

PHIA Drug Resistance Data Use Manual

Reference Guide for Using Antiretroviral Drug Resistance Genotyping Data from the Population-based HIV Impact Assessments



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List of Abbreviations

AIDS	Acquired Immunodeficiency Syndrome
ART	Antiretroviral Therapy
ARV	Antiretroviral
CDC	US Centers for Disease Control and Prevention
CD4	CD4+ T Cell
CIRB	Centre Internationale de Recherche Chantal Biya in Yaoundé, Cameroon
CSV	Comma Separated Values
DBS	Dried Blood Spot
DTS	Dried Tube Specimens
DNA	Deoxyribonucleic Acid
EA	Enumeration Area
EID	Early Infant Diagnosis
HIV	Human Immunodeficiency Virus
ID	Identification Number
ILB	International Laboratory Branch, CDC
INI	Integrase Inhibitor
LA _g OD _n	Limiting-Antigen Avidity (normalized) Optical Density
MDRI	Mean Duration of Recent Infection
mL	Milliliter
µL	Microliter
NHRL	National HIV Reference Laboratory, Tanzania
NICD	National Institute for Communicable Diseases, Zimbabwe
NNRTIS	Non-Nucleoside Reverse Transcriptase Inhibitors
NRTI	Nucleoside Reverse Transcriptase Inhibitors
PCR	Polymerase Chain Reaction
PEPFAR	U.S. President's Emergency Plan for AIDS Relief
PHIA	Population-based HIV Impact Assessment
PMTCT	Prevention of Mother-to-Child Transmission
PI	Protease inhibitor
PII	Personally Identifying Information
RBC	Rwanda Biomedical Center
Retro-CI	Retrovirus Côte d'Ivoire laboratory
RNA	Ribonucleic acid
QA	Quality Assurance
QC	Quality Control
STI	Sexually Transmitted Infection
TNA	Total Nucleic Acid
UVRI	Uganda Virus Research Institute
VL	Viral Load
VLS	Viral Load Suppression

1. Introduction

1.1 Background

The purpose of the Population-based HIV Impact Assessment (PHIA) Project is to collect nationally representative data regarding the status of the HIV epidemic in PEPFAR priority countries, including HIV prevalence, HIV incidence, and viral load suppression (VLS) among persons living with HIV, alongside social, geographic, and economic data. Antiretroviral (ARV) drug resistance (DR) testing was conducted on a non-random subset of persons living with HIV.

The objective of DR testing in PHIA conducted from 2015-2019 was to determine the proportion of HIV-1 drug resistance mutations and the level of transmitted drug resistance, among HIV-positive participants who met the Selection Criteria as described below. Transmitted drug resistance is among individuals who are recently infected and have never been on antiretroviral therapy (ART).

2. Methods

2.1 Selection Criteria

The selection criteria for drug resistance testing consisted of general and country-specific criteria determined by country needs and resource availability. Please see Table 1 for drug resistance selection criteria by PHIA country. Unless otherwise stated, the criteria apply to HIV positive adults aged 15 to the maximum age of participation for each country.

Researchers using these data should note that additional survey eligibility criteria such as sleeping in the household the night before the survey were not by default included in the selection criteria for drug resistance testing. A few HIV positive usual residents who did not sleep in the household the night before the survey are included in the data. See section 3.2 for additional details on how to identify these records.

Table 1. Drug Resistance Selection Criteria by PHIA Country

PHIA Country	Survey Year(s)	HIV-positive babies aged 0 to 17 months	Recent infection ^a and VL >1,000	Long-term infection ^b and VL >1,000 and self-reported on ART	VL 200 to <1,000	VL < 200 and self-reported on ART	Between "recent" and "long-term" LAg ODn cut-off ^c and VL > 1000
Zimbabwe	2015-2016	X*	X	X	Not tested	Not tested	Not tested
Malawi	2015-2016	X	X	X	X	X	Not tested
Zambia	2016	X	X	X	X	X	Not tested
Eswatini	2016-2017	X	X	X	X	Not tested	Not tested
Uganda	2016-2017	X	X	X	Not tested	Not tested	Not tested
Lesotho	2016-2017	X	X	X	X	X	Not tested
Tanzania	2016-2017	X	X	X	Not tested	X	Not tested
Namibia	2017	X	X	X	Not tested	Not tested	Not tested
Cameroon	2017-2018	X	X	X	Not tested	Not tested	Not tested
Côte d'Ivoire	2017-2018	X	X	X	X	X**	X
Ethiopia	2017-2018	X	X	X	X	X	Not tested
Kenya	2018	X	X	X	X	Not tested	Not tested
Rwanda	2018-2019	Not tested	X	X	X	Not tested	X***
Haiti	2019-2020	X	X	X****	Not tested	Not tested	Not tested

^a0 ≤ LAg ODn ≤ 2 except Uganda/Tanzania defined as 0 ≤ LAg ODn ≤ 1.5

^bLAg ODn > 4; Tanzania selected up to 10 adults per region; Uganda selected up to 20 adults per region

^cLAg ODn >2 and < 4

*Included 18 months

**Additional testing included those self-reported not on ART or unknown ART status

***Testing occurred only among those on ART

**** Tested irrespective of self-reported ARTstatus

2.2 Laboratory Methods

Specimens were analyzed using the Applied Biosystems™ HIV-1 Genotyping Kit (Thermo Fisher Scientific) to identify mutations within the HIV-1 *pol* gene region, which encodes amino acid substitutions known to be responsible for resistance to specific antiretroviral drugs.

Viral RNA or total nucleic acid (TNA) from samples of plasma was extracted using the NucliSens easyMAG (bioMérieux) or a Qiagen platform. For samples of dried blood spots (DBS), NucliSens easyMAG or miniMAG (bioMérieux) was used for extraction, see Table 2 for details. A 1.3- kilobase fragment, covering the protease and reverse transcriptase region of the HIV *pol* gene was amplified by one-step reverse transcription-polymerase chain reaction (RT-PCR), followed by nested PCR. Sequencing of the approximately 1.1- kilobase amplicons was performed on an Applied Biosystems Genetic Analyzer (Zhou et al. 2011).

Table 2. Extraction methods

PHIA Country	Survey Year(s)	Lab Name	Extraction Methods (PR-RT)
Zimbabwe	2015-2016	NICD	NucliSens easyMAG (bioMérieux)
Malawi	2015-2016	ILB	NucliSens EasyMAG or emag (bioMérieux)
Zambia	2016	ILB	NucliSens EasyMAG or emag (bioMérieux)
Eswatini	2016-2017	ILB	NucliSens EasyMAG or emag (bioMérieux)
Uganda	2016-2017	UVRI	bioMérieux miniMag (manual)
Lesotho	2016-2017	ILB	NucliSens EasyMAG or emag (bioMérieux)
Tanzania	2016-2017	NHRL	Qiagen
Namibia	2017	ILB	NucliSens EasyMAG or emag (bioMérieux)
Cameroon	2017-2018	CIRB	Qiagen
Côte d'Ivoire	2017-2018	Retro-CI	Qiagen
Ethiopia	2017-2018	EPHIA	Qiagen
Kenya	2018	NHRL	Plasma – Qiagen DBS – miniMag (bioMérieux)
Rwanda	2018-2019	RBC	Qiagen
Haiti	2019-2020	ILB	bioMérieux emag

The customized RECall software program was used to edit the raw sequences and generate consensus sequences (Woods, 2012). Sequences were also analyzed for potential cross-contamination by phylogenetic analysis from codon 6 of protease gene to codon 251 of reverse transcriptase gene using BioEdit (Hall, 1999) or MEGA (Tamura, 2018). Sequences with >98% homology were flagged for investigation for potential cross-contamination or possible epidemiological links. In order to validate results, internal quality assurance measures and in-house quality control standards were included in each sequencing run. After confirmation of sequence quality, drug resistance-associated mutations in the protease and reverse transcriptase genes were identified using the Genotypic Resistance Interpretation Algorithm Tool or the Calibrated Population Resistance Tool at the Stanford University Drug-Resistance Database (<https://hivdb.stanford.edu>). The assay's sensitivity has been established at 1,000 copies/mL for plasma and DBS (Zhou et al. 2011).

Subtyping of each sample was performed using the REGA HIV-1 & 2 Automated Subtyping Tool (Alcantara et al. 2009; de Oliveira et al. 2005). This BioAfrica viral subtyping tool is designed to

use phylogenetic methods in order to identify the HIV-1 subtype of a specific sequence, and is also mirrored at the Stanford University Drug-Resistance Database (<https://hivdb.stanford.edu>). The sequence is analyzed for recombination using bootscanning methods.

3. Using Drug Resistance Data

3.1 How to Access Drug Resistance Data

Researchers who are approved to use a country's drug resistance data will be provided with the data as CSV, STATA (.dta), and SAS (.sas7bdat) files via a link where they can download the approved data. Please see the PHIA data use manual for instructions for requesting and accessing the datasets, including the drug resistance data.

3.2 Merging Drug Resistance Data with Survey Data

Once downloaded, the drug resistance data can be merged with its corresponding household questionnaire, individual questionnaire, and biomarker data using the variables *householdid* or *personid* as noted in the PHIA data use manual.

The drug resistance data contain one observation per participant with a blood sample sent for drug resistance testing (see section 2.1). In addition to the identifiers, the drug resistance data contains an indicator of whether the genotyping was successful (*genotypingflag*), the HIV subtype (*subtype*), and indicators of whether each drug resistance mutation was detected. For the variable *genotypingflag*, the value of TNP corresponds to test not performed, for example in cases for which the amount of sample was insufficient.

See Attachment A. Codebook for a full list of the variables included, with descriptions and values.

3.3 Analysis Considerations

When conducting analyses with drug resistance data, the user should carefully consider the aims of their analysis in determining an analytic plan. The survey weights associated with the PHIA survey data (for example, biomarker weights) are not recommended for use in drug resistance analyses.

As noted in section 2.1, HIV positive individuals who did not sleep in the household the night before the survey are included in the drug resistance testing results. To identify such individuals, merge the adult and/or child biomarker datasets with the drug resistance data by *personid*, and these individuals will have *hivstatusfinal* = 1 and *bt_status* = 9. Researchers should take care to apply any desired inclusion criteria to the data for analyses.

For technical questions related to data analysis, please contact the CDC PHIA team at genpopreview@cdc.gov.

3.4 Dataset Specifications

The table below lists the DR dataset associated with the country specific PHIA survey and the number of observations on each DR dataset. The DR datasets contain the same 151 variables.

Each country-specific DR dataset must be requested separately. See Section 3.1 for how to access the drug resistance data.

Table 3. Dataset Specifications

PHIA Country	Survey Name	File name	Number of observations
Zimbabwe	ZIMPHIA 2015-2016	zimphia2015dr	216
Malawi	MPHIA 2015-2016	mphia2015dr	164
Zambia	ZAMPHIA 2016	zamphia2015dr	268
Eswatini	SHIMS2 2016-2017	shims22016dr	187
Uganda	UPHIA 2016-2017	uphia2016dr	269
Lesotho	LePHIA 2016-2017	lephia2016dr	227
Tanzania	THIS 2016-2017	this2016dr	275
Namibia	NAMPHIA 2017	namphia2017dr	169
Cameroon	CAMPHIA 2017-2018	camphia2017dr	101
Côte d'Ivoire	CIPHIA 2017-2018	ciphia2017dr	96
Ethiopia	EPHIA 2017-2018	ephia2017dr	137
Kenya	KENPHIA 2018	kenphia2018dr	175
Rwanda	RPHIA 2018-2019	rphia2018dr	108
Haiti	HAPHIA 2019-2020	haphia2019dr	193

4. Data Confidentiality

To protect participant confidentiality, all participant IDs are scrambled to ensure that participants cannot be identified from the data. Specifically, *householdid* variables are randomly generated and cannot be linked to the participant except via a secure link file maintained by designated data managers at Ministries of Health and ICAP at Columbia University. All other identifying information such as participant names, addresses, phone numbers, as well as identifying information provided in free-text fields have been excluded from all PHIA datasets.

The protection of participant privacy and confidentiality was maintained at each phase of PHIA data collection and processing. To ensure the protection of participant privacy and confidentiality, PHIA survey data processing encompasses various methods to reduce the risk of disclosure in the public use data. The mitigation of potential risk disclosure occurs at the household-level and individual-level and addresses both direct and indirect identifiers in the public use data.

5. References

Alcantara LC, Cassol S, Libin P, Deforche K, Pybus OG, Van Ranst M, Galvao-Castro B, Vandamme AM, De Oliveira T. A standardized framework for accurate, high-throughput genotyping of recombinant and non-recombinant viral sequences. *Nucleic acids research*. 2009 Jul 1;37(suppl_2):W634-42.

De Oliveira T, Deforche K, Cassol S, Salminen M, Paraskevis D, Seebregts C, Snoeck J, Van Rensburg EJ, Wensing AM, Van De Vijver DA, Boucher CA. An automated genotyping system for analysis of HIV-1 and other microbial sequences. *Bioinformatics*. 2005 Jan 1;21(19):3797-800.

Stanford University. Major HIV Drug Resistance Mutations. Stanford, California. <https://cms.hivdb.org/prod/downloads/resistance-mutation-handout/resistance-mutation-handout.pdf>, Accessed May 12, 2021.

Woods CK, Brumme CJ, Liu TF, Chui CK, Chu AL, Wynhoven B, Hall TA, Trevino C, Shafer RW, Harrigan PR. Automating HIV drug resistance genotyping with RECall, a freely accessible sequence analysis tool. *Journal of clinical microbiology*. 2012 Jun;50(6):1936-42.

Surveillance of HIV drug resistance in adults receiving ART (acquired HIV drug resistance). July 2014. <https://www.who.int/publications/i/item/9789241507073>, Accessed April 2, 2021.

World Health Organization. Surveillance of HIV drug resistance in populations initiating antiretroviral therapy (pre-treatment HIV drug resistance). July 2014. <https://www.who.int/publications/i/item/9789241507196>. Accessed April 2, 2021.

Zhou Z, Wagar N, DeVos JR, Rottinghaus E, Diallo K, Nguyen DB, Bassey O, Ugbeno R, Wadonda-Kabondo N, McConnell MS, Zulu I. Optimization of a low cost and broadly sensitive genotyping assay for HIV-1 drug resistance surveillance and monitoring in resource-limited settings. *PloS one*. 2011 Nov 23;6(11):e28184.

6. Attachments

Attachment A. Drug Resistance Dataset Codebook