Sentinel surveys of acquired HIV resistance to dolutegravir among people receiving dolutegravir-containing antiretroviral therapy in *[country name], [year of the survey implementation]*

* **Generic protocol version 1.0, 24 April 2023**
* **Laboratory-based sentinel 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.
* **Detectable viral load:** for the purposes of this survey, HIV RNA above the lower limit of detection of the assay used in a country for individuals receiving a dolutegravir-containing regimen.
* **Confirmed unsuppressed viral load:** for the purposes of this survey, confirmed unsuppressed viral load among individuals receiving a dolutegravir-containing regimen is defined as HIV RNA >1000 copies/mL on a second or confirmatory viral load test obtained after a first viral load test showing detectable virus and after a period of enhanced adherence counselling or other recommended adherence support, as defined by the national antiretroviral therapy programme.

# **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 a well-tolerated and highly effective antiretroviral drug recommended by WHO in the first- and second-line ART regimens (5). An important advantage of DTG is its high genetic barrier to selecting HIV drug resistance (6). DTG resistance did not emerge among ART-naive participants in clinical trials (7, 8) and, to date, has only been described in a few ART-naive people for whom DTG-based ART has failed as their first-line treatment (9). However, DTG resistance can emerge, especially among people with previous exposure to first-generation integrase inhibitors with comparatively lower genetic barriers to selecting HIV drug resistance or when used as DTG monotherapy (10). Recent ART programme information from sub-Saharan Africa documents the emergence of DTG resistance in populations for which DTG-containing regimens have failed (11, 12), thus highlighting the need for routine population-level surveillance of HIV drug resistance among people receiving DTG-containing ART regimens (3).

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, 13). Even in contexts where ART programs are optimally managed, HIV resistance to ARVs can emerge and be transmitted (14). As the number of people receiving ARVs for HIV prevention or treatment expands, HIV's resistance to ARVs will likely increase (15-17).

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 (18).

As DTG-based ART expands globally, estimating the extent to which acquired DTG drug resistance emerges in populations receiving ART is important. WHO recommends that countries scaling up DTG-containing ART accompany its roll-out with routine surveillance of HIV resistance to DTG (3, 19).

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

# **Survey outcomes**

## **Primary outcomes**

* Estimate the prevalence of HIV drug resistance to DTG among adults with confirmed viral non-suppression while receiving a DTG-containing ART regimen.
* Estimate the prevalence of HIV drug resistance to DTG among children and adolescents with confirmed viral non-suppression while receiving a DTG-containing ART regimen.

## **Secondary outcomes**

* Estimate the prevalence of HIV drug resistance to co-administered and other ARV drugs among adults with confirmed viral non-suppression while receiving a DTG-containing ART regimen.
* Estimate the prevalence of HIV drug resistance to co-administered and other ARV drugs among children and adolescents with confirmed viral non-suppression while receiving a DTG-containing ART regimen.

# **Methodology**

## **Survey design**

A sentinel survey will be implemented following WHO-recommended methods for a laboratory-based sentinel survey of acquired HIV drug resistance to dolutegravir (19).

The survey period will be three months, from *[start month]* to *[end month]*. During these three months, eligible case specimens, remnant plasma samples from viral load testing (**section 7.3.1**), will be stored at the sentinel site (**section 7.2**) following WHO recommendations for the handling of such specimens (**section 7.3.2**) until the required sample size is reached (**section** **7.4**). Selected eligible case specimens will be genotyped following WHO recommendations (**section 7.7**), and the results will be used to estimate the prevalence of HIV resistance to ARV drugs (**section 7.10**).

The survey will be stratified by adults, on the one hand, and children and adolescents, on the other. It is necessary to analyse these two groups since the prevalence of viral suppression, acquired resistance of HIV to ARVs, their determinants and the public health actions required may differ between them.

## **Sentinel sites**

*[Note to edit this section: Viral load testing laboratories serve as sentinel sites. Countries may implement the survey at one or more viral load testing laboratories. To serve as sentinel sites, laboratories must have adequate capacity to store eligible remnant viral load specimens at –20°C or –80°C. The laboratories where most confirmatory testing is conducted should be given priority for inclusion as a sentinel site.*

*Option A (only one viral load testing laboratory): [The viral load testing laboratory X will be the sentinel site for the HIVDR survey.]*

*Option B (>1 viral load testing laboratory): [The following laboratories were selected as sentinel sites for the HIVDR survey.]*

*Please include the list of selected laboratories and the site ID, a three-letter abbreviation (a three-letter abbreviation for the site, unique within the country; by default, the first three letters of the name of the viral load laboratory unless this is not unique).]*

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

* + 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 confirmed unsuppressed viral load (> 1000 copies/ml). Remnant viral load specimens eligible for the survey must meet inclusion criteria and not meet exclusion criteria. **Annexe 1** supports the identification of eligible remnant specimens.

* + - 1. **Inclusion criteria**
* The remnant specimen is from an individual receiving a DTG-containing ART regimen for at least 6 months.
* The remnant specimen is from an individual with a previous detectable viral load who received enhanced adherence counselling for three months (this is a repeat confirmatory test).
* The viral load test result of the corresponding remnant specimen has a high viral load (HIV RNA >1000 copies/mL), consequently classifying the individual as having a confirmed unsuppressed viral load.
* 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 remnant viral load specimen has an inconclusive test result.
* The remnant specimen is a “first” viral load test not obtained to confirm viral non-suppression.

### **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 (20) (**Annexe 2**).

The eligible case specimens will be frozen as soon as possible, within 48 hours after sampling. 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 (20).

## **Survey sample size**

The sample size recommended by WHO (**Annexe 3**) for sentinel surveys of acquired HIV resistance to DTG is 139 per sentinel site and per population (19). Therefore, for this survey, the sample size will be *[add if more than 1 sentinel site will be included: for each sentinel site]:*

* 139 eligible case specimens from adult
* 139 eligible case specimens from children and adolescents.

## **Sampling procedures**

Eligible specimens *[add if more than 1 sentinel site will be included: within a sentinel laboratory]* will be sampled using consecutive sampling, stopping when the target sample size of 139 is reached*.* The starting date and ending date of sampling will be recorded *[add if more than 1 sentinel site will be included: in each sentinel laboratory],* and use the ending date to estimate the total size of the eligible population over the three-month survey window. This information will be required for using finite population correction for data analysis.

If the sentinel laboratory has not achieved its target sample size of 139 by the end of the recommended three-month survey period, all eligible specimens sampled during that period will be included for HIV drug resistance testing.

## **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: sentinel acquired HIV drug resistance (SADR)
* Year the survey started: *[year]*
* Site abbreviation (**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 selected as a sentinel site in the country]* is selected as a sentinel site in Belize *[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 BLZ-SADR-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 (20). 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 (20) 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 (20). 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) (21). The sequence quality control will be done using the WHO/BCCfE HIVDR quality control tool: <https://recall.bccfe.ca/who_qc/> (22). 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**

### **7.9.1 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 (or age) (DD/MM/YYYY)
* Current ART regimen – the names of each currently prescribed antiretroviral drug
* Date of initiation of current ART regimen (DD/MM/YYYY)
* Date of initiation of a DTG-containing ART regimen (DD/MM/YYYY)
* Previous ART regimens – the names of each previously prescribed antiretroviral drug
* Date of first ART initiation (DD/MM/YYYY)
* Current HIV viral load (copies/ml)
* Previous HIV viral load (date and copies/ml)

### **7.9.2 Required sentinel laboratory-level information**

* Viral load testing laboratory name
* Viral load testing laboratory ID (**section 7.2**)
* Start date of collection of eligible samples (DD/MM/YYYY)
* Date eligible sample collection ended (DD/MM/YYYY)

## **Statistical analysis**

The *WHO/BCCfE HIVDR quality control tool* (22) 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 (23, 24). 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 sentinel surveys of acquired HIV resistance to dolutegravir (19) using STATA (StataCorp, College Station, TX, USA). 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:** Flow diagram for selecting the eligible specimens for the sentinel surveys of acquired HIV resistance to DTG among people receiving DTG-containing ART­­

Viral load testing performed by six months after ART initiation, 12 months after ART initiation and yearly thereafter

ELIGIBLE REMNANT SPECIMEN

Undetectable viral load\*

Detectable viral load\*\*

Repeat viral load testing after three months of enhanced adherence counselling

Detectable viral load

>1000 copies/ml

Detectable viral load

≤1000 copies/ml

Ineligible remnant specimen

Ineligible remnant specimen

\*Undetectable viral load: HIV viral load is **below** the lower limit of detection of the viral load assay used in the country. In most countries, the lower limit of detection is 20 copies/ml.

\*\*Detectable viral load: HIV viral load is **above** the lower limit of detection of the viral load assay used in the country. In most countries, the lower limit of detection is 20 copies/ml.

Individual receiving a DTG-containing ART regimen

## **Annexe 2:** 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 (20).

**Blood collection:** peripheral venous blood specimens will be collected in tubes with EDTA. This procedure will be carried out following universal biosecurity 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 3:** Sample size calculation

This annexe provides the statistical details of the DTG-specific HIV drug resistance survey approach.

**A1.1 Calculating required sample sizes**

For moderate sample sizes and prevalence estimates away from the boundaries (prevalence estimates away from 0% or 100%), the method for calculating a confidence interval for individual-sample surveys uses a *z*-distribution. The required sample size formula is obtained by inverting the 95% Wald confidence interval with a *z*-distribution:

$$n=\frac{z\_{0.975}^{2} \*p^{HIVDR}\*\left(1-p^{HIVDR}\right)}{L^{2}},$$

where $n$ is the required sample size; $p^{HIVDR}$ is the anticipated prevalence of DTG-specific HIV drug resistance; $L$ is the desired absolute precision; and $z\_{0.975}$ is the 97.5th quantile of the *z*-distribution.

For this survey, in the absence of more conclusive and broad evidence on the prevalence of DTG drug resistance, $p^{HIVDR}$ is set at 50%. This is the point of maximum variability and will yield the largest required sample size for this estimate. The target confidence interval width is ±10%, thus resulting in a required sample size of *n* = 97.

**A1.2 Inflating the sample size for genotypic testing failure**

The required sample size must be inflated to account for genotypic testing failure rates among specimens. Since not all specimens will have an HIV drug resistance test result to contribute to the analysis, WHO recommends incorporating an anticipated genotypic testing failure rate of 30% into sample size calculations. WHO recommends this 30% failure rate. Thus, the target sample size will be 139 ($=97/(1-0.3)$).

## **Annexe 4:** timeline

*[Adjust as needed]*

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **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 |  |  |   |   |
| 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 sentinel viral load testing laboratory to facilitate quality assurance and return of results to participants’ medical records, if desired. [↑](#footnote-ref-2)