Cross-sectional survey of acquired HIV drug resistance to ARV drugs in children, adolescents and adults receiving antiretroviral treatment in *[country name], [year of the survey implementation]*

* **Generic protocol version 1.0, 9 May 2023**
* **Nationally representative laboratory-based method**

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# **Resources**

## **Collaborating institutions**

*[Complement as appropriate]*

## **Funding sources**

*[Complement as appropriate]*

# **Abbreviations and acronyms**

|  |  |
| --- | --- |
| ART | Antiretroviral therapy |
| ARV | Antiretroviral (drug) |
| DTG | Dolutegravir |

# **Definitions**

* **Adults:** people 18 years of age and older *[adjust if needed for the country context]*.
* **Children and adolescents:** generally, people younger than 18 years of age*[adjust if needed for the country context]*.
* **Acquired HIV drug resistance:** develops when HIV mutations emerge due to viral replication in individuals receiving ARV medicines.
* **Viral load suppression:** defined for this survey as viral loads <1000 copies/ml.

# **Introduction**

HIV drug resistance emerges and is selected when the virus replicates in the presence of antiretroviral (ARV) drugs. HIV resistance to ARV drugs affects the ability of these drugs to block viral replication, negatively affecting the effectiveness of antiretroviral therapy (ART) programmes. HIV drug resistance to ARV drugs decreases the efficacy and options of ART regimens. In addition, it may reduce the prevalence of viral suppression in people with HIV receiving ART, increase the number of new HIV infections and deaths associated with advanced HIV infection, and increase ART program costs (1, 2). Therefore, WHO recommends monitoring HIV resistance to ARV drugs as a key component of a comprehensive and effective HIV response (3, 4). Surveillance of acquired HIV drug resistance provides critical information for evaluating the performance of ART programs in achieving viral suppression goals and describes patterns of HIV drug resistance among individuals receiving ART (3).

Dolutegravir (DTG) is an integrase strand transfer inhibitor (INSTI) drug. Since 2018, WHO has recommended using DTG-containing ART as a first-line regimen for adults and as a second-line preferred regimen for those receiving a non-DTG-containing ART regimen with unsuppressed VL (5). As of July 2023, DTG-based ART has been adopted as the primary first-line ART for adults and adolescents by 91% of the 127 reporting countries. Additionally, 77% of the 116 reporting countries have included DTG as part of their second-line ART for adults and adolescents (6). WHO also recommends using DTG-containing ART for children with ≥3 kg and aged ≥4 weeks. As of July 2023, DTG-containing ART regimens are preferred for initiating treatment among infants and children in 69% of the 114 reporting countries (6).

In clinical trials, the prevalence of emergent INSTI-associated drug-resistance mutations remained low among previously INSTI-naive individuals receiving DTG-containing ART regimens with unsuppressed viral load. Among ART-experienced people living with HIV on an NNRTI-containing regimen who were switched to DTG plus two NRTIs, the prevalence of acquired INSTI-associated mutations reached 1.6% by weeks 48/96. By contrast, among ART-naive individuals initiating DTG-based ART and ART-experienced individuals with suppressed viral load who were switched to a DTG-based regimen, the prevalence of INSTI-associated drug-resistance mutations was ≤0.1% (7).

The prevalence of ADR to DTG may be higher in populations that are less closely monitored than in clinical trials. Therefore, WHO recommends monitoring HIV drug resistance to INSTIs as part of the HIV drug resistance surveillance (3). According to the 2024 WHO HIV Drug Resistance Report, populations receiving DTG-containing ART have achieved high levels of HIV VL suppression (>90%) (7). However, the report highlights that levels of HIVDR to DTG observed in country-generated survey data are higher than those seen in clinical trials (7). Four cross-sectional surveys of acquired HIV drug resistance to DTG, supported by the United States President’s Emergency Plan for AIDS Relief (PEPFAR), have been conducted in Malawi, Mozambique, Uganda, and Ukraine (7, 8). These surveys found that the prevalence of DTG resistance among individuals receiving DTG-based ART with unsuppressed VL (≥1,000 copies/mL) ranged from 3.9% to 19.6%. The highest prevalence was observed among ART-experienced people who transitioned to TLD while having high HIV viral loads (7, 8).

In *[name of country]*, *[national estimate]* people are living with HIV. As of *[indicate corresponding year]*, *[national estimate]* adults and *[national estimate]* children and adolescents received ART. According to current national ART guidance, for adults and adolescents, the preferred first-line ART scheme in *[country name]* is *[include ART regimen]*, and the standard second-line scheme is *[include ART regimen]*. The preferred first-line scheme for children weighing <20kg is *[include ART regimen],* 20 to 30kg is *[include ART regimen],* and >30kg is *[include ART regimen]*.

# **Justification**

HIV drug resistance may compromise the efficacy of ARV drugs in reducing HIV-associated HIV incidence and morbidity (1, 2, 9). Even in contexts where ART programs are optimally managed, HIV resistance to ARVs can emerge and be transmitted (10). As the number of people receiving ARVs for HIV prevention or treatment expands, HIV's resistance to ARVs will likely increase (11-13).

To minimise the emergence and spread of HIV drug resistance, WHO recommends that ART and pre-exposure prophylaxis programmes be accompanied by measures to monitor the quality of ART and pre-exposure prophylaxis services, as well as surveillance for HIV resistance to ARV drugs (3, 4), including surveillance of acquired HIV drug resistance (14).

The results of this survey will be used to inform the national ART guideline and the national action plan to prevent and control HIV resistance to ARV drugs.

# **Survey outcomes**

## **Primary outcomes**

* To estimate the prevalence of HIV drug resistance to ARV drugs in adults who receive ART and are not virally suppressed (viral load ≥1000 copies/ml), regardless of their ART regimen.
* To estimate the prevalence of HIV drug resistance to ARV dugs in children and adolescents who receive ART and do not have viral suppression (viral load ≥1000 copies/ml), regardless of their ART regimen.
* To estimate the prevalence of HIV drug resistance to DTG in adults receiving DTG-containing ART regimens who are not virally suppressed (viral load ≥1000 copies/ml).
* To estimate the prevalence of HIV drug resistance to DTG in children and adolescents receiving DTG-containing ART regimens that are not virally suppressed (viral load ≥1000 copies/ml).

## **Secondary outcomes**

* To estimate the prevalence of viral suppression (viral load <1000 copies/ml) in adults receiving ART, regardless of their ART regimen.
* To estimate the prevalence of viral suppression (viral load <1000 copies/ml) in adults receiving ART, stratified by age group, sex and ART regimen.
* To estimate the prevalence of viral suppression (viral load <1000 copies/ml) in children and adolescents receiving ART, regardless of their ART regimen.
* To estimate the prevalence of viral suppression (viral load <1000 copies/ml) in children and adolescents receiving ART, stratified by age group, sex and ART regimen.
* To estimate the prevalence of HIV resistance to ARVs in adults without viral suppression (viral load ≥1000 copies/ml) and receiving ART, stratified by age group, sex, and ART regimen.
* To estimate the prevalence of HIV resistance to ARVs in children and adolescents without viral suppression (viral load ≥1000 copies/ml) and receiving ART, stratified by age group, sex and ART regimen.

# **Methodology**

## **Survey design**

A nationally representative cross-sectional survey will be conducted following WHO-recommended methods for laboratory-based acquired HIV drug resistance surveys (14).

The survey period will be three months, from *[start month]* to *[end month]*. During these three months, all eligible case specimens (**section 7.3.2**) will be stored following WHO recommendations for handling such specimens (**section 7.3.3** and **Annexe 1**). When the three-month period ends, the sample size will be calculated (**section 7.4**). The survey sampling design will use double stratification, with the viral load testing laboratory and the ART regimen (DTG-containing or non-DTG-containing) as stratifying variables. A laboratory’s contribution will be proportional to the number of eligible case specimens stratified by those receiving DTG-containing and non-DTG-containing ART regimens.

Selected eligible case specimens will be genotyped following WHO recommendations (**section 7.7.2**), and the results will be used to estimate the prevalence of HIV resistance to ARV drugs (**section 7.10**).

Because the prevalence of acquired HIV drug resistance, its determinants and public health actions may differ for adults and children and adolescents, these populations are assessed separately in simultaneous surveys. Therefore, the calculation of the sample size, the enrolment in the survey and the data analysis will be stratified for adults and, separately, for children and adolescents.

## **Selection of viral load testing laboratories to participate in the survey**

*[Option A (ideal): All viral load laboratories in the country will participate in the survey. Below is the list of laboratories and their three-letter unique identifier:*

*\*Include list\*]*

*[Alternative for countries with >10 viral load laboratories: All viral load laboratories in the country will participate in the survey, except for those that handle <10% of total viral load tests in the country. Below is the list of laboratories participating in the survey and their unique three-letter identifier. Also included is the list of laboratories that will not participate in the survey:*

*\*Include list\*]*

## **Selection and management of eligible case specimens**

* + 1. **Survey period**

The survey period will be three months, from *[start month]* to *[end month]*. All eligible case specimens (**section 7.3.2**) will be handled and stored following WHO recommendations (**section 7.3.3 and Annexe 1**) during these three months.

* + 1. **Eligibility criteria for remnant viral load specimens**

The eligible case specimens are remnant plasma specimens from the routine viral load testing from individuals with unsuppressed viral load (≥1000 copies/ml). Remnant viral load specimens eligible for the survey must meet inclusion criteria and not meet exclusion criteria.

* + - 1. **Inclusion criteria**
* The plasma specimen is from a person who has received ART for at least 6 months and is receiving ART at the time of the specimen collection for viral load testing;
* The plasma specimen has a viral load result of ≥1000 copies/mL;
* If there are several samples of an individual, only the first sample obtained from an individual in the survey period is included;
* The remnant viral load specimen is sufficient in quantity for the HIV genotyping test (at least *[the minimum volume required by the laboratory that will perform the HIV genotyping tests]* of plasma).
* The remnant viral load specimen can be linked to the minimum information needed for the survey (**section 7.9**).
	+ - 1. **Exclusion criteria**

The specimen comes from a person infected with HIV-2 or coinfected with HIV-1 and HIV-2.

### **Handling of eligible case specimens**

The plasma samples for this survey should have been collected and handled following WHO-recommended procedures for surveillance of HIV resistance to ARV drugs (15) (**Annexe 1**).

The eligible case specimens will be frozen as soon as possible, within 48 hours after blood collection. Plasma aliquots will be stored at -80°C *[alternative: -20]* in the *[name of laboratory]* until processing. Freezing and thawing plasma samples will be avoided to avoid damaging viral RNA, which could lead to failures in the HIV amplification and sequencing (15).

## **Sample size calculation**

When the three-month period ends (after collecting and storing eligible case specimens), the sample size will be calculated following the WHO-recommended method (14). The WHO web tool (<https://worldhealthorg.shinyapps.io/ADR_LabBasedMethod/>) will be used, adjusting the parameters to the context of *[country name]* (**see Annexe 2**). This WHO web tool will also be used to assign the contributing sample by each viral load testing laboratory participating in the survey. A laboratory’s contribution will be proportional to the number of eligible case specimens stratified by those receiving DTG-containing and non-DTG-containing ART regimens. The sample will be calculated separately for adults and for children and adolescents (14).

## **Sampling procedures**

Once laboratory sample sizes have been determined, each laboratory will randomly select the eligible case specimens through systematic random sampling (**see Annexe 3**). Sampling will be performed for eligible case specimens from people receiving DTG-containing and non-DTG-containing ART regimens.

## **Survey ID**

Eligible case specimens will be identified using the WHO-recommended survey ID, which is composed of the following five elements delimited by a hyphen (-):

* Country abbreviation: the International Organization for Standardization’s standard three-letter abbreviation:[[1]](#footnote-1) *[three-letter ISO code]* for *[country name]*
* Survey type: acquired HIV drug resistance (ADR)
* Year the survey started: *[year]*
* Three-letter code identifying the participating viral load laboratory (**section 7.2**)
* A four-digit unique number: that is, a consecutive unique number assigned to an eligible case specimen at that site
* Population: **a** for adults and **c** for children and adolescents

For example, if the laboratory University Lab *[change this example with the name of a viral load lab participating in the survey]* is selected to participate in the survey to be conducted among adults in the Bahamas *[change this example for the name of the country]* in 2023 *[change to the year the survey will start]*, the survey ID for the first eligible case specimen from an adult would be BHS-ADR-2023-UNI-0001-a *[change this example accordingly]*.

## **Laboratory procedures for HIV drug resistance testing**

### **Eligible case specimen transportation**

Eligible case specimens selected for the survey will be sent to the *[name of laboratory]* in a cold chain using dry ice and avoiding thawing of specimens (15). Specimens will be packed using triple packaging. The shipment of specimens (packaging, classification and labelling) will be carried out according to the regulations of the International Air Transport Association. Import and export permits will be obtained before sending the samples.

### **HIV drug resistance testing**

Eligible case specimens will be tested for HIV drug resistance. HIV drug resistance testing will follow WHO recommendations (15) at *[name of laboratory]*, a laboratory designated by WHO for the surveillance of HIV drug resistance. Drug resistance genotyping should include sequencing of the integrase, reverse-transcriptase and protease regions of the HIV-1 *pol* gene (15). The sequences will be identified using the survey ID (**section 7.6**).

The sequences will be assembled using ReCall (British Columbia Centre for Excellence in HIV/AIDS, BCCfE, Vancouver, BC) (16). The sequence quality control will be done using the WHO/BCCfE HIVDR quality control tool: <https://recall.bccfe.ca/who_qc/> (17). If pairs or groups of viral sequences with a small genetic difference (<0.5%) are identified, the HIV genotyping process will be repeated to rule out contamination errors.

## **Management of clinical and demographic data**

Required demographic and clinical data (**section 7.9**) will be obtained from electronic laboratory databases *[Alternatively: manually extracted from viral load requisition forms]. [If necessary: [if necessary, the information will be supplemented using records from the ART clinics from which the specimens were obtained.]* Demographic and clinical data will be identified using the survey ID (**section 7.6**).

A survey coordinator will evaluate the quality of the data. Discrepancies, such as inconsistent or out-of-range responses and missing data, will be reported and corrected as appropriate.

## **List of variables to be collected**

### **Required specimen-level information**

* Survey ID (**section 7.6**)
* Participant ART number (clinic ID)[[2]](#footnote-2)
* Viral load testing laboratory ID (**section 7.2**)
* Gender (female, male or other)
* Date of birth (DD/MM/YYYY)
* Age
* Date of first ART initiation (DD/MM/YYYY)
* Current ART line (first line/second line/third line)
* Current ART regimen – the names of each currently prescribed antiretroviral drug
* Date of initiation of current ART regimen (DD/MM/YYYY)
* Current HIV viral load (copies/ml)

### **Required laboratory-level information**

* Viral load testing laboratory name
* Viral load testing laboratory ID (**section 7.2**)
* Total number of eligible case specimens from people receiving a DTG-containing ART regimen from each viral load testing laboratory during the three-month survey period (disaggregated by adults and paediatric population). ***Note:*** *This variable is essential to perform statistical analysis adjusted with statistical weights according to the survey design.*
* Total number of eligible case specimens from people receiving a non-DTG-containing ART regimen from each viral load testing laboratory during the three-month survey period (disaggregated by adults and paediatric population). ***Note:*** *This variable is essential to perform statistical analysis adjusted with statistical weights according to the survey design.*
* Total number of people taking a DTG-containing ART regimen who received a viral load test (regardless of viral load results) and have all required variables from each laboratory during the survey period (disaggregated by adults and paediatric population).
* Total number of people taking a non-DTG-containing ART regimen who received a viral load test (regardless of viral load results) and have all required variables from each laboratory during the survey period (disaggregated by adults and paediatric population).
* Total number of people taking a DTG-containing ART regimen who received a viral load test, were virally suppressed, and have all required variables from each laboratory during the survey period (disaggregated by adults and paediatric population).
* Total number of people taking a non-DTG-containing ART regimen who received a viral load test, were virally suppressed, and have all required variables from each laboratory during the survey period (disaggregated by adults and paediatric population).

## **Statistical analysis**

The WHO/BCCfE HIVDR quality control tool (17) will be used to assess the quality of the HIV sequences. If pairs or groups of viral sequences with a genetic difference <0.5%, with no apparent epidemiological link, are identified, only one sequence from that group will be included in the data analysis.

HIV resistance to ARV drugs will be predicted using the Stanford University HIVdb (18, 19). The virus will be considered resistant when the HIVdb assigns a score ≥15 to a given ARV drug. HIV resistance disaggregated by ARV drugs will be reported.

Statistical analysis will be performed according to the WHO guidelines to analyse surveys of acquired HIV resistance (14) using STATA (StataCorp, College Station, TX, USA) and will account for stratification by laboratory and ART regimen (DTG-containing and non-DTG-containing ART). The statistical analysis will be performed separately for children and adolescents and for adults and will be adjusted for a finite population. Proportions and their 95% confidence intervals will be estimated.

## **Ethical considerations**

*[Select the appropriate option and edit accordingly:*

*Option A: This protocol has been evaluated and approved by the ethics committee [insert name of committee]. Since HIV genotyping testing will be performed on unidentified remnant viral load specimens, informed consent will not be required.*

*Option B: This protocol describes a surveillance activity for HIV resistance to ARV drugs. Therefore, local regulations have approved this protocol as a surveillance activity, and informed consent will not be required.]*

Staff participating in the survey will be trained on the purpose and appropriate procedures for storing and selecting eligible case specimens for the HIV drug resistance survey. The training will address maintaining the confidentiality of the patient's data whose specimens will be included in the survey.

# **Dissemination of survey outcomes**

A report will be written with the survey findings. It will be discussed within the Ministry of Health and used to develop/update the national action plan to prevent and control HIV drug resistance and the national ART guideline. The report will be shared with different Ministry of Health entities as appropriate and with partners in the national HIV response.

The survey findings will be published in scientific journals as deemed appropriate by the Ministry of Health. In addition, survey data (deidentified demographic, clinical and laboratory data) will be shared with PAHO/WHO for inclusion in regional and global analyses.

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## **Annexe 1:** Collection and handling of plasma specimens to be used for the HIV drug resistance survey

The plasma specimens will be collected, handled, stored and processed following WHO-recommended procedures for the surveillance of HIV drug resistance (15).

**Blood collection:** peripheral venous blood specimens will be collected in tubes with EDTA. This procedure will be carried out following universal biosafety precautions for blood collection.

**Handling:** Centrifugation, pipetting and aliquot preparation will follow standard biosafety precautions in the laboratory. The plasma will be separated as soon as possible within 6 hours of specimen collection. During the period between sampling and plasma separation, whole blood specimens will be kept refrigerated (4°C).

Blood specimens will be centrifuged at room temperature at 800-1600 x g for 20 minutes. The plasma will be separated using sterile transfer pipettes, generating at least three aliquots of plasma of 1 ml in cryotubes. The plasma aliquots will be kept in refrigeration (4 °C) while they are frozen (<48 hours after being collected) at -20 or -80 °C.

## **Annexe 2:** assumptions for the sample size calculation

**Survey of acquired HIV drug resistance to ARV drugs among adults**

|  |  |  |
| --- | --- | --- |
| **Assumptions** | **Everyone regardless of ART regimen** | **People receiving DTG-containing ART** |
| Expected prevalence of overall acquired HIV drug resistance | 50% | NA |
| Expected prevalence of acquired HIV drug resistance to DTG | NA | 3.5% |
| Desired absolute precision (95% confidence interval half-width) | ±6% | ±2% |
| Number of viral load testing laboratories that will participate in the survey  | *[include the number]* |
| Number of eligible case specimens from adults receiving DTG-containing ART by viral load testing laboratory | *[to be defined at the end of the 3 months of storage of eligible case specimens]* |
| Number of eligible case specimens from adults receiving non-DTG-containing ART by viral load testing laboratory | *[to be defined at the end of the 3 months of storage of eligible case specimens]* |
| Genotyping failure rate | 30% |

DTG, dolutegravir; ART: antiretroviral therapy

**Survey of acquired HIV drug resistance to ARV drugs among children and adolescents**

|  |  |  |
| --- | --- | --- |
| **Assumptions** | **Everyone regardless of ART regimen** | **People receiving DTG-containing ART** |
| Expected prevalence of overall acquired HIV drug resistance | 50% | NA |
| Expected prevalence of acquired HIV drug resistance to DTG | NA | 3.5% |
| Desired absolute precision (95% confidence interval half-width) | ±6% | ±2% |
| Number of viral load testing laboratories that will participate in the survey  | *[include the number]* |
| Number of eligible case specimens from children and adolescents receiving DTG-containing ART by viral load testing laboratory | *[to be defined at the end of the 3 months of storage of eligible case specimens]* |
| Number of eligible case specimens from children and adolescents receiving non-DTG-containing ART by viral load testing laboratory | *[to be defined at the end of the 3 months of storage of eligible case specimens]* |
| Genotyping failure rate | 30% |

DTG, dolutegravir; ART: antiretroviral therapy

## **Annexe 3:** Systematic sampling

Systematic sampling will be carried out as follows in each viral load testing laboratory:

* In each viral load testing laboratory, 4 **sampling frames** will be constructed:
	+ List of eligible case specimens from adults receiving DTG-containing ART *[at the end of the 3-month survey period, list the eligible case specimens and assign a correlative number starting at 1]*
	+ List of eligible case specimens from adults receiving non-DTG-containing ART *[at the end of the 3-month survey period, list the eligible case specimens and assign a correlative number starting at 1]*
	+ List of eligible case specimens from children and adolescents receiving DTG-containing ART *[at the end of the 3-month survey period, list the eligible case specimens and assign a correlative number starting at 1]*
	+ List of eligible case specimens from children and adolescents receiving non-DTG-containing ART *[at the end of the 3-month survey period, list the eligible case specimens and assign a correlative number starting at 1]*
* For each sampling frame, the **sampling interval** will be calculated by dividing the total number of eligible case specimens by the number of eligible case specimens to be sampled (sample size assigned to the laboratory by population -adult or paediatric- and by ART regimen -with or without DTG-). The sampling interval will be rounded so as not to include decimal places.
* A random number will then be selected to initiate systematic sampling. A random number between 1 and the sampling interval *[rounded sampling interval without decimals]* will be generated using the random number generator at <https://openepi.com/Random/Random.htm>.
* The first eligible case specimen to be selected will be the one that corresponds to the random number selected for each sampling frame.
* The sum of the initial random number and the sampling interval will correspond to the second eligible case specimen to be selected.
* The sum of the second eligible case specimen selected and the sampling interval will correspond to the third eligible case specimen to be selected and so on.

**Table A3.1.**  Form to record per each participating laboratory the sampling interval calculation for each sampling frame and the selection of the first two eligible case specimens.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **A** | **B** | **C** | **D** | **E** | **F** |
| **Sampling frame** | **Total number of eligible case specimens** | **Sample size** | **Sampling interval** | **Random number = first eligible case specimen to be selected** | **Second eligible case specimen to be selected** |
| Adults receiving DTG-containing ART | *[include the total number of eligible case specimens stored at the end of the 3-month survey period]* | *[include the sample size assigned to the laboratory]* | *[*$D=B÷C$*]* | *[random number between 1 and the sampling interval (D) using:* [*https://openepi.com/Random/Random.htm*](https://openepi.com/Random/Random.htm)*]* | *[*$F=E+D$*]* |
| Adults receiving non-DTG-containing ART | *[include the total number of eligible case specimens stored at the end of the 3-month survey period]* | *[include the sample size assigned to the laboratory]* | *[*$D=B÷C$*]* | *[random number between 1 and the sampling interval (D) using:* [*https://openepi.com/Random/Random.htm*](https://openepi.com/Random/Random.htm)*]* | *[*$F=E+D$*]* |
| Children and adolescents receiving DTG-containing ART | *[include the total number of eligible case specimens stored at the end of the 3-month survey period]* | *[include the sample size assigned to the laboratory]* | *[*$D=B÷C$*]* | *[random number between 1 and the sampling interval (D) using:* [*https://openepi.com/Random/Random.htm*](https://openepi.com/Random/Random.htm)*]* | *[*$F=E+D$*]* |
| Children and adolescents receiving non-DTG-containing ART | *[include the total number of eligible case specimens stored at the end of the 3-month survey period]* | *[include the sample size assigned to the laboratory]* | *[*$D=B÷C$*]* | *[random number between 1 and the sampling interval (D) using:* [*https://openepi.com/Random/Random.htm*](https://openepi.com/Random/Random.htm)*]* | *[*$F=E+D$*]* |

**Table A3. 2.** Example of a sampling frame and selection of eligible case specimens

|  |  |  |  |
| --- | --- | --- | --- |
| **Correlative number** | **Sample ID** | **Selection** | **Selected eligible case specimen** |
| 1 |  |  |  |
| 2 |  |  |  |
| 3 |  |  |  |
| 4 |  | 4† (first sample selected) | Yes |
| 5 |  |  |  |
| 6 |  |  |  |
| 7 |  |  |  |
| 8 |  |  |  |
| 9 |  | 4+5\*=9 | Yes |
| 10 |  |  |  |
| 11 |  |  |  |
| 12 |  |  |  |
| 13 |  |  |  |
| 14 |  | 9+5=14 | Yes |
| 15 |  |  |  |
| 16 |  |  |  |
| 17 |  |  |  |
| 18 |  |  |  |
| 19 |  | 14+5=19 | Yes |
| 20 |  |  |  |
| 21 |  |  |  |
| 22 |  |  |  |
| 23 |  |  |  |
| 24 |  | 19+5=24 | Yes |
| 25 |  |  |  |
| 26 |  |  |  |
| 27 |  |  |  |
| 28 |  |  |  |
| 29 |  | 24+5=29 | Yes |
| 30 |  |  |  |
| 31 |  |  |  |
| 32 |  |  |  |
| 33 |  |  |  |
| 34 |  | 29+5=34 | Yes |
| 35 |  |  |  |
| 36 |  |  |  |
| 37 |  |  |  |
| 38 |  |  |  |
| 39 |  | 34+5=39 | Yes |
| 40 |  |  |  |
| 41 |  |  |  |
| 42 |  |  |  |
| 43 |  |  |  |
| 44 |  | 39+5=44 | Yes |
| 45 |  |  |  |
| 46 |  |  |  |
| 47 |  |  |  |
| 48 |  |  |  |
| 49 |  | 44+5=49 | Yes |
| 50 |  |  |  |
| Total number of eligible case specimens stored during the survey period at X laboratory: | 50 |
| Sample size: | 10 |
| \*Sampling interval: | 50/10 =5 |
| †Random start generated using <https://openepi.com/Random/Random.htm>: | 4 |

## **Annexe 4:** Timeline

*[to be adjusted]*

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Activity** | **Month A** | **Month B** | **Month C** | **Month D** | **Month E** | **Month F** | **Month G** | **Month H** | **Month I** | **Month J** |
| **Planning phase** |
| Protocol development | X |  |   |   |   |   |   |   |   |   |
| Protocol approval |   | X |  |   |   |   |   |   |   |   |
| Supplies procurement |   |   | X |  |  |   |   |   |   |   |
| Training |   |   | X |  |  |   |   |   |   |   |
| Import and export permits |   |   | X | X |  |   |   |   |   |   |
| **Implementation phase** |
| Selection and storage of eligible case specimens |   |   |   | X | X | X |  |  |   |   |
| Sample size calculation and selection of eligible case specimens  |   |   |   |   |   |  | X |  |  |   |
| Shipment of eligible case specimens for HIV drug resistance testing |   |   |   |   |   |   | X |  |   |   |
| HIV drug resistance testing |   |   |   |   |   |   | X | X |  |  |
| Data analysis and draft report  |   |   |   |   |   |   |   |   | X |   |
| Dissemination of survey outcomes  |   |   |   |   |   |   |   |   |   | X |

1. ISO 3166 country codes: <https://www.iso.org/obp/ui/#search/code/> [↑](#footnote-ref-1)
2. This variable is not used in analysis; however, a code linking the assigned participant survey identification code and the participant ART number (clinic ID) should be maintained at the viral load testing laboratory to facilitate quality assurance and return of results to participants’ medical records, if desired. [↑](#footnote-ref-2)