Evaluating the Performance of Locally Purchased Malaria Rapid Diagnostic Test in the Laboratory, Using Highly Characterized Quality Control Samples

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Abstract: **- A total of 36 Malaria Rapid Diagnostic Tests (MRDTs) were evaluated in the year 2007 using four highly characterized Quality Control (QC) samples at 200p/ul and 2000p/ul dilutions to assess their performance. Twenty-three MRDTs were not locally purchased in the open market in Lagos but were gotten directly from the manufacturers. Malaria Rapid Diagnostic Tests, out of the 23 MRDTs 19 of them are Histidine rich protein based MRDTs while 4 are Plasmodium lactate dehydrogenase based MRDTs 17 (73.9%) MRDTs of the IMRDTs passed while 6 (26.1%) failed Quality Assurance (QA) testing. Thirteen were locally purchased MRDTs (LPMRDTs), only 1 (7.7%) of LPMRDTs passed, while the remaining 12 (92.3%) failed QA testing. Imported MRDTs had mean signal strength of 1.14±0.56 at 200p/µl and 2.26±0.58 signal strength at 2000p/µl, while the LPMRDTs had a mean signal strength of 0.28±0.49 at 200p/µl and0.79 ±0.75 at 2000p/µl. In the year 2013, quality assurance of locally purchased was repeated using 30 MRDTs (22 HRP2 based and 8 pLDH based) which were part of the MRDTs tested in 2007 were tested using highly characterized QC samples. All the MRDTs tested using 200p/ul and 2000p/ul dilutions passed QA testing (100% sensitive and specific). The mean signal strength at 200p/ul and 2000p/ul were high. There was no significantly difference in the signal strength and performance of the malaria RDTs (P < 0.001). For heat stability testing, all the MRDTs that had 100% sensitivity during the initial QA testing were stored at different temperatures (35°C, 40°C and 45°C) for 6 months. All the MRDTs had 100% positivity. There was a significant improvement in the performance of the locally purchased MRDTs in the year 2013 this result shows that the implementation of MRDTs product testing by WHO has made a great impact in the quality of MRDTs sold in the open market in lagos, Nigeria.**

I. INTRODUCTION

The World Health Organization estimates that 3.3 billion persons were at risk of acquiring malaria in 2006, with 247 million of these developing clinical malaria (86% in Africa), and nearly 1 million (mostly African children) dying from the disease. Malaria remains endemic in 109 countries, and while parasite-based diagnosis is increasing, most suspected cases of malaria are still not properly identified, with accurate diagnosis and disease monitoring consequently remaining elusive (WHO,2010).The adoption and use of expensive artemisinin-based antimalarial therapies in the past few years is unprecedented but has not been matched by a similar increase in parasitological confirmation of malaria diagnoses. Targeted treatment is important, not only to limit unnecessary dispensing of antimalarial treatment but also to allow judicious use of these precious, life-saving medicines, for which the supply of raw materials is decreasing because of reduced cultivation of *Artemisia annua* (Bastiaens *et al.,* 2011)

WHO recommends that malaria case management be based on parasite-base confirmation of malaria in all cases, through quality-assured diagnosis in all settings before treatment commences. Treatment solely on the basis of clinical suspicion should only be considered when a parasitological diagnosis is not accessible (WHO, 2009) . Prompt diagnostic confirmation of malaria can be achieved through good quality microscopy. Since this is not feasible in many situations, quality-assured malaria Rapid Diagnostic Tests (RDTs) represent suitable alternatives for the diagnosis of *Plasmodium falciparum* infections, and a number of product scan also detect most cases of non-falciparum malaria.Malaria RDTs are lateral-flow tests based on interactions of biological agents (antibodies and antigens attached to or flowing along a nitro-cellulose strip.

However, the functioning and accuracy of RDTs can be affected by several factors, including manufacturing defects, storage, transport, operator error (Tavrow *et al*., 2000), and antigenic polymorphism (Baker *et al*.,2005) and damage to the RDT itself with storage under field conditions or during transport. RDTs are vulnerable to extremes of temperature and high humidity, thus stability of the kit affects its sensitivity (Jorgensen *et al*., 2007). They are therefore sensitive to degradation by heat and humidity. Heat can damage RDTs through de-conjugation of the antibody-dye conjugate, detachment of the bound antibody from the nitrocellulose, loss of ability of the antibody to bind to antigen, and degradation of the nitrocellulose strip. While exposure to humidity can be prevented by packaging in moisture-proof envelopes, if RDTs are stored at temperatures exceeding the recommended temperature it is likely that loss of sensitivity will occur and the shelf-life of the RDTs will be reduced (Jorgensen *et al.,* 2007).

In addition, national health authorities should take the following fact into consideration when selecting appropriate malaria RDTs for procurement: Stability requirements at temperatures of intended storage, transport and use, ease of use and training requirements by the health workers. Once all these factors have been considered, other parameters should be also evaluated, such as completeness of the kits (e.g. inclusion of lancets and alcohol swabs). Malaria diagnostic tests need to be highly accurate because false negative and false positive diagnosis have medical , social and economic consequence such as prolongation of illness, increase in morbidity and mortality, and loss of credibility of health services (Reyburn *et al*., 2004). The storage and use of malaria rapid diagnostic tests (MRDTs) in remote areas presents a new challenge to many health systems. Exposure of MRDTs to high temperatures has the potential to degrade RDTs. Most manufacturers recommend storage between 4-30^{o}C, and shelf life is based on this assumption, but refrigeration and air-conditioning are commonly unavailable in malaria endemic areas where RDTs are intended for use (Chiodini *et al.,* 2007).

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. Hence the objectives of this study are to evaluate the performance of imported and locally purchased MRDTs and to assess the suitability and stability for use in the field before their expiration date.

II. MATERIALS AND METHODS

Study Areas

Two hospitals in two areas, Amukoko Ijora in Apapa LGA and Lekki in Etiosa LGA, with similar climatic and geographic features were selected for this study. Both areas are densely populated and are near to the Atlantic Ocean and Lagos lagoon in Lagos, Nigeria.

Study Sites

The field study for samples collection was conducted at St. Kitzito's Hospital Lekki and St Matthew Hospital Amukoko Lagos State due to high prevalence of malaria parasite in the area. The local MRDTs were purchased in the open markets (Mushin,Yaba and Isale-Eko Idumota) in Lagos, while the imported RDTs were purchased directly from the manufacturers in South Africa, India, Austria and U.S.A. Blood were collected after informed consent were obtained from all participants and laboratory analysis were carried out at WHO collaborating centre for malaria RDT, International Malaria Microscopy Training Centre, Tropical Disease Research Laboratory, Department of Medical Microbiology and Parasitology, College of Medicine, University of Lagos, idi- Araba, Lagos, Nigeria.

Ethical approvals

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 samples.

Enrolment of study participants

Patients that visited the clinic showing clinical signs of malaria and who consented to participate in the study were recruited. The study participants were given study numbers and personal identifiers removed to ensure confidentiality. Access to study records were restricted. The inclusion criteria were: (a) patient with auxiliary temperature >37.5^{o}C; (b) No history of intake of antimalaria in the last two weeks; (c) patient must be above seven years. The exclusion criteria were: (a) severe anaemia; (b) Hepatitis B/C and HIV 1& 2 positive patients; (c); Patients < seven years.

Collection of Blood Samples

Blood samples will be collected from patients that meet all the inclusion criteria. The blood will be collected from veinpuncture and at least 3mls of blood will be collected into EDTA tubes and 2mls in plain bottle. The EDTA tubes will be mixed thoroughly by gentle inversion of the tube. Each tube will be labeled with the patient number and date of blood collection (DD/MM/YY), using a marker pen. The blood will be used to prepare smears, make blood spots on filter, carry-out RDT test and antigen quantification after collection.

Laboratory Methods

Preparation of Blood Films for Parasite Diagnosis/ Quantification

Thin Smear Preparation

2µL of blood will be dropped near the frosted end of a grease free clean slide using an automated micropipette. Another "speader" slide will be held with polished edges at a 40-45^{0} angle and then drawn into the 2µL of blood. The blood will be allowed to spread almost to the width of the slide. The "spreader" slide will be rapidly and smoothly pushed to the opposite end of the slides. The smear will be allowed to dry at room temperature. The smear will have feathered edge-the area where the microscopic examination will be performed on the stained smear.

Thick Smear Preparation

Thick smear will need 12µL of blood with a diameter of approximately 12mm. The corner of another slide will be used to spread the blood using a circular motion until the proper density is achieved. The smear will be allowed to dry in a flat level position at room temperature for 8 to 12hours. Both smears will be done a slide, and each patient will have 2 slides. The slides with both smears will be labeled using a lead pencils to write the patient number and date at the tail end of the slide.

Both smears were protected from flies, dust and extreme heat. The thin smear will be fixed in methanol. Both films will be stained with 3% Giemsa stain for 45minutes. The slide will be

observed under the microscope using x100 magnification (oil immersion objective) for malaria parasites.

Determining Parasitaemia

With tally counters, one will be used for asexual stage of the parasite and the other for white blood cells (WBC) count. The parasites observed will be counted simultaneously with WBCs up to a total of 500 WBCs. A malaria blood film will be considered negative when 100 High Power Field (HPFs) is scanned and no parasite is seen.

PREPARATION OF QUALITY CONTROL SAMPLES

The preparation of quality control (QC) dilution for testing RDTs according to WHO(2008), manual requires parasite densities sufficiently high to allow preparation of dilutions of 200 up to2000 or 5000parasites/µL.The samples for dilution are parasite- free donor blood. The blood samples that will be used will be screened for malaria parasite, hepatitis Band C, HIV 1 and 2, and those that are positive will be discarded or excluded from the study. Donor preparation will commence 2-3 hours prior to dilution process. A finger prick blood sample from the donor will be used to prepare the blood smears and to perform the RDTs (WHO, 2008).

Dilution of Parasitized Blood using "PARASITE-FREE" Donor Blood

For dilution of *Plasmodium*-infected patient blood, "parasite-free" blood will be prepared by centrifuging O^- or O^+ whole blood and replacing the O^+ or O^- plasma by AB^+ plasma (this ensures compatibility with all patient blood groups). Alternatively, blood from a donor having the same blood group as the patient recruited can be used. The whole blood will be screened for plasmodium species by two microscopist, hepatitis B & C and HIV I and II before it will be used (WHO, 2008).

The parasitized blood will be characterized for species and parasite density by thick and thin film analysis by two microscopist, using a discrepancy limit of 20% and white blood cell count will be estimated. The mean parasite density will be used to calculate the dilution steps down to a high-level (2000p/µl), and a low-level parasite density (200p/µl). The dilution will be prepared initially in a small test volume of 1ml (900µl of donor blood plus 100µl of parasitized blood), to check for the absence of agglutination. If clumping occurs, another donor blood will be used to dilute the parasitized blood, and clumping is checked again. Larger volumes of dilution will be prepared, the absence of agglutination is checked again, different dilutions (200p/µL and 2000p/µL) of the blood will be prepared. The diluted blood will be aliquoted in 50µL volume into pre-lobule cryotubes and immediately frozen at -80^0C. These samples will be used as quality control samples in subsequent tests (WHO, 2008).

PRODUCT TESTING OF MALARIA RDTs USING QC SAMPLES

Malaria RDTs procured from the manufacturers and locally purchased ones will be tested immediately, prior to use in the field and long-time testing will be performed on some selected Malaria RDTs that performed well during the initial Qc testing. The RDTs that will be used for this testing are PfHRP2 and pLDH detecting RDTs (WHO, 2008).

Initial Quality Control Testing

Each test kit were checked for any sign of moisture and the colour of the dessicant that came with each kit were aslo checked for colour changes. Four different highly characterized Pf QC samples (A-D), each with dilutions of 200 p/µl and 2000 p/µl were selected. For each of the samples with dilution 200 p/µl, 2 test kits of each of the 36 different MRDTs were used, while for each of the samples with dilution 2000 p/µl one test kit of the 36 different MRDTs (32HRP2 detecting and 4pLDH detecting MRDTs) were used to perform the quality assurance test. Ten (10) negative QC samples (I-R) were used on each of the 36 different MRDTs. The procedure was strictly adhered to, according to manufacturer' instructions insert.

Heat Stability Testing of Locally Purchased and Imported MRDTs

Thirty-six MRDTs were assessed. The MRDTs that had 100% sensitivity in the initial QA testing were used for heat stability testing and they were kept at various temperatures (35^{o}C, 40^{o}C and 45^{o}C)for 60 days. At the start of the study, the incubators were stabilized at the specific or required temperature for 3 days before the MRDTs to be tested were placed inside. The MRDTs 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 MRDTs were examined using Quality Control samples. The Pf QC samples used were at 200p/µl and 2000p/µl dilution and were taken from WHO specimen bank in Department of Medical Parasitology College of Medicine, University of Lagos, Nigeria.

Data Analysis

The data generated from the study were analyzed using EPIINFO 2002 statistical software (CDC Atlanta, USA). Tests for associations and differences were done by chi square analysis. Test of statistical significance was set at P value less than 0.05 at 95% confidence interval

III. RESULT

A total of 36 Malaria RDTs were tested for their performance using known parasitized blood samples at 200p/µl and at 2000p/µl dilutions. The imported Malaria Rapid Diagnostic Tests (IMRDTs) used for the quality assurance testing were 19 (59.4%) HRP2 based and 4 (100%) pLDH based MRDTs, while the locally purchased MRDTs (LPMRDTs) used were

13(40.6%) for HRP2 based and none for pLDH based MRDTs (table1). The IMRDTs performed better than the LPMRDTs at 200 parasites/ul and at 2000parasites/ul. Most of the LPMRDTs did not show any signal strength at 200parasites/ul (fig 2 and 3). The positivity rate for IMRDTs and LPMRDTs was 17 (73.9%) and 1(7.7) respectively while the negativity rate was 6 (26.1%) and 12 (92.3) respectively (table 2). Positivity rate for HRP2 based MRDTs for IMRDTs and LPMRDTs was 15(78.9) and 1(7.7) respectively while the negativity rate was 4 (21.1) and 12 (92.3) respectively (table 3a and fig 4). For pLDH based MRDTs for IMRDTs the positivity and negativity rate was 2 (50%) and 2 (50%) respectively. There was no LPMRDTs (table 3b and fig 4). The HRP2 based MRDTs mean signal strength for IMRDTs was 1.14±0.56 at 200parasites/µl and 2.26±0.58 at 2000parasites/µl while mean signal strength for the LPMRDTs was 0.28±0.49 at 200parasites/µl and 0.79±0.75 at 2000parasites/µl (table 4).

For heat stability testing, all the MRDTs that had 100% sensitivity at the initial testing, still had 100% sensitivity after been subjected to high temperature which differs from the manufacturer recommended temperature

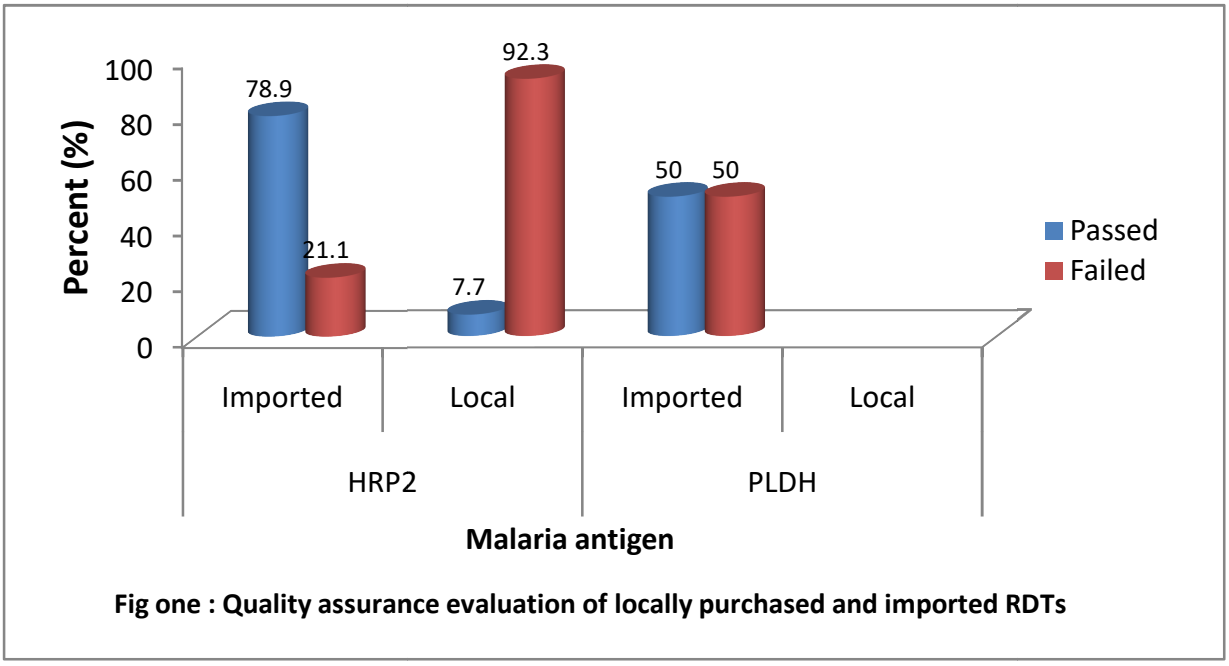


Fig one : Quality assurance evaluation of locally purchased and imported RDTs

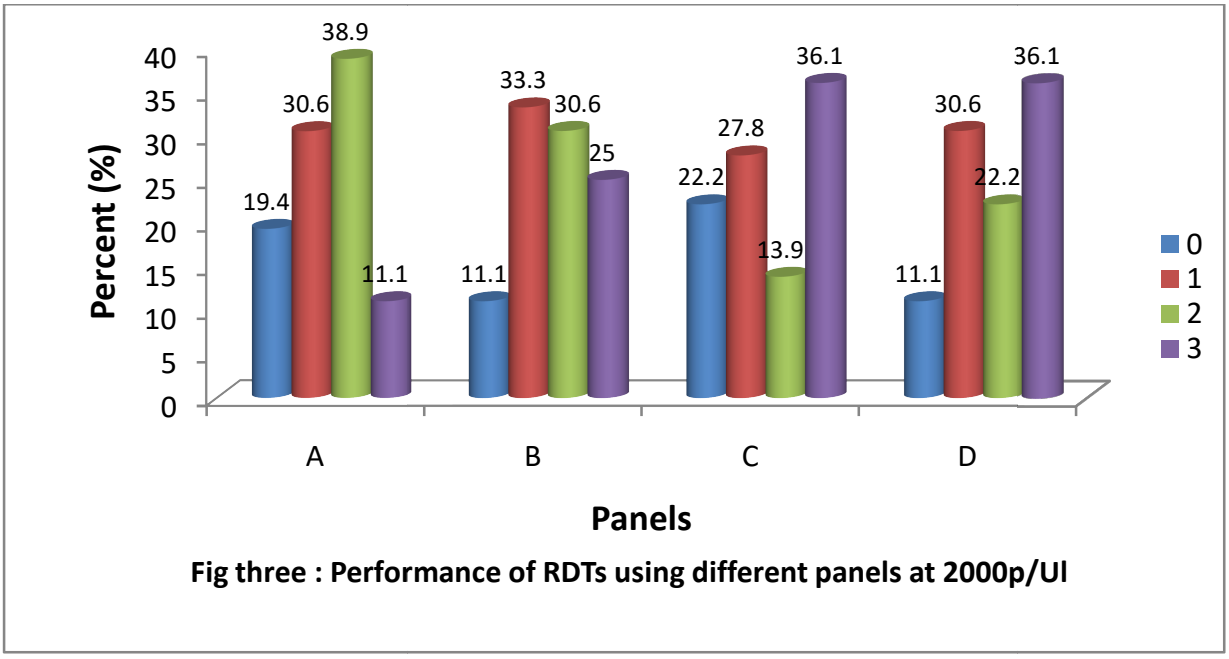


Fig three : Performance of RDTs using different panels at 2000p/Ul

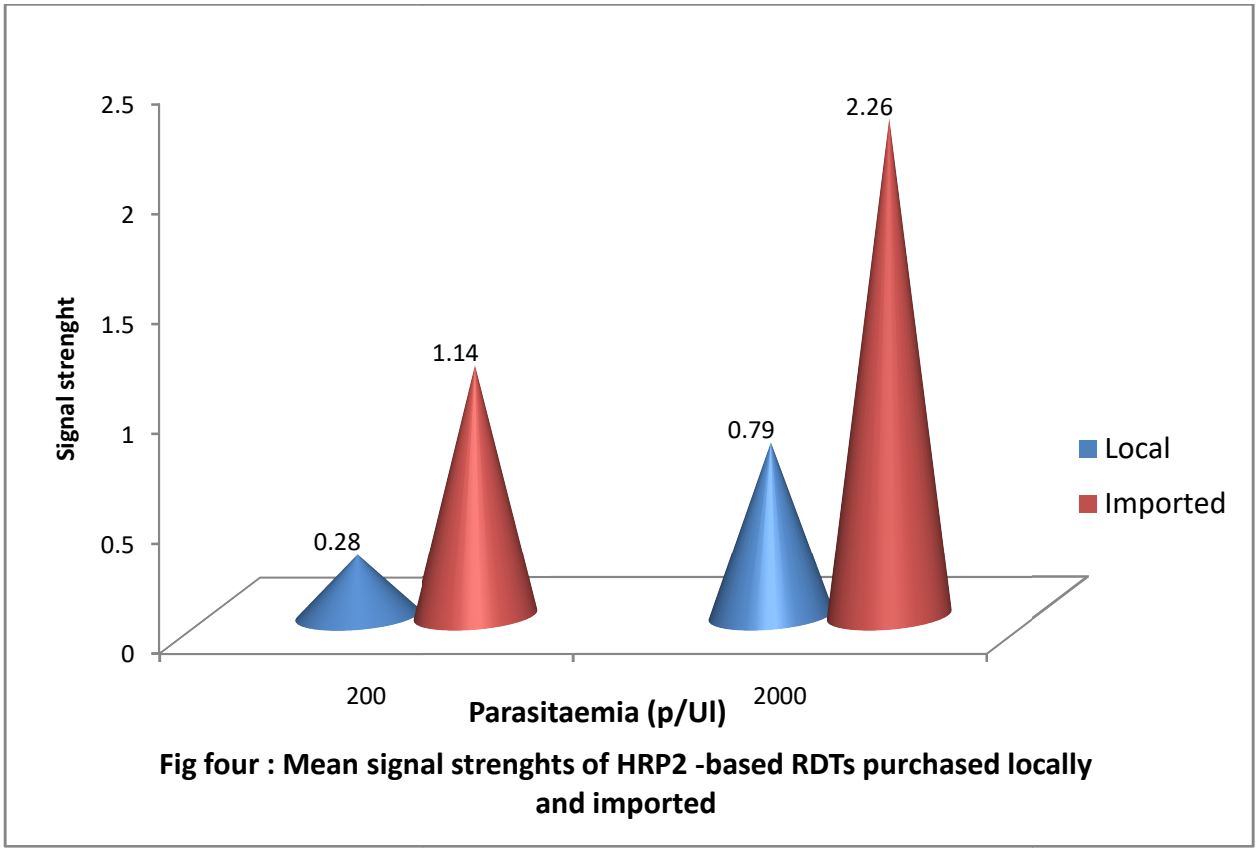


Fig four : Mean signal strenghts of HRP2 -based RDTs purchased locally and imported

Table 1: Total Number of Imported and Locally purchased MRDTs (HRP2 & pLDH)

Antigen	RDTs		Total
	Imported	Locally purchased	
HRP2	19(59.4%)	13(40.6%)	32
pLDH	4(100%)	0	4
Total	23	13	36

Table 2: Positivity and Negativity rate of Imported and Locally purchased MRDTs (HRP2 & pLDH)

Quality Assurance	RDTs		Total
	Imported	Locally purchased	
Passed	17(73.9)	1(7.7)	18
Failed	6(26.1)	12(92.3)	18
Total	23	13	36

$\chi 2 = 14.16$; df = 1; $P < 0.001$

Table 3a: Quality Assurance testing using both imported and locally purchased HRP2 MRDTs

Quality Assurance	RDTs		Total
	Imported	Locally purchased	
Passed	15(78.9)	1(7.7)	16
Failed	4(21.1)	12(92.3)	16
Total	19	13	32

$\chi 2 = 15.19$; df = 1; $P < 0.001$

Table 3b: Quality Assurance testing using both imported and locally purchased pLDH MRDTs

Quality Assurance	RDTs		Total
	Imported	Locally purchased	
Passed	2 (50.0)	0	2
Failed	2 (50.0)	0	2
Total	4	0	4

$\chi 2 = 15.19$; df = 1; $P < 0.001$

Table 4: HRP2 mean signal strength

		Signal strength	
	N	200p/µl	2000 p/µl
Local	13	0.28±0.49	0.79±0.75
Range		0 – 1.75	0-2.75
Imported	19	1.14±0.56	2.26±0.58
Range		0.25 – 2.25	1.25-3.0
F (Anova)		20.11	38.99
P		<0.001	<0.001

IV. DISCUSSION AND CONCLUSION

WHO (2008), algorithm for RDTs Quality Control (QC) testing states that for a MRDTs to pass both initial and longtime quality control testing, the percentage positivity must be 100%. However, the result from the study showed that only one out of the 13 LPMRDTs passed, while others failed the QA test at the initial QA testing. Imported MRDTs performed better than the LPMRDTs. 17 IMRDTs had 100% sensitivity while 6 had less than 100% sensitivity. Enviromental exposure may be responsible for low sensitivity observed in LPMRDTs (Moody, 2002; Murray *et al.,* 2003; 2008)

The performance of some of the LPMRDTs could be due to poor storage facility and exposure to high temperature during the course of transporting the MRDTs (Jorgensen *et al.,* 2006). This also 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; Moody, 2002) 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. 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. 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. 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 (Chiodini *et al*., 2007).

The locally purchased MRDTs were purchased in the open market, and the importers of this MRDTs are not aware of the importance of storing this MRDTs within the manufacturer recommended temperature. The effect of temperature as illustrated in this study, has shown that in our country, Nigeria and in Africa where the temperature is usually hot, MRDTs 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 MRDTs. Mismanagement could also affect the sensitivity and specificity of the MRDT and defects in the device membrane as stated by Reyburn *et al*., (2007). The heat stability test result is similar to 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.

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 of the LPMRDTs is well below acceptable levels. The test and control lines were likely to have different sensitivity to heat.

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 LPMRDTs was low because of its inability to pass 100% initial QA 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. The inclusion of a temperature effect indicator, ideally on the individual RDTs or on their packaging should be considered. The training of health workers on how to carry out the test is very important. The accuracy involve in picking the right volume of blood could also affect the performance of the test (Hanitra *et al.,* 2008; Waverly *et al.,* 2007).

This study highlights need for standardized product testing, quality control by the manufacturer and continuous external quality assessment when the MRDTs are in routine clinical use. Good storage and quality control need to be established in all situations where MRDTs are deployed. There is need to also consider the importance of stability of diagnostic test during procurement.

In conclusion, from the results obtained in the investigation carried on LPMRDTs and IMRDTs, it can be deduced that theLPMRDTs 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 of MRDTs to high temperature. The use of these LPMRDTs should be discouraged in an endemic country such as Nigeria where the consequences of failing to treat malaria can be deadly.

Therefore, to ensure good performance and reliable results from the use of MRDTs for the diagnosis of malaria especially in endemic country like Nigeria, MRDTs should be purchased from reliable source to ensure that its potency is in good state and not sub-standard.

REFERENCES

[1]. Baker, J., McCarthy, J., Gatton, M., Kyle, D. E., Belizario, V., Luchavez, J., Bell, D., Cheng, Q., (2005). Genetic diversity of *Plasmodium falciparum* histidine-rich protein-2 (PfHRP2) and its effects on the performance of PfHRP-2 based rapid diagnostic tests. *Journal of Infectious Disease* 192: 870-877

[2]. Bell D., Wongsrichanalai C., Barnwell J.W (2006). Ensuring quality and access for malaria diagnosis; how can it be achieved? *Nat .Rev. Microbiology 4: S7-S20*

[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]. Hanitra, R, Celine B, Rogelin R, Henry A, Arsene R, Laurence R, Martial J, Valerie A, and Didier M (2008). Accuracy and Reliability of malaria diagnostic technique for guiding febrile outpatient treatment in malaria-endemic countries. *American Journal of Tropical Medicine and Hygiene 78(2): 217-221*

[5]. Jennifer Luchavez, Ma Eichelle Lintag, Mary Coll-Back, Fred Back and David Bell (2007). An assessment of various blood collection and transfer methods used for malaria rapid diagnostic test. *Malaria Journal 6: 149*

[6]. 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*

[7]. Moody, A., Hunt-Cooke , A., Gabbett, E. and Chiodini, P. (2000). Performance of the OptiMAL malaria antigen capture dipstick for malaria diagnosis and treatment monitoring at the hospital for Tropical Diseases, London. *Br. J. Haematol. 109: 891-894*

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

[9]. Murray C K., Bell D., Gasser R.A., Wongsrichanalai C. (2003). Rapid diagnostic testing for malaria. *Trop Med Int Health 8: 876-883*

[10]. Murray, C. K., Robert, A. Gasser, Jr., Alan, J. Magil, and R. Scott Miller (2008). Update on Rapid Diagnostic Testing for Malaria *Microbiology Reviews* P. 97-110

[11]. Oduola, A M., Fawole, O. I, Onadeko M O. (2005). Plasmodium falciparum evaluation of lactate dehyrodegenase in monitoring therapeutic responses to standard antimalaria drugs in Nigeria. *Exp. Parasitol. 87: 283-289*

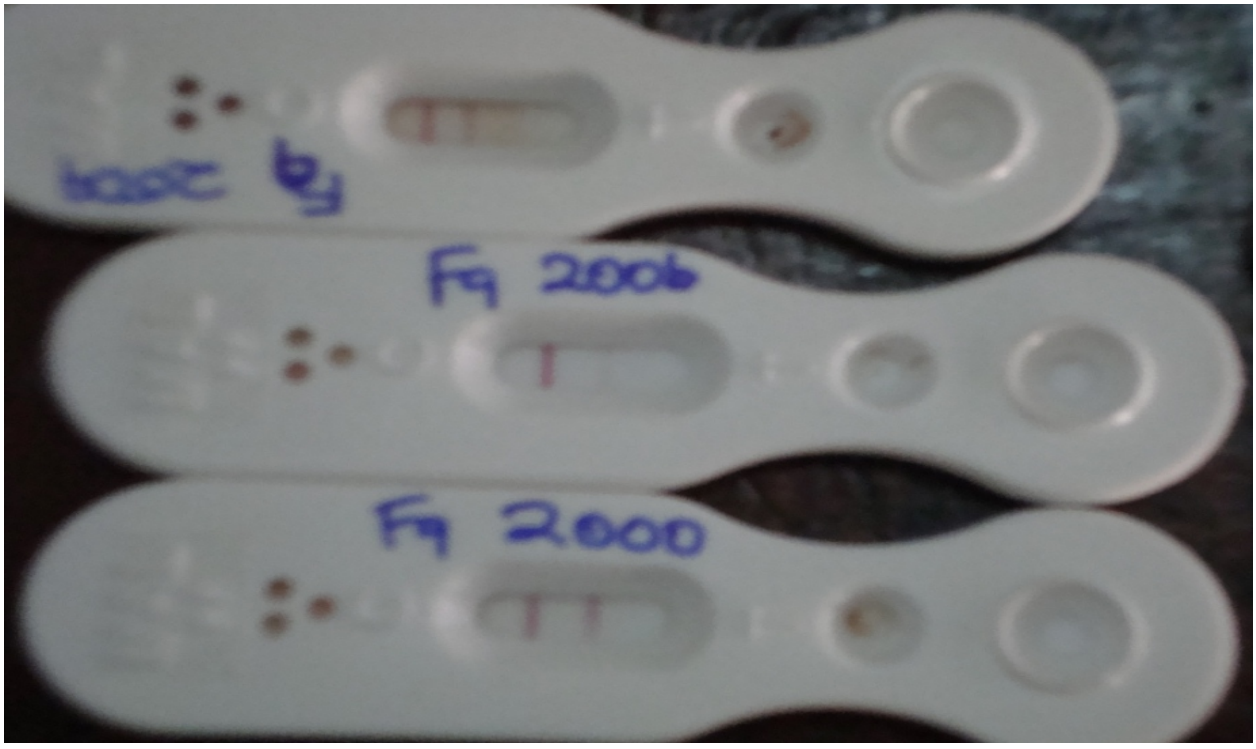
[12]. Tavrow, P., E, Knebel, and L. Cogswell. (2007). Using quality design to improve malaria rapid diagnostic tests in Malawi. Operations Research Results 1: 4

[13]. 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: 9-18*

[14]. 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

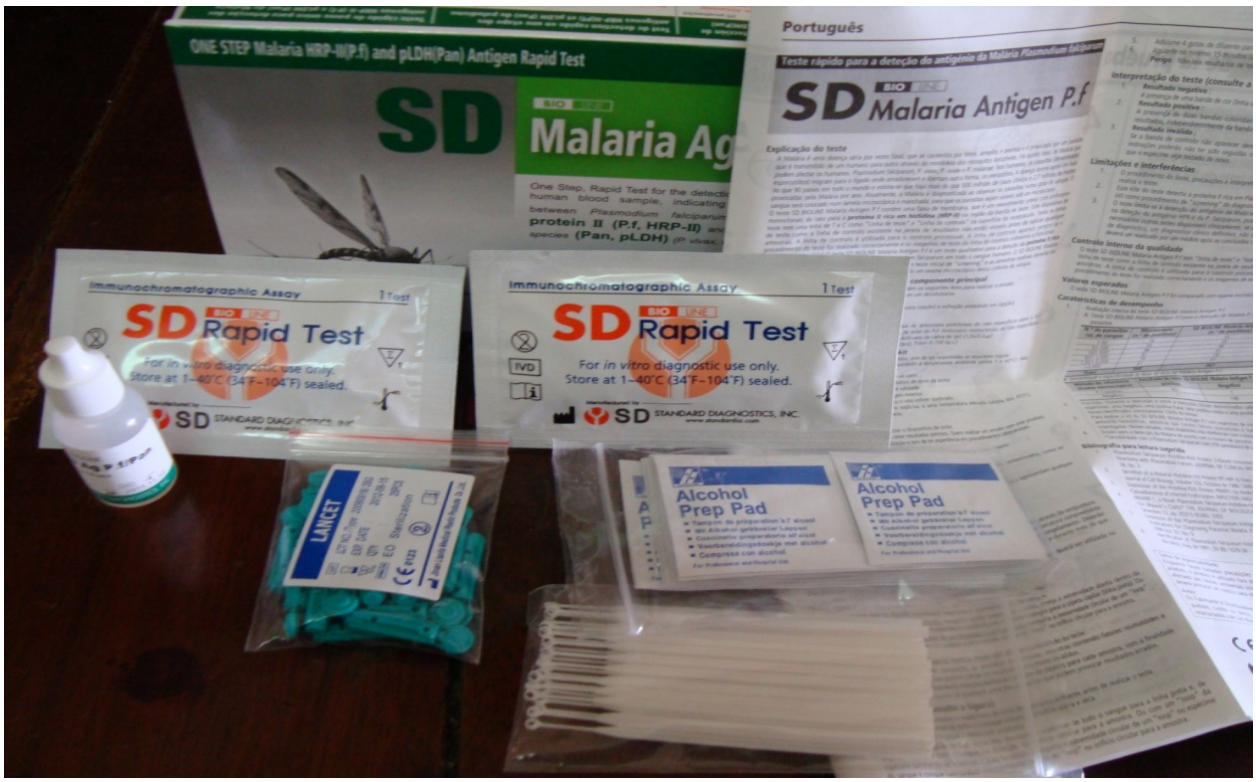
[15]. World Health Organization (2010).Methods manual for laboratory Quality Control Testing of Malaria Rapid Diagnostic Tests. UNICEF/UNDP/World Bank/WHO. Special Programme for research and training in tropical disease (TDR).

Appendix A: Example of Locally purchased MRDT that failed Quality assurance e testing (at200p/µl and 2000p/µl dilutions)



Appendix B : Example of an imported MRDT that passed Quality assurance testing at 200p/ul and 2000p/ul dilutions

Appendix C: An example of an imported HRP2 base MRDT with all its inclusions



Appendix D: An example of a Combo (HRP2 and pLDH) base MRDT with all its inclusions

APPENDIX E
DETAILED INFORMATION ON LOCALLY PURCHASED AND IMPORTED 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 Numbe	Date of initial Testing	Supplier Address
1	Global Device	25	Detects P.f antigen only(HRP2)	2^{o}C-30^{o}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^{o}C	Jan-08	Dec-09	20th May 2008	2,200	31074 31075	7th July 2008	Lascon Phar.Ltd Idumota Lagos
3	SD Bioline(Antigen)	30	Detects Antigen)	2-30^{o}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^{o}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^{o}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^{o}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^{o}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^{o}C	Sep-08	Sep-11	June,2007	4,000	W37809 08W2	4th Feb 2009	Embassy Ltd Isale-Eko,Lagos
9	ICT Combo	25	Detects all plasmodium spps (PLDH)	4-37^{o}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^{o}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^{o}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^{o}C	-	Jun-10	NIL	-	20081117	29th April 2009	Chudi Concerns Ltd Isale-Eko,Lagos.
13	BID	30	Detects Pf(HRP2)	4-30^{o}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^{o}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^{o}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^{o}C	-	Mar-11	NIL	-	MAL070909	1-Oct-09	Codix Pharma Ltd Ajao Est Lagos.
17	Acon	25	Detects Pf(HRP2)	2-30^{o}C	-	Sep-11	NIL	-	MAL9090012	1-Oct-09	Acon Laboratory Inc.

18	Bioland Pf/pv Strip	25	Detects Pf/Pv	2-30^{o}C	Oct-09	Jan-10	NIL	-	MAL030708	1-Nov-09	Nano sign Inc
19	First Response Malaria test kit	30	Detects Pf(HRP2)	2-30^{o}C	Apr-11	NIL	NIL	-	56EI709	1-Oct-09	Premier Medical I nc
20	Dr Greg's Malaria test kit	25	Detects Pf/Pv	2-30^{o}C	NIL	Sep-09	19th Nov 2009	5,200	C90910MAL	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	2-30^{o}C	Oct-09	Apr-11	NIL	NIL	MAF0210009	1-Nov-09	Nano sign Inc
24	ICT Pf Malaria test kit	25	Detects Pf (HRP2)	2_37^{o}C	NIL	Jun-11	NIL	NIL	32412	1-Nov-09	ICT Diagnostic
25	Bioland Combo	25	Detects Pf/pan	2-30^{o}C	26/10/ 09	Apr-11	Jan-10	NIL	MAL 021009	1-Nov-09	Codix Inc
26	Clearview Pf	25	Detects Pf(HRP2)	4_30°C	NIL	Aug-11	Sep-10	NIL	32556	1-Sep-10	Vision Biotech
27	Clearview Pf	25	Detects all plasmodium spps (PLDH)	4_30°C	NIL	NIL	Sep-10	NIL	NIL	1-Sep-10	Vision Biotech
28	Onsight Pf	25	Detects Pf(HRP2)	4_30°C	Jul-10	Jun-12	Sep-10	NIL	A21008	1-Sep-10	Amgenix Inc
29	Poct Pf	16	Detects Pf(HRP2)	2-30^{o}C	Feb-12	Mar-12	Mar-10	NIL	2010030226	1-Mar-10	Intec Producer Inc
30	ParaHit	10	Detects Pf (HRP2)	4_40°C	19/12/ 08	Dec-10	NIL	NIL	4000001826	1-Mar-10	NIL
31	Cortez	25	Detects Pf (HRP2)	2-30^{o}C	NIL	Jun-11	NIL	NIL	10020422	1-Apr-10	NIL
32	Tri malaria	30	Detects Pf(HRP2)	4_27°C	Feb-11	Jul-11	Mar-10	NIL	MPF1002001	April	Trinition Biotech Nig Ltd Abuja
33	Dialab	30	Detects Pf (HRP2)	2-30^{o}C	NIL	Feb-12	Apr-10	NIL	82040	April	Dialab Austria
34	BinaxNOW	20	Detecccts Pf (HRP2)	2-30^{o}C	NIL	FEB -13	Apr-11	NIL			
35	Acore malaria	1	Detects Pf (HRP2)	2-30^{o}C	NIL	MAR-13	Feb- 11	NIL			
36	IDA Paracheck	25	Detects Pf (HRP2)	4_45°C	NIL	Feb-12	Jan-12	NIL	31817	1-Jul-10	Orchid Biomedical System