Viral Load Suppression and HIV Drug Resistance Prevalence among PLHIV in Nigeria: Cross-Sectional Survey

STUDY PROTOCOL

Sponsor Institution: United States Presidents Emergency Plan for AIDS Relief (PEPFAR) through the Centers for Disease Control and Prevention

Acronyms

AIDS	Acquired Immune Deficiency Syndrome
ALTC	Asokoro Laboratory Training Center
ART	Anti-Retroviral Therapy
ARV	Anti-Retroviral
CCFN	Catholic Caritas Foundation of Nigeria
CD4	Cluster of Differentiation 4
CDC	Centres for Disease Control and Prevention
CIHP	Centres for Integrated Health Programs
CSIMS	Central Survey Information Management System
CTC	Central Technical Committee
DRM	Drug Resistance Mutation
EDC	Electronic Data Collection
EDTA	Ethylene Diamine Tetra-acetic Acid
EMR	Electronic Medical Record
FHI360	Family Health International 360
GON	Government of Nigeria
GRL	Genotyping Reference Laboratory
HIV	Human Immunodeficiency Virus
HIVDR	HIV Drug Resistance
IHVN	Institute of Human Virology, Nigeria
IRB	Institutional Review Boards
JUTH	Jos University Teaching Hospital
LUTH	Lagos State University Teaching Hospital
NHREC	National Health Research Ethical Committee
NIMR	Nigerian Institute of Medical Research
NNRTI	Non-Nucleoside Reverse Transcriptase Inhibitors
NRTI	Nucleoside Reverse Transcriptase Inhibitors
PCR	Polymerase chain reaction
PII	Personal Identifiable Information
PLHIV	People Living with HIV
PMTCT	Prevention of Mother To Child Transmission
PPS	Probability Proportional to Size
PR	Protease
RT	Reverse Transcriptase
SID	Survey Unique Identifier
SSL	Secure Sockets Layer
SST	States Supervisory Teams
UNIAIDS	The Joint United Nations Programme on HIV/AIDS
VL	Viral Load
VLS	Viral Load Suppression
WHO	World Health Organization

Table of Content

Study Overview

Study Title: Viral Load Suppression and HIV Drug Resistance Prevalence among PLHIV in Nigeria: Cross-Sectional Survey

Project Summary:

Currently, about 3.4 million people in Nigeria are estimated to be living with HIV/AIDS, and about 1.6 million are in need of ART.Anti-Retroviral Therapy (ART). In line with the Joint United Nations Programme on HIV/AIDS (UNAIDS) identification of the VLS as a key component of its Fast Track strategy targets, termed "90-90-90" and the World Health Organization (WHO) recommendation for routine viral load monitoring to diagnose and confirm treatment failure, it is critical that programs be able to monitor Viral Load Suppression (VLS) and HIV drug resistance (HIVDR) among People Living with HIV (PLHIV) receiving treatment for the purposes of program management, monitoring and evaluation (M&E), and surveillance. In addition, the routine monitoring of VLS and HIV DR allows clinicians to identify PLHIV who are failing treatment, understand the HIV drug resistance among them, and intervene, reducing the probability of poor HIV-related outcomes and preventing /minimizing the development of HIV drug resistance (HIVDR). However, accurate national estimates on these essential indicators in Nigeria are not available.

The purpose of this survey is to estimate the national and regional prevalence of VLS and of HIVDR among PLHIVs on ART in Nigeria.

Primary Objectives:

1. to estimate the national and regional prevalence of viral suppression among adult PLHIV (15 + years) receiving ART for at least 12 months,
2. to estimate the national and regional prevalence of viral suppression among paediatric PLHIV (<15 years) receiving ART for at least 12 months, and
3. to estimate the national and regional prevalence of acquired HIV drug resistance among adult and child PLHIVs receiving ART for at least 12 months with VL≥1000 copies/ml.

Secondary Objectives:

1. to describe the pattern of acquired drug resistance mutations (DRMs) among adults and children receiving ART and by regimen types, and
2. to determine clinical and demographic risk factors associated with virologic failures and HIVDR among PLHIVs on ART for at least 12 months using multivariate analysis

The survey will be a cross-sectional study of the PLHIV receiving ART for at least 12 months in selected ART facilities in Nigeria. A stratified cluster sampling design will be used. Seventy (70) ART facilities will be the study clusters and will be selected within regions using Probability Proportional to Size (PPS) from a sampling frame (list of all ART facilities in the country). The survey will be conducted among 1050 adults and 560 children, the sample sizes are determined for the separate sub-populations. The survey is expected to be completed within a period of 10 months from January to October 2018 while survey data abstraction using the electronic data collection model will occur over a 6-month study period within which the study sample sizes are expected to be reached both in adults and children.

Ethical practices such as confidentiality and informed consent will be strictly observed to minimize study risks and protect the interest of the patients. The protocol and informed consent will be reviewed and approved by the National Health Research Ethical Committee (NHREC).

Investigators Roles and Responsibilities

- Principal Investigator and Roles

Principal Investigator (s)	Affiliations	Position	Roles and Responsibilities	SEV#
Alash'le G. Abimiku MON, M.Sc; PhD	University of Maryland Program in Nigeria	Professor of Epidemiology and Executive Director, International Research Center of Excellence	Alash'le is the Implementing Partner PI, responsible for development/reviews of the protocol and other study instruments. He will oversee all aspects of the study implementation and report writing. He is overall responsible for protocol compliance.	**NA**
Sunday Aboje, MD	National HIV/AIDS Division, Federal Ministry of Health (FMOH),	National Coordinator	SA is the FMOH PI, responsible for the overall development of protocol, study implementation and guidance of the study from the government of Nigeria / host country perspective. He support writing and dissemination of study findings to relevant authorities in the country.	

- Co- Investigator(s) and Roles

Co - Investigators	Affiliations	Position	Roles and Responsibilities	SEV#
Mahesh Swaminathan, MD	US Centres for Disease Control and Prevention, Nigeria	Country Director	MS is responsible for providing technical and administrative leadership on the study	**16975**
Dalhatu Ibrahim, MD	US Centres for Disease Control and Prevention, Nigeria	Chief, Epidemiology, Strategic Information and Health Systems Strengthening Branch	DI is responsible for the overall development of protocol and guidance of the study from the CDC perspective.	**14589**
Henry Debem, PhD	US Centers for Disease Control and Prevention, Nigeria	Program Specialist - Epidemiology and Strategic Information	HD is the study Point of Contact (POC) and is responsible for the protocol development. He provides other relevant technical support to the Government of Nigeria (GoN) in the implementation of the process.	**1023**

Co - Investigators	Affiliations	Position	Roles and Responsibilities	SEV#
Dennis Onotu	US Centres for Disease Control and Prevention, Nigeria.	Branch Chief, Care and Treatment	DO supports the technical reviews and validation of the study processes as well as provides other relevant technical support to the process.	**5251**
Charles Nzelue, MD	National HIV/AIDS Division, Federal Ministry of Health (FMOH),	Head, Strategic Information Unit	CN supports the technical reviews and validation of the study processes as well as provides other relevant technical support to the process.	**NA**
McPaul Okoye	US Centres for Disease Control and Prevention, Nigeria	Branch Chief, Laboratory Services	MP supports the laboratory technical reviews and validation of the study processes as well as provides other relevant technical support to the process.	**7817**
Jahun Ibrahim, MD	US Centres for Disease Control and Prevention, Nigeria	Snr. Program Specialist - Strategic Information	JI supports the technical reviews and validation of the study processes as well as provides other relevant technical support to the process.	**19322**
Sebastian Victor, MD	US Centres for Disease Control and Prevention, Nigeria.	Snr. Program Specialist - Surveillance	SV supports the technical reviews and validation of the study processes as well as provides other relevant technical support to the process.	**7404**
Dickson Adegoke	US Centres for Disease Control and Prevention, Nigeria.	Program Specialist – Laboratory Services	DA supports the laboratory technical reviews and validation of the study processes as well as provides other relevant technical support to the process.	**5853**
Sherry Yin, MPH	US Centres for Disease Control and Prevention, Atlanta.	Contractor, Northrop Grumman	SY supports the technical reviews and validation of the study processes as well as provides other relevant technical support to the process.	**16120**
Obinna Ogbanufe	US Centres for Disease Control and Prevention, Nigeria.	Snr. Program Specialist, Care and Treatment	OO supports the technical reviews and validation of the study processes as well as provides other relevant technical support to the process.	**6566**
Sherri Pals, PhD	US Centres for Disease Control and Prevention, Atlanta.	Health Information Specialist and Statistician	SP provides technical support in the study design, sample size and statistical analysis of the study. She provides other relevant technical support to the process.	**12092**
Isiramen Olajide, MPH	UMB	Technical Advisor	IO is the study Point of Contact (POC) and is responsible for the day-to-day	**NA**

Co - Investigators	Affiliations	Position	Roles and Responsibilities	SEV#
			technical lead in the development, reviews, and conduct of the study at UMB	
Bodunde Onifade, MD	National HIV/AIDS Division, Federal Ministry of Health (FMOH),	National Lead HIV Data Mgt & ART-MIS	BO provides the leading coordination of the implementation of the survey and activities / processes; will supervise the integrity of data management including data collections and reporting.	**NA**
Manhattan Charurat, PhD	UMB	Professor and Director of the Epidemiology and Prevention Division,	MC supports Technical reviews and validation of the study processes including data analysis and reporting.	**NA**
Mercy Niyang, MPH	UMB	SI Lead and Director Health programs	MN will coordinate all data collection activities; will ensure fidelity of the data and oversee data analysis and reporting.	**NA**
Joy Musa	UMB	Epidemiologist	JM will coordinate all data collection activities; will ensure fidelity of the data and oversee data analysis and reporting.	**NA**

- Collaborating Institutions

Institutions	Roles and Responsibilities
United States Agency for International Development (USAID)	USAID provides the needed technical support as a sister agency in the support of HIV/AIDS programs in Nigeria
Jos University Teaching Hospital (JUTH) Lagos State University Teaching Hospital (LUTH) Nigerian Institute of Medical Research (NIMR) Asokoro Laboratory Training Centre	The institutions will provide the laboratory centres and expert laboratory supports needed for the survey, including conducting and supervisions of the viral load and HIV DR laboratory testing and analysis
FHI 360 APIN Public Health CIHP IHVN	The Institutions will provide the needed support in facilitation of trainings, workshops, and supervision of field implementations.

Please note that all CDC employees will not intervene or interact with participants or have access to identifiable information

Introduction

Background

Nigeria's population is estimated to be over 180 million people and Nigeria is home to about one in every six people living on the African continent, making it Africa's most populous nation [1]. Since the first AIDS (Acquired Immune Deficiency Syndrome) patient was reported in 1986, national adult Human Immuno-deficiency Virus (HIV) prevalence has risen from about 1.8% in 1991 to 5.8% in 2001, with a recent fall to about 3.0% in 2014 [2]. Currently, about 3.4 million people in Nigeria are estimated to be living with HIV/AIDS, and about 1.6 million are in need of Anti-Retroviral Therapy (ART)[3]. The ART program in Nigeria started in 2002 and the number of people placed on ART has increased through time, from 108,572 in 2006 to 983,980 in December, 2016. The number of children on treatment also increased from 6% in 2010 to 21% in 2016 [4]. However, the retention of these patients declines with time with 82%, 74%, 67% and 62% of the patients retaining on treatment at 12, 24, 36, and 48 months respectively[5].

A key clinical measure of treatment efficacy among people living with HIV (PLHIV) receiving anti-retroviral therapy (ART) is viral load suppression (VLS) i.e., viral load copies <1000 copies per microliter and emergence of HIV drug-resistance (HIVDR). The suppression of HIV viral replication by anti-retrovirals (ARVs) prevents the destruction of CD4 cells and allows for reconstitution of host immune systems, thereby greatly reducing HIV-associated morbidity and mortality among PLHIV receiving ART[6]. Furthermore, viral load suppression dramatically reduces the risk of transmission of HIV to those who are HIV-uninfected[6]. The routine monitoring of viral suppression and, possibly, HIV drug resistance allows clinicians to identify PLHIV who are failing treatment, understand the HIV drug resistance among them, and intervene, reducing the probability of poor HIV-related outcomes and preventing/minimizing the development of HIVDR. However, Nigeria, currently does not actively monitor the HIVDR at any WHO recommended time points (12 +/-3 months and > 48 months), but it monitors the VLS at 6 months (first evaluation after initiation) and 12 months (annually if suppressed).

Study Justification

National surveillance of VLS and HIVDR in PLHIV is essential in determining the quality of ART programs as well as informing effective strategies to improve VLS including selection of first-line ART combinations to reduce HIVDR. Because of the centrality of VLS in preventing transmission and measuring treatment success at the individual and population levels, the Joint United Nations Programme on HIV/AIDS (UNAIDS) has made VLS a key component of its Fast Track strategy targets, termed "90-90-90" (i.e., 90% of PLHIV to be diagnosed, 90% of diagnosed PLHIV to be on treatment, and 90% of PLHIV on treatment to achieve viral suppression). Additionally, the World Health Organization (WHO) recommends routine viral load monitoring to diagnose and confirm treatment failure[6] and the President's Emergency Plan for AIDS Relief (PEPFAR) is increasing the availability of routine viral load testing at PEPFAR sites to ensure optimal treatment outcomes[5]. As PEPFAR supports countries to increase routine viral load testing availability, it is critical that programs be able to monitor VLS among PLHIV receiving treatment for the purposes of program management, monitoring and evaluation (M&E), and surveillance.

Ideally, HIV control programs would rely upon routine program data to monitor viral load; however, this approach can be problematic in low resource settings. Most high burden countries lack highly functioning case-based surveillance or other systems that enable the reporting of complete, high quality, individual-level viral load testing data. While Nigeria is expanding access to routine viral load monitoring in the context of laboratory system strengthening, the coverage and quality of routine viral load testing remains heterogeneous in the country. Furthermore, some programs in the country are focusing on testing PLHIV on ART in select circumstances, such as patients suspected of treatment

failure because of their immunological or clinical status, rather than routinely providing viral load testing to all PLHIV on ART. In addition, in the view of strengthening the preference for TDF as the first line NRTI in terms of improved VLS and implications of DRM on the alternative/second lines of ART in limited resource settings[4], the study will be useful in providing a more nationally generalizable evidences for efficient ART lines selections for Nigeria.

Detected HIVDR in populations initiating ART may have been transmitted at the time of initial infection during treatment or acquired due to previous exposure to ARV drugs in the context of prevention of mother-to-child transmission (PMTCT), post-exposure prophylaxis, pre-exposure prophylaxis, or previously disclosed or undisclosed ART [7]. Surveillance of HIVDR in nationally representative populations receiving first-line ART is critical to assessing delivery [8]. ART program quality and informing the selection of second-line ART regimens. Suboptimal VLS and the detection of HIVDR in populations receiving ART may reflect gaps in ART program quality, including inadequate adherence assessment and counselling, interruptions in drug supply, low retention in care, or other factors contributing to sub-optimal ART service. The early detection of virologic failure and/or HIVDR will be beneficial to all people infected with HIV including pregnant women and children. Improving treatment outcomes and success of PMTCT programmes are central to the achievement of the 90-90-90 targets and the elimination of MTCT – the importance of including pregnant women and children in this study.

The overall aim of this protocol is to initiate a reliable method for monitoring the national VLS and HIVDR prevalence in Nigeria where 1) routine viral load testing services are expanding but are still not universally available and 2) VLS and HIVDR information and surveillance systems are not robust enough to leverage routine program data for this purpose. In the interest of the national programmatic and clinical benefits in determining Acquired HIV resistance among the PLHIV with virologic failure, this concept may identify efficient methods of monitoring the emergence of acquired HIV resistance among this population using different ART regimen.

Purpose and Objectives

The purpose of this survey is to estimate the national and regional prevalence of VLS and of HIVDR among PLHIVs (adult and children) on ART in Nigeria.

Primary Objectives are

- To estimate the national and regional prevalence of viral suppression among adult PLHIV (15 + years) receiving ART for at least 12 months.
- To estimate the national and regional prevalence of viral suppression among paediatric PLHIV (<15 years) receiving ART for at least 12 months.
- To estimate the national prevalence of acquired HIV drug resistance among adult and child PLHIVs receiving ART for at least 12 months with VL≥1000 copies/ml

Secondary Objective

- To describe the pattern of DRMs among adults and children receiving ART and by regimen types.
- To determine clinical and demographic risk factors associated with virologic failures and HIVDR among PLHIVs on ART for at least 12 months

Methods and Procedures

Study Design

The survey will be a cross-sectional study of the PLHIV receiving ART as per national guidelines (for at least 12 months in selected ART facilities in Nigeria. A stratified cluster sampling design will be used. ART facilities will be the study clusters and will be selected within regions using Probability Proportional to Size (PPS) from a sampling frame (list of all ART facilities in the country). All eligible patients who are enrolled for ART in the selected facilities, and consent to participate in this study, will be consecutively enrolled for the study and assigned a survey unique identifier (SID) until the predetermined sample size for each facility is achieved for adults and children.

Study Population

The study population will be PLHIV (adults and children) who are receiving ART for their own health including those implementing PMTCT option B plus (on ART for their babies and their health).

Eligibility Criteria

Survey in adults

- Inclusion criteria:
    a. Age 15 years or older.
    b. Legally able and willing to provide informed consent.
    c. Initiated ART between 12±3 and 48 months prior to date of survey enrolment.

- Exclusion criteria:
    a. All treatment Naïve individuals

Survey in children

- Inclusion criteria:
    a. Children at least 12 months but less than 15 years
    b. Parent or legal guardian gives permission and minor assents
    c. Initiated ART at least 12±3 and ≤48 months prior to date of survey enrolment

- Exclusion criteria:
    a. All treatment naïve children

Site Selection

To achieve a nationally representative estimate of VLS and HIVDR in adults and children, we will include in the sampling frame all facilities reporting at least 250 patients currently on treatment. These criteria exclude fewer than 10% of the otherwise eligible patient population, including adults and children. With disaggregated (by age) data from PEPFAR sites, we have determined that, for eligible facilities, the percentage of the total number of patients on treatment that are pediatric cases (<=15 years of age) is at least 3 in 75% of eligible facilities. Thus, the restriction to facilities with 250 or more total patients should allow us to complete our enrolment of pediatric patients.

Within each of the six regions in Nigeria, we will use probability-proportional-to-size (PPS) sampling to select sites, basing the probability on the total number of patients on treatment. The number of sites selected in each region will be roughly proportional to that region's contribution to the total population of adults on ART (see Table 1).

Table 1. Number of sites to be selected in each region

Region	% of PLHIV currently on treatment in Region	Facilities Selected in Region	% of Facilities in Region
North Central	33.89	24	34.29
North East	16.97	12	17.14
North West	11.89	8	11.43
South East	9.37	7	10.00
South South	17.02	12	17.14
South West	10.86	7	10.00
Total	100.0	70	100.0

**proportions are based on the proportion of patients in each region, including only facilities with at least 250 patients currently on treatment.*

Duration of Study

The survey is expected to be completed within a period of 10 months from January to October 2018 while survey data collection will occur over a 6-month study period within which the study sample size is expected to be reached both in adults and children. However, though very rarely, data may be collected for longer period of time if the patient volume at the selected sites is not sufficient to achieve the estimated sample size in Six months.

Sample Size

Within each of the 70 selected facilities, 15 adult patients and 8 pediatric patients will be enrolled, yielding a total sample size of 1050 adults and 560 children. Based on these initial sample sizes and varying assumptions about the percentage of adults and children who are virally suppressed and of those, the percentage who have HIVDR at 80% amplification rate[5], we estimated confidence limits for VL suppression and HIVDR. The following tables present these estimates.

- Adult VL suppression and HIVDR

Enrolled	% with VL<1000	95% CL	Number with HIVDR result*	% with HIVDR	95% CL
1050	75	71.6, 78.4	210	50	42.9, 57.1
	80	76.9, 83.1	168	50	42.2, 57.8
	85	82.2, 87.8	126	50	41.1, 58.9

*Equals number enrolled x % with VL>1000 x sample amplification rate (assumed 80%)

- Pediatric VL suppression and HIVDR

Enrolled	% with VL<1000	95% CL	Number with HIVDR result*	% with HIVDR	95% CL
560	65	60.4, 69.6	157	50	41.9, 58.1
560	70	65.6, 74.4	134	50	41.3, 58.7
560	75	70.8, 79.2	112	50	40.6, 59.4

*Equals number enrolled x % with VL>1000 x sample amplification rate (assumed 80%)

- Regional adult VL suppression

Region	Enrolled	% with VL<1000	95% CL
North Central (24 facilities)	360	75	70.1, 79.9
		80	75.5, 84.5
		85	81.0, 89.0
North East (12 facilities)	180	75	67.8, 82.2
		80	73.4, 86.6

		85	79.1, 90.9
North West (8 facilities)	120	75	66.7, 83.3
		80	72.4, 87.6
		85	78.2, 91.8
South East (7 facilities)	105	75	65.4, 84.6
		80	71.2, 88.8
		85	77.2, 92.8
South South (12 facilities)	180	75	68.3, 81.7
		80	73.8, 86.2
		85	79.5, 90.5
South West (7 facilities)	105	75	66.4, 83.6
		80	72.1, 87.9
		85	77.9, 92.1

*Equals number enrolled x % with VL>1000 x sample amplification rate (assumed 80%)

- Regional Pediatric VL suppression

Region	Enrolled	% with VL<1000	95% CL
North Central (24 facilities)	192	65	58.4, 71.6
		70	63.7, 76.3
		75	69.0, 81.0
North East (12 facilities)	96	65	55.4, 74.6
		70	60.8, 79.2
		75	66.3, 83.7
North West (8 facilities)	64	65	53.9, 76.1
		70	59.3, 80.7
		75	64.9, 85.1
South East (7 facilities)	56	65	52.2, 77.8
		70	57.6, 82.4
		75	63.4, 86.6
South South (12 facilities)	96	65	56.0, 74.0
		70	61.3, 78.7
		75	66.8, 83.2
South West (7 facilities)	56	65	53.4, 76.6
		70	58.9, 81.1
		75	64.5, 85.5

The tables show that we will have good precision for estimates of viral load suppression overall for both adults and children (+/- 5% or narrower). For regional VLS estimates for adults, precision varies by region size, but is < +/- 10% for all regions and likely values of viral load (VL) suppression. Precision is lower for estimates of VL suppression in children, but still less than +/- 10% for the largest two regions. There is a strong correlation between adult and pediatric patient population sizes in eligible facilities; thus, adult facility size was used as a proxy for pediatric facility size.

Estimation of confidence limits was done using a SAS macro that divides the sample size by the design effect, [1+(m-1)ICC], where m is the average number of patients per facility, and produces

confidence limits using SAS PROC FREQ (asymptotic method). We assumed a value of 0.05 for the intraclass correlation coefficient (ICC), based on an ICC of 0.013 estimated using unpublished data on viral load suppression among pregnant women in antenatal care (ANC) clinics [6]; we used a larger ICC to take into account the error in the estimate.

Standard Facility Flow, Standard of Care /Procedures for the Determination of Patient Virology and HIVDR (See Figure 1)

After commencement of ART, clients are usually assessed either clinically, immunologically or virologically to determine their level of response to treatment. The Nigerian 2016 National guideline for HIV prevention, treatment and care recommends that all clients initiating ART should have their viral load determined at 6months, and at 12 months. Thereafter, a 12 monthly viral load will follow, to determine the efficacy and suitability of the regimen for the individual. In suspected cases of virologic failure, viral load testing is normally repeated 3 months after an intense regime of reinforced adherence counselling and support. A viral load test of >1000 copies /ml following reinforced adherence counselling and support is indicative of virologic failure of which its persistence may lead to a change in the treatment regimen with second line treatment options

Generally, requisition for client viral load is stimulated from various points in the service delivery cascade using the viral load order and result form (Appendix 1). The medical records clerk, has the responsibility of using the client's hospital visit card to sort out the client's chart/folder and ensure that the requisite laboratory request tools are inserted into the folder, ready for completion by the clinician or trained triage nurse. For clients newly initiated on ART, their first viral load investigation at 6 months is usually tied to the their 3rd visit for drug pickup date, while other clients on treatment for longer periods, will have their yearly viral load sample collection set at a 12 months interval but still tied to a drug pick up visit .

Ideally, depending on the client's time of visit to the health facility, the client will either go first, to pick up his/her medication or go to the phlebotomy unit for sample collections so as to effectively manage time. Blood samples collected for the day will be kept at 2-25^{0}C for a period not more than 6 hours of collection before centrifugation at 800-1600g for 20 minutes at room temperature (CAP/CTM ROCHE Quantitative kit insert)- [appendix 2: COBAS Ampliprep-COBAS Taqman HIV-v2 kit insert]. The plasma is stored at 2-8^{0}C refrigerator and packaged for transportation to the PCR laboratory within 5 days. If there is any delay in shipment from collection or hub site and plasma sample stayed longer than 1 week; samples will be stored at -20^{0}C for not longer than 2 weeks and at -80^{0}C for longer storage. Depending on the turnaround time, of which will be between 2– 4 weeks, the results will be sent back to the referring facility for sorting and uploading unto the Electronic Medical Records or filing into the client's folder/chart. Subject to the outcome of the result, the client could be contacted immediately for a repeat visit at the instance of a facility multidisciplinary team to re-enforce adherence or possible regimen switch if not virally suppressed or left to be informed and encouraged to keep up a good adherence practice at the next hospital contact with a counsellor or clinician.

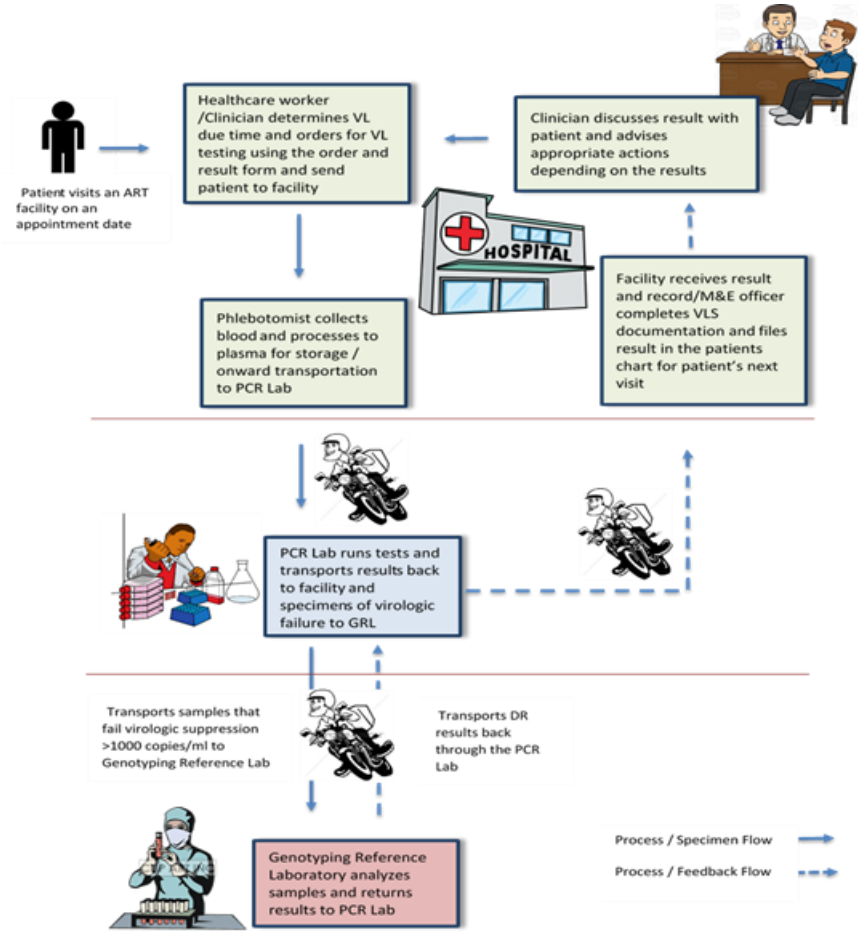

Fig 1. Standard VL testing flow in the Facility

However, since HIVDR testing is not a routine clinical practice in Nigeria, for the purpose of the survey and efficient logistic processes, all specimens with results that showed virologic failure will be transported directly to the designated genotyping reference laboratory (GRL) for sequencing/genotyping to identify possible ART resistant strains. The HIV DR results will be sent back to the facilities through the same courier system or transport network developed for the study. A multidisciplinary team, comprising of a doctor, pharmacist, lab scientist, and adherence counsellor, meets to analyse the result and take a decision as to the next line of action for the patients based on the ART national guideline. The turnaround time will be between 4 – 6 weeks from sample collection. (See figure 1).

For the virally suppressed clients, a yearlong appointment will be fixed for the next viral load monitoring test, while the unsuppressed clients will have to repeat their viral load after 3 months post re-enforced adherence counselling.

Participants Enrolment Procedures for the Study

We will have at each site, a number of clinic staff that have been trained to screen for eligibility and enrol participants in this study. Depending on the facility patient load, one of the clinic staff will be dedicated to assess patients' eligibility and enrol them for the study. This staff will have a dedicated desk next to the clinic nurse attending to patients on clinic days. Upon the visit of a potential research participant or a parent/guardian for routine care, which also includes a routine VL testing, the clinic study staff will assess the patient's eligibility based on ART duration and consistency, and age (first stage eligibility) and inform such patients about our study if the patient is eligible. Following their

willingness to participate, participant's consent will be documented using the consent, permission, and assent forms (second stage eligibility) as applicable before proceeding with subsequent clinical services. Where the patient is not eligible based on any of the eligibility stages, his/her ineligibility information/status is still documented using the EDF (appendix 3) and he/she proceeds to other clinical services.. Where a child/adolescent does not have his/her parent/guardian present to give permission, such children will not be eligible for inclusion in this study. Following site selection, a listing of the participant SIDs for each site will be generated, along with a corresponding set of participant-specific barcode labels. These labels will be used on blood collection vacuum tubes, the viral load order and result form, and specimen inventory forms. The barcode labels will contain the SIDs, facilitating linkage of plasma specimens to the enrolment sample forms. A log will be established at the site linking SIDs to the patient in order to return VL and HIVDR testing results. Patient identifying information maintained in the log will include the site identifier for the patient (i.e., medical record number) and any other person identifiable information needed by the site such as name and telephone number that the site uses to uniquely identify their patients. This log will never leave the site and will be kept in a secure locked location within the clinic. Only clinic staff designated as study staff for the purpose of the survey will have access to this log. It will be closely monitored to validate the unique combination of SID and patient personal identifier to minimize the risk of transcription errors in study identifiers.

Each survey ART clinic will select a convenient starting date. Trained staff at the selected clinics will then screen all patients attending the clinics for eligibility per the criteria above. Eligible participants will be enrolled sequentially until the desired sample size for adults and children for the survey ART clinic has been reached. Due to the very small estimated proportion of children (5%) among the PLHIV currently on treatment in Nigeria, it is expected that the sample size for adults will be reached earlier than the children; however, facilities that have enrolled the maximum sample size for adults will terminate adult enrolment but continue with the children until the required sample size for children is reached, and vice versa, depending on the situations. Full consent will be obtained from the eligible adults and mature minors; parental permission will be obtained, and assent will be sought from children of an appropriate age (12 – 17 years, as determined by national standards); consenting participants will then be assigned a SID. After a participant's enrolment, the designated clinic staff will ensure that all the variables of interest are documented appropriately in the participating patient's electronic records/charts and blood specimens will be obtained in the facility laboratory section for transportation to designated PCR reference laboratory for VL testing and storage for HIVDR. Blood collection will occur as an inclusive activity with the standard of care blood collections and testing for persons maintained on ART.

Return of Results and Linkage to Care

All PLHIV selected for the survey round (and due for their next VL test as per routine care) will receive viral load testing and the results will be returned to the patient and clinician for further determinations at least within 2 weeks of sample collection. HIVDR results will also be returned to optimize care for the affected patients. The participant SIDs generated at enrolment and the log linking SIDs to the patient will be used to ensure linkage of the results to each patient at each site.

Data Collection

Patient –Level Information

Only minimal patient – level information (listed below; and in appendices 4 & 5) relevant and sufficient to answer important programmatic and clinical questions related to the study objectives will be collected.

Complete Individual data of interest from the selected participants will be abstracted from both clinical and laboratory information systems (EMR and/or PBR). The patient – level information to be collected includes:

A. Clinical/demographic information

i. Hospital/ART ID
ii. Patient Survey ID (SID)
iii. Date and time of specimen collection (DD/MM/YYYY)
iv. Date and time of plasma processing (DD/MM/YYYY)
v. Date of ART initiation for the first time (DD/MM/YYYY)
vi. Month and year of birth (if available) (MM/YYYY)
vii. Age in years (age in months for children under 3 years)
viii. Gender (female, male)
ix. Weight (children only);
x. Current ART regimen classification (first-line /second-line/third-line/unknown)
xi. First-line ART regimen prescribed: list ARVs
xii. Date (DD/MM/YYYY) when second line ART regimen was initiated, if applicable.
xiii. Second-line ART regimen prescribed: list ARVs
xiv. Date (DD/MM/YYYY) when third-line ART was initiated, if applicable.
xv. Third-line ART regimen prescribed : list ARVs
xvi. WHO stage at ART initiation
xvii. CD4 at ART initiation
xviii. Latest CD4 at survey enrolment

B. Laboratory Information

i. Specimen ID (Barcode # on specimen label)
ii. Acceptable sample? (Y/N)
Date sample was received in the lab for testing
iii. VL testing successful? (Y/N)
Date sample was tested for viral load
iv. VL testing results available? (Y/N, N/A)
v. VL (copies/ml) result from survey blood collection
Date sample was received for HIVDR Testing
Date sample was tested for HIVDR
vi. If VL≥ 1000 copies/ml, RT region successfully sequenced? (Y/N)

vii. If VL≥1000 copies/ml, PR region successfully sequenced? (Y/N, not applicable)

viii. Drug resistance. For all drugs, choose the appropriate HIVDR level according to Stanford HIVdb algorithm interpretation: susceptible/potential low-level, low, intermediate, or high

Facility information

The following data describing key characteristics of each ART facility will be collected:

i. Facility ID

ii. Facility name

iii. Location of the facility: Region/State/LGA

v. Date when specimen collection started (DD/MM/YYYY)

vi. Date when specimen collection ended (DD/MM/YYYY)

vii. Estimated number of patients (adults/ children) who have been on ART for 12 months before or at the date of data collection during the specified 6-month period

viii. Clinic size as contained in the table used for systematic sampling

ix. If stratification is used, stratum name (e.g., urban/rural hospital) for each setting

x. Type of facility - tertiary/secondary/primary

Biological Testing

Plasma specimen collection

After obtaining consent from participants for blood collection, blood will be collected (for both adults and children) in a 10ml vacutainer tube containing ethylene diamine tetra-acetic acid (EDTA) anticoagulant by Becton Dickinson. The blood collection will be part of routine care, no additional blood draw will be required. Blood specimens will be collected by venepuncture following standard procedure and universal precautions[9]. For adults, the phlebotomist will identify the participant with the National viral load order/report form with participant's unique identifiers. The phlebotomist will place a pre-printed SID label on the 10 ml blood collection vacuum tube. Label the bottle with participant's name, date of collection; a good antecubital vein will be located and where this is not possible due to adipose tissue, the bulging vein at the back of the wrist of the hand can be used. The skin is cleaned with methylated spirit and allowed to dry for few seconds. Apply tourniquet to firm up the vein. Insert 21G steel needle attached to vacutainer barrel to the antecubital vein, release the tourniquet and insert a 10ml EDTA bottle for blood collection. Remove the needle after collection and cover the puncture site with dry cotton wool and ask the participants to hold it for 2 minutes to allow the blood flow to stop. Blood collection from paediatric and young children will require additional training for sample collection. The phlebotomist should cross check the unique identifiers on the National viral load order/report form from the parent or care giver. Ask whether the parent would like to help by holding the child. If the parent wishes to help, provide full instructions on how and where to hold the child; if the parent prefers not to help, ask for assistance from another phlebotomist. Warm up the wrist of the child. With the support of the parent or another phlebotomist, hold the child faced up on a stretcher and locate the wrist vein, clean the selected vein with methylated spirit. Insert

a butterfly winged 23G steel needle attached to vacutainer barrel and insert a 10ml EDTA bottle for blood collection. The parent or the other phlebotomist will continually pump the child's hand to serve as tourniquet to assist blood flow. Remove the needle after collection and cover the puncture site with dry cotton wool and hold it for 2 minutes to allow the blood flow to stop.

The 10ml EDTA blood is mixed gently and fill the time of blood collection. The blood will be transported to the laboratory under appropriate conditions. The blood tubes will be transported to a laboratory at 2-8 ^{o}C using cold transport box for preparation of plasma within 6 hours after sample collection. The plasma will be centrifuged at 800-1600g for 20 minutes and aliquoted aliquots prepared in triplicates, approximately 1.2ml each. Selected study facilities that do not have the capacity to process whole blood samples to plasma; spoke and hub referral network model would be initiated to transport the whole blood in a cold box maintained at 2-8 ^{0}C to the designated nearest hub that are equipped with laboratory infrastructure for centrifugation and cold storage at 2-8^{0}C for a period of 1-5 days or frozen at -20^{0}C freezer if storage is required for up to 2 weeks, before transporting to the PCR laboratory for VL test.

Specimen transport to PCR and genotyping laboratory

Each of the samples will be accompanied with National Viral load order and report form (appendix-1) and the summary of the samples will be contained on the National viral load sample manifest for identification and tracking of sample shipment. Once all specimens have been collected, the survey coordinator will inform the target reference laboratory and determine the shipment date and transportation model to be used. Plasma stored at 2-4°C will be tested within 1-5 daysafter blood collection, or those stored frozen at minus 20 to -80°C will be tested much later. Shipment shall be done according to WHO standard triple packaging for infectious samples. Plasma sample, which is the country-approved bio-medium for VL and HIVDR testing (National Guidelines for HIV Prevention, Treatment and Care 2016), is considered infectious and will be handled following WHO standard procedures. Both samples for viral load and HIVDR collected and stored at 2-8 ^{0}C after 4-5 days are shipped to the testing laboratory[9]. If testing is not conducted after plasma preparation, the plasma specimens will be stored at -20°C for not more than 2 weeks or -80°C in a non-auto-defrost freezer for a longer period.

All freezers that will be used will be in secure locations with restricted access. All the labs that will store specimens will have back-up power supply in the form of back up power generator sets and/or power inverters with solar panels.

VL measurement

Plasma specimens: VL measurement will be performed at Nigerian Institute of Medical Research (NIMR) Lagos; Jos University Teaching Hospital (JUTH) Jos; Asokoro Laboratory Training Center (ALTC) Asokoro Abuja; and Lagos University Teaching Hospital (LUTH), Idi-Araba Lagos. PCR laboratories using locally available VL testing assays Roche COBAS® AmpliPrep/COBAS® TaqMan which have been validated in the laboratory

HIVDR genotyping

For those specimens with a VL ≥1000 copies/ml, genotyping for drug resistance will be performed for the following ARVs in use according to the national guidelines using the Stanford HIV Drug Resistance Database (http://hivdb.stanford.edu) as reference:

Class	Drugs
Nucleoside/Nucleotide Reverse Transcriptase Inhibitors (NRTIs)	Abacavir (abacavir sulfate, ABC) Emtricitabine (FTC) Lamivudine (3TC) Tenofovir Disoproxil fumarate (TDF) Zidovudine (AZT,ZDV)
Non-Nucleoside/Nucleotide Reverse Transcriptase Inhibitors (NNRTIs)	Efavirenz (EFV) Etravirine (ETR) Neverapine (NVP) Rilpirivine (RPV)
Protease Inhibitors (PIs)	Atazanavir (ATV) Darunavir (DRV) Lopinavir (LPV) Fasomprenavir (FPV) Indinavir (IDV) Ritonavir (RTV) Saquinavir (SQV) Tipranavir (TPV)

Drug resistance mutations will be categorized, scored for susceptibility to all the above listed ARVs and interpreted using the Stanford HIVdb Program – a genotypic resistance interpretation algorithm (HIVdb version 8.4) (Liu TF, Shafer RW(2006). Web Resources for HIV type 1 Genotypic-Resistance Test Interpretation. Clin Infect Dis 42(11):1608-18. Epub 2006 Apr 28). Mutations will be interpreted as "Susceptible" and "Resistant" as described by Conan et al 2012 (*Woods CK, Brumme CJ, Chui CKS et al. Automating HIV Drug Resistance Genotyping with RECall, a Freely Accessible Sequence Analysis Tool.* J. Clin. Microbiol. June 2012 50:6 1936-1942). For patients failing first line, the most common NRTI mutations were M184V (89.1%) and thymidine analog mutations (TAMs). The most common NNRTI mutations were Y181C (49.7%), K103N (36.4%), G190A (26.3%), and A98G (19.5%). Among NNRTI mutations, subtype G patients had an increased risk for A98G (AOR = 2.40, $p = 0.036$) and V106I (AOR = 6.15, $p = 0.010$), whereas subtype CRF02_AG patients had an increased risk for V90I (AOR = 3.16; $p = 0.003$) and a decreased risk for A98G (AOR = 0.48, $p = 0.019$) B. Chaplin, G. Essien, J. Idoko et al (2011). Impact of HIV Type 1 Subtype on Drug Resistance Mutations in Nigerian Patients Failing First-Line Therapy. *AIDS Research and Human Retroviruses, Vol. 27, No. 1/Virology.* https://doi.org/10.1089/aid.2010.0050.

The following principles/procedures will be used in the process of the HIVDR genotyping: Reverse Transcription and Polymerase Chain Reaction (RT-PCR), Nested Polymerase Chain Reaction (Nested PCR), Cycle sequencing, Automated Sequencing and Sequence Analysis.

Sample preparation: The HIV-1 genotyping kit will accept RNA or total nucleic acid (TNA) that has been extracted by numerous automated and manual methods ATCC. The kit has been tested and valeted with the following methods: BioMerieux easyMag (DBS and plasma), BioMerieux miniMag (DBS and plasma), Qiagen QIAamp Viral RNA (Mini Kit (plasma) and Abbott Molecular's m2000sp (plasma).

Laboratory method: Patient plasma samples collected at the initial high VL (F1) (i.e., VL>1,000 cp/mL) will beanalysed; if we are unable to amplify and sequence the virus from that sample, the plasma sample collected at the second confirmatory high VL>1000 cp/mL (FC) will be used. HIV RNA will be extracted from plasma using the Qiagen Viral RNA Kit (Qiagen Inc, Valencia, CA, USA). HIV-RNA will be reverse transcribed and amplified using ATCC® HIV-1 Drug Resistance Genotyping Kit (ATCC, Applied Biosystems by Thermo Fisher Scientific- appendix6) kit module 1. PCR products will be purified using ExoSAP-IT enzyme and cycle sequencing done using ATCC (Applied Biosystems by Thermo Fisher Scientific- appendix 6) kit module 2. AB1 files will be assembled and edited using ReCall 2.25 software (University of British Columbia, Canada). Sequence identity matrix will be performed using BioEdit® (*ibis Therapeutics*) software to check for contamination and fasta files analysed with Stanford HIVDB Calibrated Population Resistance "QA details" to confirm base calls (Gifford RJ, Liu TF, Rhee SY, et al 2009). HIV drug resistance profiles will then be determined using HIVdb algorithm version 8.2 (Liu TF, Shafer RW (2006), while subtyping will be done using REGA HIV-1 subtyping tool - version 3.0 on Stanford HIVDB website. Using the five-level resistance profile from HIVdb algorithm, genotype sensitivity score (GSS) will be calculated per individual drug and cumulated to obtain GSS for the patient's prescribed 2L regimen (Conan KW, Chanson JB,et al 2012). Scores will be assigned as follows: susceptible = 1.0, potential low-level resistance = 0.75, low-level resistance = 0.5, intermediate resistance = 0.25, and high-level resistance = 0.0. These categories were further simplified into susceptible (i.e., potential low-level, low-level, and susceptible) or resistant (i.e., intermediate- or high-level resistance).

The HIV-1 genotyping(genotyping of the HIV-1 pol gene, including protease (PR) and reverse transcriptase (RT) gene regions) will be performed at the PEPFAR supported laboratories that are in the process of designation by WHO into WHO/HIVResNet Laboratory Network for HIV genotyping; these are Jos University Teaching Hospital Laboratory, the National Institute for Medical Research (NIMR) laboratory and Asokoro Training Laboratory. All these laboratories are certified and meet quality control standards. They have consistently passed the assessments Stepwise Laboratory Improvement Process Towards Accreditation (SLIPTA) scheme developed by the Centers for Disease Control and Prevention and WHO. All laboratories except the "Asokoro lab" have recently received the SANAS (South African National Accreditation System) making them certified public health laboratories to conduct a number of tests including HIV-1 drug resistance testing. The ATCC genotyping kits include internal controls; both negative and positive controls will be used to ensure reliability of the results. However, 10% of the samples will be retested in CDC Atlanta ILB as an assurance check of the performance of the assays carried out locally. Plasma specimens, which are considered infectious, will be handled and shipped frozen on dry ice following international regulations on shipment of infectious agents (*CLSI. 2007. Procedures for the collection of diagnostic blood specimens by venipuncture; Approved Standard - Sixth Edition. CLSI document H3-A6 Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA).* A material transfer agreement (see sample MTA as appendix 7) duly signed between the donor laboratories in Nigeria and the ILB in Atlanta and approved by the Nigerian National Health Research

Ethics Committee will be used to facilitate the shipment of the samples for this assay performance testing in Atlanta.

Samples will be shipped to ILB frozen at -80 ^{0}C using" shippers"World courier or other couriers that have IATA certification for shipping biological samples will be used for sample shipment.

Principles of assay:
Reverse Transcription Polymerase Chain Reaction (RT-PCR): The HIV-1 Genotyping kit amplifies a 1.1 kb region of the HIV-pol gene that spans the entire protease gene and two-thirds of the RT gene, this procedure followed the sample preparation stage. This stage is followed by Nested Polymerase Chain Reaction (Nested PCR) to amplify the DNA. This is followed by Cycle Sequencing, before this stage, confirmation of PCR products is required to conserve reagents and save the technician's time. To confirm PCR reaction results, a 1% (w/v) agarose gel with a nucleic acid stain is used to separate out the PCR product and the DNA ladder before visualizing on an image system. This procedure allows the technician to validate the PCR product being of the correct size for both specimen and positive control, and the negative and reagent controls having no band present with little or no smearing within gel bands (appendix 8- HIVDR ATCC Testing SOP pg 7, 8, 9).

Cycle sequencing: Single stranded DNA templates are replicated by a process that uses DNA polymerases in which nucleotides are added to a growing chain, known as product extension. Chain extension takes place at the 3" end of a primer that has annealed to its complimentary template. The dNTP that is added to the growing extension product is selected by base pair matching to the template. The chain is extended by the formation of Phosphodiester Bridge between the 3'-hydoxyl group at the growing end of the primer and the 5'-phosphate group of the incoming deoxynucleotide. Growth is thus in the 5' to 3' direction.

Purifying the Sequences: It is essential to remove the unincorporated BigDye Xterminators from the samples so that they do not interfere with the sample sequencing analysis. The HIV-1 Genotype kit is compatible with three options for purifying sequence reactions: Applied Biosystems BigDye Xterminator Solution, Princeton Seoarations Centri-Sep Spin Columns or plates, Qiagen DyeEx Spin Kit or Plates and Ethanol/EDTA precipitation. For the best sequence data, it is recommended that the BigDye Xterminator be used. The purification quality of Ethanol/EDTA precipitation is poor and not recommended where other sequence purification methods are available.

Automated Sequence Detection: The applied Biosystems ABI automated DNA sequencers are the only sequencer that are compatible with the HIV-1 Genotyping kits. The DNA sequences detect fluorescence from four different dye terminators that are used to identify the A, C, G and T extension/termination reactions. Each dye emits light at a different wavelength when excited by an argon ion laser. All the four colours and therefore all four bases are detected and distinguished in a single capillary. The purification quality of Ethanol/EDTA precipitation is poor and not recommended where other sequence purification methods are available (appendix 8- HIV DR ATCC Testing SOP).
Sequence Analysis Software: RECall sequence editing software: Is recommended because it is a customized software package that will basecall, trim, align and produce consensus sequence which is compared to a known reference strain, HXB-2 to identify points of variance (appendix 8-HIV DR ATCC Testing SOP pg 10) .

10

Left-over specimen storage

After specimens have been processed for viral load testing and/or drug resistance testing, any leftover specimens will be stored in the central storage facility after laboratory processing is complete. All

stored samples from this study will be disposed-off, after 3 years of study completion, following standard procedures and good laboratory practice requirements. Any additional tests on these specimens within the 3-year-storage period will be limited to the test approved in this protocol. However, any future tests on the stored specimens besides that in this protocol shall be guided by an approved amendment of this protocol or submission of another protocol specifying the purpose and procedures of the tests.

Data Management

Paper Based Records or Electronic Medical Records (where available) will be the primary source documents. Demographic and clinical data from these sources required for this study will be abstracted unto will be abstracted directly unto an electronic form using an android-based handheld device. On not more than weekly basis, data will be transmitted to a central server. All data transmitted from the field will be stored in a secure PostgreSQL database, or comparable system, located on the central survey server. All files received by the server's software are securely stored.

Individuals enrolled in the survey will be assigned a unique SID number. This number will be used to identify the patient as well as the VL and viral sequence generated by the genotyping laboratory. This will ensure the ability to link patient clinical and demographic information with the laboratory results.

A log will be established at the site linking SIDs to the patient in order to return VL and DR testing results. Personal identifiable information (PII) recorded in the log will consist of the minimum necessary to confidentially identify the medical record associated with the study participant assigned the corresponding SID. This log will never leave the site and will be kept in a secure location within the facility. If a paper log book is used, it will be physically secured in a locked cabinet or room. If an electronic file is used, it will be protected by a user password and stored on a secure network or local drive, with access limited to only authorized individuals. If stored on a network, this will be by virtually restricting access to the drive. If stored on a local drive such as a work station (e.g., desk top or lap top), the local drive will be physically secured (e.g., kept in a locked room or a locked cabinet). Individuals accessing the list to update or validate it will ensure that the activity is not visible by unauthorized individuals. Authorized individuals will be those having routine access to patient medical records or staff assigned to the survey and having signed the survey confidentiality agreement. The list will be closely monitored each time a new entry is made to minimize the risk of transcription errors and mistakes in assigning SIDs by having a second individual verify entry of SID and by validating that no SID is associated with more than one patient and vice versa. The list will be deleted at the end of the study in case we need to go back for verifications during the study process.

Once all specimen have been processed and data captured, the data will be assessed for missing data (Appendix 9). If the participating facility is responsible for the missing data, attempts will be made to resolve the issue by directly contacting the designated facility data manager. Recurrent issues of missing data will be handled as described in Appendix 9. Once data collection is completed, an analysis taking the estimates of overall VLS, along with their respective confidence intervals, will be calculated. Data will be weighted for analyses, taking into account observed facility-level patient accrual.

In the event there is no network or sudden network failure in the site, the completed forms will be saved and protected in the device and taken to a nearby pre-designated and secured data management facility where there is internet network and be uploaded to the Central Survey Information Management System (CSIMS). If the internet network available for data entry on-site is unsecured or not trusted, the electronic data file will be encrypted prior to transfer/upload. Furthermore, the PDAs will be digitally locked on transit to protect the content in case of theft or loss.

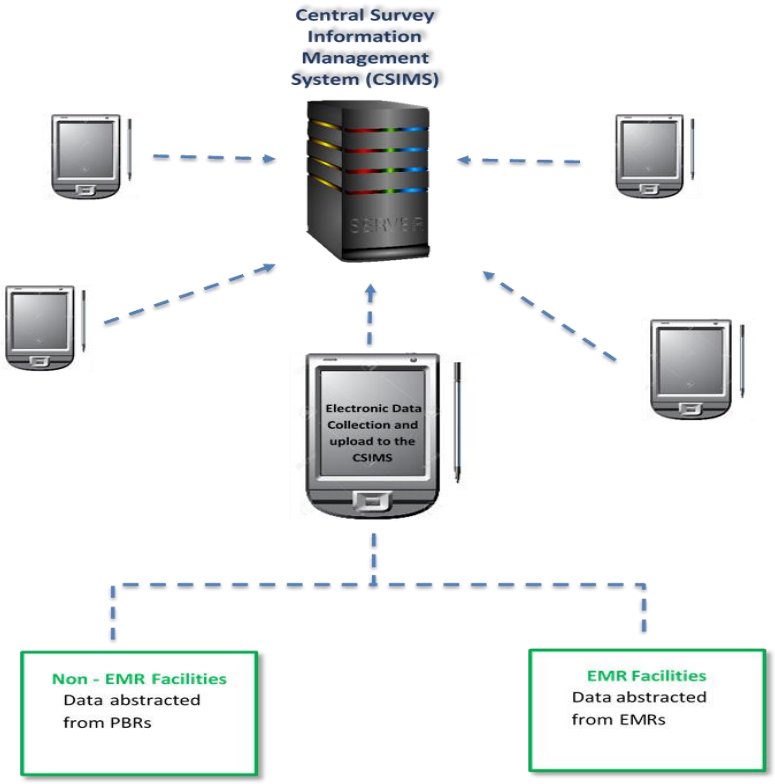

Fig 2: Data Management Flowchart

Data Quality Management

Data quality checks to minimize data entry errors will be built into the Electronic Data Collection (EDC) tool. Data will be reviewed daily at the central point by the designated central data manager (with secured access) to identify any illogical and/or entry errors not checked at the site/entry level. If any, the data manager will call the attention of the field study officer to validate the error from the source records on-site and confirm if it is a source record error or data entry error. If true error is established, the data manager will correct it and document the error in a daily data management activity log.

The data management designee will be responsible for ensuring that data is checked for quality and the data handling process is secure. This includes password protection and role-based access to the encrypted electronic database and or Secure Sockets Layer (SSL) protocol (or equivalent) as well as locked cabinets for paper forms. The data collection devices (Personal Digital Assistants/tablets) will be locked on password access to the authorized users only before use, and all the data / information transfers to the central database be encrypted for additional security and quality of the data. This also includes ensuring daily backup of the database to a secure and separate location and processes for authorized recovery.

Data Analysis

Data analysis will be done using SPSS, STATA, SAS, R, or similar statistical package. All analyses will account for stratification and clustering within site, and where appropriate, weights will be applied to produce nationally representative estimates, given the survey design. We will estimate the percentage of enrolled participants with suppressed viral load and 95% confidence limits around this estimate. Domain estimation will be done to estimate the percentage of patients with HIVDR, among those with non-suppressed viral load. Confidence limits will be estimated for this percentage, and sensitivity analyses performed to assess the impact of samples that did not amplify and were excluded from the primary analysis.

Analysis will be completed to fulfil the survey objectives. In line with the survey objectives (refer to pg.2), the following outcomes will be determined for adults and children separately:

a. The prevalence of VLS among adults and children receiving ART for at least 12 months

b. The prevalence of VLS among adults and children receiving ART for at least 12 months by ART lines

c. The prevalence of HIVDR among adults and children receiving ART for at least 12 months with VL ≥1000 copies/ml

d. The prevalence of DRMs among adults and children receiving ART by regimen types at least 12 months with VL ≥1000 copies/ml

Weights can be used to adjust the individual-level selection probabilities for children, yielding a nationally representative estimate. It is pertinent to note that we considered the country's programmatic relevance and circumstances facilitating the study design (chances of generating sufficient sample size with good precision) to estimate the VLS and HIVDRM rates at only >=12 months for recruiting participants. However, the analysis will attempt to estimate the parameters at >48 months (according to the WHO guidance (ref) based on the samples generated but with likely low precision. Other relevant demographic analyses supporting the objectives of the study to inform programmatic decisions would be performed (Appendices 10 - 12).

HIVDR will be categorised as low, intermediate or high levels of resistance according to the Stanford HIVdb (www.https://hivdb.stanford.edu) to one or more of the following drugs or drug classes: NNRTI (nevirapine [NVP] and efavirenz [EFV]), any NRTI and PI (boosted darunavir [DRV/r], boosted lopinavir [LPV/r], or boosted atazanavir [ATZ/r]. Sequences classified as susceptible and potential–low level resistance are all classified as susceptible. Lastly, we will conduct analysis to estimate the relationship between the binary outcome of of DRobserved in the study and some demographic and clinical characteristics that may serve as risk factors.

Study Management Plan

The study proposes a three level management and three level supervisory structure. The Government of Nigeria (GON) would have the overall management and coordination responsibility through the Lead IP who will have the responsibility for direct operations,conduct, and implementation of the study. The Lead IP will work directly with the selected facilities to ensure that the study is implemented according to the protocol/plan. CDC would provide the central TA to both the GON and the Lead IP.. The CTC provides the central supervison of the study, particularly, during trainings, workshops, data collection, data management, report writing, and disseminations. The CTC will provide field supervision during the data collection period. The lead partner will provide more regular supevision of the field workAt the facility level, while the Facility Supervsiory Teams (FSTs)

will see to the daily activities and conduct weekly supervisory meetings to review and report the progress of the study in their facilities (see fig 3 below).

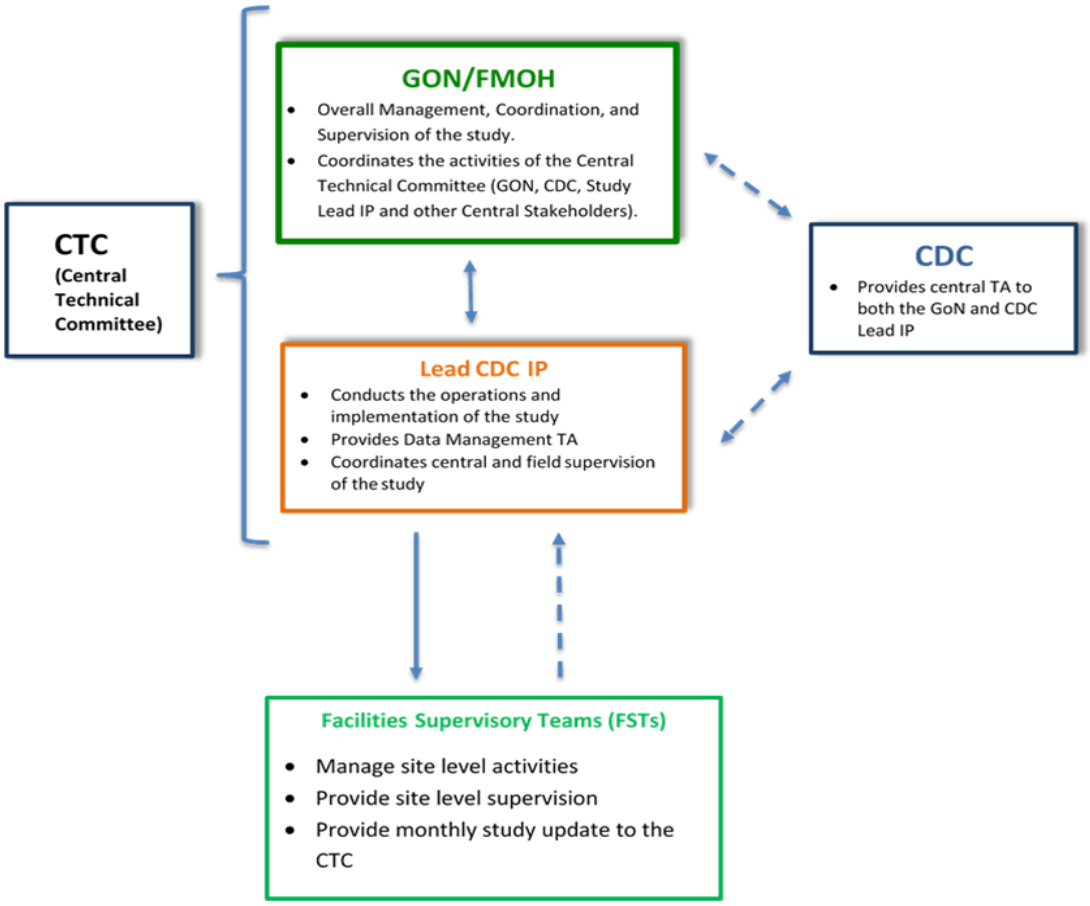

Fig 3. Study Management Flowchart

Dissemination, Notification and Reporting of Results

Individual laboratory test results from the study (i.e., viral load and HIV drug resistance) will be returned to the site to inform respective patient care (within 4 weeks for VL and maximum of 6 weeks for HIV DR from the date of sample collection) following the same study and clinical procedures afore-described. The facility will confirm receipt of results and link the SID of each patient from the report and the patient log before notifying the patient. This should be done in line with the facility's procedures for maintaining confidentiality of patient medical information. Overall, VL test results will be returned between 2-4 weeks and HIVDR results between 4-6 weeks from the time of blood collection.

A survey report will be prepared to share findings with all stakeholders, which includes participating and non-participating states, considering the national implications of the findings. Conference abstracts and manuscripts will be developed for dissemination as deemed appropriate by the investigators and implementing institutions.

De-identified survey datasets will be shared with the CDC; however, data sharing with CDC would bound by a standard agreement template (Appendix 13). Analyses will be governed by the protocol. Ownership of the data remains with Government of Nigeria. Additional uses of the information not described in this protocol will receive separate permission from the Government of Nigeria.

Ethical Considerations

Informed consent

This survey will be implemented primarily to improve public health service delivery in the area of HIV treatment and care. Patients will be required to provide written informed consent prior to study participation. Standard consent forms (Appendix 14: adult consent; appendix 14A: parental permission; appendix 14B: child assent) will be used that describes the purpose of the study, the procedures involved, potential risks and benefits, and assurance of confidentiality of collected information. This consent form may be read aloud by the study staff at the patient's request. To facilitate readability, the consent/assent forms have been designed to at most be at Flesch-Kincaid level 8.6 reading level. Furthermore, consent forms will be translated and back-translated to and from the target language for all study staff that will be involved in the consent process at each site during the pre-survey training.

The age of majority in Nigeria is 18 years. For participation in this study, individuals must have attained the age of 18 years to grant consent, except for mature minors (children aged less than 18 years who are married, pregnant, parents or sexually active…and can consent independently to receive HIV services)[1], who can give consent in their own right. For participants aged 12-17 years, other than mature minors, the permission of a parent or legal guardian (Appendix 14A) is required together with the assent of the child (Appendix 14B). For all children aged less than 12 years, only the consent/permission of the parent or legal guardian is required[2] (Appendix 14A). Study participants are free to opt out of the study at any time.

Confidentiality/Privacy Protections

No personally identifiable information (PII) such as name, hospital/ART ID, photographs and address collected at the facility level will be shared with others involved in the survey at any stage or included in the survey database. However, a log will be established at the site linking SIDs and patient personal identifiable information in order to return VL and HIVDR testing results and will be kept in a secure locked location within the facility along with all informed consents and any other study related material with personal identifying information. Each facility will be notified of the genotyping results for their individual study participants, so that the information can be used to optimize individual patient management. Once this notification step has been completed for all survey participants, all study related materials including data capture forms, logs, and informed consents, will be transferred to a secure locked fire-proof cabinet at a central study site where they will be kept and be transferred to the government of Nigeria (GON) principal investigator within 3 years of the study completion. Other study files such as the main analysis files will follow similar transfer process to the GON.

All individuals involved in the study implementation and management will be required to show evidence of completion of the Collaborative Institutional Training Institute (CITI) and will be required to complete a statement of intent to maintain confidentiality (Appendix 15) as part of the ethical considerations.

[1] https://globalhealth.washington.edu/sites/default/files/AIDS_Law_Brief-Age_of_Consent_for_HIV%20Testing_and_Counseling_in_Nigeria.pdf

[2] http://nhrec.net/nhrec/Final%20NHREC%20Policy%20Statement%20on%20Enrollment%20of%20Children%20in%20Research.pdf

Benefits/Risks/Discomforts to Participants

There is no payment or material compensation for survey participation, however, patient genotype results will be returned, and the availability of this additional information may lead to earlier detection of failure, HIVDR, and an ART regimen switch, as appropriate. Patients will not be subjected to additional risks beyond any associated with the routine clinical viral load testing process/monitoring in the facility such as bleeding and minor pain from needle insertion.

Additional Safeguard for Vulnerable Populations

All paper questionnaires will be kept in secure, locked locations during field work at participating ART facilities. Blood samples and their respective results will be coded. Electronic data files and computers will be password protected. The focal person for this survey at the facilities will be in charge of overall data management, including data quality monitoring, analysis and linking/de-linking of personal identifiers from the main dataset. No identifying information will be included in the data that any of the co-investigators receive.

Institutional Review Boards

This protocol and all other supporting documents including informed consent forms will submitted to the National Health Research Ethical Committee (NHREC) of Nigeria and the CDC for review and appropriate determination.

Sponsor Monitoring

As the study sponsor, the Centres for Disease Control (CDC) may conduct monitoring or auditing of study activities to ensure the scientific integrity of the study and to ensure the rights and protection of study participants. Monitoring and auditing activities may be conducted by:

- CDC staff (“internal”)
- Authorized representatives of CDC (e.g., a contracted party considered to be “external”)
- Both internal and external parties

Monitoring or auditing of study activities may be performed by means of on-site visits to the selected facilities or through other communications such as telephone calls or written correspondence. The visits will be scheduled at mutually agreeable times, and the frequency of visits will be at the discretion of CDC. During the visit, any study-related materials may be reviewed and the Investigator along with the study staff should be available for discussion of findings.

The study may also be subjected to oversight by regulatory authorities (CDC CGH and/or NHREC) to review compliance and regulatory requirements before, during and post implementation

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Appendices

List of appendices for this study is provided below. Please see detailed appendices in a separate attachment:

Appendix 1: National Viral Load Order and Result Form
Appendix 2: COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, Version 2.0.
Appendix 3: Participant Eligibility Determination Form
Appendix 4: Acquired HIVDR (ADR) Data Collection Form (Adults)
Appendix 5: Acquired HIVDR (ADR) Data Collection Form (Children <15)
Appendix 6: ATCC, Applied Biosystems by Thermo Fisher Scientific (flier)
Appendix 7: Prototype Material Transfer Agreement
Appendix 8: HIVDR ATCC Testing SOP pg 7, 8, 9
Appendix 9: Missing data and its consequences for analysis
Appendix 10: Other Possible Variables
Appendix 11: Examples of table shells to be used in survey reports
Appendix 12: Sampling, sample size and analysis examples
Appendix 13: Data sharing agreement
Appendix 14: Sample Informed Consent for VLS/ADR in Adults
Appendix 14A: Parental Consent/Permission Form (Flesch-Kincaid level 8.6)
Appendix 14B: Child assent Form (Flesch-Kincaid level 8.6)
Appendix 15: Confidentiality Agreement Form