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

* **Generic protocol version 1.0, 8 January 2025**
* **Nationally representative laboratory-based method, enhanced approach**

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# **Summary**

**Title:** Cross-Sectional Survey of Acquired HIV Drug Resistance in Children, Adolescents, and Adults Receiving Antiretroviral Treatment in *[Country Name]*, *[Year of Survey Implementation]*

**Approach:** Nationally Representative Laboratory-Based Method, Enhanced Approach. This protocol outlines a nationally representative cross-sectional survey to assess acquired HIV drug resistance among children, adolescents, and adults receiving antiretroviral therapy (ART) in *[Country Name]* during *[Year]*. Conducted in alignment with WHO recommendations, the survey employs an enhanced laboratory-based method suitable for settings with ≥60% viral load (VL) testing coverage but <80% availability of required survey variables.

**Objectives:** The primary objectives are to estimate the prevalence of HIV drug resistance to antiretroviral (ARV) drugs among individuals on both DTG-containing and non-DTG-containing ART regimens with unsuppressed viral load. Secondary objectives include assessing viral suppression rates and identifying resistance patterns stratified by age, sex, and ART regimen.

**Methodology:** The survey utilises a double-stratified sampling design, targeting both laboratories and ART regimens (DTG-containing and non-DTG-containing). Clinics within these laboratories catchment area will be randomly chosen using simple random sampling without replacement to ensure representativeness. Eligible case specimens, defined as remnant plasma from individuals with VL ≥1000 copies/mL, will be systematically sampled and stratified by ART regimen and population group (adults vs. children and adolescents). Specimens will be handled and stored following WHO guidelines to preserve viral RNA integrity. The selected eligible case specimens will undergo HIV drug resistance testing.

**Data Collection and Management:** Intensive interventions, including training and supervision, will support sampled clinics in completing requisition forms accurately and transferring data efficiently. Data will be managed through the WHO HIV drug resistance database, ensuring robust quality control and confidentiality.

**Outcomes:** The survey will provide critical insights into the prevalence and patterns of HIV drug resistance, informing national ART guidelines and action plans. Additionally, the survey will contribute to global HIV drug resistance surveillance efforts.

**Ethical Considerations:** As this protocol involves using de-identified remnant specimens collected as part of programmatic health-care monitoring, informed consent is not required. The confidentiality of participant data will be strictly maintained throughout the study.

**Dissemination:** Results will be disseminated through national reports, presentations to the Ministry of Health, scientific publications, and shared with WHO for inclusion in regional and global analyses.

# **Resources**

## **Collaborating Institutions**

*[Complete as appropriate]*

## **Funding Sources**

*[Complete as appropriate]*

# **Abbreviations and Acronyms**

|  |  |
| --- | --- |
| ART | Antiretroviral therapy |
| ARV | Antiretroviral (drug) |
| DTG | Dolutegravir |
| INSTI | Integrase strand transfer inhibitor |

# **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]*. *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].* In *[year]*, *[name of country]* will implement a nationally representative survey of acquired HIV drug resistance among populations receiving ART, following WHO recommendations, as detailed in this protocol.

# **Justification**

HIV drug resistance may compromise the efficacy of ARV drugs in reducing HIV-associated HIV incidence and morbidity (1, 2, 9). Resistance may develop even within well-managed ART programs, potentially impacting long-term treatment success and public health goals. As the use of ARV drugs for both prevention and treatment continues to expand, monitoring HIV drug resistance becomes crucial to maintaining ART efficacy and adapting treatment protocols as needed.

WHO recommends that ART programs incorporate measures to monitor service quality and implement surveillance systems for HIV drug resistance (3, 4), including surveillance of acquired HIV drug resistance (10). This survey will be relevant for informing national ART guidelines and shaping action plans to prevent and control HIV drug resistance in *[country name]*. The data generated will offer evidence-based insights into the current landscape of HIV drug resistance. Understanding the prevalence and determinants of HIV drug resistance enables healthcare providers and policymakers to optimise ART programs, ensuring sustained viral suppression among individuals living with HIV. This optimisation contributes to reducing HIV transmission rates and enhancing public health outcomes. Additionally, accurate data on HIV drug resistance will guide resource allocation, ensuring that efforts to monitor and address resistance are prioritised and adequately funded, particularly in resource-limited settings.

Aligning national surveillance efforts with WHO recommendations also supports broader global health initiatives aimed at controlling the HIV epidemic. By contributing to the global understanding of HIV drug resistance, *[country name]* can play a significant role in shaping international strategies and collaborations.

In summary, this survey is a key component of a comprehensive approach to managing HIV drug resistance, providing essential data to inform national policies, enhance ART program effectiveness, and support ongoing efforts to reduce HIV-related morbidity and mortality.

# **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 (10), following an enhanced approach recommended by WHO when the national viral load testing coverage is ≥60%, but the availability of required survey variables is <80%.

The sampling design uses double stratification, with both the laboratory and the regimen (DTG-containing or non-DTG-containing) as stratifying variables. A sample of clinics within each viral load testing laboratory catchment area will be randomly chosen. A random sample of eligible case specimens, stratified by DTG-containing versus non-DTG-containing regimens, will be sampled from these clinics only. There are two design phases (**Figure 1**).

The first design phase will determine the required sample sizes and the number of clinics to sample for each laboratory (**section 7.3**). Sampled clinics will receive intensive training and oversight from the national ART programme to improve the completeness of data on the laboratory requisition forms and thereby the completeness of the required survey variables (**section 7.12**). Laboratories will prospectively store epidemiological data and remnant samples from eligible case specimens (**section 7.6.2**) collected from the sampled clinics during a three-month survey period, from *[start month]* to *[end month]*. Eligible case specimens will be stored following WHO recommendations for handling specimens for HIV drug resistance testing (**section 7.10.1** and **Annex 3**).

The second design phase will happen after the three-month study period and will involve determining the per-clinic sample size (**section 7.7**), stratified by DTG- and non-DTG eligible case specimens. The samples from DTG eligible case specimens will be used to estimate the prevalence of acquired HIV drug resistance to DTG among people taking DTG-containing regimens, and the combined samples (those for DTG and non-DTG eligible case specimens) will be used to estimate the overall prevalence of acquired HIV drug resistance.

A laboratory’s contribution will be proportional to the number of eligible case specimens per participating clinic and stratified by those receiving DTG-containing and non-DTG-containing ART regimens. Eligible specimens will be selected from the sampled clinics using systematic random sampling (**section 7.8**). Selected eligible case specimens will be genotyped following WHO recommendations (**section 7.10.2**), and the results will be used to estimate the prevalence of HIV resistance to ARV drugs (**section 7.13**).

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.

**Figure 1.** Enhanced Approach for Implementing WHO Laboratory-Based Acquired HIV Drug Resistance Surveys in Settings with ≥60% Viral Load Coverage and <80% Variable Availability

## **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\*]*

**Table 1.** List of Viral Load Testing Laboratories to Participate in the Survey

|  |  |  |
| --- | --- | --- |
| **Region** | **Laboratory Name** | **Laboratory ID[[1]](#footnote-1)** |
| *[Complete accordingly]* | *[Complete accordingly]* | *[Complete accordingly]* |
| *[Complete accordingly]* | *[Complete accordingly]* | *[Complete accordingly]* |
| *[Complete accordingly]* | *[Complete accordingly]* | *[Complete accordingly]* |

## **Determining the Required Sample Sizes and Number of Clinics to Sample**

The required sample size and the number of clinics to sample per laboratory will be determined following the WHO-recommended method (10). The WHO web tool (<https://worldhealthorg.shinyapps.io/ADR_LabBasedMethod_2/>) will be used, adjusting the parameters to the context of *[country name]* described in **Annexes 1-2**. Sample sizes and the number of clinics will be calculated separately for adults and for children and adolescents to ensure adequate representation of each population group (10).

The required sample sizes per target population are described in **Table 2**. The number of clinics to be sampled per Laboratory’s catchment area is described in **Table 3** and **4**.

**Table 2.** Required Sample Sizes for DTG and Non-DTG Case Specimens in Adult and Paediatric Populations

|  |  |  |
| --- | --- | --- |
| **Sample Type** | **Adults** | **Children and adolescents** |
| Sample Size for DTG Case Specimens | *[Complete based on the sample size calculator output]* | *[Complete based on the sample size calculator output]* |
| Sample Size for Non-DTG Case Specimens | *[Complete based on the sample size calculator output]* | *[Complete based on the sample size calculator output]* |
| Total Sample Size | *[Complete based on the sample size calculator output]* | *[Complete based on the sample size calculator output]* |

**Table 3.** Required Number of Clinics to be Sampled per Laboratory’s Catchment Area for Adults

|  |  |  |  |
| --- | --- | --- | --- |
| **Laboratory No.** | **Laboratory Name** | **Number of Clinics in the Catchment Area** | **Number of Clinics to be Sampled** |
| 1 | *[Complete accordingly]* | *[Complete accordingly]* | *[Complete based on the sample size calculator output]* |
| 2 | *[Complete accordingly]* | *[Complete accordingly]* | *[Complete based on the sample size calculator output]* |
| 3 | *[Complete accordingly]* | *[Complete accordingly]* | *[Complete based on the sample size calculator output]* |

**Table 4.** Required Number of Clinics to be Sampled per Laboratory’s Catchment Area for Paediatric Population

|  |  |  |  |
| --- | --- | --- | --- |
| **Laboratory No.** | **Laboratory Name** | **Number of Clinics in the Catchment Area** | **Number of Clinics to be Sampled** |
| 1 | *[Complete accordingly]* | *[Complete accordingly]* | *[Complete based on the sample size calculator output]* |
| 2 | *[Complete accordingly]* | *[Complete accordingly]* | *[Complete based on the sample size calculator output]* |
| 3 | *[Complete accordingly]* | *[Complete accordingly]* | *[Complete based on the sample size calculator output]* |

## **Selection of ART Clinics to Participate in the Survey**

For each laboratory, the allocated number of clinics will be sampled from the total number of clinics in their catchment area using simple random sampling without replacement. Simple random sampling without replacement is employed to ensure that each clinic within the target population has an equal probability of being selected for the survey, and that once a clinic is chosen, it is not eligible for selection again. This method minimises selection bias and enhances the sample's representativeness by providing an unbiased selection process. Therefore, the diversity and characteristics of the entire population of clinics in *[country name]* will be accurately reflected by the sampled clinics through simple random sampling without replacement.

## **Interventions and Specimen Collection for Sampled Clinics**

A needs assessment will be conducted to identify the most effective interventions for optimising the completion of requisition forms. Throughout the study period, each sampled clinic will receive intensive support to ensure that all specimens collected for viral load testing are accompanied by properly completed requisition forms. These interventions will include training sessions and ongoing supervision focused on accurately completing viral load requisition forms and efficiently transferring data from clinics to the viral load laboratory.

## **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 7.6.2**) will be handled and stored following WHO recommendations (**section 7.10.1** and **Annex 3**) during these three months.

* + 1. **Eligibility Criteria for Remnant Viral Load Specimens**

The eligible case specimens will be remnant plasma specimens from routine viral load testing of individuals with unsuppressed viral load (≥1000 copies/mL). Remnant viral load specimens eligible for the survey must meet inclusion the following inclusion criteria:

* The plasma specimen is from a person who has received ART for at least six 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;
* 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 information needed for the survey (**section 7.12**);
* If there are several samples of an individual meeting the criteria above, only the first eligible specimen obtained from an individual in the survey period is included.

### **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 (11) (**Annex 3**).

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 (11).

## **Sample Size Allocation Across Sampled Clinics**

Upon completion of the three-month specimen collection and storage period, the target sample size will be distributed among the sampled clinics. For each ART regimen (DTG-based and non-DTG-based), the allocation will be proportional to the number of eligible case specimens each clinic contributed during the study period, in accordance with the WHO-recommended method (10). Sample sizes for each ART regimen will be automatically allocated to each clinic using the WHO web tool (<https://worldhealthorg.shinyapps.io/ADR_LabBasedMethod_2/>) based on the number of eligible specimens stored at each laboratory during the survey period. Sample size allocation will be conducted separately for adults and for children and adolescents to ensure appropriate representation across different population groups (10).

## **Sampling** **of stored eligible case specimens**

After determining the sample sizes for each clinic, laboratories will employ systematic random sampling to select eligible case specimens (**see Annex 4**). The sampling process will be stratified by ART regimen, ensuring separate samples for DTG-containing and non-DTG-containing regimens. This stratification guarantees that each ART regimen is adequately represented within the sample. Additionally, sampling will encompass specimens from each clinic and will be conducted separately for adults and for children and adolescents to ensure appropriate representation across different population groups (10).

## **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:[[2]](#footnote-2) *[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 Uganda *[change this example for the name of the country]* in 2025 *[change to the year the survey will start]*, the survey ID for the first eligible case specimen from an adult would be UGA-ADR-2025-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 (11). 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 (11) 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 (11). The sequences will be identified using the survey ID (**section 7.9**).

The sequences will be assembled using ReCall (British Columbia Centre for Excellence in HIV/AIDS, BCCfE, Vancouver, BC) (12). The sequence quality control will be done using the WHO/BCCfE HIVDR quality control tool: <https://recall.bccfe.ca/who_qc/> (13). 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, and the WHO-designated laboratory will link with the country to ascertain for possible epidemiological linkages of sequence pair(s).

## **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 7.9**).

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.

The WHO HIV drug resistance database will be the primary platform for managing survey data, ensuring robust epidemiological and sequence data quality assurance (14). Deidentified data will be entered using a standardised Excel-based upload template (available at: <https://www.who.int/teams/global-hiv-hepatitis-and-stis-programmes/hiv/treatment/hiv-drug-resistance/hiv-drug-resistance-surveillance/surveillance-of-acquired-hiv-drug-resistance-in-populations-receiving-art>), and corresponding HIV drug resistance genotypes in FASTA format will also be submitted to the WHO HIV drug resistance database (14). This web-based system consolidates survey-level and deidentified participant-level data, performing automated quality checks on epidemiological and sequence information before analysis. The WHO HIV drug resistance database reviews sequence data following the WHO HIV drug resistance laboratory operational framework (11).

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

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

* Survey ID (**section 7.9**)
* Participant ART number (clinic ID)[[3]](#footnote-3)
* 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 sampled clinic 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 sampled clinic 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 sampled clinic 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 sampled clinic 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 sampled clinic 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 sampled clinic during the survey period (disaggregated by adults and paediatric population).

## **Statistical analysis**

The WHO/BCCfE HIVDR quality control tool (13) 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 (15, 16). 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 (10) using STATA (StataCorp, College Station, TX, USA) and will account for stratification by laboratory and ART regimen (DTG-containing and non-DTG-containing ART). These analyses will also account for including a random sample of clinics rather than all clinics. The statistical analysis will be performed separately for children and adolescents and 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 WHO for inclusion in regional and global analyses.

# **References**

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## **Annex 1:** 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

## **Annex 2:** Assumptions for determining the number of clinics to sample

**ADULTS**

Total number of clinics in each laboratory's catchment area

* How many viral load laboratories are there in your country?
* What are the total number of clinics served by each laboratory?

|  |  |  |
| --- | --- | --- |
| **Laboratory No.** | **Laboratory Name** | **Number of Clinics in the Catchment Area** |
| 1 |  |  |
| 2 |  |  |
| 3 | *[Add rows as needed]* |  |

Available historical data

* What is the anticipated proportion of patients with all required survey variables?
* What type of national-level historical data is available?

[ ]  Number of patients with viral non-suppression among patients on DTG-containing and non-DTG-containing regimens. In the table below, input the national-level historical data.

|  |  |  |
| --- | --- | --- |
| **National-level historical data** | **Those on DTG-containing regimens** | **Those on non-DTG-containing regimens** |
| Number of individuals, in a recent three-month period, who were receiving ART, underwent **viral load testing**, and had viral non-suppression. | *[Complete as needed. Must be a whole number]* | *[Complete as needed. Must be a whole number]* |

[ ]  Number of patients who underwent viral load testing among patients on DTG-containing and non-DTG-containing regimens. In the table below, input the national-level historical data.

|  |  |  |
| --- | --- | --- |
| **National-level historical data** | **Those on DTG-containing regimens** | **Those on non-DTG-containing regimens** |
| Number of individuals, in a recent three-month period, who were receiving ART and underwent **viral load testing**.  | *[Complete as needed. Must be a whole number]* | *[Complete as needed. Must be a whole number]* |
| Anticipated proportion of those receiving viral load tests who also have viral non-suppression.  | *[Complete as needed. Must be between 0 and 1]* | *[Complete as needed. Must be between 0 and 1]* |

[ ]  Number of patients receiving ART among patients on DTG-containing and non-DTG-containing regimens. In the table below, input the national-level historical data.

|  |  |  |
| --- | --- | --- |
| **National-level historical data** | **Those on DTG-containing regimens** | **Those on non-DTG-containing regimens** |
| Number of individuals, in a recent three-month period, who were **receiving ART**.  | *[Complete as needed. Must be a whole number]* | *[Complete as needed. Must be a whole number]* |
| Anticipated proportion of those receiving ART who receive viral load test. | *[Complete as needed. Must be between 0 and 1]* | *[Complete as needed. Must be between 0 and 1]* |
| Anticipated proportion of those receiving viral load tests who also have viral non-suppression. | *[Complete as needed. Must be between 0 and 1]* | *[Complete as needed. Must be between 0 and 1]* |

**Children and adolescents**

Total number of clinics in each laboratory's catchment area

* How many viral load laboratories are there in your country?
* What are the total number of clinics served by each laboratory?

|  |  |  |
| --- | --- | --- |
| **Laboratory No.** | **Laboratory Name** | **Number of Clinics in the Catchment Area** |
|  |  |  |
|  |  |  |
|  | *[Add rows as needed]* |  |

Available historical data

* What is the anticipated proportion of patients with all required survey variables?
* What type of national-level historical data is available?

[ ]  Number of patients with viral non-suppression among patients on DTG-containing and non-DTG-containing regimens. In the table below, input the national-level historical data.

|  |  |  |
| --- | --- | --- |
| **National-level historical data** | **Those on DTG-containing regimens** | **Those on non-DTG-containing regimens** |
| Number of individuals, in a recent three-month period, who were receiving ART, underwent **viral load testing**, and had viral non-suppression. | *[Complete as needed. Must be a whole number]* | *[Complete as needed. Must be a whole number]* |

[ ]  Number of patients who underwent viral load testing among patients on DTG-containing and non-DTG-containing regimens. In the table below, input the national-level historical data.

|  |  |  |
| --- | --- | --- |
| **National-level historical data** | **Those on DTG-containing regimens** | **Those on non-DTG-containing regimens** |
| Number of individuals, in a recent three-month period, who were receiving ART and underwent **viral load testing**.  | *[Complete as needed. Must be a whole number]* | *[Complete as needed. Must be a whole number]* |
| Anticipated proportion of those receiving viral load tests who also have viral non-suppression.  | *[Complete as needed. Must be between 0 and 1]* | *[Complete as needed. Must be between 0 and 1]* |

[ ]  Number of patients receiving ART among patients on DTG-containing and non-DTG-containing regimens. In the table below, input the national-level historical data.

|  |  |  |
| --- | --- | --- |
| **National-level historical data** | **Those on DTG-containing regimens** | **Those on non-DTG-containing regimens** |
| Number of individuals, in a recent three-month period, who were **receiving ART**.  | *[Complete as needed. Must be a whole number]* | *[Complete as needed. Must be a whole number]* |
| Anticipated proportion of those receiving ART who receive viral load test. | *[Complete as needed. Must be between 0 and 1]* | *[Complete as needed. Must be between 0 and 1]* |
| Anticipated proportion of those receiving viral load tests who also have viral non-suppression. | *[Complete as needed. Must be between 0 and 1]* | *[Complete as needed. Must be between 0 and 1]* |

## **Annex 3:** 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 (11).

**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.

## **Annex 4:** Systematic sampling

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

* For each participating clinic, **four** **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 clinic 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. Record the outcome in **Table A3.1. Column D**.
* 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>. Record the outcome in **Table A3.1. Column E**.
* 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. Record the outcome in **Table A3.1. Column F**.
* 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 (see example in **Table A3. 2.**).

**Table A3.1.** Sampling Interval Calculation and Specimen Selection for Each Clinic within the Laboratory Catchment Area

|  |
| --- |
| **Laboratory Name:** |
| **Clinic Name:** |
| **A** | **B** | **C** | **D** | **E** | **F** |
| **Sampling frame** | **Total number of eligible case specimens from the clinic** | **Required sample size for the clinic** | **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 |

## **Annex 5:** Timeline

*[Adjust accordingly]*

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Activity** | **Month 1** | **Month 2** | **Month 3** | **Month 4** | **Month 5** | **Month 6** | **Month 7** | **Month 8** | **Month 9** | **Month 10** |
| **Planning phase** |
| Protocol development | X |  |   |   |   |   |   |   |   |   |
| Protocol approval |   | X |  |   |   |   |   |   |   |   |
| Supplies procurement |   |   | X |  |  |   |   |   |   |   |
| Training |   |   | X |  |  |   |   |   |   |   |
| Import and export permits |   |   | X | X |  |   |   |   |   |   |
| **Implementation phase** |
| Intensive support for clinics to ensure availability of required data |   |   |   | X | X | X |  |  |   |   |
| Selection and storage of eligible case specimens |  |  |  | X | X | X |  |  |  |  |
| Sample size allocation 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. Three-letter code identifying the participating viral load laboratory [↑](#footnote-ref-1)
2. ISO 3166 country codes: <https://www.iso.org/obp/ui/#search/code/> [↑](#footnote-ref-2)
3. 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-3)