these factors have been considered, other parameters are: completeness of the kits (e.g. inclusion of lancets and alcohol swabs) and price. Price alone should not be the determining factor for the procurement of RDTs (WHO, 2012).

The absolute reliance of these tests remains a problem due to the uncertainty of the quality of the test and lack of confidence since there is no regulation and proper quality control testing. Hence the objectives of this study are to assess the performance and carry out a stability testing of some selected malaria RDTs using quality control samples.

II. MATERIAL AND METHODS

2.1 Study Site and Sample Collection

The malaria RDTs used for this study were commercially procured from the open market: Ojax Medics Ltd Iga idunganran, Isale-Eko, idumato- Lagos, Lagos state, Ubastic itire road, Surulere, Codix Pharma Ajao Isolo, Acon laboratory inc, Bundi international diagnostic Ltd, Lascon Pharma Ltd, Global Nig Ltd. Some were imported directly from the manufacturer: SD Bioline and ICT are from South Africa. The quality assurance for these MRDTs were carried out at 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.

2.2 Ethical Consideration

The protocol received ethical approval from the ethic and experimental committee of the College of Medicine University of Lagos and signed informed consent was sought from each individual who donated their blood for the preparation of *P. falciparum* quality control panels.

2.3: Laboratory-based initial Quality Control testing of MRDTs from commercial outlets using four Quality Control Panels

The MRDTs used for this study were procured from commercial outlets namely; Mushin, Idumota, Yaba, Isale Eko, Ajao Estate and Isolo in Lagos State (table one). Each MRDT kits were checked for any sign of moisture and the colour of the dessicant that came with each kit was checked for colour changes. Four different Pf highly characterized archived QC panels (A-D), each at dilutions of 200 parasites/µL of blood and 2000 parasites/µL of blood were selected. For each of the samples with dilution 200 parasites/µL of blood, 2 malaria RDTs of each of the different types were used, while for each of the samples with dilution 2000 parasites/µL of blood one Malaria RDT of the different types were used to perform malaria test. Also ten (10) highly characterized archive malaria negative QC panels (I-R) were used for each of the different RDTs (Figure one). The QC panels were made from clinical blood samples of *P. falciparum* that were diluted to 200p/µL and 2000p/µL using standard protocol (WHO, 2012). The procedure for performing the malaria RDTs was strictly adhered to, according to manufacturer' instructions and results were interpreted.

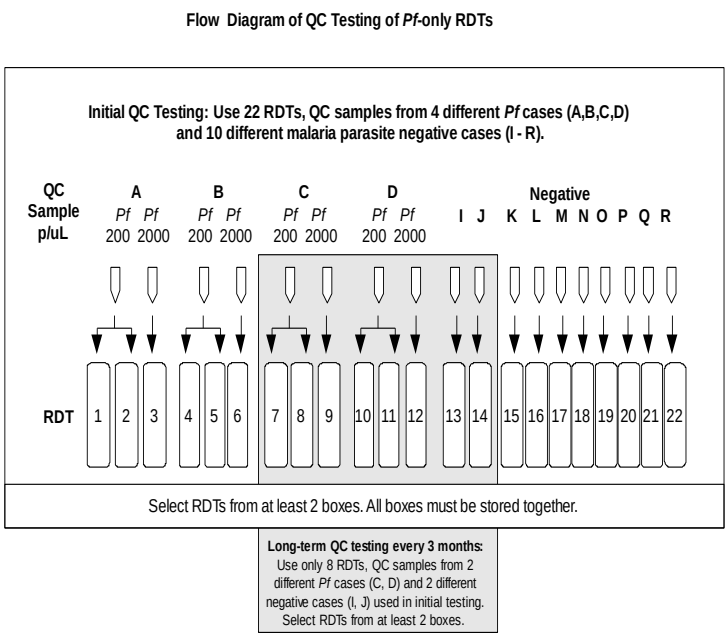

Figure one: Flow Diagram of QC Testing of Pf-only MRDTs

Source: WHO-FIND, 2008

Table one: Manufacturer's Information on Procured MRDTs from the Open Market in Lagos, Nigeria

S/No	Name of MRDTs	Number of Test	Principle	Storage Temp (°C)	Manufac-turer's Date	Expiry Date	Lot Number	Manufacturer's Address
1	CTK	25	Detects pLDH	2-30	March 2007	Sept 2008	F0327PZ	Lascon Pharmaceutical Ltd Lagos
2	Wondfo	50	Detects HRP2	4-30	July 2007	October 2009	W377WIW	46/48 Iga Str. Idumota, Lagos
3	Paracheck	25	Detects HRP2	4-45	Jan 2008	Dec 2009	31074	Lascon Pharmaceutical Ltd Lagos
4	Global	25	Detects HRP2	2-30	October 2005	Oct 2008	MAL710002	Lascon Pharmaceutical Ltd Lagos
5	SD Bioline	25	Detects MSP	2-30	Nov 2009	May 2011	018090	Lascon Pharmaceutical Ltd Lagos
6	ICT (Stock)	30	pLDH	4-30	Jan 2009	Dec 2010	0189060	C.C. Obi Ltd Yaba, Nigeria

2.3.1. Performing the malaria RDTs

Test the RDTs as per manufacturer instructions. Use a timer to record all steps exactly as per manufacturer instructions. Read RDT results within the manufacturer recommended time. Results were interpreted according to colour intensity of the test lines. The colours were graded as follows: 0= No test line observed (Negative), 1+ (Positive with very faint test line), 2+ (Positive equal to test line), 3+ (Strong positive, greater than test line). All results must show the control line for the results to be considered valid. A result without the control line showing is considered an invalid result and should be repeated immediately

2.3.2 Preparation of a blood film for malaria microscopy

Malaria diagnosis by microscopy was carried out in accordance with WHO protocol (WHO, 2000) with minor modifications. Thin and thick blood films were made on the same frosted end grease free slide, with date, participant study 1D number (DD/MM/YY) written on the frosted end of the slide using soft lead pencil. For thick film, 12µL of blood taken with an adjustable micropipette (P20 Pipetman, Gilson)

was spread over a diameter of 15mm, while 2µL of blood taken with an adjustable micropipette (P20 Pipetman, Gilson) was used for thin film. Duplicate slides were made and labelled appropriately. Thin film was fixed with absolute methanol and air dried. Malaria Blood Films (MBFs) were stained after 24 hours with 3% Giemsa stain working solution (pH 7.2). Allow the thick film to dry in a flat, level position protected from flies, dust and extreme heat. Malaria blood stained films were read at ANDI-Centre of Excellence, College Medicine University Lagos, using standard protocol.

The absolute parasite density per µl of blood was calculated using the formula below.

$$\frac{\text{\# of parasites counted x Total leukocytes count}}{\text{\# of leucocytes counted}}$$

2.4 Heat Stability Testing on Commercially Procured MRDTs

Five RDTs were studied. These RDTs were kept at various temperatures for 50 days. At the start of the study, the incubators were stabilized at the specific or required temperature for 3 days before the malaria RDTs to be tested were placed inside. Three RDTs: CTK Biotech®, Global device® and Wondfo® were kept at 40°C and 45°C while Paracheck® was kept at 45°C and 50°C and Bioline® at 40°C. After 50 days, the RDTs were removed from the incubator and were brought to room temperature for 1-2 hours immediately before testing, and were tested according to the manufacturers' instructions. The performances of the RDTs were examined using negative Quality Control samples. The Pf QC samples used were of dilution 200p/µl and 2000p/µl taken from WHO specimen bank at ANDI Center of Excellence for Malaria Diagnosis/WHO-FIND Malaria Specimen Collection Site, Department of Medical Parasitology College of Medicine, University of Lagos, Nigeria.

III. RESULTS

3.1 Performance of Procured MRDTs

Five MRDTs procured from the open market were evaluated using highly characterized quality control panels. None of the five evaluated MRDTs gave 100% sensitivity at dilutions 200p/µl and 2000p/µl for the initial quality control testing. The five RDTs; Paracheck®, Wondfo®, Global device®, CTK Biotech® and SD Biotech® gave percentage (%) positivity of 16.7%, 33.3%, 33.3%, 8.3% and 83.3% respectively. The negative QC samples were 100% negative for all the RDTs (Paracheck®, Wondfo®, Global device ®and CTK Biotech®) except for SD Bioline® which was 80% percentage negative

Table 3: Initial Quality Control Testing of Procured MRDTs using Highly Quality Control Panels

QC Samples	A			B			C			D				Negative Samples
QC Samples Dilution	200a	200b	2000	200a	200b	2000	200a	200b	2000	200a	200b	2000	Control	Negative Samples MNI – MN10
Types of RDTs														
ICT(Stock RDT) Combo	Pf2+ Pan 1+	Pf2+ Pan 1+	Pf3+ Pan 1+	Pf2+ Pan 1+	Pf2+ Pan 1+	Pf2+ Pan 1+	Pf2+ Pan 2+	Pf2+ Pan 2+	Pf3+ Pan 2+	Pf3+Pan 2+	Pf3+ Pan 2+	Pf3+ Pan 3+	3+	-ve
Global Device	-ve	1+	-ve	-ve	-ve	1+	-ve	1+	-ve	-ve	-ve	1+	3+	-ve
Paracheck	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	1+	3+	-ve
SD Bioline (Antibody)	1+	1+	1+	1+	-ve	-ve	1+	-ve	-ve	1+	1+	1+	3+	-ve
CTK Biotech Malaria Ag Test	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	3+	-ve
Wondfo Biotech	-ve	-ve	1+	-ve	1+	1+	-ve	-ve	1+	-ve	-ve	1+	3+	-ve

Table 4: Percentage Positive /Negative of initial QC testing of the five malaria rapid diagnostic tests (MRDTs)

	Positive Samples		Negative Samples (%)	Initial Total Positive (%)
RDTs	200p/µl (%)	2000p/µl (%)		
CTK	0	25	100	8.3
Paracheck	0	50	100	16.7
Wondfo	12.5	75	100	33.3
Global	25	50	100	33.3
SD Bioline	5	100	80	83.3
(Stock)	100%	100%	100%	100%

Table 5: Percentage Positive of Heat Stability test for 5 MRDTs on QC samples of 200p/µL and 2000p/µL dilutions at various temperatures above the manufacturers' recommended temperatures

Parasite/µL		200p/µL			2000p/µL	
	40°C %	45°C %	50°C %	40°C %	45°C %	50°C %
CTK Biotech ® (CTK Biotech Inc. USA)	0	0	-	0	0	-
Paracheck Pf ® (Orchid Biochemical Systems, India)	-	0	0	-	0	0
SD Bioline ® (Standard Diagnostics Inc. Korea)	12.5	-	-	50	-	-
Global Device ® (Global device Ltd USA)	0	0	-	0	0	-
Wondfo ® Biotech Co Ltd	0	0	-	0	0	-

IV. DISCUSSION

WHO (2008), algorithm for RDTs Quality Assuranc (QA) testing states that for a RDT kit to pass ,both initial and longtime quality control testing, the percentage positive must be 100%. However, the result from the study showed that the five locally purchased malaria rapid diagnostic test MRDTs failed the QA test at the initial QA testing. Among the 5 MRDTs used (Paracheck®, Global device®, Wondfo biotech®, CTK Biotech®, and SD Bioline®), SD Bioline® (MSP-based) RDTs showed some false negative result on the *Plasmodium falciparium* (Pf) QC samples at 200p/µl dilution and these samples were positive on ICT Combo® (Stock) RDT. This suggests that the threshold of detection of the MSP-RDTS was low compared to the stock RDT, this was also stated by WHO. (2008). SD Bioline also gave the highest percentage positive of 83.3% compared to Paracheck®, Global device® Wondfo biotech®, and CTK Biotech® RDTs that gave percentage positive of 16.7%, 33.3%, 33.3% and 8.3% respectively.

The Merozoite Surface Protein-based RDT also gave a false positive result of 20% with the some of the negative QC panels that were confirmed by microscopy (being the "gold standard") and ICT Combo (Stock RDT) to be negative while the other procured MRDTs were all negative. The explanation to this is that the SD Bioline® RDT detects antibodies and there is possibility of long persistence of antibody in the circulation after treatment which gave a false positive result unlike the other 4 MRDTs that detects antigens (pLDH and HRP2). There was a decline of the percentage positivity during the stability testing for all the 5 MRDTs used. This suggests that the deterioration could be as a result of substandard materials used in producing these kits or poor storage facility and exposure to heat which could possibly reduce sensitivity and shell life of the kit (Tekola *et al*., 2008)

In heat stability testing of 5 procured MRDTs, the MSP-based RDTs stored at 40°C showed percentage positive of 25% while Wondfo biotech®, Global device® and CTK Biotech® stored at 40°C and 45°C and Paracheck® stored at 45°C and 50°C all showed percentage positive of 0%. This result is different from the study carried out by Chiodini *et al*., (2007) where HRP2 based RDT tested gave 100% positivity and pLDH based RDT fell well below 80% positivity/ sensitivity. This poor performance may be due to the fact that heat induces denaturation of antibodies in the test membrane and this may thus prevent their binding to the target antigen (Chiodini *et al*., 2007). Another possibility is damage to the nitrocellulose membrane forming the strip, changing its flow characteristics or causing the antibody to detach from the membrane. Damage of the membrane could be the cause of reduced sensitivity. Different membrane products may account for some between-product variability (Bell *et al.*, 2006; Chiodini *et al*., 2007; Okangba, 2019b).

The effect of temperature as illustrated in heat stability study, has shown that in our country, Nigeria and in Africa where the temperature is usually hot, RDTs stored in warehouses and other storage facilities tend to lose their sensitivity, and this might be as a result of deterioration of monoclonal antibody and antibody dye used in the production of the RDTs. Mismanagement could also affect the sensitivity and specificity of the RDT and defects in the device membrane as stated by Reyburn *et al*. (2007).

Through-out this study, the controls bands were seen in all the MRDTs. This confirms the integrity of the antigen gold conjugate indicates the visible lines, but does not confirm the ability of the RDTs parasite antigen and antibodies. The appearance of the control line in all the MRDT kit can mislead the end user with the fact that the test was working satisfactorily, when in fact, its performance is well below acceptable levels. The test and control lines were likely to have different sensitivity to heat in the case of these five locally purchased RDTs. Among the locally purchased MRDTs, MSP-based antibody detection malaria RDT can only be useful in providing epidemiological information on community wide exposure, to malaria, particularly in low transmission area for evaluating the effectiveness of control program. However, using MSP-based RDTs for the diagnosis of malaria in hospitals is not reliable, as it was seen that the test gave false positive and false negative results. These false results have medical consequences such as wrong diagnosis by the clinicians which can result to loss of credibility of health services, prolongation of illness, abuse of drugs, drug resistance and increase in morbidity and mortality.

Malaria RDTs can only be considered for use in the diagnosis of malaria in the field if they are able to work day in day out

at a high level of reliability under the prevalent conditions, notably high ambient temperature. Generally, specific factors that need to be considered in introducing MRDTs should include performance characteristics, operational characteristics and cost (Murray *et al*., 2008) However, the results from this study shows that the performance characteristics of the five local MRDTs was low because of its inability to pass 100% initial QC testing and also their operational characteristics were poor because of the difficulty encountered in the course of carrying out this test, and the MRDTs inability to withstand high temperature.

Further work is required for all the five local MRDTs to define the temperature and true parameters within which the MRDTs can be expected to perform satisfactorily. The inclusion of a temperature effect indicator, ideally on the individual RDTs or on their packaging should be considered. This study also highlights need for standardized product testing, quality control by the manufacturer and continuous external quality assessment when the RDTs are in routine clinical use. Good storage and quality control need to be established in all situations where RDTs are deployed. There is need to also consider the importance of stability of diagnostic test during procurement.

V. CONCLUSION

In conclusion, from the results obtained in the investigation carried on five locally purchased MRDTs, it can be deduced that the five locally MRDTs purchased are not suitable for malaria diagnosis. This may be due to lack of regulation governing the importation of these kits, suppliers are not health personnel and they do not know the implication of misdiagnosis and exposure to high temperature. The use of these RDTs should be discouraged in an endemic country such as Nigeria where the consequences of failing to treat malaria can be grave.

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