FEDERAL MINISTRY OF HEALTH

LABORATORY BASED (PHASE I) EVALUATION OF HIV RAPID TEST KITS IN NIGERIA

ROUND 2

DECEMBER, 2011

FOREWORD

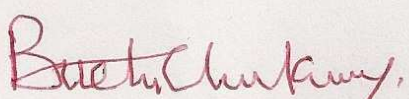
The intervention measures such as Prevention of Mother to Child Transmission (PMTCT), Antiretroviral therapy (ART), HIV Counseling and Testing (HCT), Blood safety and HIV Surveillance put in place by the Government of Nigeria and Partners for the control of HIV/AIDS infection largely depend on the establishment and provision of accurate and reliable diagnosis. Detection of specific antibodies and antigens in the blood or other body fluids is the main method of testing for HIV and the standard procedures for diagnosis of HIV infection. Rapid test, one of the assays used in detecting HIV specific antibodies are evaluated and placed in appropriate combinations (Testing Algorithm) for reliable diagnosis of HIV infection.

Recognizing the importance of establishing an algorithm in the country, the Federal Ministry of Health (FMOH) in collaboration with United State Government (USG) President Emergency Plan For AIDS Relief (PEPFAR) group and United States Centers for Disease Prevention and Control (CDC) in 2007, carried out the maiden laboratory evaluation (Round 1 phase I evaluation) of some non-cold chain dependent test kits where six test kits performed well and three algorithms in serial testing were recommended and approved by the Honourable Minister of Health. In the course of implementation of these algorithms, it was found that the test kits in the algorithms were limited for a large country like Nigeria. The government then found it necessary to expand the Algorithms in order to accommodate more rapid test kits. To this end, a National HIV Laboratory Quality Assurance Team (NHLQAT) with members drawn from different relevant organizations was inaugurated by the Honourable Minister of Health to carry out evaluation of HIV rapid test kits where 15 performed well out of 22 test kits evaluated. The team made recommendations to government on the test kits combinations that formed the expanded HIV National Testing Algorithm.

Meanwhile, the Second phase of the round 1 evaluation of the six test kits has been concluded and the result will soon be published. The results of the two phases of the evaluations will be used side by side.

The parallel testing algorithm was in use before the serial testing was recommended in the Round 1 phase I evaluation since there was no significant difference in performance between parallel and serial testing. The serial testing has an advantage of cost over the parallel testing. This informed the use of serial testing in HCT.

I hereby endorse the recommendations of the National HIV Laboratory Quality Assurance Team and approve that the results of this report be used for assessing the HIV status in Nigeria.



Professor C. O. Onyebuchi Chukwu
Honourable Minister of Health

ACKNOWLEDGEMENTS

The Federal Ministry of Health wishes to appreciate the Evaluation technical working group and the National HIV Laboratory Quality Assurance Team on the successful completion of the process of round 2 phase 1 evaluation of HIV rapid test kits from the planning to the completion of the exercise.

We extend our gratitude to all organizations and individuals who contributed to the success of the exercise including the National Agency for the Control of AIDS (NACA), Nigerian Institute of Medical Research (NIMR), National Agency for Food and Drug Administration and Control (NAFDAC), Medical Laboratory Science of Nigeria (MLSCN), Nigerian Institute of Pharmaceutical Research and Development (NIPRD), the World Health Organization (WHO), the United State Government (USG), United States Agency for International Development (USAID), African Health Project (AHP), Axios Foundation, Institute of Human Virology-Nigeria (IHVN), AIDS Prevention Initiative in Nigeria(APIN) and Supply Chain Management System (SCMS).

Our sincere thanks go to the Centre for Disease Control and Prevention-Global AIDS Programme (CDC-GAP) for providing technical support.

We also like to acknowledge the contributions of the staff and management of the National Laboratory External Quality Assessment Centre, Saye-Zaria (Managed by the MLSCN and Axios Foundation Nigeria) for allowing access for their laboratory to be used for the exercise.

Dr. Mansur Kabir
Head, Department of Public Health

ABBREVIATIONS

Ab	Antibody
Ag	Antigen
AHP	African Health Project
AIDS	Acquired Immune Deficiency Syndrome
ANC	Antenatal Clinic
APHL	Association of Public Health Laboratories
ART	Antiretroviral Therapy
CDC-GAP	Centre for Disease Control and Prevention, Global AIDS Program
CPHL	Central Public Health Laboratories
EDTA	Ethylene Di-amino Tetra-acetic Acid
EIA	Enzyme immunoassay
EQA	External Quality Assurance
FGON	Federal Government of Nigeria
FMOH	Federal Ministry of Health, Nigeria
GON	Government of Nigeria
HAD	HIV/AIDS Division
HCT	HIV counseling and testing
HIV	Human Immunodeficiency Virus
IHVN	Institute of Human Virology, Nigeria
IRB	Institutional Review Board
NACA	National Agency for the Control of HIV and AIDS
NAFDAC	National Agency for Food, Drug Administration and Control
NASCP	National AIDS and STIs Control Programme
NBTS	National Blood Transfusion Service
NGO	Non-Government Organization
NHALQAT	National HIV/AIDS Laboratory Quality Assurance Team
NHREC	National Health Research Ethics Committee
NIMR	Nigerian Institute for Medical Research
NIPRD	National Institute for Pharmaceutical Research and Development
NPV	Negative Predictive value
NTBL	National Tuberculosis and Leprosy
OD	Optical Density
PEPFAR	President's Emergency Plan for AIDS Relief
PMTCT	Prevention of Mother-To-Child Transmission
PPV	Positive Predictive Value
RTK	Rapid Test Kit
SBFAF	Safe Blood for Africa Foundation
SCMS	Supply Chain Management System
SFH	Society for Family Health
SOP	Standard Operating Procedure
TOT	Training of Trainers
UATH	University of Abuja Teaching Hospital
UCH	University College Hospital
USAID	United State Agency for International Development

USG	United State Government
WB	Western Blot
WHO	World Health Organization

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EXECUTIVE SUMMARY

Development of Human Immunodeficiency Virus (HIV) Rapid Testing algorithm is considered critical at ensuring the quality of HIV testing in support of HIV diagnostics and screening programs such as HIV Counseling and Testing (HCT) and Prevention of Mother to Child Transmission (PMTCT). Most countries in Africa have made this a priority and have evaluated each assay to determine their performance characteristics and suitability for use within their own setting. The formal evaluation of HIV rapid tests for the development of an algorithm was carried out in Nigeria in 2007 with the laboratory based evaluation (Phase 1) of HIV rapid test kit. A total of nine (9) HIV rapid test kits were evaluated and rated, out of which six (6) namely; Determine, Double-Check Gold, Sure check, Bundi, Statpak, and Unigold passed the evaluation criteria.

The experience from the use of algorithm reveals that despite the years of the establishment, only a sizeable number of stakeholders are adhering to the algorithm. This could be traced, in part, to a limited circulation of these kits in the country. Realizing also that health is on the concurrent list and the current algorithm does not enjoy wide usage, adherence to the algorithm by stakeholders other than the Federal Government and Partners have been very difficult. Hence, various HIV rapid kits are used by various state governments, Non- Governmental Organizations (NGOs), private hospitals etc to screen their clients. Rather than allowing this practice to go on without any enforcement, it was considered necessary to evaluate some of these kits and bring up a matrix of combination algorithms that these stakeholders can buy into.

Applications were received for the evaluation of thirty two (32) HIV rapid test kits, out of these; twenty two (22) met the criteria for evaluation. The protocol developed for the evaluation was reviewed and approved by the National Health Research Ethics Committee. The plasma samples for the evaluation were collected from the six geopolitical zones of the country. Before commencement of sample collection, all the sites personnel received training on their respective roles. The samples collected were characterized using two (2) Enzyme Immunoassays (EIAs) and Western blot as gold standard. All the test kits were tested with the characterized samples at the National Laboratory External Quality Assessment Centre, Saye, Zaria. The results of the evaluation of the test kits with plasma and oral fluids were analyzed. Testers rating together with the laboratory performance characteristics formed the basis upon which suitable test kits were selected and appropriate testing algorithm proposed.

Fifteen (15) out of the 22 test kits evaluated met the selection criteria and are therefore recommended.
This recommendation is based on the "WHO Guidelines on Appropriate Evaluations of HIV Testing technologies in Africa" which recommends that, for confidence interval of 95%, test kits must have a minimum sensitivity and specificity of 98%. The fifteen (15) test kits selected met these criteria in addition to others such as global tester ratings and composite scores of the individual test kits.

The recommended kits and the proposed algorithm table is presented below:

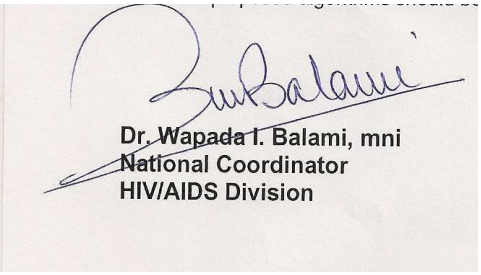
ALGORITHM TABLE		
1st Line	**2nd Line**	**Tie Breaker**
SD Bioline	Retrocheck	Colloidal Gold
Dialab	Rapid Signal	Insti
Determine Combo	Core Instant	
Retroscreen	Advanced Quality	
Vikia	HIV Status	
HIV Quick Check		
Oraquick		
DPP		

Note:

1. ***These kits in this algorithm are valid for only Whole blood, Serum or Plasma.***
2. ***Any of the test kits in the first line can be use for screening, any of the second line test kits can be used for confirmation while any kit in the tie breaker column can be used to resolve discordance.***

Based on the result of the evaluation, the following recommendations are made:

- The performance characteristics of DPP and Oraquick test kits using oral fluid as specimen was poor and therefore HIV testing using oral fluid is not recommended.
- Multiple testing algorithms is hereby proposed thereby creating multiple options that testing programs in the country could use.
- A robust monitoring system including but not limited to post market validation and random field sampling of test kits should be developed to ensure maintenance of quality at all levels of testing.
- Considering the fact that evaluation of HIV rapid test kit performance is an on-going process and this evaluation is a laboratory based validation of individual test kit; a field testing and monitoring of the kits in the proposed algorithms should be embarked upon.



1.0 Background

The Human Immunodeficiency Virus (HIV) burden has continued to pose a serious challenge to the socioeconomic growth and health infrastructure of Nigeria. A significant progress has been made in reducing the prevalence of the infection from 5.8% in 2001 to 4.6% in 2008 and 4.1% in 2010. Improving the quality of life for the infected and affected population will require an effective and efficient implementable plan to consolidate on this gain and prevent new infections.

In 2003, Nigeria adopted and used successfully in HIV Antenatal care (ANC) surveys, a serial algorithm using Capillus (for screening) followed by Genie II (for all Capillus positive specimens), and Determine as a tie-breaker (in cases where discordant results were seen between the initial two tests). The first two tests in this algorithm require refrigeration, and it was apparent that this hindered expansion of HCT beyond tertiary and secondary healthcare facilities. As a result of this in early 2006 a temporary move was made toward a non-cold chain dependent testing algorithm where parallel testing was suggested using any two of the following tests: Determine, Stat-Pak, Bundi, Double Check Gold or Inocheck. None of these tests require refrigeration which eased the burden of cold chain during transport to and storage at testing sites and also allowed Nigeria to move toward the use of trained, non-laboratory staff for HIV diagnostic testing at HCT sites.

In 2007, the Federal Government of Nigeria (FGN), in collaboration with the President Emergency Plan For AIDS Relief (PEPFAR) Program, implemented and completed the Phase 1 laboratory-based evaluation of the available rapid test kits (RTKs) with specific focus on non-cold chain dependent HIV RTKs to suit both infrastructure and the varied national skilled levels. The use of HIV rapid testing has dramatically increased the proportion of tested individuals who receive their results. Prior to the availability of rapid testing, same-day results were not available, and an estimated one-third of those tested did not return to learn their HIV status. The FGN is currently working to expand quality HIV counseling and testing (HCT) services as a prevention intervention, and as an entry to care and treatment. Therefore, the need for well-evaluated, reliable testing products whose performance and use is quality-assured is essential and urgent.

Health care facilities and Non-Governmental organizations (NGOs) in Nigeria are currently providing HIV rapid testing for HCT, prevention of mother-to-child transmission (PMTCT), emergency blood transfusions and clinical diagnosis. When rapid testing is provided in settings where people learn their status, a multiple test algorithm is used. Enzyme immunoassays (EIA) and Western Blot (WB) technologies have been available in Nigeria; however cost and the required infrastructure have limited their availability. HIV rapid tests offer a cheaper, simpler and faster alternative.

The formal evaluation of HIV rapid tests for the development of an algorithm in Nigeria was concluded in 2007 with the laboratory based evaluation (Phase 1) of HIV RTKs. A total of nine (9) HIV RTKs were evaluated and rated out of which six (6); Determine, Double-Check Gold, Sure check, Bundi, Statpak, and Unigold passed the evaluation criteria. At the moment, 5 of the test kits are undergoing phase II process.

2.0. Rationale

The current HIV testing algorithm has been in use by the stakeholders involved in HIV/AIDS programme especially the partner supported sites since 2008. Although periodic post-marketing surveys of the kits are carried out, there has not been comprehensive review of the kits in relation to other kits coming into the country. The experience from the development of the current algorithm reveals that despite the years of the establishment, only a sizeable number of stakeholders are adhering to the algorithm. This could be traced, in part, to a limited circulation of these kits in the country. Realizing also that health is on the concurrent list and the current algorithm does not enjoy wide usage, adherence to the algorithm by stakeholders other than the Federal Government and Partners have been very difficult. Hence, various HIV rapid kits are used by various state governments, Non- Governmental Organization (NGOs), private hospitals etc to screen their clients. Rather than allow this practice to go on without any enforcement, it is considered necessary to evaluate some of these kits and bring up a matrix of combination algorithms that these stakeholders can buy into. These will create a wider choice for testers, reduce the overdependence on narrow choice HIV rapid test kit as constituted in the current algorithm, and will enable a more robust monitoring and advisory process.

It is assumed that non cold chain HIV rapid testing is a minimum package for use within Nigeria and this is being utilized without bias to other organization involved in higher level of HIV testing e.g. National Blood Transfusion Service (NBTS). However, the test kits in the national testing algorithm are limited in number such that a problem with one kit in the algorithm can almost cripple the testing activities across the country. Also, the limited number of kits in the algorithm has not given room for competition thus creating monopoly which has potential quality and financial implications.

Evaluation of test kits is a continuous process that should be done from time to time. It is now about 4 years when the first evaluation was done and several others are seeking the opportunity to present their products for evaluations which they claim are cheaper and with higher performance. Therefore, based on the aforementioned, the FGN in collaboration with some development partners carried out a second round of phase 1 evaluation of the available kits in the country to expand the algorithm.

3.0. Objectives

3.1. General Objective

To evaluate 32 test kits by testing their performances based on internationally accepted test standards in order to determine their suitability for use in the national HIV rapid testing algorithm in Nigeria.

3.2. Specific Objective:

- To evaluate 32 HIV RTKs available in the country and increase the number of test kits valid for the algorithm using well characterized plasma/serum samples in the laboratory
- To provide reliable combinations of kits under the current serial algorithm for national HIV testing.

4.0. METHODOLOGY

4.1. Establishment of an Evaluation Working Group

In August 2005 a multi-agency working group was established by the FGN for the maiden evaluation of HIV RTKs. This group was expanded to accommodate other relevant stakeholders; and used for this second round phase 1 evaluation. This includes Federal Ministry of Health, National Agency for the Control of AIDS (NACA), Development Partners and other stakeholders (See Annex I). The National HIV Laboratory Quality Assurance Team (NHLQAT) was constituted from the expanded multi agency working group. NHLQAT carried out the Round 2 Phase 1 evaluation.

4.2. Protocol Development

The protocol for this evaluation was developed from Guidelines for Appropriate Evaluations of HIV Testing Technologies in Africa, jointly developed by the CDC and the African Regional Office of the WHO (WHO/AFRO). This document outlines a three-phase strategy for evaluating HIV rapid tests and monitoring the performance of a testing algorithm. The process starts with a laboratory-based evaluation using well-characterized specimens to determine test performance and results in proposed algorithms (Phase I). This is followed by a field evaluation using whole blood at a few points of service to generate data on test kits performance under field condition, (Phase II). Once a new algorithm is developed it is monitored indefinitely through a National external quality assurance (EQA) program, post market validation or surveillance (Phase III) (2007 HIV Kits Evaluation Report). (See list of contributors in the protocol development Annex II).

4.3. Ethical Clearance:

The Protocol for this evaluation was reviewed by the National Health Research Ethics Committee (NHREC) of the Federal Ministry of Health. The approval with NHREC number NHREC/01/01/2007 was granted to conduct the evaluation between 09/02/2011 and 08/02/2012 (Annex III).

4.4. Test Kits Selection Criteria

A number of test kits were selected using a set of criteria ranked by order of importance as listed below. The first six were the most important.

1) **Registered with NAFDAC**
2) **Ability to detect HIV-1, HIV-2 and HIV- type O subtypes**
3) **Long shelf life (at least one year) and robust**
4) **Test results provided in less than 30 minutes**
5) **Packaging (existence of manufacturer's address, kit insert)**
6) **Manufacturer's representative (known office address, certificate of incorporation etc).**

7) Ability of manufacturers to produce and provide adequate numbers of testing kits to meet the needs of testing programs in Nigeria
8) Storage criteria, cold chain/ non-cold chain)
9) Ability to test a range of samples (whole blood, plasma or serum)
10) Easy to perform and interpret
11) Low cost price (cost per test)
12) Prior experience and evaluation- documented performance in Nigeria and other African countries-
13) Guaranteed safety in the use of test devices during assay
14) Do not require additional equipment to run tests or read results
15) Packaging of test kits not excessively bulky
16) Number of test per kit.
17) Volume of blood/plasma required for testing
18) Provision of required quantity of test kits for the evaluation by the vendor.

4.5. Advertisement for submission of HIV test kits for evaluation

Announcement notice for submission of test kits for evaluation for the purpose of algorithm development was placed on the Federal Ministry of Health notice boards.

4.6. Sites and Source of Specimens :

The two stage panel production strategy was used for specimen collection for this evaluation
(i) Blood samples from National Blood Transfusion Service (NBTS) centre and
(ii) Samples from health facilities from the six zones. For this evaluation, specimen panel collected from the Health facilities in the six geopolitical zones of the country were used. The sites that contributed panel had the capacity of storing samples at -20ºC (See sites in table 2). All specimens that were included in this evaluation were unlinked and anonymized prior to inclusion. Seven (7) to Ten (10) ml of blood was drawn and oral fluid specimen collected from individual volunteer after obtaining consent from amongst clients presenting at HCT and ART centres.

All specimens in this evaluation were checked for haemolysis, fungal or bacterial contamination/growth. The specimens received at the sites were stored at between -20ºC to -80ºC until transportation to the reference laboratory for characterization and evaluation. For oral fluid the test were conducted at sample collection point and left over specimen discarded appropriately.

Table 1: Sample collection sites and number of samples

Zone	Site	Number of samples
South South	University of Uyo Teaching Hospital.	169
South East	Ebonyi State University Teaching Hospital, Abakaliki	147
South West	University College Hospital, Ibadan	167
North West	Aminu Kano Teaching Hospital, Kano	117
North East	Federal Medical Centre, Gombe	166
North Central	University of Abuja Teaching Hospital, Gwagwalada	167
	TOTAL	**933**

4.7 Training

Prior to the commencement of the exercise, different categories of personnel were trained on the conduct of the exercise.

The first training was training of trainers (TOT) involving staff of FMOH and Implementing Partners supporting the respective sites. The trainers then carried out on-site training for site personnel (Site coordinator, counselors and lab scientists) on their respective roles which include consecutive sampling, counseling, oral fluid collection and testing, blood sample collection and processing, and storage.

The Laboratory Scientists that carried out the reference testing and testing of the RTKs were provided with background information on the evaluation, refresher training on Good Laboratory Practices, orientation to the data entry forms (Annex IV a-b) and testing procedures on each test kits.

4.8 Specimen collection and oral fluid testing

Consecutive sampling was used for sample collection. Oral Fluid was collected with oral swab provided by the manufacturer of the 2 oral fluid based test kits (Oraquick and DPP) from consenting (see annex V) individuals and tested for HIV antibodies using the two Oral fluid based test kits submitted for the evaluation. Also, 10 ml of whole blood was collected in K3 EDTA vacutainer tubes and forwarded to the site lab lead that centrifuge and separate the plasma into two (2) aliquots. These aliquots were then stored at -20ºC freezers. No information was received on the patient as the focus was the test performance for sensitivity and specificity.

Two (2) test kits; OraQuick and DPP were tested by trained Laboratory Scientists and result recorded in Oral fluid test result data sheet (Annex IVa). The results sheet was sent to the National Laboratory External Quality Assessment Centre where the results were compared with the corresponding plasma EIA results.

4.9 Site for Laboratory-Based Validation.

All laboratory work on plasma associated with this evaluation was carried out at the National Laboratory External Quality Assessment Centre, NTBLTC, Saye-Zaria. This Centre was chosen for the reason that it is the current National HIV External Quality Assurance Laboratory and training facility. It has constant electrical power, appropriate infrastructure for reference testing (EIA equipment) and adequate specimen storage space at 4°C and -20°C. Storage of samples from sites, characterization of the samples and evaluation of the test kits were done at this laboratory.

4.10 Sample Size

Consecutive sampling was used during sample collection from the consenting individuals (see Annex V for consent form) at the six zones. A total of nine hundred and thirty three (933) individuals were sampled for blood and oral fluids out of 1000 samples proposed. The oral fluids were tested using the oral fluid test kits (Oraquick and DPP) at the collection sites. However, only eight hundred and eighty two (882) of the plasma samples collected from the individuals were retrieved to the reference laboratory, while 51 samples did not come with duplicate and were not sufficient to run EIA with the 22 test kits therefore they were excluded.

Out of 882 samples tested with EIA, 358 EIA positive samples were tested with New Lav Blot 1 where 250 WB positive samples were selected as positive panel for the evaluation. Three hundred (300) EIA concordant negative samples were selected as negative panel for the evaluation of the test kits (making a total of 550 panels consisting of 300 negatives and 250 positives). During evaluation of the test kits 15 positives and 43 negatives were insufficient and were excluded from the analysis leaving 235 positive and 257 negative samples totaling 492 samples which were used for the evaluation. This sample size was sufficient to provide 95% confidence interval (± 2%) for calculating sensitivity and specificity.

4.11 Testing Procedure

A total of nine hundred and thirty three (933) samples were collected and tested using the oral fluid test kits (Oraquick and DPP) at the collection sites out of 1000 samples proposed. Eight hundred and eighty two (882) specimens were retrieved to the National Laboratory Quality Assessment Centre for reference testing.

4.12 Reference Testing

All calibration and validation procedures of the reference laboratory were assured using existing procedure at the National Laboratory External Quality Assessment Centre (NLEQAC) before characterization. Also a validation plate testing which was used to guarantee the equipment and reagents performance as well as personnel dexterity was carried out.

All the 882 specimens retrieved from the sites were tested using two EIA test kits (Vironostika HIV Ag/Ab test kit from Biomerieux and HIV Genscreen Ultra). New Lav Blot 1 test kits from Bio-Rad were used to test EIA concordant positive specimens. Reactive EIA specimens without duplicate samples and insufficient samples were excluded from the western blot testing. Specimens that had discordant EIA or indeterminate WB results were excluded from the panel. The EIA concordant samples with low Optical Density (OD) and WB positives were selected to form the panel for the evaluation. The combination of 2 EIA test kits and WB was used as **Gold Standard** in this evaluation. All reference testing were conducted as per the manufacturer's instructions. Kit controls and in-house positive and negative controls developed in Nigeria were included on all EIA plates.

The results that were concordant with the two EIA test kits were used in comparing the results of the two oral fluid based test kits conducted at the sites. Out of the 882 samples that were tested with EIA, seven hundred and eighty nine (789) were used for the analysis of the two oral fluid test kits while ninety three (93) that had discordant EIA test results were excluded.

4.13 Kit Evaluation

All the 933 specimens were characterized using 2 EIA test kits the positive EIA were further characterized with WB. 550 specimens were then selected for the evaluation. These were made of 250 positives consisting of both high and low EIA antibodies titre and 300 negatives. They were randomized and new ID numbers between 1 and 550 were assigned. Due to large number of test kits that were evaluated some panel were not sufficient to go round the specimen and therefore excluded from the analysis. The number of samples that formed the panel were 492 consisting of 235 positives and 257 negatives. This panel size provides a 95% confidence interval (± 2%) for calculating sensitivity and specificity. The order of positive and negative specimens was mixed to allow for blinded testing.

The NHLQAT (qualified Medical Laboratory Scientists) experienced in HIV serology were used for the evaluation (See Annex VI list of NHLQAT). They were provided with background information on the evaluation, refresher training on Good Laboratory Practices and orientation to the data entry forms (Annex IV). Job aids were provided for each rapid test and each test was demonstrated by an experienced scientist. Under the supervision of a senior member of the evaluation working group, the laboratorians practiced on control specimens prior to evaluating the test kits. They

worked in pairs, each pair evaluating a total of 110 specimens per test product. Specimen sets were rotated between them. Each test result was read by five laboratorians independently. After each test product was evaluated, all of them completed a questionnaire (Annex VII) concerning various aspects of the rapid test they had just evaluated in other to assess the ease of performance.

The ease of performance of each of the test kit was assessed by the five (5) teams of laboratory Scientists. Performance attributes that were considered include; ease of collecting and delivering the correct volume of plasma/sera, adding diluent, ease of reading and interpreting results. Also reported on were the packaging size, waste generation and safety of the testing procedures. Each of the performance characteristics was assigned the ranking scale of 1 - 5 in which 5 represented the easiest and 1 the most difficult (cumbersome) (Annex VII). The scores of each of the performance characteristics were then summed to yield global scores and the RTKs were ranked according to their global scores. This was done in an effort to capture information, in addition to accuracy, which is also critical in identifying tests for an algorithm.

4.14 Quality Assurance Measures

Several QA measures were put in place to ensure quality conduct of the exercise. These measures include the following: -

i. Training of all levels of personnel that were involved in this exercise
ii. Monitoring of the exercise by the various IPs supporting each site.
iii. Central monitoring of the sample collection by officers from FMOH
iv. Production and use of SOPs during oral fluid testing, reference kits testing and testing of 22 test kits
v. Inclusion of in-house control specimen in all EIA plates during reference testing
vi. Use of sample retrieval forms during sample retrieval from the sites.
vii. Use of qualified Laboratory personnel in characterization and testing of the 22 test kits
viii. Use of three different scientists to cross-check the identity of samples selected as reference panel with the corresponding EIA and WB results.
ix. Use of senior members of the Evaluation Working Group to monitor the testing at the reference laboratory.
x. Use of the delta value in determining the cut-off during EIA testing.

4.15 Limitations

Some limitations in this evaluation include the following:

i. Insufficient sample volume collected from the sites as well as missing samples with oral testing results leading to the exclusion of these samples from the testing panel.

ii. Less than proposed sample number were collected from the different sites.

iii. New Lav Blot 2 was not available for the sample characterization.

4.16 Data Collection and Analysis

Several key parameters were evaluated for each assay: sensitivity, specificity, positive and negative predictive values using gold standard. The sensitivity and specificity of each assay are calculated using the gold standard; this was analyzed as follows:

		Results of Gold Standard assay		
		Positive (+)	**Negative (-)**	
Results of assay	**+**	A True-positives	B False positives	A + B
under evaluation	**-**	C False-negatives	D True-negatives	C + D
		A + C	B+D	

Sensitivity is defined as the ability of an assay being evaluated to correctly detect specimens containing antibody to HIV. In other words, sensitivity is the percentage of true positive HIV specimens identified by the assay under evaluation as positive (A), divided by the number of specimens identified by the reference assays as positive (A+C).

Specificity is defined as the ability of an assay being evaluated to correctly detect specimens that do not contain antibody to HIV. In other words, specificity is the percentage of true negative specimens identified by the assay being evaluated as negative (D), divided by the number of specimens identified by the reference assays as negative (B+D).

Positive Predictive Value (PPV): is the probability that when the test is reactive, the specimen actually contains antibody to HIV. PPV is calculated as follows: A/(A+B). PPV can also be calculated as follows:

$$PPV = \frac{(\text{prevalence})\,(\text{sensitivity})}{(\text{prevalence})\,(\text{sensitivity}) + (1\text{-prevalence})\,(1\text{-specificity})}$$

Negative Predictive Value (NPV): is the probability that when a test is negative, a specimen does not have antibody to HIV. NPV is calculated as follows: D/(C+D) or as:

$$NPV = \frac{(1\text{-prevalence})(\text{specificity})}{(1\text{-prevalence})(\text{specificity}) + (\text{prevalence})(1\text{-sensitivity})}$$

Data analysis was conducted by FMOH HIV/AIDs Division, with assistance from a Consultant Statistician. The data was analyzed using SPSS software that determined performance of each test kit and various test kit combinations based on their specificity, sensitivity, predictive values including overall onsite performance and comments. Exact 95% confidence intervals were determined for all combinations in various serial testing combinations. The analysis also included determining the sensitivity and specificity of concordance of tests performance in combination and evaluated their performances with discordance results requiring a tiebreaker. The ranked algorithms based on the highest performance (highest sensitivity and specificity) and composite score (accuracy and global mean tester rating) was then recommended.

5.0 RESULTS

Thirty two (32) applications requesting for evaluation of HIV rapid test kits and placement into the national algorithm were received for the round 2 phase 1 evaluation of HIV rapid test kits. Of these, 22 (69%) of the test kits were qualified for the evaluation. Out of the 22 test kits, two (2) had the ability to use oral fluid specimen for testing in addition to whole blood, serum or plasma which all of them had.

Table 2: List of HIV test kits requested for evaluation

S/N	Product name
1	HIV Q Spot
2	HIV Status
3	HIV Quick-Check
4	Colloidal gold (HIV Test kit Lot VII)
5	Antec HIV Test kits
6	Biosystems HIV spot
7	Biosystem HIV Triline kits
8	Red Dot HIV1 & II
9	SD Bioline HIV 1&2 Rapid test
10	SMART tube HIV and HCV
11	Rapid Signal HIV 1&2
12	Advance Quality HIV test
13	Insti HIV-1/HIV-2 Antibody
14	RetroCheck HIV
15	Diagnostic Kit for HIV (1+2) Ab
16	Vitest HIV 1, HIV 2 Subtype O
17	Immunocomb II HIV 1&2 Trispot(Ag-Ab)
18	Isotest Card HIV 0,1,2
19	Dialab HIV1&2y
20	EZ-Trust HIV1&2
21	Retro Screen HIV kits (1&2)
22	Oral Quick HIV test kits(oral fluid, Whole blood, serum/plasma)
23	First response HIV test kits
24	DPP Test kit (oral fluid, Whole blood, serum/plasma)
25	Vikia HIV Test kit
26	Care Start HIV 1-2-0
27	I Care HIV test kits
28	Genie III HIV test kits
29	Complete HIV 1/ 2
30	Core HIV
31	Determine combo
32	Pareekshak HIV

The findings of the evaluation of the test kits are presented below.

5.1 Individual Test Results

The sensitivity, specificity, positive and negative predictive values and the accuracy of individual test were calculated as shown below:

5.1.1 Sensitivity of the test kits:

The sensitivity as shown in table 3 of the test kits ranged from 97.4% to 99.1%. No single test kit had a sensitivity of 100%.

Table 3. Sensitivity and specificity of individual test kits based on plasma test

S/N	Name of Test Kit	No of Positives by test	Sensitivity		No. of negatives by test	Specificity
1	Advanced Quality	231	98 .3		255	99 .2
2	Antec	233	99 .1		242	94. 2
3	Carestat	231	98 .3		246	95 .0
4	Colloidal Gold	231	98 .3		254	98. 8
5	Core Instant	231	98 .3		255	99 .2
6	Determine Combo	233	99. 1		254	98. 8
7	Dialab	231	98. 3		254	98. 8
8	DPP	233	99. 1		254	98. 8
9	First Response	231	98. 3		251	97. 7
10	Genie lll	230	97 .9		255	99 .2
11	HIV Quick Check	231	98. 3		254	98. 8
12	HIV Status	231	98. 3		254	98. 8
13	Icare	230	97. 9		256	99 .6
14	Insti	231	98. 3		254	98. 8
15	OraQuick	232	98 .7		255	99 .2
16	Pareekshak	229	97 .4		253	98. 4
17	Rapid Signal	231	98. 3		255	99 .2
18	Retrocheck	231	98. 3		256	99 .6
19	Retro Screen	232	98 .7		254	98 .8
20	SD Bioline	233	99. 1		255	99 .2
21	Vikia	231	98. 3		255	99 .2
22	Vitest	229	97 .4		253	98 .4

(Total number of test positive by Gold Standard =235; Total number of test negative by Gold Standard = 257)

Table 4: Sensitivity and specificity of individual oral fluid based test kits using oral fluid

S/N	Name of kit	No of Positives by test	Sensitivity		No. of negatives by test	Specificity	Accuracy
1	DPP	322	91.8		436	100.0	96.3
2	Oraquick	324	91.2		436	100.0	96.1

*(Total number of test positive by Gold Standard =**353**; Total number of test negative by Gold Standard = **436**)*

Both test kits were highly specific but their sensitivities were much less than those obtained with blood samples as shown in table 3.

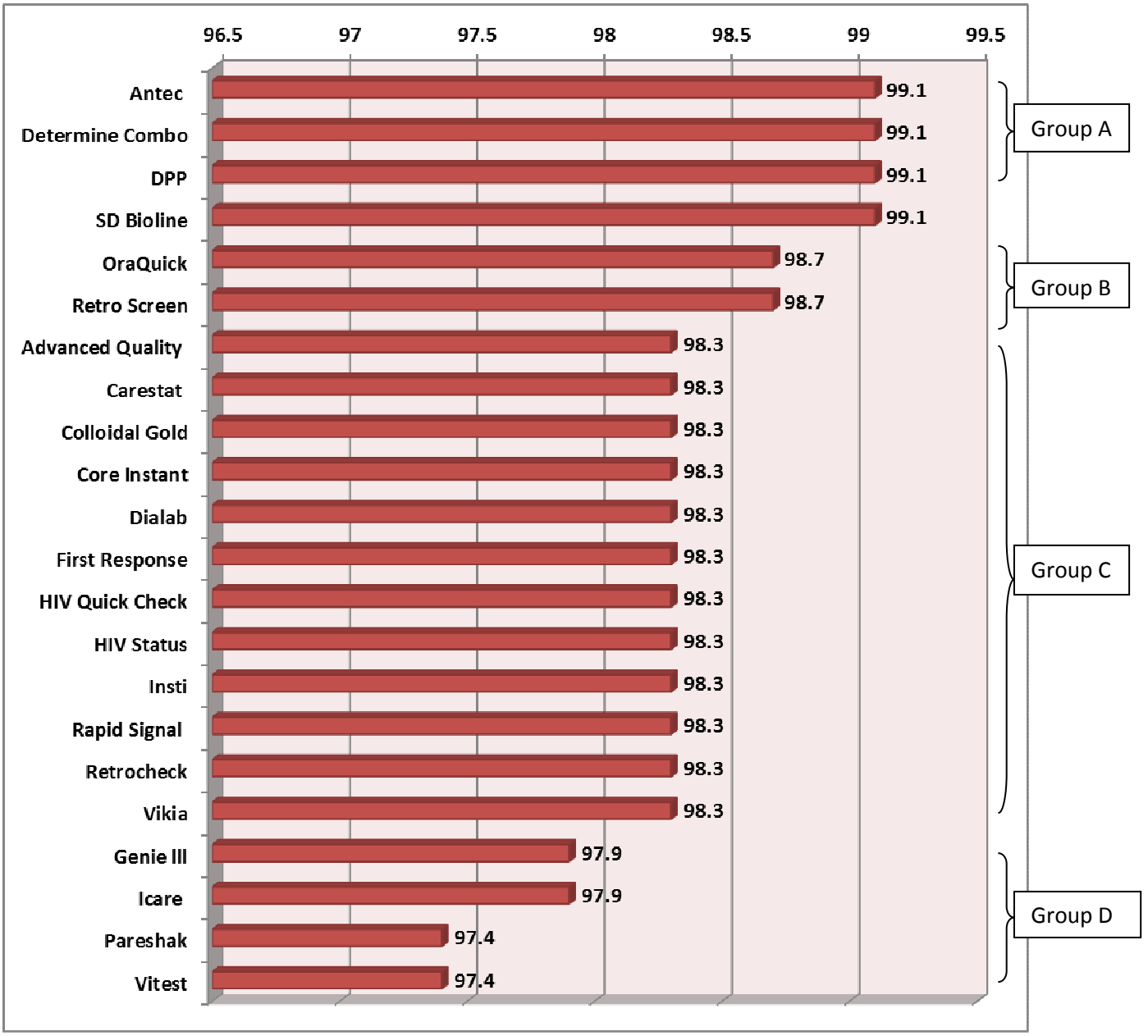
5.1.2: Classification of Rapid Test Kits sensitivity based on plasma

The test kits could be categorized into four groups (A-D). Those in group A, B and C had sensitivities of 99.1%, 98.7% and 98.3% respectively while those in group D had sensitivity of 97.4%-97.8%.

Table 5: Classification of Rapid Test Kits sensitivity based on plasma.

GROUP	NAME OF RTK	SENSITIVITY
A(se)	SD Bioline	99.1%
	DPP	
	Determine Combo	
	Antec	
B(se)	Retroscreen	98.7%
	Oraquick	
C(se)	Vikia	98.3%
	Retrocheck	
	Rapid Signal	
	Insti	
	HIV Status	
	HIV Quick Check	
	First Response	
	Dialab	
	Colloidal Gold	
	Core Instant	
	Carestat	
	Advanced Quality	
D(se)	Icare	Below 98% (97.4%-97.8%)
	Genie III	
	Vitest	
	Pareekshak	

Figure 1: Groupings of test kits sensitivity based on plasma



5.1.3 Specificity of test kits

The test kits were classified into four groups (A-D). Group A and Group B had specificities of 99.6% and 99.2% respectively while the test kits in Group C and Group D had specificities of 98.4%-98.8% and 94.2%- 97.7% respectively. Antec had the lowest specificity (94.2%). The difference between the specificity of Antec and most of the other kits was statistically significant

Table 6: classification of rapid test kits specificity based on plasma

GROUP	NAME OF RTK	SPECIFICITY
A (sp)	1. Retrocheck	99.6%
	2. Icare	
	3. Vitest	
B (sp)	1. SD Bioline	99.2%
	2. Oraquick	
	3. Vikia	
	4. Rapid Signal	
	5. Core Instant	
	6. Advanced Quality	
	7. Genie III	
C(sp)	1. DPP	98.4%-98.8%
	2. Determine Combo	
	3. Retroscreen	
	4. Insti	
	5. HIV Status	
	6. HIV Quick Check	
	7. Dialab	
	8. Colloidal Gold	
	9. Pareekshak	
D(sp)	1. First Response	Below 98% (94.2%-97.7%)
	2. Carestat	
	3. Antec	

Figure 2: Groupings of test kits specificity based on plasma

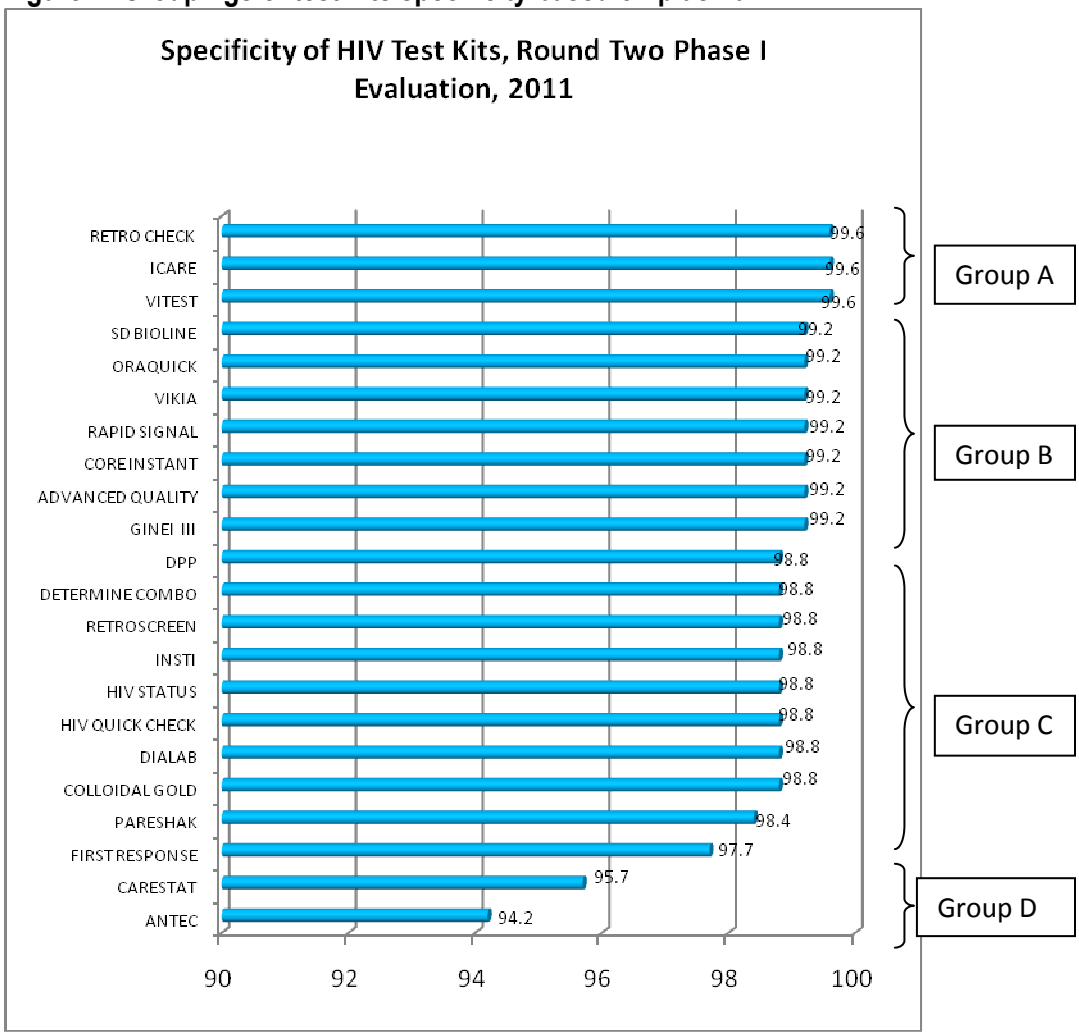


Table 7: Predictive Values of evaluated HIV test kits based on plasma

S/N	Name of Test Kit	Positive Predictive Value	Negative Predictive Value
1	Advanced Quality	99 .1	98 .5
2	Antec	94 .0	99 .2
3	Carestat	95. 5	98. 4
4	Colloidal Gold	98. 7	98 .4
5	Core Instant	99. 1	98 .5
6	Determine Combo	98 .7	99. 2
7	Dialab	98. 7	98. 4
8	DPP	98. 7	99. 2
9	First Response	97. 5	98. 4
10	Genie lll	99 .1	99 .2
11	HIV Quick Check	98 .7	98 .4
12	HIV Status	98. 7	98. 4
13	Icare	98. 1	99 .6
14	Insti	98. 7	98 .4
15	OraQuick	99. 1	98. 8
16	Pareekshak	98 .3	97. 7
17	Rapid Signal	99 .1	98 .5
18	Retrocheck	99 .6	98 .5
19	Retro Screen	98 .7	98 .8
20	SD Bioline	99 .1	99 .2
21	Vikia	99 .1	98 .5
22	Vitest	98 .3	97 .7

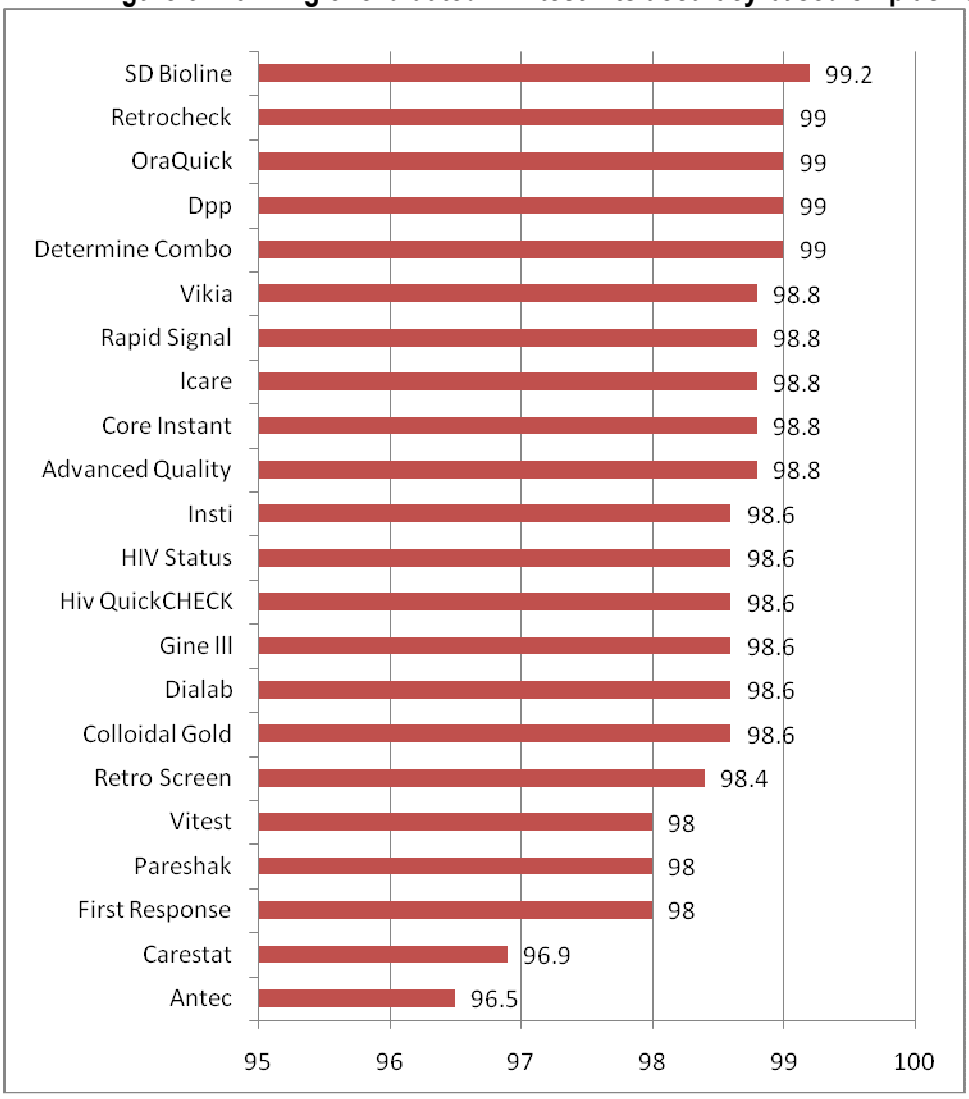
The Positive Predictive Value (PPV) was between 94.0%- 99.6% while the Negative Predictive Value (NPV) was 97.7% - 99.6%.

Table 8: Ranking of evaluated RTKs accuracy based on plasma

S/N	Name of Test Kit	Accuracy (%)
1	SD Bioline	99.2
2	Determine Combo	99.0
3	DPP	99.0
4	OraQuick	99.0
5	Retrocheck	99.0
6	Advanced Quality	98.8
7	Core Instant	98.8
8	Icare	98.8
9	Rapid Signal	98.8
10	Retro Screen	98.8
11	Vikia	98.8
12	Colloidal Gold	98.6
13	Dialab	98.6
14	Genie lll	98.6
15	HIV Quick Check	98.6
16	HIV Status	98.6
17	Insti	98.6
18	First Response	98.0
19	Pareekshak	98.0
20	Vitest	98.0
21	Carestat	97.0
22	Antec	96.5

The accuracy ranged between 96.5% - 99.2%.

Figure 3: Ranking of evaluated HIV test kits accuracy based on plasma



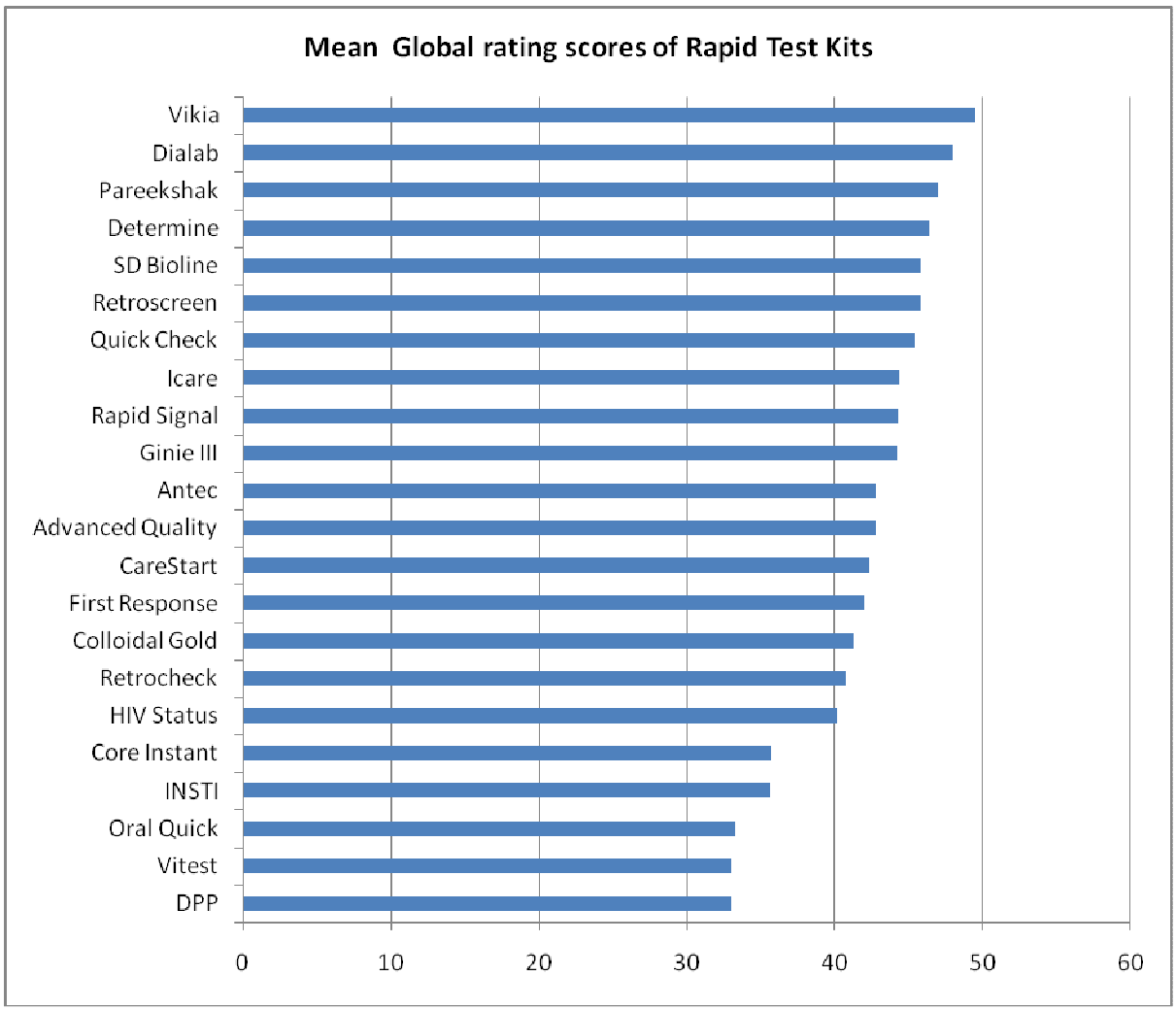
5.1.4 Testers Ratings:

The global scores of each evaluated RTK based on testers rating on ease of performance were used as one of the criteria critical in identifying the testing algorithm. Table 9 below shows the ranking of the evaluated HIV rapid test kits based on the mean global score of testers rating.

Table 9: Mean Global score of testers rating of the evaluated HIV rapid test kits

S/N	Name of test kits	Mean rating score (maximum = 55)	Mean rating score (%)
1	Vikia	49.5	90.0
2	Dialab	48.0	87.3
3	Pareekshak	47.0	85.5
4	Determine	46.4	84.4
5	Retroscreen	45.8	83.3
6	SD Bioline	45.8	83.3
7	Quick Check	45.4	82.6
8	Icare	44.4	80.7
9	Rapid Signal	44.3	80.6
10	Genie III	44.2	80.4
11	Advanced Quality	42.8	77.8
12	Antec	42.8	77.8
13	CareStart	42.3	76.9
14	First Response	42.0	76.4
15	Colloidal Gold	41.3	75.1
16	Retrocheck	40.8	74.2
17	HIV Status	40.2	73.1
18	Core Instant	35.8	65.1
19	INSTI	35.6	64.7
20	Oral Quick	33.3	60.6
21	DPP	33.0	60.0
22	Vitest	33.0	60.0

Figure 4: Mean global rating scores of testers rating of evaluated HIV rapid test kits



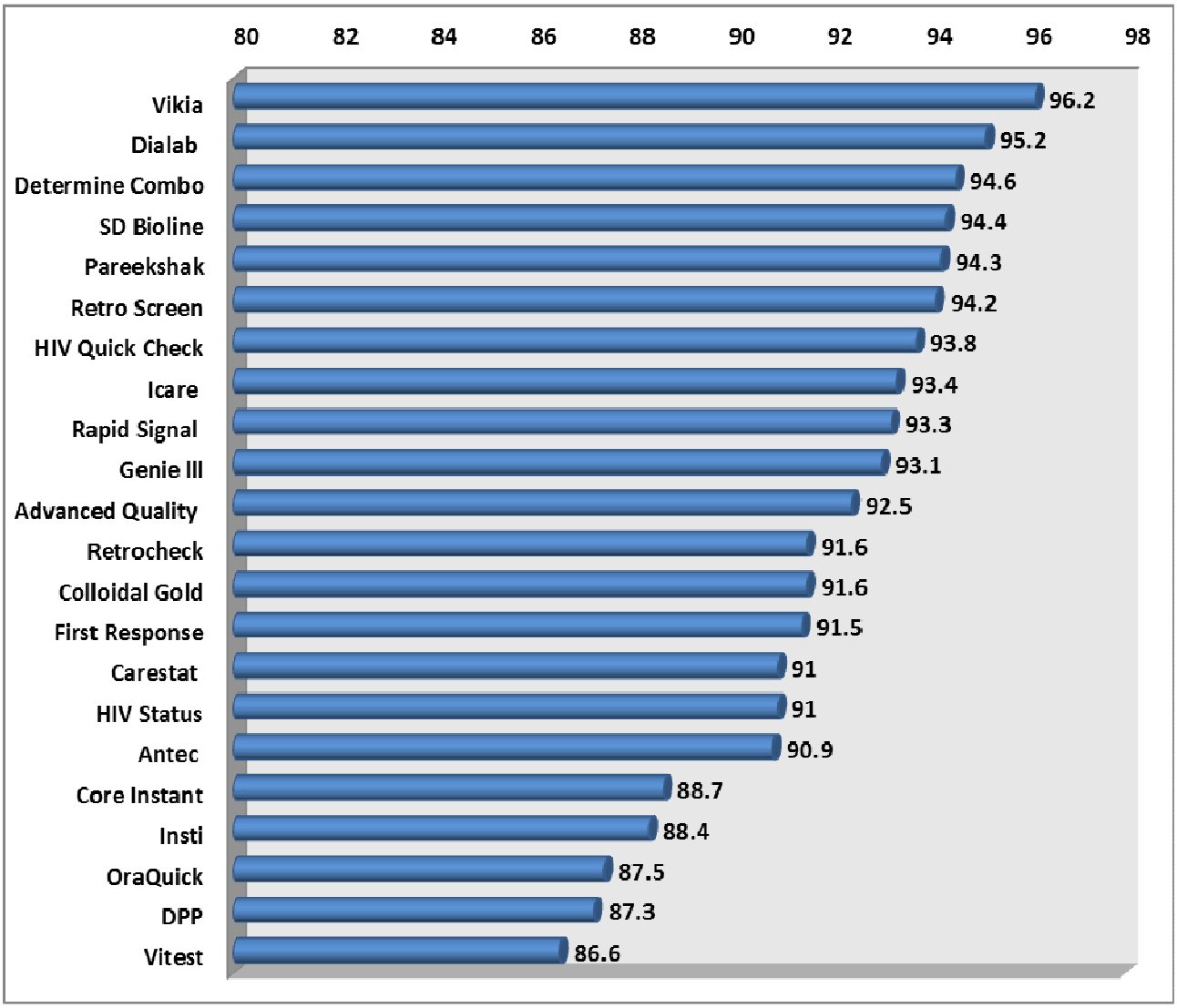
5.1.5 Composite score

The accuracy of each test was assigned a weight of 70% while the global score (based on performance characteristics) was assigned the weight of 30%. A composite score was determined as weighted mean of accuracy and global score. Similarly, the composite score was also determined for the various combinations of the RTKs, (with the accuracy being based on the combination thereof).

Table 10: Global rating showing composite scores using testers ratings and accuracy of RTKs.

S/N	Name of RTK	Composite scores (%)
1	Vikia	96.2
2	Dialab	95.2
3	Determine Combo	94.6
4	SD Bioline	94.4
5	Pareekshak	94.3
6	Retro Screen	94.2
7	HIV Quick Check	93.8
8	Icare	93.4
9	Rapid Signal	93.3
10	Genie lll	93.1
11	Advanced Quality	92.5
12	Retrocheck	91.6
13	Colloidal Gold	91.6
14	First Response	91.5
15	Carestat	91.0
16	HIV Status	91.0
17	Antec	90.9
18	Core Instant	88.7
19	Insti	88.4
20	OraQuick	87.5
21	DPP	87.3
22	Vitest	86.6

Figure 5: Ranking of RTKs using a composite score of testers ratings and accuracy



6.0 DISCUSSION

General performance of test kits depends on several parameters which includes sensitivity, specificity, positive and negative predictive values, accuracy of individual test kits including overall onsite performance and comments. Consideration was also made on 3 test combination algorithms with highest performance (Sensitivity and Specificity) and high composite score (Accuracy and global mean tester rating).

The result of the test with plasma in this evaluation showed that no single test had a sensitivity of 100%. Sensitivities ranged from 97.4%-99.1% (95%CI) (Table 3). Similarly, in oral fluid based test kits sensitivities of the two tests were 91.8% and 91.2% for DPP and Oraquick respectively (Table 4).

Based on the results of test with plasma, the kits could be categorized into four groups (A-D) in terms of their sensitivities referred to $A_{(se)}$-$D_{(se)}$ as shown in table 5. Those in group $A_{(se)}$, $B_{(se)}$ and $C_{(se)}$ had sensitivities of 99.1%, 98.7% and 98.3% respectively while those in group $D_{(se)}$ had sensitivity of 97.4%-97.8% (95%CI) (figure 1). Statistically, none of the test kits was significantly more sensitive than the other since the confidence intervals overlapped each other. However, operationally the observed difference in sensitivity is useful in constructing the algorithm.

The individual specificity of the test kits ranged from 94.2%-99.6%; test kits were classified into four groups (A-D) referred to $A_{(sp)}$-$D_{(sp)}$ as shown in Table 6. Group $A_{(sp)}$ and Group $B_{(sp)}$ had specificities of 99.6% and 99.2% respectively while the test kits in group $C_{(sp)}$ and Group $D_{(sp)}$ had specificities of 98.4%-98.8% and 94.2%- 97.7% respectively (figure 2).

It is possible that test kits with similar sensitivities may yield different discordant results when compared with the gold standard. In this evaluation, the test kits with similar sensitivity showed a high degree of concordance with each other hence, we had sufficient confidence that results obtained with kits from the same group would be the same as those obtained from any kit of the same groupings.

Based on the WHO Guidelines on Appropriate Evaluations of HIV Testing technologies in Africa recommending a sensitivity and specificity of 0.98 for 95% confidence interval, it was decided that test kits in this evaluation with both sensitivity and specificity of 98% and above would be selected. In this evaluation, 15 test kits had both sensitivity and specificity of 98% and above.

The predictive values are relevant in field situation. In this evaluation pre-determined or selected plasma samples were used, therefore the results of predictive values shown in table 7 are artificial. However, the accuracy of individual test which is a function of sensitivity and specificity were determined and ranked as shown in figure 3 and table 8.
Further consideration was given to the global ratings of test kits (Table 9) which include ease of performance of the test, ease of reading and interpreting results as well as the amount of waste

generated as rated in figure 4. The accuracy of the selected kits was combined with the testers rating scores to arrive at composite scores (Table 10). The composite scores were then used to rank the RTKs (figure 5).

7. 0 CONCLUSION

Based on the laboratory performances showing the sensitivity and specificity, the global mean tester rating of the evaluated HIV rapid test kits and in turn the composite scores, the following algorithms are recommended.

- **First line test kits:** These include SD bioline, Dialab, Determine Combo, Retroscreen, Vikia, HIV Quick Check, Oraquick and DPP. Any of these kits can be used as the screening test in this algorithm.
- **Second line test kits:** These include Retrocheck, Rapid Signal, Core Instant, Advance Quality, HIV Status. Any of these kits can be used for confirmation of positives from the first line tests.
- **Tie-Breaker:** These include Colloidal Gold, and Insti. Any of these kits may be used to resolve discordance arising from first and second line tests.

The recommended algorithm table is shown below:

ALGORITHM TABLE		
1st Line	**2nd Line**	**Tie Breaker**
SD Bioline	Retrocheck	Colloidal Gold
Dialab	Rapid Signal	Insti
Determine Combo	Core Instant	
Retroscreen	Advanced Quality	
Vikia	HIV Status	
HIV Quick Check		
Oraquick		
DPP		

8.0 RECOMMENDATIONS

Following the result of the evaluation, the following recommendations were made: -

1. **Multiple algorithms testing.**
   In view of the fact that 15 test kits performed well in the evaluation, the use of these test kits in constructing algorithm will create multiple options of algorithms that testing programmes in the country could use. Therefore, multiple algorithms should be used in the country.

2. **Phases of Evaluation**
   The process of evaluation involves three phases starting with phase I which is laboratory based evaluation. The recommended algorithm is used and moved to the second phase referred to as phase II which involves testing the algorithm in the field. The performance of the algorithm constructed with this phase is monitored through phase III. For this evaluation therefore, the phase II should be conducted immediately within the period of 6 months from commencement of the implementation.
3. **Performance Monitoring**
   There is the need for a system to be put in place for monitoring the performances of these test kits within the recommended algorithm, including Post Market Validation (PMV) and Quarterly Field Evaluation which should be co-ordinated by HAD.
4. **Dissemination:**
   This should be carried out at all state and federal levels to get all the relevant stakeholders to buy into the program.
5. **Enforcement of adherence**
   The Federal Ministry of Health (FMOH) should liaise with relevant regulatory agencies to ensure and enforce adherence to the recommended algorithms.
6. **Training**
   On adoption of the algorithms, FMOH and other relevant stakeholders including test kit manufacturers, SMOH, NGOs and IPs should commence immediate training on test kits within the recommended algorithms. Furthermore, test kit manufacturers and vendors should be responsible for training the trainers that will step down the training on the use of their test kits.
7. **Verification of existence of the authentic manufacturer's representatives and addresses.**
   One of the factors required in maintaining quality of test kits once they are in the algorithm is to ensure good manufacturing practice of the test kits and good storage condition. The manufacturers or their representatives in Nigeria should be visited at the addresses indicated in annex VIII to ensure that they have appropriate storage facilities. Vendors without appropriate storage capacity should not be patronized.

8. **HIV Oral Testing**
   The sensitivity of Oral fluid based test kits in testing with oral fluid was very low. Testing with oral fluid is thus not recommended.
9. **Funding of Evaluation**
   The Process of evaluation for Algorithm development is capital intensive and as recommended by World Health Organization, manufacturers of test kits to be evaluated should be made to contribute to the funding of the evaluation. This process took a longer time to complete due to non-availability of funds while needed. We recommend therefore that for subsequent evaluation vendors must pay for evaluation following the guidelines that is to be developed by the FMOH.
10. **Use of Tie-breaker**
    Considering the non-significant difference in the specificity and sensitivity of the evaluated test kits, the use of a tie breaker is more or less a repetition of the first line high sensitivity or 2nd line high specificity test kit. Hence, the use of tie breaker should be linked with an effective referral system where discordance can be effectively resolved.
11. **The Preferred Gold Standard**
    With the attendant variations in western blot result acceded to by the high indeterminate results observed and documented, Polymerase Chain Reaction (PCR) should be used as the gold standard in subsequent evaluations.
12. **Implementation of Round 2 Phase I evaluation**
    This evaluation is without prejudice to the earlier one done in 2007 and the consequent algorithm thereof. The implementation of this report is therefore an addition to the existing HIV testing algorithm in Nigeria.

IMPLEMENTATION PLAN FOR THE PROPOSED ALGORITHMS

S#	Activity	Responsible	Time frame
1	Endorsement of the report by the HMH	HMH	Nov, 2011
2	Printing & dissemination of report	FMOH/SMOH/IPs/NGOs	Feb – April, 2012
3	Training on the use of the algorithms	FMOH/IPs	April-July, 2011
4.	Commencement of Phase II evaluation	FMOH	6 months after commencement of use
4	Pre/Post market validation of new kits	FMOH/SMOH/IPs/NGOs	On-going
5	Periodic Monitoring	FMOH/SMOH/IPs/NGOs	3mths,6mths,12moths

9.0 REFERENCES

1) Bharat S. P, Mireille B. K,George A., Chin-Yih O., Guy-Michel Gershy-Damet and John N. N. Scalling up HIV Rapid Testing in Developing Countries; Comprehensive Approach for Implementating Quality Assurance 2010. Am. J Clin Pathol 134:573-584

2) CDC; WHO, APHL. Draft Guidelines for Appropriate Evaluation of HIV Testing Technologies in Africa

3) Federal Ministry of Health Nigeria: Laboratory based HIV Rapid Test Validation (Phase 1) in Nigeria. April 2007

4) Federal Ministry of Health Nigeria: Protocol for field evaluation of recommended HIV rapid test kits for formulation of national HIV testing algorithm

ANNEXES

Annex 1: Evaluation Working Group:

1) HIV/AIDS Division (HAD),
2) National Agency for the Control of AIDS (NACA),
3) Central Public Health Laboratories (CPHL),
4) National Agency for Food, Drug Administration and Control (NAFDAC),
5) National Institute for Pharmaceutical Research and Development (NIPRD)
6) Nigeria Institute of Medical Research (NIMR),
7) National Blood Transfusion Service (NBTS),
8) Medical Laboratory Science Council of Nigeria (MLSCN),
9) World Health Organization (WHO),

International Donor Organizations; specifically Centre for Disease Control and Prevention, Global AIDS Program (CDC-GAP), some partners implementing the PEPFAR program in Nigeria and private organizations with international experience in rapid test evaluations such as Safe Blood for Africa Foundation and African Health Project (AHP),

Annex II

Names and Contacts of Contributors to protocol development

S/N	NAME	CONTACT ADDRESS
1	Dr. Wapada I. Balami mni	National Coordinator, HIV/AIDS Division, FMOH
2	Manason Rubainu (Chairman)	UATH,Gwagwalada
3	Prof. D. Olaleye	UCH, Ibadan
4	Dr.Dauda Oladepo	NIPRD, Idu, Abuja
5	Dr. Ali Onoja	AHP, Garki, Abuja
6	Dr. Adedeji A. A.	CPHL, Lagos
7	Dr. Rosemary Audu	NIMR, Lagos
8	Idris Saliu	SBFAF, Wuse II
9	Chief Chris Elemuwa	NPHCDA, Abuja
10	Kachiro Yakubu.	NASCP, Abuja
11	Asukwo Uwah	NASCP
12	Envuladu O.A	NASCP
13	Ofaka E.C	NASCP
14	Dr.Fatima Damagum (Corper)	NASCP
15	*Dr. Wurie Isata*	CDC-GAP, Nigeria
16	Jelpe Tapdiyel	CDC-GAP, Nigeria
17	Theo Faruna	Axios Foundation, Abuja
18	Ibrahim Mohammed Murtala	SFH, Abuja

Annex III: National Health Research Ethics Committee Approval

NHREC Protocol Number NHREC/01/01/2007-22/11/2010
NHREC Approval Number NHREC/01/01/2007-09/02/2011b
Date: February 10, 2011
Re: Round 2 Phase 1 Evaluation Of HIV Rapid Test Kits For The Expansion Of The Scope Of The Current Interim National HIV Rapid Testing Algorithm
Health Research Ethics Committee (HREC) assigned number: NHREC/01/01/2007
Name of Co-Principal Investigator: Dr. Wapada Balami
Address of Principal Investigator: National Coordinator
National AIDS and STI Control Program (NASCP)
Department of Public Health,
Federal Ministry of Health, Abuja
Date of receipt of valid application: 22-11-2010
Date when final determination of research was made: 09-02-2011
Notice of Full Committe Approval
This is to inform you that the research described in the submitted protocol and the amendment sought, the consent forms, advertisements and other participant information materials have been reviewed and *given full committee approval by the National Health Research Ethics Committee.* This approval dates from 09/02/2011 to 08/02/2012. If there is delay in starting the research, please inform the HREC so that the dates of approval can be adjusted accordingly. Note that no participant accrual or activity related to this research may be conducted outside of these dates. *All informed consent forms used in this study must carry the HREC assigned number and duration of HREC*
approval of the study. In multiyear research, endeavor to submit your annual report to the HREC early in order to obtain renewal of your approval and avoid disruption of your research.
The National Code for Health Research Ethics requires you to comply with all institutional guidelines, rules
and regulations and with the tenets of the Code including ensuring that all adverse events are reported
promptly to the HREC. No changes are permitted in the research without prior approval by the HREC
except in circumstances outlined in the Code. The HREC reserves the right to conduct compliance visit your
research site without previous notification.
Signed
Clement Adebamowo BMChB Hons (Jos), FWACS, FACS, DSc (Harvard)
Honorary Consultant Surgeon, Director, West African Center for Bioethics and
Chairman, National Health Research Ethics Committee of Nigeria (NHREC)

Annex IV (a) **Round 2 Phase 1evaluation of RTKs**
Standard Log book for POC testing of Oral Fluid

Name of Site: _____ Zone_____

Client ID Number	Date Tested (dd/mm/yy)	Oral Quick HIV test kit Lot No._____ Expiration Date _____/_____/__ (Circle one)	DPP HIV Test Kit Lot No._____ Expiration Date _____/_____/__ (Circle one)	HIV Test Kit Name_____ Lot No._____ Expiration Date _____/_____/__ (Circle one)	Comments
		NR R INV	NR R INV	NR R INV	
		NR R INV	NR R INV	NR R INV	
		NR R INV	NR R INV	NR R INV	
		NR R INV	NR R INV	NR R INV	
		NR R INV	NR R INV	NR R INV	
		NR R INV	NR R INV	NR R INV	

Annex I V (b)

Data collection sheet (Nigeria HIV Rapid Test Evaluation)

Name of Rapid Test ----------

Reporting Code
Please use the codes below for reporting test results.
P = Positive
N = Negative
I = Invalid
Ins = Insufficient Sample volume

Date Tested (time and date): .....

Kit Lot: .....

Kit Exp Date: .....

Lab Scientist: .....

Pos and Neg Control Worked: **Yes** **No**

Room Temperature Reading:

Specimen ID#	Test Result	Specimen ID#	Test Result	Specimen ID#	Test Result	Specimen ID#	Test Result

Annex V Consent Form

(to be administered orally)

Hello, my name is _____ and I am a staff in this clinic.

We are evaluating some HIV rapid test kits presently in use in Nigeria. This exercise will help us to determine the quality and performance of the kits; which would ensure the proper diagnosis of HIV. This is a national project conducted by the Federal Ministry of Health. Our facility is one of the recruitment sites for sample collection for the evaluation. We are recruiting individuals who are both HIV negative and positive. Participation is voluntary. If you decide not to participate, however, you will still receive services from the clinic like other people and it will not affect the quality of services you receive. Any information you provide for this survey will be confidential and anonymous. If you agree to take part, then we will:

1. Draw 10ml of blood with a needle and syringe. The blood collection might cause some pains and discomfort at the site of blood collection.
2. Take oral fluid with an oral fluid swap. Little pressure will be applied during collection which might cause some discomfort.
3. Test the blood and the oral fluid with different HIV rapid test kits and confirmatory test.
4. We will not record your name or address on any form or result. Only the staff who tested you will know your result.
5. The results of the evaluation may be written into a report which will not have any reference to you as a person. The report will be used to improve HIV testing quality in Nigeria.
6. All left over specimen will be discarded after the testing at the collection site for oral fluid at quality control laboratory for blood samples.

Do you have any questions?

If you have other concerns about this project implementation, please contact Dr. Wapada I. Balami: 08033299779 and/or Mr. Yakubu Kachiro: 0803 324 6011 HIV/AIDs Division, FMOH, Edo House, Central Area, Abuja; and for issues regarding rights of participation should contact NHREC office, Federal Secretariat Complex; Shehu Shagari Way, Central Area, Abuja: +234-9-523 8367.

I, _____, confirm that I have given the information sheet to the participant with ID number: _____ and answered his/her questions to his/her satisfaction.

The participant has agreed to participate .

Signature/thumbprint of the participant _____Date _____

Signature/date of staff documenting __

Annex VI

Members of National HIV Laboratory Quality Assurance Team (NHLQAT) that participated at the evaluation

SN	Name	Designation	Organization
1	Mr. Manason Rubainu	Chairman, EWG	UATH, Abuja
2	Mr. Kachiro Yakubu	CSO (Lab Lead)	FMOH, HIV/AIDS
3	Mr. Asukwo Uwah	ACMLS	FMOH, HIV/AIDS
4	Mr. Tony Adonye	SMLT/Lab	FMOH, HIV/AIDS
5	Mr. Samuel Adeyemi	CMLS	CPHL, Yaba
6	Mr. Agba Janet	PMLS	NBTS
7	Mr. Ngamnori S.C.	PLT	NAFDAC
8	Mr. Uchuno Gregory	CMLS	MLSCN,
9	Mr. Busari Olusegun	PMLS	MLSCN, NLEQAC
10	Mrs Aniedobe Maureen.	Research Fellow	NIMR, Yaba
11	Mr. Theophilus Faruna	LPM	Axios Foundation
12	Enuma Joseph		SCMS
	Observer members		
13	Mr. Tapdiyel Jelpe		CDC
14	Mrs. Odafen Oke		CDC
15.	Mr. Obinna Nnadozie		CDC
	Support staff from NEQAL, Zaria		
16	Olumide Okunoye	SMLS	NEQAL
17	Julius Kwata	MLS	NEQAL
18	Okeke Onyekachukwu F. I.	MLS	NEQAL
18	Henry	MLT	NEQAL

ANNEX VII

TESTERS' RATINGS TOOL FOR RAPID TEST KITS (RTKS) DURING LABORATORY EVALUATION

Instructions:
The purpose of this questionnaire is to find out how you rate the RTKs currently under lab evaluation. You will be given this questionnaire after you have evaluated each rapid test kit. You do not need to put your name on the form. Please be open and honest. Along with other information, your feedback on this questionnaire will help form decisions on which RTKs will be recommended for use in Nigeria. Please take 5-10 minutes to complete this form. After completing the form, please give it to one of the lab supervisors.

1. What test kit did you just run?
_____

Please rate each of the RTKs on the following criteria by circling the most appropriate response using this scoring system:

1	2	3	4	5
Very easy	Easy	Neither	Difficult	Very Difficult

2. Collecting and delivering the **correct volume of plasma/sera** onto the device:
1 2 3 4 5

3. Adding **diluent/ wash/ chase buffer** correctly onto the device:
1 2 3 4 5

4. Reading the test result within the **correct time period**:
1 2 3 4 5

5. Reading the test result (was it easy or difficult to **read the lines, was the line dark Enough?**):
1 2 3 4 5

6. Interpreting the test (deciding whether the test positive/ negative based on lines or clumping):
1 2 3 4 5

7. Learning how to perform the test (was it easy for you to learn how to perform this test, would it be easy to train others how to perform this test?):
1 2 3 4 5

8. Overall ease of use:

1 2 3 4 5

9. Design of the test device for writing patient ID number (was it easy for you to write the ID number, was adequate space provided?):
1 2 3 4 5

10. How often did you obtain an invalid test result ?(test control line not present or no results were generated): ***Please state number of invalid test results you got during the testing period. If none, please write 0.***

I had _____ invalid tests out of a total of _____ specimens.

11. Did you find any **defective test devices or accessory supplies?** ***Report how many or thetotal number of specimens tested***
I found _____ defective tests while testing _____ specimens.

12. Were there any problems **with any of the RTKs during the study period (in particular around ease of learning how to use the test, how to perform the test and how to interpret the test)?**

_____
_____

13. Would you recommend the use of this test kit? Yes/No
If NOT, give all your reasons?
Please list all of the reason(s) that apply.

_____
_____
_____

14. What is your opinion of the test kit packaging? ***Rate each aspect by circling one answer:***
What did you think of the **size of the test kit box/package**?

1	2	3	4	5
Very Bulky,	Bulky,	Moderate,	Compact,	Very Compact

How **rough is the packaging**?

1	2	3	4	5
Very Flimsy	Flimsy	OK	Robust	Very Robust

How much **waste** was generated in running your set of specimens?

1	2	3
Very Much Waste	Much Waste	Minimal Waste

Annex VIII: GENERAL CHARACTERISTICS OF TEST KITS EVALUATED

S/N	Name of kit	Manufacturer, Country of origin	Local Vendor; Name and address	NAFDAC Registered	Assay Type	Antigen type / (Solid phase)	Specimen Type
1	ADVANCED QUALITY	In Tec Products, Inc. USA	Afrimed Ltd, Suit G160, Lekki Lagos	No Reg. No.	Chromatographic Lateral flow - Recombinant antigen (Gp41, p24)	Recombinant protein	Whole blood, serum/plasma
2	ANTEC	Sunrise Labs - Intech products, California USA	Antec Diagnostics Nig. Ltd. 22 Anazonwu Street, Onitsha, Anambara state	03-0731	Chromatographic Lateral flow -	Recombinant antigen (Gp41, p24)	Whole blood, serum/plasma
3	CARE STAT	Access Bio, Inc, USA	Access Bio Inc, 7 Cairo Street off Adetokunbo Ademola Crescent, Wuse 2, Abuja	03-1726	Immunoassay, Lateral flow	Recombinant proteins	WB, Serum/plasma
4	COLLOIDAL GOLD	Shanghai Kehua Bio-Engineering co, Lt	IDA Foundation, Kaduna		Immunochromatographic Lateral flow -	Recombinant proteins	WB, Serum/plasma
5	CORE INSTANT	Core Technology, Beijin China	Unidal Facts Ventures Ltd, 14 Tola Adewunmi Street, Lagos		Chromatographic Lateral flow - Recombinant antigen (Gp41, p24)	Recombinant antigen (Gp41, p24	White Blood (WB), Serum/plasma, Oral fluid
6	DETERMINE COMBO	Inverness Medical Innovations	Lab Assist Nig. Ltd. House 10 Bua Court, 15 Dar-Esalam Street off Aminu Kano Crescent, Wuse 2 Abuja	No Reg. No.	Lateral flow Ab p24 test		WB, Serum/plasma, Oral fluid
7	DIALAB	Dialab Produktion, Austria	Darlez, 10 Lingua Crescent, off Aminu Kano Crescent,		Chromatographic lateral flow	Recombinant antigen	WB, Serum/plasma,

			Wuse 2 Abuja				Oral fluid
8	DPP	Chembio Diagnostic System, Inc. 3661Horseblock Road, Medford NY 11763, USA	Chembio Diagnostics Nig. Ltd, House 9 A close, 24 Crescent Gwarinpa Estate, Abuja		Lateral flow Chromatography	Recombinant	WB, Serum/plasma, Oral fluid
9	FIRST RESPONSE	Premire Med Corporation Ltd, India	SHI Logistics, Dominion House- Ground Floor, 40 Asheik Jama Strreet, off Mike Akhigbe Way, Jabi Lake, Abuja		Immunochromatography	Recombinant antigen (Gp41, p24	WB, Serum/plasma
10	GINIE III	Bio-Rad, France	Sola-Wunmi Enterprises Bagada Lagos	03-0646	Chromatographic lateral flow	Recombinant antigen	WB, Serum/plasma
11	HIV QUICK CHECK	Hi-tech, 2 Chief Festus Dhiri Road, Odunmara, Obi-Orodo Mbaitoli LGA Imo State, Nigeria	Hi-Tech Diagnostics Ltd, 175 Dauglas Road, Owerri, Imo State	03-0989	Chromatographic lateral flow	Recombunant	WB, Serum/plasma
12	HIV STATUS	Hi-tech 2 Chief Festus Dhiri Road, Odunmara, Obi-Orodo Mbaitoli LGA Imo State, Nigeria	Hi-Tech Diagnostics Ltd, 175 Dauglas Road, Owerri, Imo State	03-0990	Chromatographic lateral flow		WB, Serum/plasma
13	ICARE	JAL Innovation Singapore Pte Ltd www.jalinnovation.com	Darlez, 10 Lingua Crescent, off Aminu Kano Crescent, Wuse 2 Abuja		Chromatographic lateral flow	Recombinant antigen	WB, Serum/plasma

14	INSTI	BioLytical Laboratories, Commerce Parkway, Richmond, British Columbia, Canada	BioLytical Nig Ltd, 304 Abisogun-leigh Street, Ogba Ikeja Lagos	03-1175	Flow through		WB, Serum/plasma
15	ORAQUICK	OraSure Technologies, Inc. ,Bethlehem PA 18015 USA	Bolingo Hotel & Towers Suit 205, Independent Avenue, Area 10, Garki, Abuja	03-1442	Leteral Flow	Recombinant antigen	WB, Serum/plasma, Oral fluid
16	PAREEKSHAK	BHAT BIO-TECH INDIA (P) LTD			Leteral Flow Chromatographic Assay	Recombinant antigen	
17	RAPID SIGNAL	Orgenics Ltd, Israel	Rilwan Rilwane Co Ltd, 11 Prince Sulaimon Taiwo Crt, Jakande Estate, Isolo Lagos	03-1338	Immunochromatography lateral flow	Recombinant proteins	WB, Serum/plasma
18	RETRO CHECK	Qualpro Diagnostics, India	IDA Foundation, Kaduna		Immunochromatography lateral flow	Recombinant antigen (Gp41, p24	WB, Serum/plasma
19	RETRO SCREEN	Qualpro Diagnostics, India	Zayo Sigma Chemicals Ltd. Zayo House, Yakubu Gowon Way Jos, Plateau State.	03-0613	Immunochromatography lateral flow	Recombinant/Synthetic Peptide	WB, Serum/plasma
20	SD BIOLINE	Standard Diagnostics Inc, Korea	C.C. Obi Nigeria Ltd, 42/44 Ashogbon Street Lagos Island Lagos	03-0616	Chromatographic lateral flow	Recombinant antigen (Gp41, p24 &gp36	WB, Serum/plasma
21	VIKIA	BioMerieux, France	C.I.O and Sons Merchants Nig. Ltd. 24 Murtala Mohammed Way, Jos		Chromatographic lateral flow by capilary	Synthetic peptides	WB, Serum/plasma