World Health Organization

HIV DRUG RESISTANCE

LABORATORY-BASED SURVEY OF ACQUIRED HIV DRUG RESISTANCE USING REMNANT VIRAL LOAD SPECIMENS

APRIL 2021

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ACKNOWLEDGEMENTS

ABBREVIATIONS AND ACRONYMS

ART   antiretroviral therapy

ATV/r   atazanavir/ritonavir

DBS   dried blood spots

DE   design effect

DRV/r   darunavir/ritonavir

DTG   dolutegravir

EFV   efavirenz

HIV   human immunodeficiency virus

INI   integrase inhibitor

LPV/r   lopinavir/ritonavir

NRTI   nucleoside reverse-transcriptase inhibitor

NNRTI   non-nucleoside reverse-transcriptase inhibitor

NVP   nevirapine

PI   protease inhibitor

PR   prevalence ratio

/r   with a ritonavir boost

DEFINITIONS

Adult: generally, people 18 years of age and older; however, the age cut off may vary from country to country.

Children and adolescents: generally, individuals younger than 18 years of age; however, the age cut-off may vary from country to country.

Case specimen: a remnant viral load specimen from individuals meeting survey inclusion criteria and from whom required survey variables are available for analysis.

Overall data completeness: availability of required patient-level data.

Viral load testing coverage: the proportion of all people receiving antiretroviral therapy who have at least one annual viral load test with classifiable results. Viral load testing coverage is usually derived from programmatic data. Importantly, for this survey, the estimate of viral load testing coverage must exclude viral load testing being performed at the point of care, since no remnant specimens are available for HIV drug resistance testing.

EXECUTIVE SUMMARY

The survey method outlined in this document uses a nationally representative design to estimate the prevalence of acquired HIV drug resistance among (1) adults and (2) children and adolescents receiving antiretroviral therapy (ART) with viral non-suppression (≥viral load 1000 copies/mL) by leveraging remnant specimens obtained during routine viral load monitoring and stored in national viral load laboratories. The primary survey outcomes are:

- outcome 1: the prevalence of acquired HIV drug resistance among individuals with viral non-suppression and receiving ART, regardless of the ART regimen; and

- outcome 2: the prevalence of acquired resistance to dolutegravir (DTG) among individuals with viral non-suppression and receiving a DTG-containing ART regimen.

The secondary survey outcomes are:

- outcome 3: prevalence of acquired HIV drug resistance among individuals with viral non-suppression and receiving ART, stratified by age band, sex, ART regimen (DTG-based versus non-DTG-based regimen), ART line, previous ART regimen, pregnancy status and breastfeeding status, if known;

- outcome 4: prevalence of viral load suppression (viral load <1000 copies/mL) among individuals receiving ART; and

- outcome 5: prevalence of viral load suppression (viral load <1000 copies/mL) among individuals receiving ART, stratified by age band, sex, ART regimen, ART line, previous ART regimen, pregnancy status and breastfeeding status, if known.

Briefly, countries must assess their readiness to implement this survey method by assessing three criteria:

- mandatory criteria (subsection 2.1), including (1) the existence of national policies establishing routine viral load testing and storage of remnant specimens, (2) data systems enabling viral load testing coverage to be estimated and (3) adequate laboratory infrastructure;

- required availability of laboratory-level data (subsection 2.2); and

- viral load testing coverage and patient-level data completeness (subsection 2.3): (1) national viral load testing coverage, which must be greater than 60%, excluding viral load testing performed at the point of care and (2) the availability of required patient-level data must be greater than 80%.

In countries in which mandatory pre-existing conditions are met (subsection 2.1) and both viral load testing coverage and the availability of the required variables meet or exceed minimum thresholds (subsections 2.2 and 2.3), a three-month survey collection period is chosen during which all remnant viral load specimens from people with viral load ≥1000 copies/mL on routine clinical testing are prospectively stored at all viral load testing laboratories in a country. Remnant viral load specimens with viral load ≥1000 copies/mL are stored for adults, children and adolescents.

The survey sampling design uses double stratification, with both the laboratory and the regimen (DTG-containing or non-DTG-containing) as stratifying variables. All viral load testing laboratories in a country contribute remnant specimens for HIV drug resistance genotyping. When the three-month period during which specimens have been prospectively stored at all viral load laboratories ends, the contribution of each laboratory with respect to sample size allocation is defined. A laboratory's contribution is proportional to the actual number of eligible case specimens, defined as remnant viral load specimens with viral load ≥1000 copies/mL having all required variables associated with it and recorded in the laboratories during the defined three-month specimen collection period, stratified by those receiving DTG and non-DTG regimens.

- A random sample is drawn from adults receiving DTG-containing regimens. The results from this sample yield an estimate of the burden of DTG resistance among adults taking DTG-containing regimens in a country.

- A second random sample includes only adults taking a non-DTG-containing regimen. This sample, combined with the first, will provide an estimate of the overall burden of acquired HIV drug resistance in the country, regardless of the regimen.

Subsection 3.5 describes sample size calculation and sampling of eligible case specimens.

The acquired HIV drug resistance survey of children and adolescents is identical to that described above for adults. However, two important scenarios may warrant special considerations in the design phase (Annex 5): the overall number of children receiving DTG-containing regimens may be too few to support a stratified design, and the available budget may necessitate estimating only the overall acquired HIV drug resistance prevalence among children and adolescents.

Specimens are tested for HIV drug resistance to the HIV reverse transcriptase inhibitor, protease inhibitor (PI) and integrase inhibitor (INI) drug classes at laboratories designated by WHO for the purpose of HIV drug resistance surveillance. De-identified patient variables, viral load and HIV drug resistance genotyping results are linked, and analyses performed to obtain estimates for primary outcome. In addition, de-identified patient-level information linked to all viral load tests regardless of viral load results during the three-month survey period is abstracted to estimate viral load suppression overall and by regimen. The data can be used for further subgroup analysis, yielding prevalence estimates of both HIV drug resistance and viral load suppression.

1. INTRODUCTION

As antiretroviral therapy (ART) for the treatment of HIV expands, it is essential to estimate, in a standardized and nationally representative manner, the extent to which acquired HIV drug resistance emerges in populations receiving therapy. The overarching purpose of acquired HIV drug resistance surveillance is to generate a nationally representative prevalence estimate among adults (and children and adolescents) with viral non-suppression. 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.

In 2014, WHO published a nationally representative survey method for assessing acquired HIV drug resistance among people receiving ART *(1)*. This 2014 ART clinic-based survey approach uses a two-stage cluster design. The first stage consists of a random sample of ART clinics in the country where the survey is conducted; the second is a sample of patients attending the selected clinics. ART clinics are chosen using probability proportional to size sampling. Subsequently, consecutive patients meeting the survey inclusion criteria are enrolled at sampled clinics and receive a viral load test and an HIV drug resistance test if the viral load ≥1000 copies/mL as part of the survey.

WHO recommends that viral load testing be performed early after initiating ART (within six months), at 12 months and annually thereafter to detect treatment failure *(2)*. Patient-level HIV drug resistance genotyping is neither recommended as part of the public health model of ART delivery nor widely available in low- and middle-income countries; continuous surveillance of HIV drug resistance through analysis routine genotyping results is therefore not feasible. However, since an increasing number of people receive at least one viral load test per year, this guidance describes an approach to estimate the prevalence of acquired HIV drug resistance using routinely collected remnant viral load specimens.

Briefly, HIV drug resistance testing is conducted on a random sample of remnant viral load specimens collected from people with viral non-suppression (defined as viral load ≥1000 copies/mL[1]) in the context of routine viral load monitoring and stored in national viral load testing laboratories.

This method accommodates the transition to dolutegravir (DTG)-based first-line ART in most countries with a high burden of HIV infection and recognizes that the pace of transition varies by country and respects that transition to DTG-based therapies may not occur in the near future in some countries. Equally, the method acknowledges that global attention and concern is focused on the emergence of DTG resistance; thus, this method will yield robust estimates to facilitate the monitoring of trends of acquired DTG resistance over time. Simultaneously, the method yields prevalence estimates of acquired HIV drug resistance among people taking non-DTG-based regimens (such as ART based on a ritonavir-boosted protease inhibitor (PI/r) or non-nucleoside reverse-transcriptase inhibitor (NNRTI)) and viral load suppression estimates, by regimen, since this information remains vital for strategic planning at the national, regional and global levels.

1.1   Advantages of the survey method

As countries increase routine viral load testing, there are several advantages to genotyping remnant viral load specimens for the purpose of acquired HIV drug resistance surveillance. These include reduced financial and human resource costs, ability to increase the frequency of surveillance activity, and provision of an on-going impetus to support viral load scale-up and strengthening of data systems to optimize patient care. Moreover, sampling at the laboratory level increases the likelihood of having representation from across most ART clinics, compared with a random sample of clinics for inclusion and therefore resulting in increased precision. Finally, sampling from amongst those with viral non-suppression increases the precision of the drug resistance outcome compared to the 2014 clinic-based approach *(1)*.

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[1] A threshold of 1000 copies/mL is chosen to be consistent with the lower limit of detection for viral load quantification when using dried blood spots specimens and because of standard requirements minimum input copy number for genotyping assays.

1.2   Potential challenges related to the feasibility of the survey method

Under ideal conditions, all individuals eligible for a viral load test should receive it, and all remnant specimens with viral load ≥1000 copies/mL should be properly handled and preferably stored at −80°C (plasma) or −20°C (dried blood spots (DBS)). All viral load requisition forms, including patients' epidemiological and clinical history, should be fully completed and should accompany the viral load specimens to the laboratory, where information should be duly entered in a local database and linked to the viral load results.

In some countries, however, because of limited testing capacity or other barriers, only a subset of the eligible population may receive viral load testing, and this subset may not be random (viral load testing may be more likely among some people or in some clinics). In addition, remnant viral load specimens may not have been collected, handled and stored properly and thus may be unsuitable for HIV drug resistance testing. Further, viral load requisition forms and related information systems may not capture the required patient-level variables necessary to interpret a drug resistance test result and viral load test result. Under such circumstances, using routinely available remnant viral load specimens and genotypes generated from a random sample of specimens with detectable virus may yield biased resistance estimates, thus compromising the appropriateness of this approach for policy-making and for assessing trends in acquired HIV drug resistance over time within and between countries.

In other instances, countries may not have access to the required laboratory-level variables needed in the design and/or analysis phases. It is therefore critical that countries assess readiness before the survey is planned to verify the country's ability to implement this method.

The challenges described above can potentially limit the feasibility of implementing this survey method. However, readiness assessments and identifying potential gaps at the country level affords an opportunity for capacity-building to increase viral load testing coverage and for supporting laboratory and data systems to improve population-level care and facilitate the future implementation of this method.

1.3   Overview

This document has five sections:

- survey overview and introduction (Section 1);

- survey planning: assessment of country readiness to implement this survey based on the conditions described in the assessment framework (Section 2);

- survey methods: survey design, sampling, and analysis methods (Section 3);

- implementation considerations and a practical guide to implementation (Section 4); and

- an alternative approach for countries meeting some but not all the requirements listed in the assessment framework (Section 5).

2. SURVEY PLANNING: FRAMEWORK FOR ASSESSING COUNTRY READINESS TO IMPLEMENT SURVEYS OF ACQUIRED HIV DRUG RESISTANCE LEVERAGING REMNANT VIRAL LOAD SPECIMENS

Using the framework described, countries evaluate their readiness to implement this survey method by critically assessing their policies and data system through a three-step process:

As a first step, countries critically appraise mandatory criteria that must be met before this survey method can be planned (subsection 2.1). The elements include:

- availability of national policy recommending:
  - o routine viral load testing
  - o storing and using remnant specimens;
- availability of data systems that:
  - o capture required patient-level variables
  - o capture data needed at the laboratory level for design and analysis.
  - o use unique patient identifiers; and
- adequate laboratory infrastructure and appropriate standard operating procedures, specifically:
  - o adequate national guidance and standard operating procedures for specimen collection, handling, transport, and storage
  - o adequate storage capacity of remnant DBS or plasma specimens after viral load testing.

If all mandatory elements are in place, countries can complete the second step (subsection 2.2), which includes assessing the extent to which the required laboratory-level data needed in the design and/or analysis phases are available. Finally, in the third step (subsection 2.3), countries must assess (1) whether national viral load testing coverage is adequate and (2) the extent to which the required patient-level data are available.

2.1 First step: assess the mandatory criteria

The elements listed below are required to be in place before a country can consider using this survey method. Annex 1 provides a checklist to be completed by countries to determine whether they meet the mandatory criteria necessary for implementing this acquired HIV drug resistance survey method.

2.1.1 National policy

A. Viral load testing policy. A national policy requiring that all people receiving ART routinely receive at least one viral load test per year should be in place before using this survey method.

Risk of bias: If national policy does not require that all people receiving ART receive at least one viral load test per year, the genotyping of a random sample of remnant specimens from people with viral non-suppression may yield biased estimates of acquired HIV drug resistance. For example, if national policy recommends a "targeted viral load approach" –

that is, only a subset of people (such as with CD4 cell counts below a certain predefined threshold or those for whom ART is suspected of clinical failure) are offered viral load testing – remnant viral load specimens clearly cannot be used to make nationally representative statements on acquired HIV drug resistance.

Likewise, when the national policy on the routine viral load testing of all eligible people is in place but is not fully implemented (viral load testing coverage is patchy, such as high coverage in urban but not in rural areas or in specific geographical regions of a country), using remnant viral load specimens to make nationally representative estimates of acquired HIV drug resistance is not advisable. In fact, it can be hypothesized that patient care may be suboptimal in clinics and geographical or administrative regions of a country with limited uptake of viral load testing, which in turn is associated with a higher risk of acquired HIV drug resistance.

B. Policy on storage and use of remnant specimens. A national policy and guidance to store remnant viral load specimens with viral load ≥1000 copies/mL during the survey period for the purpose of HIV drug resistance testing must be in place at all viral load testing laboratories in a country. In addition, policies[1] for genotyping remnant specimens should be established before implementing the survey, permitting testing of a random sample of stored specimens for the purpose of public health surveillance. Finally, viral load laboratories should have sufficient freezer capacity to store remnant viral load specimens with viral load ≥1000 copies/mL during the three-month survey window.

If a national policy is not in place to store remnant viral load specimens for resistance testing from everyone with viral loads ≥1000 copies/mL, ethical requirements for using remnant specimens and decisions around informed consent and return of results must be clearly established before undertaking a survey using remnant viral load specimens.

2.1.2   Data systems

Data systems should capture the required patient-level variables characterizing the person from whom a viral load result has been obtained. Subsection 2.3 defines the required and desirable patient-level variables.

Data systems should also use unique patient identifiers (such as ART identification number) included in laboratory databases that enable the viral load test result, any relevant laboratory-assigned accession numbers, and demographic and clinical patient-level data to be linked for analysis.

Risk of bias: If data systems do not enable specimens to be linked to the minimum required data at all viral load testing laboratories, bias may be introduced by sampling a subset of specimens with the available data. For example, the specimens with minimum required data may originate from clinics with better resources than from those not completing them and may be associated with different levels of viral load suppression and acquired HIV drug resistance.

2.1.3   Laboratory infrastructure and specimen collection, handling, transport, and storage

Successful genotyping of remnant viral load specimens from people with viral non-suppression requires properly collecting, processing, handling, and storing the specimens. Laboratory requirements for remnant viral load specimens destined for HIV drug resistance testing as part of acquired HIV drug resistance surveillance are broadly consistent with those required for successful viral load testing. The requirements presented here apply to all ART clinics obtaining specimens for viral load testing and to all viral load testing laboratories in a country, since each laboratory will contribute some proportion of eligible case specimens (subsection 3.3) to the overall national sample size for a survey.

Collection, handling, transport, and storage of specimens from the time of collection to the time of arrival at the national viral load laboratory before viral load testing is performed. Collection of either plasma or DBS specimens should follow universal precautions, and any centrifugation, pipetting or aliquoting should follow standard laboratory biosafety precautions. Annex 1 provides a checklist for countries to complete to determine whether they meet the mandatory criteria necessary for successfully implementing this survey method, including the requirements for collecting, processing and storing specimens destined for possible HIV drug resistance genotyping.[2] Moreover, when planning surveys of acquired HIV drug resistance using this method, countries are encouraged to seek support from WHO and its designated HIV drug resistance testing laboratories to ensure that the specimen collection, processing, storage and handling procedures of viral load testing laboratories meet minimum standards *(3,4)*. Remnant plasma or remnant DBS are the recommended specimen types. Because of high levels of amplification failure during genotyping observed with dried plasma spots, they are not a recommended specimen type for this survey. When exploring the possibility of using remnant

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[1]   This survey method does not use written or verbal informed consent but does enable HIV drug resistance test results to be returned to patients' medical records. Since there is no informed consent, ART programmes may promulgate a policy and inform people receiving ART that acquired HIV drug resistance surveillance may be performed on a random sample of specimens from people with viral non-suppression and, if their specimen has been sampled, the results will be returned to their medical record and may or may not be used by their doctor to make treatment decisions.

[2]   More detailed information about required specimen collection, processing storage and handling requirements is available in the *WHO/HIVRESNET HIV drug resistance laboratory operational framework (available at: https://www.who.int/publications/i/item/978-92-4-000987-5)* and the WHO *Manual for HIV drug resistance testing using dried blood spot specimens (Available at: https://www.who.int/publications/i/item/9789240009424).*

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specimens for acquired HIV drug resistance surveillance, countries may identify laboratory infrastructure or procedures that need to be established or strengthened before being able to implement the method outlined here.

Storage capacity of remnant DBS or plasma specimens after viral load testing. Countries planning to implement this survey method should also have adequate capacity available in all viral load laboratories in a country to store eligible remnant viral load specimens at −20°C or −80°C. Countries may use a mix of specimen types such as plasma and DBS specimens, although this is not ideal from an HIV drug resistance testing standpoint.

Risk of bias: At clinics with poor performance (for example, because of insufficient staff), specimens may not be properly collected, processed, and shipped for viral load testing and possible subsequent HIV drug resistance genotyping. Poorly collected specimens may lead to poor amplification rates and may bias viral load and drug resistance estimates.

2.2   Second step: assess laboratory-level data availability

A set of required viral load testing and national level variables is required in the design and analysis phases of the survey. Countries need to ensure that the following information will be available and can be retrieved from the information systems:

Laboratory-level required data for sample size calculation (planning phase) and for data analysis

1. The total number of eligible case specimens (subsection 3.3) from people taking a DTG-containing regimen received in each viral load testing laboratory during the three-month survey period.

2. The total number of eligible case specimens (subsection 3.3) from people taking a non-DTG-containing regimen received in each viral load testing laboratory during the three-month survey period.

3. The total number of eligible case specimens (subsection 3.3) from all people tested for viral load (taking a DTG-containing regimen, taking a non-DTG-containing regimen or overall) nationally during the survey period. Importantly, these numbers do not need to be explicitly collected; rather, they can be calculated by summing up the number of case specimens, by laboratory.

Laboratory-level required data for analysis (available after the three-month survey period)

1. Total number of eligible case specimens (subsection 3.3) disaggregated by patient-level data (subsection 2.3.2) for each laboratory during the survey period.

2. Total number of people taking DTG-containing ART who received a viral load test (regardless of the results) and who have all required variables (subsection 2.3.2) from each laboratory during the survey period.

3. Total number of people taking non-DTG-containing ART who received a viral load test (regardless of the results) and who have all required variables (subsection 2.3.2) for each laboratory during the survey period.

4. Total number of people with the required variables (subsection 2.3.2) who were tested during the survey period, disaggregated by patient-level data (subsection 2.3.2) and for each viral load laboratory.

5. Total number of people with the required variables (subsection 2.3.2) who were tested and had suppressed viral loads during the survey period, disaggregated by patient-level data (subsection 2.3.2) and for each laboratory.

Annex 3, Table A3.1 describes in detail the data needed for designing and analysing the results generated.

During the readiness assessment, each laboratory must demonstrate that systems are currently in place or that they will be put in place for reporting these numbers. If no system is available, a simple spreadsheet tool which laboratories can use to track specimens that are processed during the survey window is 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.

2.3   Third step: assess viral load testing coverage and patient-level data completeness

Ideally, both (1) viral load testing coverage and (2) patient-level data completeness should be 100%, meaning that (1) all eligible individuals should receive viral load testing and remnant specimens from everyone with viral load ≥1000 copies/mL should be stored and available for possible sampling for HIV drug resistance testing in all viral load laboratories (100% viral load testing coverage); and (2) all required epidemiological and clinical information should be completed for everyone tested for viral load and should be available in exploitable local databases, linked to viral load test results though a unique identifier (100% completeness of required patient-level data). However even the best programmes will have imperfect viral load testing coverage and incomplete required patient-level variables. For this reason, perfection is not required for a country to move forward with this survey activity. Rather, thresholds for data completeness have been established that are acceptable (having minimal bias) and will provide informative estimates. Here, we present the process of assessment and recommended thresholds. Annex 2 describes the motivations for these recommended thresholds.

2.3.1   Viral load testing coverage

For a country to implement this survey method, viral load testing coverage must be equal to or exceed 60% in the population surveyed. Viral load testing coverage is assessed in each population separately, adults and children and adolescents, and must exceed 60% in either population to implement the survey in the respective population. Viral load testing coverage is calculated as:

- numerator: number of people within the denominator below with successful viral load test results during the previous calendar year or over a recent three- or six-month period; and

- denominator: number of adults or number of children and adolescents receiving ART during the previous calendar year or over a recent three- or six-month period.

Viral load testing coverage is generally known through routine programme monitoring and the associated evaluation processes since this is a globally required ART programme indicator. Viral load testing coverage in the year before the survey can be used as a proxy measure of coverage during the survey period.

2.3.2   Patient-level data completeness

The availability at the national level of four required variables must be equal to or exceed 80% in the population surveyed. Data completeness is assessed in each population separately, adults and children and adolescents, and must exceed 80% in either population to implement the survey in the respective population. Table 1 shows the required variables. The proportion of data completeness is calculated as:

- numerator: among those in the denominator below, the number with a viral load test, regardless of result, with all four required survey variables reported and linked; and

- denominator: number of viral load tests performed in the specific survey population over a defined period, such as over a recent three- or six-month period.

For example, a country defines a recent three-month period, interrogates relevant databases at all viral load testing laboratories (or at the national level if the information is available centrally) and extracts information from all viral load requisition forms (regardless of results) to quantify the overall availability of the required four survey variables.

There are also highly desirable variables (Table 1) whose completeness is not quantified at this stage, since their availability is not critical for deciding on the readiness to implement this survey method; however, highly desirable variables will be extracted from national databases or viral load requisition forms at the time of the survey and used in analysis. This may mean that some viral load test results (and HIV drug resistance test results) will have different and varying quantities of patient data available for subanalysis.

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Table 1. Patient-level survey variables

Type of variable	Variable
Required variables[a]	Name of viral load testing laboratory where the specimen has been tested
	Unique patient identifier
	Date of birth or age
	Current ART regimen (drugs)
Highly desirable variables	Date of ART start
	Current ART line (first-line, second-line, etc.)
	Previous ART regimen (drugs)
	Gender (male, female or other)
Optional variables	Breastfeeding status (yes, currently breastfeeding; no, not currently breastfeeding; unknown)
	Pregnancy status (yes, currently pregnant; no, not currently pregnant; unknown)

[a] In countries in which viral load testing is performed before initiating ART, the country has an additional fifth required variable: viral load test obtained from a person receiving ART (yes, no or unknown), in which only viral load testing performed while a person is receiving treatment is considered.

2.4 Determining readiness to implement acquired HIV drug resistance surveys leveraging remnant viral load specimens

Once mandatory criteria are in place and verified (first step, subsection 2.1) and the availability of required laboratory-level variables has been established (second step, subsection 2.2), a country should proceed to the third step to estimate (1) viral load testing coverage and (2) patient-level data completeness. If the result of the assessment is that viral load testing coverage is ≥60% and the data completeness of required variables is ≥80% (third step, subsection 2.3), the country can proceed using the standard laboratory-based acquired HIV drug resistance survey method outlined in here (Fig. 1). An alternative approach, presented in Section 6, is recommended for countries with viral load testing coverage ≥60% but availability of required survey variables <80%. Countries with viral load testing coverage <60% should implement a standard WHO-recommended acquired HIV drug resistance clinic-based survey *(1)*.

In addition, countries unable to implement laboratory-based acquired HIV drug resistance surveys are encouraged to amend national viral load policy and/or strengthen data systems, data collection and completeness and viral load requisition forms and harmonize laboratory procedures with WHO guidance regarding plasma or DBS specimens destined for HIV drug resistance testing. To guide successful transition away from standard clinic-based acquired HIV drug resistance surveys to laboratory-based surveys, policy changes and capacity building should be accompanied by ongoing assessment of data completeness and verification. Gaps or shortfalls, if identified, should be addressed well before acquired HIV drug resistance surveys that use remnant viral load specimens. A country should allow for ample time for any needed changes in policy, data or laboratory systems to be implemented at all levels before remnant viral load specimens are leveraged for acquired HIV drug resistance surveys.

Fig.1. Steps involved in a country readiness assessment to implement acquired HIV drug resistance surveys using remnant viral load specimens

FIRST STEP:
Assess mandatory criteria

HAVE ALL MANDATORY CRITERIA BEEN MET?
1. National policy for routine viral load testing and storing remnant specimens
2. Data systems capture the required patient- and laboratory-level variables with unique patient identifiers
3. Adequate laboratory infrastructure and appropriate standard operating procedures for collecting, handling, transporting and storing specimens

YES   NO

SECOND STEP:
Assess laboratory-level data availability

Are laboratory-level data available?

YES   NO

THIRD STEP:
Assess viral load testing coverage and patient-level data completeness

How complete are viral load testing coverage and patient-level data?

- Viral load testing coverage $\geq$60%
- Patient-level data completeness $\geq$80%

- Viral load testing coverage $\geq$60%
- Patient-level data completeness <80%

Viral load testing coverage <60%

Implement acquired drug resistance laboratory-based survey (Section 3)

Implement enhanced acquired drug resistance laboratory-based survey (Section 6)

Implement clinic-based acquired drug resistance survey

Implement actions to meet acquired drug resistance laboratory-based survey

3. SURVEY METHODS

3.1 Overview

The survey has been designed to precisely estimate the prevalence of acquired HIV drug resistance among people with viral non-suppression regardless of ART regimen and of acquired HIV drug resistance to DTG among people with viral non-suppression while taking DTG-containing regimens. The sampling design uses double stratification with both the viral load testing laboratory and the regimen (DTG versus non-DTG) as stratifying variables.

Subsection 3.3 defines the eligible case specimens. In this design, two types of samples are taken concurrently from each viral load testing laboratory, one from eligible case specimens from people receiving DTG-containing regimens (DTG eligible case specimens) and the other from eligible case specimens from people not receiving DTG-containing regiments (non-DTG eligible case specimens). The samples from DTG eligible case specimens are used to estimate the prevalence of acquired HIV drug resistance to DTG among people taking DTG-containing regimens, and the combined samples (taken from DTG and non-DTG eligible case specimens) are used to estimate the overall prevalence of acquired HIV drug resistance.

This approach has several benefits, including convenience and efficiency. Since the numbers of DTG and non-DTG eligible case specimens from each viral load testing laboratory are known in advance, all sampling necessary for estimating the overall prevalence of both acquired HIV drug resistance and DTG-specific acquired HIV drug resistance can be done simultaneously.

Important points on implementing the laboratory-based acquired HIV drug resistance survey method are summarized below.

- The methods described in this section can be implemented in countries reaching the threshold of ≥60% viral load testing coverage and ≥80% patient-level data completeness of four required variables. As described in subsection 2.3, these thresholds were chosen to minimize the amount of bias in estimates; not achieving these thresholds will require alternative approaches, such as the one described in Section 6.

- The survey method is a retrospective assessment of stored remnant viral load specimens with documented viral non-suppression, defined as a viral load ≥1000 copies/mL from people taking ART, collected as part of routine clinical care during a defined survey period and for whom the essential data elements are available.

- The recommended survey period is three months.

- The inferences for outcomes 1 and 2 are specific to people receiving ART with collected viral load specimens, viral non-suppression and the required variables during the defined survey period.

- The required patient-level survey variables and highly desirable patient-level survey variables are extracted from laboratory requisition forms, with no patient-level identifying information being recorded for analysis; however, a link between the survey identification number and the ART number is kept at the viral load laboratory to facilitate the return of results, if desired.

- Remnant specimens corresponding to at least two DBS or 0.5 mL of plasma collected from each patient at the time of routine viral load testing should be available for acquired HIV drug resistance surveillance purposes.

- All viral load testing laboratories in the country should contribute eligible case specimens (subsection 3.3) to the survey, with the number contributed per laboratory proportional to the number of eligible case specimens collected during the three-month study period (subsection 3.6.6).

- The specimens collected should be tested in WHO-designated HIV drug resistance genotyping laboratories. These laboratories are members of the WHO HIVResNet Laboratory Network and undergo a rigorous quality assurance process and participate in annual proficiency panel testing *(3)*. Using WHO-designated laboratories guarantees quality-

assured results for the purpose of public health surveillance. If a country does not have a WHO-designated laboratory for HIV drug resistance testing, it is encouraged to send specimens to a WHO-designated regional or specialized laboratory. A list of WHO-designated laboratories is available at the WHO HIV drug resistance website (https://www.who.int/teams/global-hiv-hepatitis-and-stis-programmes/hiv/treatment/hiv-drug-resistance/laboratory-network).

The reverse transcriptase, protease and integrase regions of the HIV-1 *pol* gene are sequenced using standard sequencing methods generating drug resistance information for HIV-1 nucleoside reverse-transcriptase inhibitor (NRTI), NNRTI, PI and INI. The HIV-1 integrase region of case specimens from people taking non-DTG regimens is genotyped to provide information related to polymorphism and possible circulating integrase resistance in a country.

3.2   Survey outcomes

3.2.1   Primary outcomes

Outcome 1: prevalence of acquired HIV drug resistance among individuals with viral non-suppression and receiving ART, regardless of the ART regimen.

Outcome 2: prevalence of acquired HIV drug resistance to DTG among individuals with viral non-suppression and receiving ART who are taking a DTG-containing regimen.

These are the primary outcomes of the survey; having sufficient precision for these outcomes drives the overall design and sample size calculations.

3.2.2   Secondary outcomes

Outcome 3: prevalence of acquired HIV drug resistance among individuals with viral non-suppression and receiving ART, stratified by age band, gender, ART regimen (DTG-based versus non-DTG-based regimen), ART line, previous ART regimen, pregnancy status and breastfeeding status, if known.

Outcome 4: prevalence of viral load suppression (viral load <1000 copies/mL) among individuals receiving ART.

Outcome 5: prevalence of viral load suppression (viral load <1000 copies/mL) among individuals receiving ART, stratified by age band, gender, ART regimen, ART line, previous ART regimen, pregnancy status and breastfeeding status, if known.

In the absence of electronic laboratory information systems, which make the calculation of outcomes 4 and 5 very difficult, countries may consider not reporting them.

3.3   Eligibility criteria for remnant specimens

An eligible case specimen is defined as a specimen meeting survey inclusion criteria.

3.3.1   Inclusion criteria

- The specimen is from a person receiving ART.

- The specimen has a viral load result ≥1000 copies/mL;

- Only the first specimen obtained from an individual in the survey period is included if more than one specimen is obtained.[1]

- The specimen has been properly stored for HIV drug resistance testing (subsection 2.1 and Annex 1);

- The specimen has sufficient material available for HIV drug resistance testing (subsection 2.1 and Annex 1).

- The specimen has all four required survey variables available (subsection 2.3) to characterize it either on viral load requisition forms or in electronic databases.

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[1] If de-duplication is not possible, this fact should be reported as a limitation when reporting results. However, it is not anticipated than any individual would have more than one viral load test in any given three-month survey period.

3.3.2 Exclusion criterion

A remnant specimen known to be from a person infected with HIV-2 or coinfected with HIV-1 and HIV-2.

3.4   Interpreting the survey results

In this survey design, eligible case specimens collected during the survey period are the population to which inferences apply. Technically, the inferences should be stated as acquired HIV drug resistance prevalence "among people with viral non-suppression receiving ART with remnant specimens and all required survey variables collected during the defined survey period." There are some important considerations for this inference. These estimates do not include those who should have had viral load testing but did not nor do they include those for whom the four required variables were not collected. Using the estimate from this survey to generalize to those who were not tested or who did not have these variables collected could result in bias and should not be done. The thresholds of ≥60% viral load testing coverage and ≥80% completeness of required patient-level variables were set to minimize this bias, as described in subsection 2.3 and Annex 2. Even in the absence of this bias, the precision of survey outcomes is calculated based on the population being restricted to: (1) those with viral load tests; (2) those with required variables; and (3) those with specimens during the three-month survey period. Extrapolating inference beyond this population will likely lead to results stated with greater precision than warranted.

3.5   Survey sample size

3.5.1   Sample size design parameters

Table 2 summarizes the initial parameters for calculating the sample size. These assumptions determine the necessary sample sizes for acquired HIV drug resistance prevalence estimates in the overall sample and the prevalence of DTG resistance for the population taking DTG-containing ART.

For overall prevalence estimates, the expected prevalence of acquired HIV drug resistance varies widely depending on the context and regimens in use in the country. For this reason, the expected acquired HIV drug resistance prevalence is set at 50%, the point of maximum variability. This means that the resulting required sample size, if the expected prevalence is set at 50%, will yield a confidence interval no wider than ±6%, regardless of the actual observed prevalence. Using the formulas presented in Annex 3, subsection A3.1 results in a required overall sample size of 267 eligible case specimens.

The prevalence of DTG resistance among people receiving DTG-containing ART with viral non-suppression is unknown but is anticipated to be low based on data from clinical trials. For this survey, the anticipated prevalence has been set at 3.5%, the upper range of values suggested by the advisory group.[2] Designing the survey using a 3.5% expected prevalence of DTG resistance ensures a confidence interval no wider than ±2% for any prevalence of 3.5% or lower. This results in a required sample size of 325 eligible case specimens from people receiving DTG-containing ART.

Table 2. Assumptions used to calculate sample size for acquired HIV drug resistance among adults or among adolescents and children

Assumptions	Everyone regardless of regimen	People receiving DTG-containing ART
Expected prevalence of overall acquired HIV drug resistance	50%	–
Expected prevalence of acquired HIV drug resistance to DTG	–	3.5%
Desired absolute precision (95% confidence interval half-width)	±6%	±2%
Sample size	267	325
Sample size after 30% inflation for expected genotyping amplification success rate	347	422

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[2] A DTG resistance prevalence of 3.5% is the upper limit of anticipated DTG resistance prevalence as established by the WHO HIVResNet Surveillance and Monitoring Working Group and recommended for use in this acquired HIV drug resistance survey.

3.5.2   Country-specific sample sizes

Countries should tailor the sample sizes in subsection 3.5.1 to the number of eligible case specimens collected during the three-month survey window. This will be especially advantageous for countries with few eligible case specimens, since the target level of precision can be obtained with considerably smaller required sample sizes. However, these adjustments, made by applying a finite population correction, are appropriate for any country to implement.

Table 3 provides examples of how these sample sizes change depending on the population sizes. The full calculation is available in Annex 3, subsection A3.2. WHO developed an application in which countries can specify their own population sizes, by laboratory, to obtain a country-specific sample size. The online sample size calculator is available at https://worldhealthorg.shinyapps.io/ADR_LabBasedMethod/. Annex 4 provides an example of sample size calculations and allocation across strata using the online WHO sample size calculator.

Table 3. Required sample sizes based on the number of eligible case specimens

Number of eligible case specimens, either: • Overall • From people receiving DTG	Overall sample size of eligible case specimens from people receiving ART (regardless of regimen used)	Sample size of eligible case specimens from people receiving DTG-containing ART
100	73	77
500	174	197
1000	211	245
5000	254	305
Infinitely large population	267	325

3.5.3   Allocating samples across DTG and non-DTG strata

The required sample sizes are calculated for eligible case specimens from people receiving DTG-containing ART and all eligible case specimens. However, the stratified sampling for this survey will pull samples from eligible case specimens from people receiving DTG- and non-DTG-containing ART. To calculate the required sample size of eligible case specimens from people receiving non-DTG-containing ART, we multiply the overall required sample size by the proportion of eligible case specimens from people receiving non-DTG containing regimens. The total required sample size is the combination of the sample size of eligible case specimens from people receiving DTG- and non-DTG-containing ART.

The full calculation is available in Annex 3, subsection A3.3. The allocation of sample size across DTG and non-DTG strata and the total required sample size are automatically calculated and reported in the online sample size calculator, available at https://worldhealthorg.shinyapps.io/ADR_LabBasedMethod/. Annex 4 provides an example of sample size calculations and allocation across strata using the online WHO sample size calculator.

3.5.4   Sample size inflation for genotyping failure

Since not all specimens will successfully amplify, the required sample sizes must be inflated to account for the genotyping failure rate. WHO recommends that an anticipated genotyping failure rate of 30% be used in this calculation. The full calculation is available in Annex 3, subsection A3.4. The sample size inflation for genotyping failure is automatically calculated and reported in the sample size calculator, available at https://worldhealthorg.shinyapps.io/ADR_LabBasedMethod/. Annex 4 provides an example of sample size calculations and allocation across strata using the online WHO sample size calculator.

3.5.5   Allocating samples across all viral load testing laboratories in a country

Once the target sample sizes¬ are determined, the sample sizes are distributed across all viral load testing laboratories in a country. If a country has only one viral load laboratory, all case specimens will be sampled from that laboratory. If the country has more than one viral load laboratory, the sample sizes will be distributed across all viral load testing laboratories in a manner proportional to the number of eligible case specimens from each laboratory during the survey period.

Ideally, all viral load laboratories in a country participate. However, in countries with many viral load laboratories (such as more than 10), a viral load laboratory or a combination of laboratories can be dropped from the sampling frame if they have <10% of potential case specimens during a defined 3-month survey period. If viral load testing laboratories are dropped from the sampling frame, this fact should be mentioned as a caveat when reporting survey data.

Importantly, the allocation of specimens from people taking DTG and non-DTG regimens for each laboratory do not need to be the same proportional allocation. For example, a laboratory with 10% of eligible case specimens from people taking DTG-containing regimens and 40% from people taking non-DTG-containing regimens will provide 10% of the DTG sample and 40% of the non-DTG sample.

Annex 3, subsection A3.5 fully describes of how samples are allocated across all viral load testing laboratories in a country. The sample size calculator automatically calculates and reports the allocation of samples across all viral load testing laboratories in a country and is available at https://worldhealthorg.shinyapps.io/ADR_LabBasedMethod/. Annex 4 provides an example of sample size calculations and allocation across strata using the online WHO sample size calculator.

3.5.6   Survey among children and adolescents

The acquired HIV drug resistance survey should be implemented twice simultaneously: once for adults and once for children and adolescents (younger than 18 years of age). The sample size calculations for children and adolescents are implemented following the same steps described in subsections 3.5.2 to 3.5.5 but using the numbers of eligible case specimens from children and adolescents. Annex 5 describes two unique situations that should be considered when replicating this survey among children and adolescents.

3.6   Sampling procedures

Once the target sample sizes per laboratory have been determined, accounting for the inflation due to anticipated genotyping failure, each laboratory randomly samples the eligible case specimens for inclusion in the survey using systematic sampling. Sampling is performed at the same time for both specimens from people taking DTG and non-DTG-containing regimens. Annex 3, subsection A3.7 describes the systematic sampling steps in detail.

3.7   Observed sample size

Not all sampled specimens will successfully be genotyped. The observed sample size is the number of eligible case specimens from people taking DTG- and non-DTG-containing ART across each viral load testing laboratory that are successfully genotyped and for whom HIV drug resistance genotyping results are available for analysis. All of these samples are included in the analysis.

Ideally, the observed sample size is at least as large, if not larger, than the required sample size to meet the constraints defined in the design (Table 2). If the observed sample size is larger than the required sample size, all viable results are included in the analysis. If the observed sample size is smaller than the required sample size, because the genotyping failure rate is larger than 30%, then the confidence intervals may be wider than specified in the design. The analysis below remains statistically correct whether or not the observed sample size is larger or smaller than the required sample size.

3.8   Data analysis

The statistical analysis for the five outcomes will account for stratification by laboratory, stratification by DTG and non-DTG eligible case specimens and the fact that samples are drawn from finite populations. Full formulas and Stata code that automates these calculations are provided in Annex 3, subsection A3.8.

For outcomes 1–3, prevalence estimates and corresponding 95% confidence intervals are calculated. For outcomes 4 and 5, the prevalence of viral load suppression is reported with complete certainty, since data are reported from all individuals tested during the survey period. Therefore, using this design, there will be no reported variance estimates for these measures.

4. IMPLEMENTATION CONSIDERATIONS

4.1 Convention for assigning survey identification numbers

All viral load tests performed during the three-month survey period are assigned a unique survey identification number. A logbook should be kept in the viral load laboratory that links the survey identification number to the case specimens' original identification numbers (such as unique patient clinic identifiers and often laboratory accession numbers). The survey identification number is used as a link when extracting de-identified patient information and when matching HIV drug resistance genotyping results. The survey identification number comprises the following five elements, delimited by a hyphen ("-"):

• country abbreviation: the standard three-letter abbreviation, as defined by the International Organization for Standardization (ISO 3166);[1]

• survey type: acquired HIV drug resistance (ADR);

• year when survey was initiated;

• viral load testing laboratory abbreviation: a three-letter abbreviation for the laboratory performing the viral load testing, unique within the country (by default, the first three letters of the laboratory name, unless this is not unique);

• a unique four-digit patient number: a consecutive unique patient number assigned to a case specimen sampled at a laboratory for this survey; and

• suffix denoting adults or children and adolescents. A lower case "-a" denotes adults and a lower case "-c" denotes children and adolescents.

For example, if the "University HIV Laboratory" contributed cases specimen in an acquired HIV drug resistance survey of adults conducted in South Africa leveraging specimens collected in 2020, the survey identification number for the first case specimen would be: ZAF-ADR-2020-UNI-0001-a. Using this unique survey identifier is required if using the WHO HIV drug resistance database, which supports cleaning and quality assurance of both de-identified patient data and HIV sequences, thus enabling data analysis and quality-assured results.

4.2 Data extraction

Information should be downloaded from electronic laboratory databases using validated queries or extracted manually from viral load requisition forms accompanying viral load specimens. If necessary, information may be supplemented by using records or registers maintained at the ART clinics from which specimens were obtained. The same unique survey identification number must be assigned to the de-identified patient-level data and the patient's remnant viral load specimen. Data should be entered into Excel-based data capture tools developed by WHO for this purpose (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).

4.3 Reporting data

All countries are encouraged to report to WHO a dataset consisting of (1) individual de-identified patient-level information, (2) viral load laboratory-level data and (3) HIV sequences in FASTA file format. It is recommended that sequence identification numbers and eligible case specimen identifiers be identical and follow WHO convention as defined in subsection 4.1. An Excel data upload template is available for download from within the WHO HIV drug resistance database (https://www.who.int/teams/global-hiv-hepatitis-and-stis-programmes/hiv/treatment/hiv-drug-resistance/hiv-drug-resistance-surveillance).

In countries choosing not to report individual de-identified patient information and sequences, survey outcomes and additional data on the prevalence of HIV drug resistance in various subpopulations should be reported in an aggregated fashion. An Excel data collection and reporting tool is available for download from within the WHO HIV drug resistance

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[1] Country codes – ISO 3166 are available at: https://www.iso.org/obp/ui/#search/code.

database (https://www.who.int/teams/global-hiv-hepatitis-and-stis-programmes/hiv/treatment/hiv-drug-resistance/hiv-drug-resistance-surveillance). Prevalence data should be accompanied by numerator, denominator, standard error of prevalence, standard error of numerator and standard error of denominator to enable regional and global data to be pooled.

When reporting HIV drug resistance by a specified antiretroviral drug, sequences classified as having predicted low-level, intermediate, or high-level resistance (according to the Stanford HIVdb) should be classified as drug resistant. Equally, this classification applies when reporting resistance to all drug classes.

When reporting HIV drug resistance by drug class, the following operational definitions for drug class are used.

- Any HIV drug resistance is defined as resistance to nevirapine (NVP), efavirenz (EFV), any NRTI, darunavir/ritonavir (DRV/r), lopinavir/ritonavir (LPV/r), atazanavir/ritonavir (ATV/r) or any INI.

- Resistance to the NNRTI drug class is defined as resistance to NVP or EFV.

- Resistance to the NRTI class is defined as resistance to any NRTI.

- Resistance to the ritonavir-boosted protease inhibitor drug class is defined as resistance to DRV/r, LPV/r or ATV/r.

- Resistance to the INI drug class is defined as resistance to any INI.

4.4   Implementation overview: practical guide

This section provides a practical overview to implementation. In this example, a national HIV drug resistance working group in a country meets on 1 January 2021 and plans to implement an acquired HIV drug resistance survey. Funding is available until December 2021. The working group follows the steps below.

- Assess the feasibility of implementing a survey by reviewing the assessment framework for country readiness (first, second and third steps as outlined in Section 2).

- Estimate the sample sizes for both adults and children and adolescents and budget for the survey based on country data. Annex 8 provides an example of a budget.

- Decide whether the available budget permits the implementation of a simultaneous survey among children and adolescents and whether or not the survey in children and adolescents will assess overall acquired HIV drug resistance or DTG resistance and overall acquired HIV drug resistance.

- Define the survey period. If all viral load laboratories in the country have stored remnant specimens with viral load ≥1000 copies/mL at –20°C or below that have been collected over a previous period of at least three months, the specimens can be sampled and genotyped. In contrast, if storage has not been conducted or not been conducted properly, a three-month specimen collection period is established (all viral load testing laboratories in the country will store all specimens with viral load ≥1000 copies/mL after viral load testing starting from a predetermined date (such as 1 March 2021). This process will ensure proper storage capacity and will ensure that sufficient remnant material is available for HIV drug resistance testing from all viral load testing laboratories.

- Start collecting all remnant specimens with viral load ≥1000 copies/mL. From 1 March 2021 or the agreed study period start date, all remnant specimens with viral load ≥1000 copies/mL in all viral load testing laboratories are stored for a period of three months.

- The sample sizes for people taking DTG-containing ART and non-DTG-containing ART for each population (adults and children and adolescents) are calculated as defined in subsection 3.5.

- Sample the eligible case specimens and HIV drug resistance genotyping. On 1 June 2021 or the first day after the agreed study period of three months, storage is completed, and specimens can be sampled for HIV drug resistance genotyping.

- HIV drug resistance genotyping is performed in a WHO-designated laboratory, and de-identified patient information is entered into upload templates provided by WHO (subsection 4.3) for use with the WHO HIV drug resistance database.

- De-identified patient data and HIV drug resistance genotypes are uploaded into the WHO HIV drug resistance database for cleaning and quality assurance of both de-identified demographic information and sequences.

- The data are analysed.

- A national report is written, and data are disseminated and used for public health and ART programme policy-making.

5. ALTERNATIVE APPROACH FOR COUNTRIES WITH VIRAL LOAD COVERAGE ≥60% BUT AVAILABILITY OF REQUIRED SURVEY VARIABLES <80%

5.1 Overview

The methods described in this section should be implemented in countries reaching the threshold of ≥60% viral load testing coverage (subsection 2.3.1) but not achieving the threshold of ≥80% required patient-level survey variables (subsection 2.3.2). Similar to the primary approach described in Section 3, the survey is implemented simultaneously in two populations: once among adults and once among children and adolescents. The survey outcomes are identical to those described in subsection 3.2.

As described in Section 3, the sampling design uses double stratification, with both the laboratory and the regimen (DTG-containing or non-DTG-containing) as stratifying variables. However, in this adaptation for countries with viral load coverage ≥60% but availability of required survey variables <80%, a sample of clinics within each viral load testing laboratory catchment area will be randomly chosen and 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.

The first design phase will determine the required sample sizes and the number of clinics to sample for each laboratory (subsection 5.3). 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 (subsection 5.4). Laboratories will prospectively store epidemiological data and remnant samples from eligible case specimens[1] collected from the sampled clinics during the three-month survey period.

The second design phase will happen after the three-month study period and will involve determining the per-clinic sample size, stratified by DTG- and non-DTG eligible case specimens (subsection 5.5). 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.

5.2 Viral load laboratory- and country-level data needed to implement this survey

5.2.1 Required data for the first design phase

- The number of individuals, in a recent three-month period, who were receiving ART, underwent viral testing and had viral non-suppression, obtained both for those receiving DTG-containing regimens and those receiving non-DTG-containing regimens.

- For each clinic, the number of people, in a recent three-month period, who were taking ART, underwent viral testing and had viral non-suppression (viral load ≥1000 copies/mL), with information obtained for those taking DTG-containing and non-DTG-containing regimens.

- The anticipated proportion of people with all required survey variables. This will ideally be ≥80% after interventions are implemented to ensure that all viral load tests are sent to the laboratory with completed requisition forms (subsection 5.4).

- The number of clinics served by each laboratory (also used in the analysis phase).

Annex 6, Table A6.1 provides alternatives that use other data in the design phases in case the variables listed in subsections 5.2.1. to 5.2.3 are not available.

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1 Subsection 3.3 defines the eligible case specimens.

5.2.2   Required data for the second design phase

Total number of eligible case specimens from people taking DTG- and non-DTG-containing ART during the survey period from each sampled clinic (these data are also used in the analysis phase).

5.2.3   Required data for the analysis phase

- Total number of people taking DTG- and non-DTG-containing ART who had viral load tests performed during the survey period and who had suppressed viral loads (viral load <1000 copies/mL) from each sampled clinic.

- Total number of viral load tests submitted from people taking DTG- and non-DTG-containing ART from each sampled clinic.

- Number of people taking DTG- and non-DTG-containing ART who had viral load tests during the survey period and had suppressed viral loads (viral load <1000 copies/mL), from each sampled clinic, disaggregated by patient-level data (subsection 2.3.2).

- Total number of viral load tests submitted from people taking DTG- and non-DTG-containing ART from each sampled clinic that also have complete data disaggregated by patient-level data (subsection 2.3.2).

Annex 6, Table A6.1 describes in detail the data that will be needed for the design and/or analysing the results generated when using this adapted method.

5.3   First design phase: determining the required sample sizes and number of clinics to sample

The first design phase focuses on determining the required sample sizes and the number of clinics to sample. This requires the following steps:

- determining the required sample sizes, overall and by DTG- and non-DTG eligible case specimens;

- determining the target sample sizes by inflating the required sample sizes to account for increased variance because of sampling in clusters and genotyping failure rates;

- determining the number of clinics to sample to be able to achieve the target sample sizes; and

- allocating the number of sampled clinics for each laboratory.

5.3.1   Determining the required sample sizes, overall and by DTG- and non-DTG eligible case specimens

The required sample sizes for this adapted approach are driven by the same parameters and assumptions as specified in Table 2 in subsection 3.5.1. Once again, if the number of eligible case specimens is anticipated to be large, the required sample size for estimating the overall prevalence of acquired HIV drug resistance is 267 and the required sample size for estimating the prevalence of acquired HIV drug resistance to DTG is 325.

Similar to subsection 3.5.2, the required sample size can be adjusted downward using the finite population correction. However, since the sample sizes are calculated before the study period and the actual accrual of eligible case specimens, this downward adjustment must be made based on historical numbers. In the ideal scenario, a country has historical data at the national level on the number of people with viral non-suppression (viral load ≥1000 copies/mL) among people taking DTG-containing and non-DTG-containing regimens over a recent three-month period. If this information is available, the eligible population size can be approximated by multiplying these numbers by the anticipated proportion of individuals with all required variables. If this information is available over a year-long period, assume uniform distribution of patients and divide the numbers by four.

However, if these data are unavailable, a country can use the alternatives detailed in Annex 6, subsection A6.1.1.

The sample size for estimating the prevalence of acquired HIV drug resistance to DTG among individuals taking DTG-containing ART is likely to be larger than the sample size for estimating the overall prevalence of acquired HIV drug resistance, and the final required sample size will be between the sample size to estimate the prevalence of acquired HIV

drug resistance to DTG among individuals taking DTG-containing ART and the sum of the two sample sizes. The final required sample size will depend on the proportion of people receiving DTG-containing regimens, which is discussed more in subsection 5.3.4.

To determine the required sample sizes for non-DTG eligible case specimens, we must estimate the anticipated proportion of eligible case specimens that are from people receiving non-DTG-containing regimens. We will approximate this using historical data, and we can use the parameters available to account for finite populations, as described above. For example, if historical data on the number of people with viral non-suppression is available, the proportion would be approximated by the number of people with viral non-suppression among people receiving anon-DTG-containing regimens over a recent three-month period divided by the number of people with viral non-suppression among all people (receiving DTG-containing and non-DTG-containing regimens combined) over a recent three-month period.

The full calculation to determine the required sample size, incorporating available historical data, is available in Annex 6, subsection A6.1. The required sample size is determined automatically in the WHO sample size calculator, which is 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. Annex 7 provides an example of sample size calculations using this online WHO sample size calculator.

5.3.2   Determining the target sample sizes

The required sample sizes must undergo two inflations. The first inflation is for increased variance because of sampling of clinics (clusters) within laboratories. We inflate the sample size to offset this increased variance by incorporating the design effect (DE). For this activity, we will set DE at 1.5, which corresponds to anticipating that the variance of our estimates when using cluster sampling will be 50% greater than the variance when using simple random sampling of individuals. We provide code to support the calculation of the actual design effect during the analysis phase, and future iterations of this global HIV drug resistance protocol may update the design effect based on these estimates.

The second inflation accounts for the fact that not all samples will successfully amplify. Similar to the inflation detailed in subsection 3.5.4, we account for an anticipated 30% genotyping failure rate. The resulting target sample sizes account for these two adjustments and are automatically reported in the WHO online sample size calculator (available at https://worldhealthorg.shinyapps.io/ADR_LabBasedMethod_2/). The full calculation is available in Annex 6, subsection A6.2.

5.3.3   Determining the number of clinics to sample

From previous acquired HIV drug resistance survey experiences, the number of clinics to sample is recommended to be between 20 and 40 *(1)*. Although sampling more clinics is always preferred from a statistical perspective, it comes at increased costs. Enough clinics should be sampled to ensure that at least two clinics are sampled per laboratory, for all laboratories that serve two or more clinics. This enables direct estimation of within-laboratory variation for the acquired HIV drug resistance prevalence estimates.

Another concern is having sufficient clinics to ensure enough eligible case specimens to achieve the target sample sizes. This is nearly impossible to guarantee under this design; despite best efforts, the random sample of clinics may include only small clinics, resulting in fewer than desired eligible case specimens being collected during the study period. Fortunately, even if this were to happen, the analyses and inferences would remain correct. The consequence is that the confidence intervals would possibly be wider than desired. Therefore, this scenario should be avoided as much as possible.

When determining the number of clinics to be sampled, decision-making may be aided by using historical data at the design stage. In the most ideal situation, countries have access to historical viral non-suppression data for each clinic. Then, one helpful piece of information to consider is the median size of clinics – specifically, the median number of individuals, per clinic, who received viral load tests in a recent three-month period and have viral non-suppression, stratified by regimen (DTG versus non-DTG). The information will be used to ensure that the number of clinics recommended for sampling is greater than the target sample size divided by the median number of eligible case specimens from each clinic. For a more conservative approach, the 25th percentile value can be used instead of the median, but this will result in recommending sampling more clinics.

The mechanics of this process are detailed in Annex 6, subsection A6.3 and are automated in the WHO online sample size calculator (available at https://worldhealthorg.shinyapps.io/ADR_LabBasedMethod_2/). As in subsection 5.3.2, if the number of individuals with viral non-suppression in a recent three-month period for all ART clinics is unknown, then an alternative value that can be used is the number of individuals undergoing testing or the number of individuals receiving ART, with the same necessary adjustments as described in subsection 5.3.2.

If none of this information is available at the clinic level, then the number of clinics to be sampled can be aided by national-level historical data on the total number of individuals with viral non-suppression for either DTG- or non-DTG regimens over a recent three-month period, as described in subsection 5.3.1. This can be used to calculate the mean clinic size by dividing by the total number of clinics in the country. This approach is simpler because it does not require clinic-level data that may be difficult to obtain and it has been automated in the WHO online sample size calculator; however, it is less robust against extreme scenarios in which the sizes of clinics vary substantially within a country.

5.3.4   Allocating the number of clinics to sample across laboratories

The last step of the first design phase is allocating the number of clinics for sampling across laboratories. The number of clinics sampled from a laboratory will be proportional to the number of clinics that send samples to the laboratory for viral load testing. The number of clinics to be sampled from each laboratory can be calculated using the number of clinics planned to be to sampled multiplied by the number of clinics that submit samples for testing to that laboratory divided by the total number of clinics in the viral load laboratory catchment area, rounded to the nearest integer. For each laboratory, the allocated number of clinics will be sampled from the total number of clinics using simple random sampling without replacement.

The full calculation is available in Annex 6, subsection A6.4. The sample size and the allocation of clinics to be sampled across laboratories are automatically calculated in the WHO online sample size calculator (available at https://worldhealthorg.shinyapps.io/ADR_LabBasedMethod_2/). Annex 7 provides an example of sample size calculations and the number of clinics to sample per laboratory using the online WHO sample size calculator.

5.4   Interventions and specimen collection for sampled clinics

Before and during the study period, each sampled clinic should receive intensive interventions to make sure that all viral loads are sent to the laboratory with completed requisition forms. This may include training and supervision on completing viral load requisition forms and transferring data from clinics to the viral load laboratory. Individual countries will need to perform a needs assessment to best target the development of appropriate interventions to optimize the completion of requisition forms.

At the laboratories, all submitted specimens from the sampled clinics will be tracked and tested for viral non-suppression. Specimens with completed requisition forms and viral non-suppression will be considered eligible case specimens. Each laboratory will then report the total number of eligible case specimens during the study period, stratified by regimen (DTG-containing or non-DTG-containing), from the sampled clinics.

5.5   Second design phase: allocating sample sizes across sampled clinics

After the three-month study period, the target sample sizes must be allocated across each of the sampled clinics. For each regimen, the target sample size will be allocated to each sampled clinic proportional to the number of eligible case specimens that clinic contributed during the study period. This can be entered into the WHO online sample size calculator (available at https://worldhealthorg.shinyapps.io/ADR_LabBasedMethod_2/) and allocated automatically.

5.6   Sampling procedures

Sampling will be done systematically, as described in subsection 3.7 for the primary acquired HIV drug resistance surveillance activity. In this case, however, the process will apply to each clinic rather than each laboratory, and sampling will be among the batch of eligible case specimens from the clinic, stratified by regimen.

5.7   Analysis

The statistical analysis for the five outcomes will consider stratification by laboratory, stratification by DTG and non-DTG eligible case specimens and the fact that the samples are drawn from finite populations. These analyses will also account for the inclusion of a random sample of clinics, rather than all clinics. Annex 6 provides the full formulas and Stata code that automates these calculations.

For outcomes 1–3, the results will include prevalence estimates and 95% confidence intervals. As in the primary acquired HIV drug resistance survey methods, outcomes 4 and 5 will be based on totals rather than individual-level data; however, there will now be a variance estimate and resulting confidence intervals because the data will only come from the clinics that participated in the survey activity.

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ANNEX 1. CHECKLIST TO ASSESS COUNTRY READINESS TO IMPLEMENT ACQUIRED HIV DRUG RESISTANCE SURVEYS LEVERAGING REMNANT VIRAL LOAD SPECIMENS

This annex provides a national-level checklist that can be used to assess whether or not a country meets the criteria needed to conduct acquired HIV drug resistance surveillance using remnant viral load specimens. For each required element on the first and second steps, the "yes" column should be ticked, signalling that the require element is in place, before the country proceeds to quantitatively assessing available data (subsection 2.3 and Annex 2).

If countries find that they do not meet all mandatory criteria, completing the checklist will have identified gaps in policy and or laboratory and data system infrastructures, which can be filled to optimize population-level HIV care and treatment, and once closed, facilitate the use of the acquired HIV drug resistance survey method described in this publication.

Table A1.1. Checklist to assess country readiness to implement surveillance of acquired HIV drug resistance using remnant viral load specimens

First step: Assess mandatory criteria					
Policy			Yes	No	Comments
National policy to provide viral load testing: at least one viral load test annually to all people receiving ART is implemented through the country					
National policy to store remnant viral load specimens from all viral load testing laboratories is in place					
Ethical principles for use of remnant specimens established					
Data systems			Yes	No	Comments
Viral load requisition form captures all required acquired HIV drug resistance survey variables and is generally filled					
Viral load laboratory and a national electronic viral load database is in place: • to capture the required variables for the acquired HIV drug resistance survey; and • to link viral load test results to patient-level variables to avoid double counting, to the extent possible, of the same person within the same laboratory and the same person across different clinics or viral load testing laboratories					
Data systems	Plasma	DBS	Yes	No	Comments
Specimen collection	• Venepuncture • Collected using standard precautions	• Aliquot of whole blood obtained by venepuncture preferred over DBS obtained by finger prick • Collected using standard precautions			
Storage conditions of whole-blood specimen from time of collection to preparation and aliquoting	Plasma should be separated from whole blood: • within six hours at room temperature; or • within 24 hours at 4°C	DBS should be spotted freshly: • Within six hours at room temperature; or • Within 24 hours at 4°C			

First step: Assess mandatory criteria					
DBS drying procedure	Not applicable	≥3 hours in a drying rack, in a biosafety cabinet or on a laboratory bench. Drying time depends on air circulation and ambient humidity; longer times may be needed in high-humidity environments			
Storage conditions (Since routine programme data and remnant specimens are designed to provide contemporary and actionable data for ART programmes, it is not anticipated that specimens be frozen for prolonged periods of time before HIV drug resistance genotyping; the maximum survey specimen collection period is three months)	Plasma aliquot frozen to –80°C within 48 hours of collection. Alternatively, specimens may be stored at –20°C but ideally not for longer than one month	• If time since collection ≤14 days: ambient temperature. During the maximum of 14 days at ambient temperature, use of gas impermeable bags with desiccant is required. Humidity indicator cards must be inspected daily and used desiccant changed when indicator cards turn pink. • If >14 days: –20°C or below.			
Shipping conditions	Dry ice	Specimen cards should be maintained in the original gas-impermeable plastic bag with desiccant until time of transport. If previously stored frozen at –20°C or below, specimens should remain frozen and be shipped on dry ice. If shipment on dry ice is not possible, DBS may be removed from the freezer and be allowed to thoroughly equilibrate to room temperature for a minimum of 30 minutes before opening the bag. After equilibrating, the outer bag should be opened, and the desiccants contained in each of the small plastic bags should be replaced with fresh desiccant for shipping. The equilibrated DBS should be placed in a new plastic bag containing a humidity indicator and sufficient desiccant to last the anticipated time of shipping and should then be shipped at ambient temperature			
Required quantity for HIV drug resistance genotyping	≥0.5 mL (larger volumes may be needed when viral load is low)	Minimum of two spots of 70–75 µL each (additional spots required for repeat testing)			
Viral load requisition form	High level of completeness	High level of completeness			
Viral load testing laboratory infrastructure			Yes	No	Comments
All viral load testing laboratories in a country must be able to participate and contribute case specimens to the survey sample					

First step: Assess mandatory criteria			
Storage conditions: • Specimens should be left at +4°C for a maximum of 48 hours before viral load testing is performed • As soon as viral load testing is completed, all remnant viral load specimen with viral load ≥1000 copies/mL are stored at –20°C or below			
National-level viral load database	**Yes**	**No**	**Comments**
Database: a national-level database captures all required acquired HIV drug resistance survey variables from all viral load testing laboratories in a country. Alternatively, the national level is able to query databases in all viral load testing laboratories to complete the viral load and HIV drug resistance cascade or individual viral load laboratories have databases that capture required information that can be extracted and sent to the national level.			

Second step: assess laboratory-level data availability			
Assess required laboratory-level data needed for design and/or analysis phase as defined in subsection 2.2	**Yes**	**No**	**Comments**
Required viral load laboratory variables available at all viral load testing laboratories			

Third step: assess viral load testing coverage and patient-level data completeness			
Viral load testing coverage	**Yes**	**No**	**Comments**
Viral load testing coverage ≥60%			
Availability of required survey variables	**Yes**	**No**	**Comments**
Availability of required survey variables ≥80%			

ANNEX 2. MODELLED IMPACT OF MISSING DATA ON ACQUIRED HIV DRUG RESISTANCE PREVALENCE ESTIMATES

To examine how imperfect data availability could potentially bias acquired HIV drug resistance prevalence estimates, sensitivity analysis was performed. Several scenarios were created in which the true prevalence of acquired HIV drug resistance varies between 5% and 90% (Fig. A2.1). The models posits that people living with HIV receiving ART for whom no information is available (not sampled or missing) could have a prevalence of acquired HIV drug resistance that is between 30% less and 30% more than the observed prevalence among those sampled (that is, a prevalence ratio that varies between 0.7 and 1.3). The prevalence ratio (PR) is defined as the prevalence of acquired HIV drug resistance among those missing from the sample divided by the prevalence of acquired HIV drug resistance among those sampled. Thus, a PR of 0.7 means that those missing from the sample have 30% less resistance than the sampled population. Conversely, a PR of 1.3 means that those missing from the sample have 30% more resistance than the sampled population. On each graph, the true acquired HIV drug resistance prevalence is presented as coloured lines. Some lines are broken because some scenarios cannot produce such results (not internally consistent).

If the prevalence of acquired HIV drug resistance in the sampled population is greater than the prevalence among those missing from the sample (PR <1), an acquired HIV drug resistance survey leveraging remnant viral load specimens in this scenario would lead to overestimating the acquired HIV drug resistance over a range of true acquired HIV drug resistance prevalence estimates. Likewise, if the prevalence of acquired HIV drug resistance in the sampled population is less than the prevalence in the unsampled population (PR >1), an acquired HIV drug resistance survey leveraging remnant viral load specimens in this scenario would lead to underestimating the acquired HIV drug resistance over a range of true acquired HIV drug resistance prevalence estimates.

To further illustrate the impact of absolute bias on the prevalence of acquired HIV drug resistance, if 80% available data is assumed, PR = 0.7 and the true acquired HIV drug resistance prevalence is 20%, the absolute bias is less than 5%. The absolute bias rises to 10% at a true acquired HIV drug resistance prevalence of 80%. This means that if the true acquired HIV drug resistance prevalence in a population is 80%, a survey leveraging remnant viral load specimens may overestimate acquired HIV drug resistance by about 10%. Conversely, if the PR is equal to 1.3 and 80% available data is assumed, a survey leveraging remnant viral load specimens may underestimate acquired HIV drug resistance by about 5%.

Since the results of acquired HIV drug resistance surveys are used to inform the selection of second- and third-line ART and to follow trends of acquired HIV drug resistance within and between countries over time and can be tied to a programme and public health actions, precision in resulting estimates is required for confident decision-making. The results of the sensitivity analysis suggest that at overall data availability, defined as the product of viral load coverage and proportion of remnant specimens with viral load ≥1000 copies/mL with available required survey variables of 50% or more, the absolute bias is less than 10% for most scenarios. This bias is likely to be acceptable and balances a desire for high precision with feasibility.

In 2019, the number of viral load tests run in low- and middle-income countries surpassed 20 million for the first time, representing a global coverage rate of approximately 70 percent[1] – suggesting that viral load testing coverage of >60% is a feasible threshold for this survey. Viral load testing coverage is defined as the proportion of the population (adults or children and adolescents) receiving ART with routine viral load testing performed and for whom classifiable test results are available. This information is generally available from routine programme sources and is a common global reporting indicator. As stated in subsection 2.3.2, a threshold of ≥80% availability of the four required survey variables is recommended because, below this threshold of data availability overall data completeness, the risk of increased absolute bias increases across most scenarios.

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[1] 2020 HIV Market Report. Available at: https://3cdmh310dov3470e6x160esb-wpengine.netdna-ssl.com/wp-content/uploads/2020/09/2020-CHAI-HIV-Market-Report.pdf

Fig. A2.1. Sensitivity analysis: modelled impact of missing data on acquired HIV drug resistance prevalence estimates
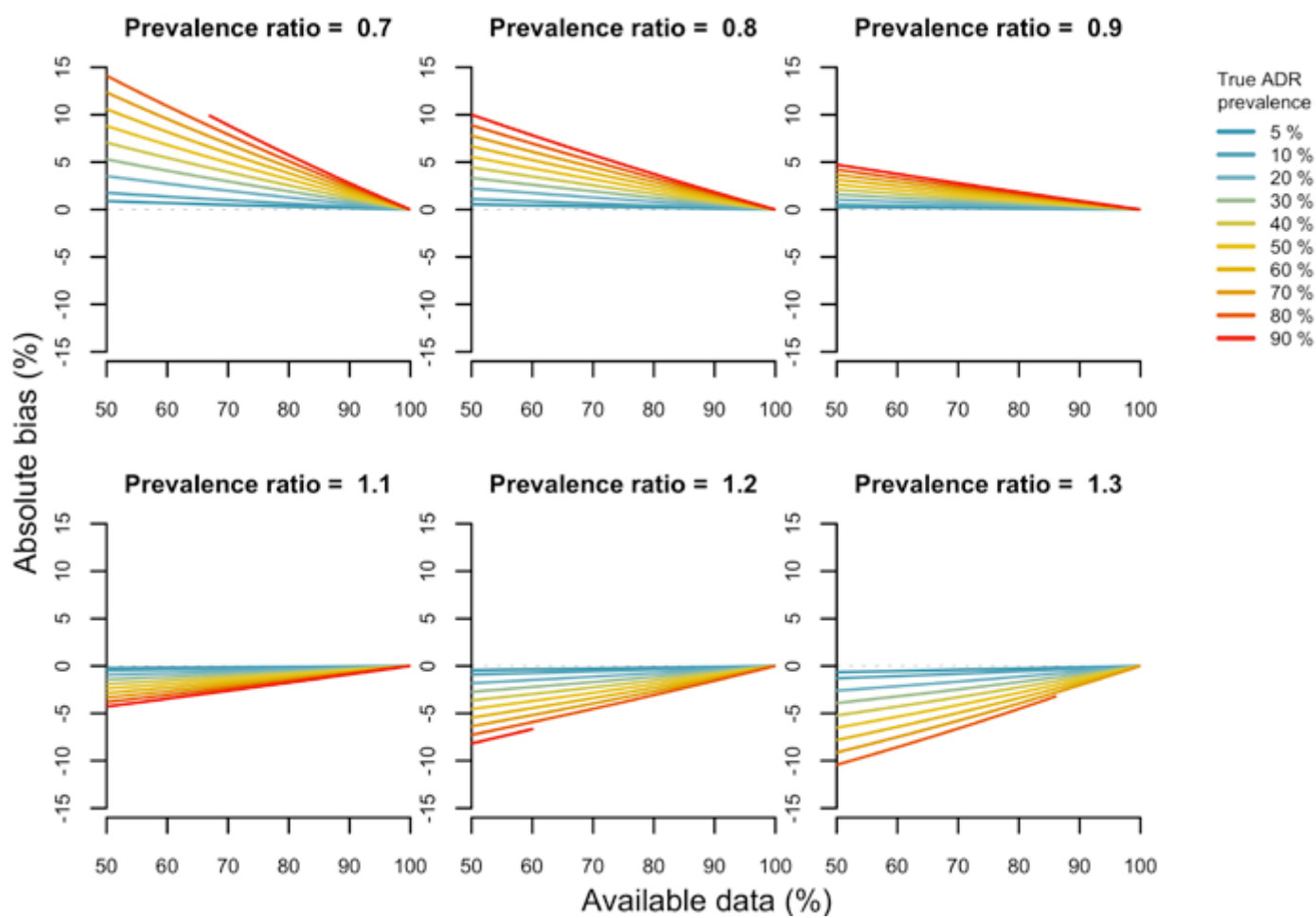
ANNEX 3. STATISTICAL METHODS

This annex provides the statistical details of the primary survey activity described in Section 3.

Table A3.1 describes the laboratory-level data that will be needed for the design and/or the analysis of this survey. Laboratories need to report these data at the end of the survey window. For each element in Table A3.1, we indicate whether or not this element is needed during the design or analysis phases. Even though some of the variables are only required in the analysis phase, we recommend collecting these data during the design phase along with the variables required by the design, since these processes overlap and are more efficiently collected at the same time.

Table A3.1. Required laboratory-level data needed to implement the acquired HIV drug resistance survey method outlined in Section 3

Notation	Explanation	Required for design or analysis phase
$N_{DTG,j}$	Total number of eligible case specimens from people receiving a DTG-containing regimen received in each viral load testing laboratory, j, during the three-month survey period	Design, analysis
$N_{nonDTG,j}$	Total number of eligible case specimens from people taking a non-DTG-containing regimen received in each viral load testing laboratory, j, during the three-month survey period.	Design, analysis
$N_{DTG}, N_{nonDTG}, N_{overall}$	Total number of eligible case specimens from all people tested for viral load (taking a DTG-containing regimen, taking a non-DTG-containing regimen or overall), nationally, during the survey period. Importantly, these numbers do not need to be explicitly collected. Rather, they can be calculated by summing up the number of eligible case specimens ($N_{DTG,j}$ and $N_{nonDTG,j}$) across laboratories.	Design, analysis
$T_{DTG,j}$	Total number of people taking a DTG-containing regimen who received a viral load test (regardless of viral load results) and have all required variables from each laboratory, j, during the survey period.	Analysis
$T_{nonDTG,j}$	Total number of people taking a non-DTG-containing regimen who received a viral load test (regardless of viral load results) and have all required variables for each laboratory, j, during the survey period.	Analysis
$T^{VS}_{DTG,j}$	Total number of people taking a DTG-containing regimen who received a viral load test, were virally suppressed, and have all required variables from each laboratory, j, during the survey period	Analysis
$T^{VS}_{nonDTG,j}$	Total number of people taking a non-DTG-containing regimen who received a viral load test, were virally suppressed and have all required variables for each laboratory, j, during the survey period	Analysis
$N_{i,a,j}$	Total number of eligible case specimens from people receiving regimen i (DTG or non-DTG) and subgroup a (for example, a = female) for each laboratory, j, during the survey period	Analysis
$T_{i,a,j}$	Total number of people receiving regimen i (DTG or non-DTG) and subgroup a (for example, a = female) tested and with required variables for each laboratory, j, that were tested during the survey period	Analysis
$T^{VS}_{i,a,j}$	Total number of people receiving regimen i (DTG or non-DTG) and subgroup a (for example, a = female) tested and with required variables for each laboratory, j, that were tested and had suppressed viral loads during the survey period	Analysis

A3.1. Calculating standard sample sizes

These calculations are based on an infinitely large population and can be used for budgetary purposes without considering country specificity. The required sample sizes for estimating either the prevalence of acquired HIV drug resistance to DTG among people taking DTG-containing regimens or the prevalence of overall acquired HIV drug resistance among people receiving any regimen follow from the parameters specified in Table 2, using the following formula:

$$n_i = \left\lceil \frac{1.96^2 * p_i * (1 - p_i)}{L_i^2} \right\rceil,$$

where i indicates either people receiving DTG-containing regimens or any regimen (overall), n_i is the required sample size for group i, p_i is the expected prevalence of resistance among those with viral non-suppression for group i (acquired HIV drug resistance if i=overall and acquired HIV drug resistance to DTG if i=DTG) and L_i is the desired absolute precision for group i.

Using the assumptions from Table 2, the required sample size for estimating the prevalence of overall acquired HIV drug resistance is 267 ($n_{overall}$ = 267) and for estimating the prevalence of acquired HIV drug resistance to DTG among people receiving DTG-containing regimens is 325 (n_{DTG} = 325). Notably, the DTG sample size is larger than what is needed for the overall prevalence estimate. The final required sample size used for the overall prevalence estimate will include these 325 sampled specimens from people receiving DTG-containing regimens combined with sampled specimens from people receiving non-DTG-containing regimens. The necessary number of case specimens to be sampled from people taking non-DTG-containing regimens will be equal to $n_{overall}$ multiplied by the proportion of eligible case specimens from people taking non-DTG-containing regimens ($prop_{nonDTG}$, described in subsection A3.3) and will therefore be less than or equal to $n_{overall}$. Thus, the total required sample size – comprising the DTG and non-DTG samples—will be $n_{DTG}+ (n_{overall} * prop_{nonDTG})$, which is between the required DTG sample size (n_{DTG} = 325) and the sum of the required DTG and overall sample sizes ($n_{DTG}+ n_{overall}$ = 592). This is described further in subsection A3.3.

A3.2. Calculating country-specific sample sizes

All countries are advised to use the online WHO sample size calculator for determining their country-specific sample sizes; it includes a finite population correction, which will benefit countries with relatively fewer case specimens during a three-month survey period. The sample size formula is thus adjusted as:

$$n_i = \left\lceil \frac{N_i * 1.96^2 * p_i * (1 - p_i)}{L_i^2 * N_i + 1.96^2 * p_i * (1 - p_i)} \right\rceil,$$

where the terms are defined as in subsection A3.1 with the additional term, N_i, defined as the number of eligible case specimens for group i.

In this case, the final required sample size will depend on the number of individuals in the eligible population, $N_{overall}$ and N_{DTG}. Table 3 provides some examples of how these sample sizes change, depending on the population sizes. WHO developed an application in which countries can specify their own population sizes to obtain a country-specific sample size. The online sample size calculator is available at https://worldhealthorg.shinyapps.io/ADR_LabBasedMethod/.

The non-DTG required sample sizes will be $n_{nonDTG}=n_{overall} * prop_{nonDTG}$ and the total sample size will be $n_{DTG}+ (n_{overall} * prop_{nonDTG})$. Obtaining n_{nonDTG} is discussed further in subsection A3.3.

A3.3. Allocating samples across DTG and non-DTG strata

As noted above, the survey will be implemented by stratifying samples between people taking DTG-containing regimens and people taking non-DTG-containing regimens. As a first step, $prop_{DTG}$ and $prop_{nonDTG}$ need to be calculated, which are the proportions of all eligible case specimens in each group (DTG or non-DTG). This can be done by using the laboratory-

level information already collected as part of the design, namely $N_{overall}$, the total number of eligible case specimens nationally, and n_{DTG}, the total number of DTG eligible case specimens nationally. From this we have:

$$prop_{DTG} = \frac{N_{DTG}}{N_{overall}}, \text{ and}$$

$$prop_{nonDTG} = \frac{N_{nonDTG}}{N_{overall}} = 1 - prop_{DTG}.$$

The number sampled from the population taking DTG-containing ART will remain n_{DTG}, as determined in subsection A3.1 (or subsection A3.2 in small populations). However, the number sampled from the non-DTG regimen population will be $n_{nonDTG} = n_{overall*} prop_{nonDTG}$. Combining these two gives the total required sample size, $n_{DTG} + n_{nonDTG}$. Sample sizes may also be automatically calculated using the sample size calculator, available at https://worldhealthorg.shinyapps.io/ADR_LabBasedMethod/.

A3.4 Sample size inflation for genotyping failure

The required sample sizes above – n_{DTG} and n_{nonDTG} – are the sample sizes needed to achieve the specified design constraints outlined in Table 2. However, not all samples will successfully amplify, so these samples sizes must be inflated to account for the genotyping failure rate, g. The recommendation is that sample sizes be inflated to account for genotyping failure such that the target sample sizes drawn, m_{DTG} and m_{nonDTG}, are the original sample sizes inflated by a factor of $(1 - g)$. That is, for i equal to DTG or non-DTG,

$$m_i = \frac{n_i}{(1-g)}.$$

WHO recommends setting g at 30%, with the target sample sizes for laboratory j being: $m_{DTG} = n_{DTG}/(1 - 0.3)$ and $m_{nonDTG} = n_{nonDTG}/(1 - 0.3)$. The sample size inflation for genotyping failure is automatically calculated and reported in the sample size calculator, available at https://worldhealthorg.shinyapps.io/ADR_LabBasedMethod/.

A3.5. Allocating samples across all viral load testing laboratories in a country

Once m_{DTG} and m_{nonDTG} are determined, the sample sizes are distributed across all viral load testing laboratories in a country, proportional to the number of eligible case specimens at each laboratory. To minimize bias, all viral load testing laboratories in a country should contribute case specimens to the survey proportional to the number of eligible case specimens from each laboratory during the survey period. So, m_{DTG} is allocated to each laboratory relative to each laboratory's percentage of the total number of DTG case specimens, and m_{nonDTG} is allocated to each laboratory relative to each laboratory's percentage of the total number of non-DTG case specimens.

Importantly, for each laboratory j, $m_{DTG,j}$ and $m_{nonDTG,j}$ do not need to be the same proportional allocation. For example, a laboratory may have 10% of DTG eligible case specimens and 40% of non-DTG eligible case specimens, resulting in: $m_{DTG,j} = m_{DTG}*(0.1)$ and $m_{nonDTG,j} = m_{nonDTG}*(0.4)$. The allocation of samples across all viral load testing laboratories in a country is automatically calculated and reported in the sample size calculator, available at https://worldhealthorg.shinyapps.io/ADR_LabBasedMethod/.

A3.6. Example: sample size calculations and allocation across strata using the formulas detailed in this annex

Annex 4 provides an example of sample size calculations and allocation across strata using the online WHO sample size calculator. Here we compute these same values by hand using the formulas detailed in this annex.

As an example, suppose a country has three viral load testing laboratories with a total of 20 000 eligible case specimens during the survey period – 12 800 of which are from people receiving DTG-containing regimens and 7200 of which are from people receiving non-DTG-containing regimens (Table A3.2). This gives us:

$$N_{overall} = 20\ 000, N_{DTG} = 12\ 800 \text{ and } N_{nonDTG} = 7200.$$

A3.6.1 Calculating sample sizes

First, the required sample sizes for the DTG estimate and for the overall estimate are calculated separately, each using the assumptions summarized in Table 2 (the expected prevalence of drug-specific resistance is 3.5% for people taking DTG and 50% overall; the desired absolute precision is ±2% for people taking DTG and ±6% overall). Using the sample size formula for small populations described in subsection A3.2, the required sample size for people receiving DTG-containing ART is 317, and the required sample size for people receiving ART (regardless of the regimen) is 264:

$$n_{DTG} = 317 \text{ and } n_{overall} = 264$$

A3.6.2 Allocating sample sizes across DTG and non-DTG strata

Next, to allocate sample sizes across DTG and non-DTG strata, the proportion of the population in each group as described in subsection A3.3 is calculated:

$$prop_{DTG} = \frac{N_{DTG}}{N_{overall}} = \frac{12\ 800}{20\ 000} = 0.64, \text{ and}$$

$$prop_{nonDTG} = \frac{N_{nonDTG}}{N_{overall}} = \frac{7200}{20\ 000} = 0.36.$$

The number sampled from the DTG population remains n_{DTG}=317, and the number sampled from the non-DTG population will be $n_{nonDTG} = n_{overall} * prop_{nonDTG}$=264*0.36=96. All sample sizes are rounded up to the nearest whole number. This gives a total sample size of 317+96=413.

A3.6.3 Inflating by genotyping failure rates

The DTG and non-DTG required sample sizes must then be inflated to account for a 30% genotyping failure rate, using the steps in subsection A3.4. Here:

$$m_{DTG} = \frac{n_{DTG}}{(1-0.3)} = \frac{317}{0.7} = 453; \text{ and}$$

$$m_{nonDTG} = \frac{n_{nonDTG}}{(1-0.3)} = \frac{96}{0.7} = 138.$$

A3.6.4 Allocating sample sizes across laboratories

Finally, using the steps described in subsection A3.5, the samples are allocated across laboratories proportional to each laboratory's share of the overall DTG or non-DTG case specimens. For the three viral load testing laboratories in the country in this example, suppose:

- laboratory 1 has eligible case specimens from 6000 people in the survey period, 3000 of which are DTG and 3000 of which are non-DTG;

- laboratory 2 has 10 000 case specimens, 9000 DTG and 1000 non-DTG; and

- laboratory 3 has 4000 case specimens, 800 DTG and 3200 non-DTG.

The proportion of DTG eligible specimens that are from laboratory 1 is 3000/12 800 = 0.234, and the proportion of non-DTG eligible specimens from laboratory 1 is 3000/7200 = 0.417.

Therefore, we sample $m_{DTG} * 0.234 = 453 * 0.234 \approx 107$ DTG case specimens and $m_{nonDTG} * 0.417 = 138 * 0.417 \approx 58$ non-DTG case specimens from laboratory 1.

The same calculations can be done for laboratories 2 and 3, and Table A3.2 displays the results.

Table A3.2. Sample sizes allocated across laboratories

	Laboratory 1	Laboratory 2	Laboratory 3	Total
Number of eligible case specimens from people receiving DTG, $N_{DTG,\,j}$	3000	9000	800	12 800
Number of eligible case specimens from people receiving non-DTG, $N_{nonDTG,j}$	3000	1000	3200	7200
Prop size DTG, $prop_{DTG}$	3000/12 800 = 0.234	9000/12 800 = 0.703	800/12 800 = 0.063	1
Target DTG sample size, $m_{DTG,j}$	0.234*453 = 107	0.703*453 = 319	0.063*453 = 29	455[a]
Prop size non-DTG, $prop_{nonDTG}$	3000/7200 = 0.417	1000/7200 = 0.139	3200/7200 = 0.444	1
Target non-DTG sample size, $m_{nonDTG,j}$	0.417*138 = 58	0.139*138 = 20	0.444*138 = 62	140[b]
Total recommended sample, m_j	107+58 = 165	319+20=339	29+62=91	595

[a] Slightly larger than the target sample size of 453, due to rounding up for laboratory-specific sample sizes.
[b] Slightly larger than the target sample size of 138, due to rounding up for laboratory-specific sample sizes.

A3.7. Sampling procedures and observed sample size

As described in subsection A3.5, the number of sampled specimens allocated to each laboratory for each regimen, $m_{i,j}$, will be randomly sampled from the $N_{i,j}$ eligible case specimens using systematic sampling. Operationally, this is done as follows for eligible case specimens from both people receiving DTG- and non-DTG-containing ART.

- Eligible case specimens collected during the survey period at each laboratory constitute the original set, with two sets – one DTG set and one non-DTG set.

- Within each set, the sampling interval, s, is the number of eligible case specimens ($N_{i,j}$) divided by the number of samples allocated to that laboratory for that set ($m_{i,j}$), so that $s = N_{i,j} / m_{i,j}$. For example, if there are 1,000 eligible case specimens in the set, and the laboratory must sample 100 specimens, the sampling interval is $s=1000/100=10$. The laboratory must sample every 10th specimen. If the sampling interval includes a decimal, it can be rounded down to the nearest whole number.

- The laboratory selects a specimen from the first s specimens in the set as a random starting point and then samples every sth sample thereafter. From above, a specimen in the first 10 specimens is randomly chosen (such as the fifth specimen), then every 10th specimen (such as the 15th specimen, the 25th specimen, etc.) is sampled until the sample size is achieved.

In accordance with subsection 3.7, the observed sample size, $m^*_{i,j}$, is defined as the number of individuals receiving regimen i from laboratory j that successfully amplified and for whom HIV drug resistance results are available for analysis. All these samples are included in the analysis.

A3.8. Analysis

Statistical details for each of the five outcomes are described in detail in this section. Stata code is provided to automate these analyses.

A3.8.1 Notation

We use the following notation throughout this subsection.

i = subscript for regimen, where i=DTG, non-DTG, or overall.

j = subscript for viral load laboratory. j=1,…,J, where J is the total number of viral load laboratories.

h = subscript for stratum. h=1,…,H, where H is the total number of strata.

l = subscript for individual case specimens.

a = subscript for membership in the subgroup of interest.

$p_{overall}$ = estimated prevalence of acquired HIV drug resistance among all case eligible specimens.

p_{DTG} = estimated prevalence of acquired HIV drug resistance among case eligible specimens from people receiving DTG regimens.

$p_{DTG,j}$ = estimated prevalence of acquired HIV drug resistance among case eligible specimens from people receiving DTG regimens from laboratory j.

p^*_{DTG} = estimated prevalence of acquired HIV drug resistance to DTG among case eligible specimens from people receiving DTG regimens.

p_{nonDTG} = estimated prevalence of acquired HIV drug resistance among case eligible specimens from people receiving non-DTG regimens.

$p_{nonDTG,j}$ = estimated prevalence of acquired HIV drug resistance among case eligible specimens from people receiving non-DTG regimens from laboratory j.

$q_{overall}$ = prevalence of viral load suppression among all viral load tests with the required variables recorded.

q_{DTG} = prevalence of viral load suppression among all viral load tests with the required variables recorded among people receiving DTG regimens.

q_{nonDTG} = prevalence of viral load suppression among all viral load tests with the required variables recorded among people receiving non-DTG regimens.

$N_{overall}$ = total number of eligible case specimens.

N_h = total number of eligible case specimens in stratum h.

N_{DTG} = total number of eligible case specimens from people receiving DTG regimens.

N_{nonDTG} = total number of eligible case specimens from people receiving non-DTG regimens.

$N_{DTG,j}$ = total number of eligible case specimens from people receiving DTG regimens in laboratory j.

$N_{nonDTG,j}$ = total number of eligible case specimens from people receiving non-DTG regimens in laboratory j.

$m^*_{DTG,j}$ = observed number of sampled individuals on DTG regimens with acquired HIV drug resistance results in laboratory j.

$m^*_{nonDTG,j}$ = observed number of sampled individuals on non-DTG regimens with acquired HIV drug resistance results in laboratory j.

m^*_h = observed number of sampled individuals with acquired HIV drug resistance results in stratum h.

$T_{DTG,j}$ = total number of people taking a DTG-containing regimen who received a viral load test (regardless of results) and have all required variables from each laboratory, j, during the survey period.

$T_{nonDTG,j}$ = total number of people taking a non-DTG-containing regimen who received a viral load test (regardless of results) and have all required variables from each laboratory, j, during the survey period.

$T^{VS}_{DTG,j}$ = total number of people taking a DTG-containing regimen who received a viral load test, were virally suppressed and have all required variables from each laboratory, j, during the survey period.

$T^{VS}_{nonDTG,j}$ = total number of people taking a non-DTG-containing regimen who received a viral load test, were virally suppressed and have all required variables from each laboratory, j, during the survey period.

$T_{i,a,j}$ = total number of people receiving regimen i (DTG or non-DTG) and subgroup a (for example, a=female) tested and with required variables for each laboratory, j, that were tested during the survey period.

$T^{VS}_{i,a,j}$ = total number of people receiving regimen i (DTG or non-DTG) and subgroup a (for example, a=female) tested and with required variables for each laboratory, j, that were tested and virally suppressed during the survey period.

A3.8.2 Analysis for outcomes 1 and 2

Outcome 1 is defined as: prevalence of acquired HIV drug resistance among individuals with viral non-suppression and receiving ART, regardless of the ART regimen.

Outcome 2 is defined as: prevalence of acquired HIV drug resistance to DTG among individuals with viral non-suppression receiving ART and who are taking a DTG-containing regimen.

Prevalence and variance of DTG-specific resistance among people receiving DTG-containing regimens

Prevalence

An unbiased estimate of acquired HIV drug resistance to DTG among eligible case specimens from individuals receiving DTG-containing regimens can be obtained using the standard proportion estimate for stratified sampling. Recall that the stratifying variables are regimen (DTG or non-DTG) and viral load testing laboratory. If there are J laboratories, each denoted by $j,j=1,\ldots,J$, for each laboratory j, $N_{DTG,j}$ is the number of eligible case specimens from people receiving DTG-containing regimens with viral non-suppression, $m^*_{DTG,j}$ is the observed sample size (those of the $m_{DTG,j}$ that were successfully genotyped) and $X_{DTG,j,l}$ is the binary outcome variable for DTG-specific resistance and is equal to 1 if person l has DTG-specific resistance and 0 if not. The prevalence estimate is given by

$$\hat{p}^*_{DTG} = \frac{\sum_{j=1}^{J}\left(\frac{N_{DTG,j}}{m^*_{DTG,j}}\right)\sum_{l=1}^{m^*_{DTG,j}}X_{DTG,j,l}}{N_{DTG}} = \sum_{j=1}^{J}\left(\frac{N_{DTG,j}}{N_{DTG}}\right)\left(\sum_{l=1}^{m^*_{DTG,j}}\frac{X_{DTG,j,l}}{m^*_{DTG,j}}\right) = \sum_{j=1}^{J}\left(\frac{N_{DTG,j}}{N_{DTG}}\right)\left(\hat{p}^*_{DTG,j}\right)$$

Variance

The variance formula is the typical stratified variance formula that includes the finite population correction for each laboratory, j. The formula involves the stratum-specific estimate for prevalence of DTG-specific resistance, given by

$$\hat{p}^*_{DTG,j} := \frac{\sum_{l=1}^{m^*_{DTG,j}}X_{DTG,j,l}}{m^*_{DTG,j}}.$$

$$\widehat{Var}(\hat{p}^*_{DTG}) = \sum_{j=1}^{J}\left(1 - \frac{m^*_{DTG,j}}{N_{DTG,j}}\right)\left(\frac{N_{DTG,j}}{N_{DTG}}\right)^2 \frac{\hat{p}^*_{DTG,j}(1 - \hat{p}^*_{DTG,j})}{m^*_{DTG,j} - 1}$$

Prevalence and variance of any resistance stratified by DTG- and non-DTG-containing regimens

Prevalence

An unbiased estimate of the prevalence of acquired HIV drug resistance among eligible case specimens, overall and stratified by DTG- and non-DTG-containing regimens, can be obtained with simple weighting for stratified estimates. $Y_{DTG,j,l}$ is the binary outcome variable for any resistance, equal to 1 if person l receiving DTG has any HIV drug resistance and 0 if HIV drug resistance. The prevalence estimate is given by:

$$\hat{p}_{DTG} = \frac{\sum_{j=1}^{J}\left(\frac{N_{DTG,j}}{m^*_{DTG,j}}\right)\sum_{l=1}^{m^*_{DTG,j}} Y_{DTG,j,l}}{N_{DTG}} = \sum_{j=1}^{J}\left(\frac{N_{DTG,j}}{N_{DTG}}\right)\left(\sum_{l=1}^{m^*_{DTG,j}} \frac{Y_{DTG,j,l}}{m^*_{DTG,j}}\right) = \sum_{j=1}^{J}\left(\frac{N_{DTG,j}}{N_{DTG}}\right)(\hat{p}_{DTG,j}).$$

Similarly, an unbiased estimate of the prevalence of acquired HIV drug resistance among those receiving non-DTG regimens is given by:

$$\hat{p}_{nonDTG} = \frac{\sum_{j=1}^{J}\left(\frac{N_{nonDTG,j}}{m^*_{nonDTG,j}}\right)\sum_{l=1}^{m^*_{nonDTG,j}} Y_{nonDTG,j,l}}{N_{nonDTG}} = \sum_{j=1}^{J}\left(\frac{N_{nonDTG,j}}{N_{nonDTG}}\right)(\hat{p}_{nonDTG,j}),$$

where, for each laboratory j, $N_{nonDTG,j}$ is the total number of eligible case specimens on non-DTG-containing regimens with viral non-suppression, $m^*_{nonDTG,j}$ is the observed sample size and $Y_{nonDTG,j,l}$ is the binary outcome variable of acquired HIV drug resistance for person l, among those receiving non-DTG-containing regimens.

Variance

For estimating the variance of the DTG and non-DTG estimates, we can use the typical variance formula for stratified sampling. For each laboratory j, $N_{DTG,j}$ (or $N_{nonDTG,j}$) is the population size, $m^*_{DTG,j}$ (or $m^*_{nonDTG,j}$) is the observed sample size and $\hat{p}_{DTG,j}$ (or $\hat{p}_{nonDTG,j}$) is the prevalence estimate for any drug resistance (acquired HIV drug resistance). The stratified variance formulas include the finite population correction and are given by:

$$\widehat{Var}(\hat{p}_{DTG}) = \sum_{j=1}^{J}\left(1 - \frac{m^*_{DTG,j}}{N_{DTG,j}}\right)\left(\frac{N_{DTG,j}}{N_{DTG}}\right)^2 \frac{\hat{p}_{DTG,j}(1 - \hat{p}_{DTG,j})}{m^*_{DTG,j} - 1}$$

$$\widehat{Var}(\hat{p}_{nonDTG}) = \sum_{j=1}^{J}\left(1 - \frac{m^*_{nonDTG,j}}{N_{nonDTG,j}}\right)\left(\frac{N_{nonDTG,j}}{N_{nonDTG}}\right)^2 \frac{\hat{p}_{nonDTG,j}(1 - \hat{p}_{nonDTG,j})}{m^*_{nonDTG,j} - 1},$$

where $\left(1 - \frac{m^*_{DTG,j}}{N_{DTG,j}}\right)$ (or $\left(1 - \frac{m^*_{nonDTG,j}}{N_{nonDTG,j}}\right)$) is the stratum-level finite population correction for laboratory j, and N_{DTG} (or N_{nonDTG}) is the total number in the eligible case specimens from individuals receiving DTG (or non-DTG) regimens.

Prevalence and variance of any resistance overall

Prevalence

An unbiased estimate for acquired HIV drug resistance prevalence among all eligible case specimens is of the same form as the DTG and non-DTG estimates, but it uses data from all sampled specimens because all strata are considered. For simplicity, we use H to denote the total number of strata, and we index each stratum by $h, h=1,\ldots,H$. Given that the stratifying variables are laboratory and regimen, and regimen is split into DTG and non-DTG, H is simply $2 \times J$. N_h and m^*_h are the stratum-level population size and observed sample size, respectively, $Y_{h,l}$ is the outcome variable of any drug resistance for person l in stratum h and N is the total eligible population size (defined as the total number of people with viral non-suppression during the defined survey period and across all viral load laboratories in the country). The overall prevalence estimate is given by:

$$\hat{p}_{overall} = \frac{\sum_{h=1}^{H} \frac{N_h}{m_h^*} \sum_{l=1}^{m_h^*} Y_{h,l}}{N} = \sum_{h=1}^{H} \left(\frac{N_h}{N}\right) \sum_{l=1}^{m_h^*} \frac{Y_{h,l}}{m_h^*} = \sum_{h=1}^{H} \left(\frac{N_h}{N}\right) \hat{p}_h$$

Variance

To make inferences on the overall acquired HIV drug resistance prevalence in the population, we need to estimate the variance. To do this, we apply basic variance properties to obtain:

$$\widehat{Var}(\hat{p}_{overall}) = \sum_{h=1}^{H} \left(1 - \frac{m_h^*}{N_h}\right) \left(\frac{N_h}{N}\right)^2 \frac{\hat{p}_h(1 - \hat{p}_h)}{m_h^* - 1},$$

where $\hat{p}_h$ is the stratum-specific prevalence estimate for any drug resistance.

A3.8.3 Analysis for outcome 3

Outcome 3 is defined as: prevalence of acquired HIV drug resistance among individuals with viral non-suppression and receiving ART, stratified by other key variables.

Subgroup analysis can be performed to estimate the prevalence of acquired HIV drug resistance in subgroups determined by other key variables such as age, gender, ART regimen, ART line, previous ART regimen, pregnancy status and breastfeeding status. Each of these analyses will involve a complete-case analysis that excludes missing data. Those in the subpopulation and not missing the variable of interest are used to calculate the prevalence estimate, and all individuals are used to calculate the standard errors. This allows the randomness of the subgroup size in the sample to be incorporated into variance estimation.

Note that the methods in this section pertain to subgroups in which membership is not defined by a stratifying variable. For subgroup analyses in which membership in the subgroup is defined by a stratifying variable, such as viral load laboratory or regimen (DTG, non-DTG or overall), then analysis can proceed as in outcome 1, restricting analysis to the subgroup of interest and using the outcome relevant to the type of resistance desired.

Prevalence and variance for subgroup estimation

Prevalence
Prevalence estimates for the subgroup of interest involve a ratio estimator: the denominator is the number of sampled individuals belonging to the subgroup, and the numerator is the number of sampled individuals belonging to the subgroup with acquired HIV drug resistance.

Recall that the strata are defined by regimen (DTG or non-DTG) and by laboratory, so there are $H=2J$ strata in total, each defined by a specific regimen group and laboratory. These strata can be labelled by $h, h=1,\ldots,H$. Suppose the subgroup of interest is denoted by a, with membership in the subgroup labelled through an indicator variable, $I_{h,l}(a)$, equal to 1 if individual l in stratum h belongs in the subgroup, and equal to 0 otherwise.

The estimated prevalence of drug resistance among those within the subgroup is:

$$\hat{p}_a = \frac{\sum_{h=1}^{H} \frac{N_h}{m_h^*} \sum_{l=1}^{m_h^*} I_{h,l}(a) Y_{h,l}}{\sum_{h=1}^{H} \frac{N_h}{m_h^*} \sum_{l=1}^{m_h^*} I_{h,l}(a)},$$

where $Y_{h,l}$ is the binary outcome indicator of acquired HIV drug resistance for individual l in stratum h.

Variance
Variance estimation for the subgroup prevalence is approximated using a linearized variance estimator. The denominator in the prevalence estimate is not fixed because the number of sampled individuals belonging to the subgroup is random and depends on the specific sample that is chosen. To account for this additional sample-to-sample variability, linearized variance estimation incorporates all observations but multiplies the weights of those not in the subgroup by 0. Note that

the validity of linearization depends on having a sufficiently large size of the subgroup in the sample. If the subgroup sample size is too small, one might consider composite or model-based estimators or decide not to conduct the subgroup analysis.

For simplicity, let $\hat{p}_a = \frac{\hat{z}}{\hat{v}}$, where $\hat{z} = \sum_{h=1}^{H} \frac{N_h}{m_h^*} \sum_{l=1}^{m_h^*} I_{h,l}(a) Y_{h,l}$ and $\hat{v} = \sum_{h=1}^{H} \frac{N_h}{m_h^*} \sum_{l=1}^{m_h^*} I_{h,l}(a)$. The linearized variance estimator is given by

$$\widehat{Var}(\hat{p}_a) = \frac{1}{\hat{v}^2}\left(\hat{p}_a^2 \widehat{Var}(\hat{v}) + \widehat{Var}(\hat{z}) - 2\hat{p}_a \widehat{Cov}(\hat{z}, \hat{v})\right),$$

where $\widehat{Var}(\hat{v}), \widehat{Var}(\hat{z})$, and $\widehat{Cov}(\hat{z}, \hat{v})$ can be estimated by the corresponding sample variances and covariances, which are also obtained through linearization and incorporate the finite population correction.

These methods are given for overall prevalence and variance estimates of resistance among a subgroup of interest. For subgroup estimates restricted to a certain regimen or laboratory, simply restrict the data first and then apply these estimation and inference methods.

A3.8.4 Analysis for outcomes 4 and 5

Outcome 4 is as follows: prevalence of viral load suppression (viral load <1000 copies/mL) among individuals receiving ART.

These estimates will be based on information collected during the analysis stage: (1) $T_{i,j}$, the total number of people receiving regimen i tested and with required variables from laboratory j during the study period and (2) $T^{VS}_{i,j}$ the number of these tests that are virally suppressed.

With the data, we can estimate q_{DTG}, the prevalence of viral load suppression among people receiving DTG-regimens for whom all required variables were reported, as:

$$q_{DTG} = \frac{\sum_{j=1}^{J} T^{VS}_{DTG,j}}{\sum_{j=1}^{J} T_{DTG,j}}.$$

Similarly, for non-DTG and overall, we have:

$$q_{nonDTG} = \frac{\sum_{j=1}^{J} T^{VS}_{nonDTG,j}}{\sum_{j=1}^{J} T_{nonDTG,j}} \qquad \text{and} \qquad q_{overall} = \frac{\sum_{j=1}^{J} (T^{VS}_{DTG,j} + T^{VS}_{nonDTG,j})}{\sum_{j=1}^{J} (T_{DTG,j} + T_{nonDTG,j})}.$$

Because we are restricting our population to eligible case specimens during the study period, we can report the prevalence of viral load suppression in these groups with complete certainty. Therefore, under this design, there will be no reported variance estimates for these measures.

Outcome 5 is as follows: prevalence of viral load suppression (viral load <1000 copies/mL) among individuals receiving ART, stratified by other key variables.

We will use the same approach as described above to estimate the prevalence of viral load suppression in subgroup a with: (1) $T_{i,a,j}$, the total number of people receiving regimen i tested and with required variables in subgroup a from laboratory j during the study period and (2) $T^{VS}_{i,j}$, the number of those tested in that subgroup that are virally suppressed. We will use these quantities to estimate:

$$q_{i,a} = \frac{\sum_{j=1}^{J} T^{VS}_{i,a,j}}{\sum_{j=1}^{J} T_{i,a,j}} \text{ for a specific regimen } i,$$

$$\text{or} \quad q_a = \frac{\sum_{j=1}^{J} (T^{VS}_{DTG,a,j} + T^{VS}_{nonDTG,a,j})}{\sum_{j=1}^{J} (T_{DTG,a,j} + T_{nonDTG,a,j})} \text{ for overall viral load suppression in subgroup } a.$$

Like the above, these will be reported without variance estimates.

A3.9 Stata code for the standard method described in Section 3

User-friendly instructions are provided below for data analysis in Stata. To use the code, the data must follow the format described in subsection 4.3, with patient-level and laboratory-level information following the configuration of the Excel data upload template and HIV drug resistance sequences in FASTA file format. Sequence identification numbers and eligible case specimen identifiers must be identical and follow WHO convention.

In Stata, estimation and inference can be implemented using the svy package. The default variance estimation used is linearization (based on a first-order Taylor series linear approximation). All variance computations include finite population corrections. Alternative statistical packages can be used to analyse data as long as they properly adjust for survey weights, clustering and stratification (if necessary). All statistical packages are expected to yield identical point estimates, but not all statistical packages are expected to yield identical standard error estimates and confidence intervals. Statistical packages that do not allow users to specify the finite population correction at each stage of sampling will overestimate the standard error, especially in countries with small eligible populations.

Subsections A3.9.1–A3.9.6 below provide Stata code for processing and combining the two datasets, and subsections A3.9.7–A3.9.10 provide code for analysing the survey outcomes. All code displayed can be found in a downloadable Stata do-file that can run all pre-processing and analysis instructions at once.

A3.9.1 Import viral load laboratory and patient-level data into Stata

Begin by importing viral load laboratory and patient-level data from the Excel data capture tool.

1. To start, one may choose to create a do-file so that commands can be saved and then run. Click on the notepad icon corresponding to NEW DO-FILE EDITOR on the top-left corner of the Stata viewer, then save the do-file that is created.

2. Clear any previous output and set the working directory to the directory containing the data files. For example, if the directory is C:/DOCUMENTS, run the following code:

```
clear
```

```
cd "C:/Documents"
```

3. Import each sheet of the Excel data capture tool file, storing the first row as headers, changing the header names to uppercase, and saving each sheet as its own .dta file using uppercase letters. Given the Excel file name "patient_data_A1.xlsx", run:

```
import excel using "patient_data_A1.xlsx", describe

forvalues sheet=1/`=r(N_worksheet)' {

    local sheetname = r(worksheet_`sheet')

    import excel using patient_data_A1, sheet("`sheetname'") firstrow case(upper)

    local sheetname = upper(subinstr("`sheetname'", " ", "_", .))

    save "`sheetname'", replace

    clear

}
```

The Excel file should contain five sheets titled: (1) survey information, (2) VL lab information, (3) configuration, (4) survey participants and (5) participant treatments.

A3.9.2 Import HIV drug resistance data into Stata

1. Import the HIV drug resistance data, storing the first row as headers and changing all header names to uppercase. Given the file name "FASTA_A1.xlsx", run:

```
import excel using "FASTA_A1.xlsx", sheet("ResistanceSummary") firstrow case(upper)
```

The resistance data file should be an Excel file containing one sheet titled "ResistanceSummary".

2. Rename SEQUENCENAME as PARTICIPANTID. Drop all cells without a subject ID. Drop all unnecessary variables. Replace NAs as missing.

```
rename SEQUENCENAME PARTICIPANTID

drop if missing(PARTICIPANTID)

drop *SCORE ALGORITHM* STRAIN GENES PI* NRTI* NNRTI* INSTI*

destring, ignore("NA") replace
```

3. For each of the resistance level variables, reclassify the variable as a binary resistance indicator, with levels 1–2 corresponding to susceptible (no HIV drug resistance) and levels 3–5 corresponding to HIV drug resistance. Rename the resistance type variables.

```
ds *LEVEL

local plist = r(varlist)

foreach i of local plist {

    replace `i' = 0 if `i' < 3 & !missing(`i')

    replace `i' = 1 if `i' >= 3 & !missing(`i')

}

rename *LEVEL *_RES
```

4. Generate variables of DTG-specific resistance, any boosted PI resistance, any NRTI resistance, any NNRTI resistance, any INI resistance and any acquired HIV drug resistance, according to the definitions in subsection 4.3.

```
gen DTG_ADR = DTG_RES

egen ANY_PI = rowmax(ATVR_RES DRVR_RES LPVR_RES)

egen ANY_NRTI = rowmax(ABC_RES AZT_RES D4T_R DDI_RES FTC_RES TDF_RES)

egen ANY_NNRTI = rowmax(EFV_RES NVP_RES)

egen ANY_INI = rowmax(BIC_RES DTG_RES EVG_RES RAL_RES)

egen ANY_ ADR = rowmax(ANY_PI ANY_NRTI ANY_NNRTI ANY_INI)
```

5. Save the modified HIV drug resistance data as a .dta file. In this example, we save the data as "RESISTANCE_SUMMARY.dta".

```
save RESISTANCE_SUMMARY, replace
```

A3.9.3 Prepare viral load laboratory data

1. Remove the previous dataset, then load the viral load laboratory data, stored as "VL_LAB_INFORMATION.dta", and rename the variables for total DTG and non-DTG case specimens.

```
clear
```

```
use VL_LAB_INFORMATION.dta
```

```
rename NELIGIBLEDTGCASESPECIMENS NDTG
```

```
rename NELIGIBLENONDTGCASESPECIMENS NNONDTG
```

2. Save the modified data as a .dta file. In this example, we save the data as "VL_LAB_INFORMATION.dta".

```
save VL_LAB_INFORMATION, replace
```

A3.9.4 Prepare patient-level data on ARV treatment regimen

1. Remove previous dataset, then load the treatment regimen data, stored as "PARTICIPANT_TREATMENTS.dta".

```
clear
```

```
use PARTICIPANT_TREATMENTS.dta
```

2. Exclude observations missing a subject ID or corresponding to past ART. Drop unnecessary variables and rename ARV drug types so that all variable names begin with a letter.

```
drop if missing(PARTICIPANTID) | upper(CURRENTARTYN) == "N"
```

```
drop OTHERARVDRUG CURRENTARTYN
```

```
replace ARVDRUG = "ARV_" + ARVDRUG
```

3. Generate an indicator variable of DTG-based ART, equal to 1 if a person is receiving a DTG-containing regimen and 0 if a person is receiving a non-DTG-containing regimen.

```
gen TEMP_DTG = cond(inlist(ARVDRUG, "ARV_DTG", "ARV_TLD", "ARV_JUL"), 1, 0)
```

```
by PARTICIPANTID, sort: egen DTG = max(TEMP_DTG)
```

```
drop TEMP_DTG
```

4. Reformat the ARVDRUG variable so that each ARV drug type is created as a new binary variable, set to 1 if the person's regimen includes the drug, and 0 if not.

```
gen ON = 1
```

```
reshape wide ON, i(PARTICIPANTID) j(ARVDRUG) string
```

```
rename ON* *
```

5. Save the modified data as a .dta file. In this example, we save the data as "PARTICIPANT_TREATMENTS.dta".

```
save PARTICIPANT_TREATMENTS, replace
```

A3.9.5 Prepare patient-level data on other variables

1. Remove the previous dataset, then load the patient-level data, stored as "SURVEY_PARTICIPANTS.dta". Drop observations with missing subject ID or with children and adolescents.

```
clear
```

```
use SURVEY_PARTICIPANTS.dta
```

```
drop if missing(PARTICIPANTID) | substr(PARTICIPANTID, 23, 1) != "a"
```

2. Generate a variable named "VLLABCODE" that corresponds to the viral load laboratory for each person.

```
gen VLLABCODE = substr(PARTICIPANTID, 14, 3)
```

3. Recode unknown values as missing.

```
recode DATE* (9999 = .)
```

```
recode AGE (-9 = .)
```

```
foreach var of varlist PRIORART BREASTFEEDINGSTATUS PREGNANCYSTATUS CURRENTART {
```

```
    replace `var' = "." if `var' == "UNK"
```

```
}
```

4. Save the modified data as a .dta file. In this example, we save the data as "SURVEY_PARTICIPANTS.dta".

```
save SURVEY_PARTICIPANTS.dta, replace
```

A3.9.6 Merge all datasets

1. Use a many-to-one merge to merge the viral load laboratory data on total case specimens and laboratory metadata.

```
merge m:1 VLLABCODE using VL_LAB_INFORMATION, keepusing(NDTG NNONDTG) keep(match) nogenerate
```

2. Merge in the treatment regimen data by subject ID.

```
merge 1:1 PARTICIPANTID using PARTICIPANT_TREATMENTS, keep(match) nogenerate
```

3. Merge in the HIV drug resistance data by subject ID.

```
merge 1:1 PARTICIPANTID using RESISTANCE_SUMMARY, keep(match) nogenerate
```

4. Save the combined and reorganized data as a .dta file. In this example, we save the data as "ALL_DATA.dta".

```
save ALL_DATA.dta, replace
```

A3.9.7 Create survey weights and other necessary variables and declare the survey design

1. Remove the previous dataset and load in the combined data.

```
clear
```

```
use ALL_DATA.dta
```

2. Generate the variable for total DTG and non-DTG case specimens per laboratory. This corresponds to the stratum populations.

```
gen STRAT_POP = cond(DTG == 1, NDTG, NNONDTG)
```

3. Generate the sampling weights, calculated as the stratum totals divided by the number of sampled case specimens per stratum.

```
by VLLABCODE DTG, sort: gen WEIGHTS = STRAT_POP/_N
```

4. Stratify the data by laboratory and DTG versus non-DTG regimen.

```
egen STRATA = group(VLLABCODE DTG)
```

5. Set the stratified one-stage survey design with finite population correction. If there exists a stratum or multiple strata with only one unit sampled, sampling errors cannot be estimated for all strata independently, and Stata will report a missing standard error. We recommend addressing this by setting the standard errors for single-unit strata to be the average of the standard errors for other strata. This is represented by the single unit (scaled) term in the code below.

```
svyset [pweight = WEIGHTS], strata(STRATA) fpc(STRAT_POP) singleunit(scaled)
```

A3.9.8 Analyses for outcomes 1 and 2

1. Obtain estimates and confidence intervals for the prevalence of overall drug resistance among everyone. In the output, the point estimate, standard error and 95% confidence interval of interest are located in the row labelled "1".

```
svy: proportion ANY_ADR
```

This command gives confidence intervals expressed on the logit scale. For Wald confidence intervals, simply add citype(wald) to the end:

```
svy: proportion ANY_ ADR, citype(wald)
```

2. Obtain estimates and confidence intervals for the prevalence of overall drug resistance among people receiving DTG regimens.

```
svy, subpop(if DTG==1): proportion ANY_ ADR
```

3. Obtain estimates and confidence intervals for the prevalence of overall drug resistance among individuals receiving non-DTG regimens.

```
svy, subpop(if DTG==0): proportion ANY_ ADR
```

4. Obtain estimates and confidence intervals for the prevalence of DTG-specific drug resistance among individuals receiving DTG-containing regimens.

```
svy, subpop(if DTG==1): proportion DTG_ ADR
```

A3.9.9 Analyses for outcome 3

Some examples of subgroup analyses are given below.

1. Obtain prevalence and variance estimates of overall drug resistance among men.

```
svy, subpop(if GENDER == "M"): proportion ANY_ ADR
```

2. Obtain prevalence and variance estimates of any NRTI drug resistance among individuals receiving DTG regimens.

```
svy, subpop(if DTG==1): proportion ANY_NRTI
```

A3.9.10 Analyses for outcome 4

1. Remove previous dataset and then load the viral load laboratory data.

```
clear
```

```
use VL_LAB_INFORMATION
```

2. Drop irrelevant variables and collapse data into only the column sums, which are the totals across all laboratories.

```
drop NAMEOFVLLAB VLLABCODE
```

```
collapse (sum) _all
```

3. Obtain estimate for the prevalence of viral load suppression among individuals receiving DTG-based ART. No confidence intervals are necessary.

```
di TDTGVS/TDTG
```

4. Obtain the estimate for the prevalence of viral load suppression among individuals receiving non-DTG-based ART. No confidence intervals are necessary.

```
di TNONDTGVS/TNONDTG
```

5. Obtain the estimate for the prevalence of viral load suppression among all individuals receiving ART. No confidence intervals are necessary.

```
di (TDTGVS + TNONDTGVS)/(TDTG + TNONDTG)
```

A3.9.11 Analyses for outcome 5

Some examples of subgroup analyses are given below.

1. Obtain the prevalence of viral load suppression among men receiving DTG-based ART. No confidence intervals are necessary.

```
di TDTGVSMEN/TDTGMEN
```

2. Obtain prevalence of viral load suppression among individuals 0−9 years old. No confidence intervals are necessary.

```
di (TDTGVSAGE09 + TNONDTGVSAGE09)/(TDTGAGE09 + TNONDTGAGE09)
```

ANNEX 4. EXAMPLE: SAMPLE SIZE CALCULATIONS AND ALLOCATION ACROSS STRATA USING THE METHODS DESCRIBED IN SECTION 3

WHO developed an application by which countries can specify their own population sizes, by laboratory, to obtain a country-specific sample size. This annex provides an example of how to calculate the sample size and allocate it across strata using the WHO online sample size calculator (available at https://worldhealthorg.shinyapps.io/ADR_LabBasedMethod/).

Suppose a country has three laboratories, with the number of eligible case specimens from adults as indicated in Table A4.1. In the online sample size calculator, the user can specify the number of laboratories and then enter in the number of eligible case specimens per laboratory (Fig. A4.1). For clarity, the user can input the laboratory names in the input table to make it easier to track the laboratory sample allocation. After this information is entered, the user clicks "submit".

Table A4.1. Example of eligible case specimens from adults across viral load testing laboratories by ART regimen

	Laboratory 1	Laboratory 2	Laboratory 3	Total
Number of eligible case specimens from people receiving DTG-containing ART	3000	9000	800	12800
Number of eligible case specimens from people receiving non-DTG-containing ART	3000	1000	3200	7200

Fig. A4.1. Online sample size calculator: example input number of eligible case specimens from adults across viral load testing laboratories by ART regimen

This online calculator automatically calculates all the details for sample size calculation. The user can first click on the OUTPUT: SAMPLE SIZES tab. On this page (Fig. A4.2), the required sample sizes for DTG (n_{DTG}) and overall ($n_{overall}$) are reported, equal to n_{DTG} = 317 and n_{overal} = 264 in this example. The online calculator also calculates the proportion of the eligible case specimens that are from non-DTG eligible case specimens ($prop_{nonDTG}$ = 0.36) and the required sample size from non-DTG samples, n_{nonDTG} = 96. Therefore, the total required sample size is $n_{DTG} + n_{nonDTG}$ = 413. Finally, on this page, all sample sizes are inflated for a 30% genotyping failure rate, resulting in final target sample sizes of m_{DTG} = 453, m_{nonDTG}=138 and the total: $m_{DTG} + m_{nonDTG}$ = 591.

Table A4.2. Online sample size calculator: example output of sample size of eligible case specimens from adults on DTG-containing ART, non-DTG-containing ART and the total sample size
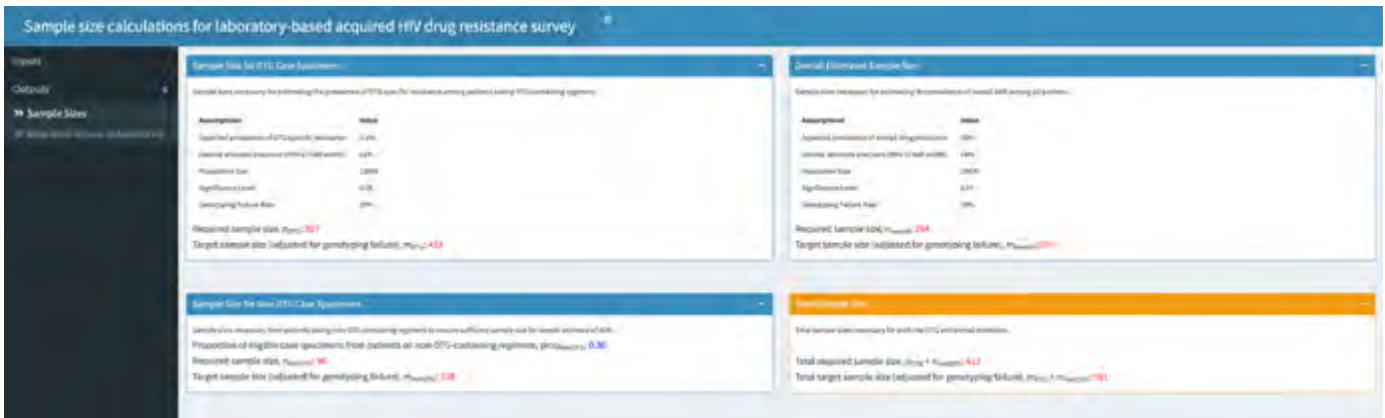
Next, the user can click on the OUTPUT: ALLOCATION ACROSS LABORATORIES button (Fig. A4.3). This page indicates the number of samples for each laboratory. For example, laboratory 1 will need to sample 107 DTG eligible case specimens and 58 non-DTG eligible case specimens ($m_{DTG,Lab1}$=107 and $m_{nonDTG,Lab1}$=58). For clarity, the total sample sizes might be slightly different that those reported on the sample sizes tab due to rounding up; for example, 455 versus 453 for the DTG target sample size.

Table A4.3. Online sample size calculator: example allocation of eligible case specimens from adults across viral load testing laboratory

Table A4.2 summarizes all the relevant input and output from this example illustrated in Fig. A4.1 to A4.3.

Table A4.2. Example of sample sizes allocated across viral load testing laboratories

	Laboratory 1	Laboratory 2	Laboratory 3	Total
Number of eligible case specimens from people receiving DTG-containing ART	3000	9000	800	12 800
Number of eligible case specimens from people receiving non-DTG-containing ART	3000	1000	3200	7200
Prop size DTG, $prop_{DTG}$	3000/12 800 = 0.234	9000/12 800 = 0.703	800/12 800 = 0.063	1
Target DTG sample size, $m_{DTG,j}$	0.234*453 = 107	0.703*453 = 319	0.063*453 = 29	455[a]
Prop size non-DTG, $prop_{nonDTG}$	3000/7200 = 0.417	1000/7200 = 0.139	3200/7200 = 0.444	1
Target non-DTG sample size, $m_{nonDTG,j}$	0.417*138 = 58	0.139*138 = 20	0.444*138 = 62	140[b]
Total recommended sample, m_j	107+58 = 165	319+20 = 339	29+62 = 91	595

[a] Slightly larger than the target sample size of 453, due to rounding up for laboratory-specific sample sizes.
[b] Slightly larger than the target sample size of 138, due to rounding up for laboratory-specific sample sizes.

ANNEX 5. SAMPLE SIZE CALCULATIONS AND ALLOCATION ACROSS STRATA AMONG CHILDREN AND ADOLESCENTS: SPECIAL CONSIDERATIONS

Ideally, the sample sizes for children and adolescents are calculated following the same steps described in subsections 3.5.2 to 3.5.5 but with the numbers of eligible case specimens from children and adolescents. However, two unique situations should be considered when replicating this survey in children and adolescents. These situations are: (1) few children and adolescents are taking DTG-containing regimens and (2) budget considerations preclude implementing this dually stratified design among adults and children. These scenarios are addressed in subsections A5.1 and A5.2, respectively.

A5.1   The number of children and adolescents receiving DTG-containing regimens is few

In many countries, few children or adolescents have transitioned to DTG-containing regimens, raising concerns about implementing a survey design that stratifies by DTG and non-DTG-containing regimens. In these settings, when using the online WHO sample size calculator that considers the finite population correction in the design phase, as described in subsection 3.5.2, the number of eligible case specimens from children/adolescents taking DTG-containing regimens is specified. Likely, this number is small, and the resulting recommendation will be to sample all these individuals. This will ensure that those taking DTG-containing regimens, even if few, are included to provide insight into their acquired HIV drug resistance outcomes. Fig. A5.1 to A5.3, taken from the online calculator, illustrate this example. The online sample size calculator is available at https://worldhealthorg.shinyapps.io/ADR_LabBasedMethod/.

Fig. A5.1. Online sample size calculator: example input number of eligible case specimens from children/adolescents across viral load testing laboratories by ART regimen if few children and adolescents are receiving DTG-containing regimens

Fig. A5.2. Online sample size calculator: example output of sample size of eligible case specimens from children and adolescents receiving DTG-containing ART, non-DTG-containing ART and the total sample size if few children and adolescents are receiving DTG-containing regimens
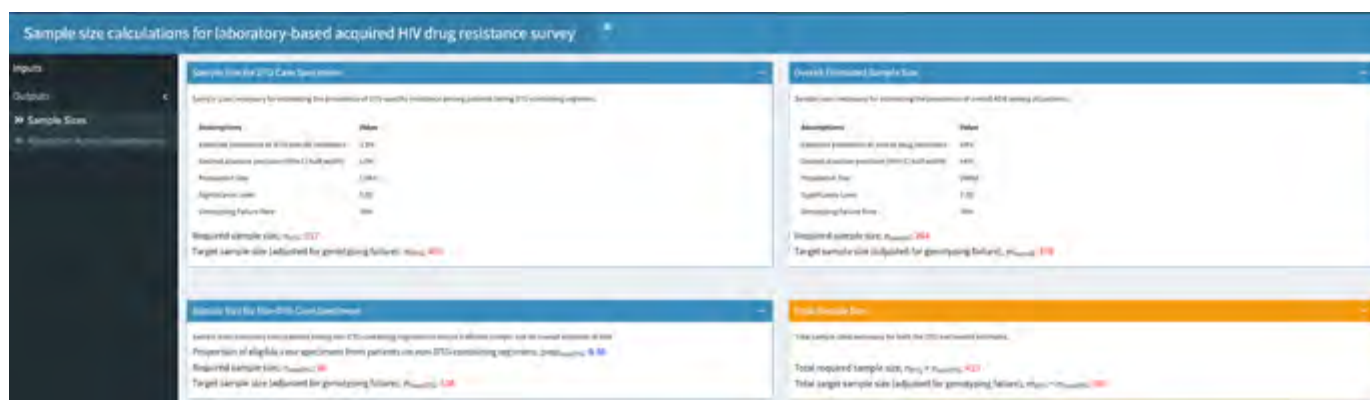

Fig. A5.3. Online sample size calculator: example allocation of eligible case specimens from children and adolescents across viral load testing laboratories if few children and adolescents are receiving DTG-containing regimens

A5.2   Budget considerations preclude assessing survey outcome by DTG and non-DTG-containing regimens among children and adolescents

Costs may also influence whether a country can afford to implement this design for children and adolescents, since the total sample size will be greater than if designing only for the first outcome (acquired HIV drug resistance prevalence overall). If finances are limited, WHO recommends that countries pursue only the overall estimate of acquired HIV drug resistance among children and adolescents. To implement this in practice, using the online sample size calculator, set the number of DTG eligible case specimens for each laboratory to 0 (N_{DTG}=0) and enter the combined number of DTG and non-DTG eligible case specimens into the number of non-DTG eligible case specimens. This will trick the app into ignoring the stratified design and will not specify a DTG-specific sample size. Importantly, the final sample size should be randomly sampled from all eligible case specimens and not just non-DTG eligible case specimens.

Fig. A5.4 to A5.6, taken from the online calculator, illustrate this example. The online sample size calculator is available at https://worldhealthorg.shinyapps.io/ADR_LabBasedMethod/.

Fig. A5.4. Online sample size calculator: example of how to enter all eligible case specimens (DTG and non-DTG) as non-DTG case specimens from children/adolescents if planning only to estimate overall acquired HIV drug resistance prevalence

Fig. A5.5. Online sample size calculator: example output of sample sizes of eligible case specimens when estimating only overall acquired HIV drug resistance prevalence among children and adolescents

Fig. A5.6. Online sample size calculator: example allocation of eligible case specimens across all viral load testing laboratories when estimating only overall acquired HIV drug resistance among children and adolescents

ANNEX 6. STATISTICAL METHODS FOR ALTERNATIVE SURVEY ACTIVITY DESCRIBED IN SECTION 5

This annex provides the statistical details of the alternative survey approach described in Section 5.

Table A6.1 describes the data that will be needed for designing and/or analysing the results generated using the adapted method described in Section 5. For each variable, we indicate whether or not this element is needed during the design (D) or analysis (A) phases. The design will occur over two distinct phases, delineated by D1 and D2, with D1 occurring at the initial stages to inform budget, planning and clinic sampling and D2 occurring after the three-month study period during which eligible case specimens from sampled clinics are collected. Even though some of these variables are only required in the analysis phase, we recommend collecting these data during the D2 phase along with the required variables for the design phase, since these processes overlap and are more efficiently collected at the same time.

Table A6.1. Required laboratory- and country-level data needed to implement the acquired HIV drug resistance survey method adaptation outlined in Section 5

Notation	Explanation	Required for design (D1, D2) or analysis (A) phase
Ideally $N^h_{VNS,DTG}$ $N^h_{VNS,nonDTG}$ Alternatively $N^h_{VT,DTG}$ $N^h_{VT,nonDTG}$ $q_{VNS,DTG}$ $q_{VNS,nonDTG}$ or $N^h_{ART,DTG}$ $N^h_{ART,nonDTG}$ $q_{VNS,DTG}$ $q_{VNS,nonDTG}$ $q_{VT,DTG}$ $q_{VT,nonDTG}$	The number of individuals, in a recent three-month period, who were receiving ART, underwent viral testing and had viral non-suppression, obtained both for those receiving DTG-containing regimens and those receiving non-DTG-containing regimens. The superscript, h, is to indicate that this is from a historical period. These two parameters can be used to estimate the total sample size (subsection 5.3.1) and, if necessary, to estimate the mean clinic size used to recommend the number of clinics for sampling (subsection 5.3.3). Alternatively, if these numbers are not available, one can use either: (1) the number of individuals, in some previous period, who were receiving ART and underwent viral testing; or (2) the number of individuals, in some previous period, who were receiving ART. If this is the information available, then these alternative parameters must be adjusted before use in subsection 5.3.1 or 5.3.3. $q_{VNS,i}$ and $q_{VT,i}$ are used in the adjustments. For each regimen i (i=DTG or non-DTG), $q_{VNS,i}$ is the national proportion of those receiving viral load tests who also have viral non-suppression, and $q_{VT,i}$ is the proportion of those receiving ART who receive viral load tests.	D1

Notation	Explanation	Required for design (D1, D2) or analysis (A) phase
Ideally $M^h_{VNS,DTG,k}$ $M^h_{VNS,nonDTG,k}$ Alternatively $M^h_{VT,DTG,k}$ $M^h_{VT,nonDTG,k}$ $q_{VNS,DTG}$ $q_{VNS,nonDTG}$ or $M^h_{ART,DTG,k}$ $M^h_{ART,nonDTG,k}$ $q_{VNS,DTG}$ $q_{VNS,nonDTG}$ $q_{VT,DTG}$ $q_{VT,nonDTG}$	For each clinic, k, the median number of individuals, in a recent three-month period, who were receiving ART, underwent viral testing and had viral non-suppression, obtained for DTG-containing and non-DTG-containing regimens. The superscript, h, indicates that this is from a historical period. This parameter is used to recommend the minimum number of clinics for sampling (subsection 5.3.3). If these numbers are not available, then alternatively, one can use either: (1) for each clinic, the median number of individuals who were receiving ART and underwent viral testing in a recent three-month period; or (2) for each clinic, the median number of individuals who were receiving ART in a recent three-month period. If this is the information available, then these alternative parameters must be adjusted before use in subsection 5.3.3. $q_{VNS,i}$ and $q_{VT,i}$ are used in the adjustments. For each regimen i (i=DTG or non-DTG), $q_{VNS,i}$ is the national proportion of those receiving viral load tests who also have viral non-suppression, and $q_{VT,i}$ is the proportion of those receiving ART who receive viral load tests. If none of these values are available, then the national-level numbers in the previous table entry can be used to calculate the mean size per clinic and used alternatively in subsection 5.3.3.	D1
$prop_{complete}$	Anticipated proportion of people with all required survey variables.	D1
C_j	The number of clinics served by each laboratory j.	D1, A
$N_{i,j,k}$	Total number of eligible case specimens from people during the survey period, for i=DTG or non-DTG, from each sampled clinic, k.	D2, A
$T^{VS}_{i,j,k}$	Total number of people who had viral load tests and required variables- during the survey period and had viral suppression, for i=DTG or non-DTG, from each sampled clinic, k.	A
$T_{i,j,k}$	Total number of viral load tests submitted from people with complete variables, for i=DTG or non-DTG, from each sampled clinic, k.	A
$T^{VS}_{i,a,j,k}$	Number of people who had viral load tests and required variables during the survey period and had viral suppression, for i=DTG or non-DTG, from each sampled clinic, k, with characteristic of interest a (for example, a=female).	A
$T_{i,a,j,k}$	Total number of viral load tests submitted from people, for i=DTG or non-DTG, from each sampled clinic, k, that also have complete data and with characteristic of interest a (for example, a=female).	A

A6.1   Determining the required sample sizes, overall and by DTG and non-DTG eligible case specimens

A6.1.1 Incorporating available historical data

For the alternative survey approach for countries with viral load coverage ≥60% but availability of required survey variables <80%, the sample sizes are determined before the study period and the actual accrual of eligible case specimens. Consequently, the total number of eligible case specimens is unknown when the sample size is calculated and must be estimated using historical data that is readily accessible from HIV treatment programmes. We use N_{DTG} and N_{nonDTG} to denote the anticipated number of eligible case specimens among people receiving DTG-containing regimens and people receiving non-DTG-containing regimens, respectively. These are also referred to as the anticipated eligible population sizes. To estimate N_{DTG} and N_{nonDTG} using historical data, countries must first provide the anticipated proportion of individuals with all required variables, denoted as $prop_{complete}$. Countries must also supply national-level historical data on people with viral non-suppression, undergoing viral load testing or receiving ART.

In the ideal scenario, historical data are available documenting the number of people in the country over a recent three-month period who were receiving ART, underwent viral testing and had viral non-suppression. These quantities are denoted by $N^h_{VNS,DTG}$ and $N^h_{VNS,nonDTG}$, respectively, with the superscript h used to denote that these are historical data. Then the anticipated eligible population size can be approximated by

$$N_{DTG} \approx N^h_{VNS,DTG} * prop_{complete} \text{ and}$$

$$N_{nonDTG} \approx N^h_{VNS,nonDTG} * prop_{complete}.$$

However, if historical viral non-suppression data are not available, a country may use corresponding historical information on the total number of people in the country (taking DTG-containing regimens and taking non-DTG-containing regimens) during a recent three-month period who were receiving ART and who underwent viral load testing $N^h_{VT,DTG}$ and $N^h_{VT,nonDTG}$. An additional adjustment must be made using the anticipated proportion of people receiving viral load tests who also have viral non-suppression by regimen type ($q_{VNS,DTG}$ and $q_{VNS,nonDTG}$). The anticipated eligible population size can be approximated by

$$N_{DTG} \approx N^h_{VT,DTG} * q_{VNS,DTG} * prop_{complete} \text{ and}$$

$$N_{nonDTG} \approx N^h_{VT,nonDTG} * q_{VNS,nonDTG} * prop_{complete}.$$

If viral non-suppression and viral load testing data are not available, a country may use corresponding historical information on the number of people in the country, during a recent three-month period, who were receiving ART by regimen type ($N^h_{ART,DTG}$ and $N^h_{ART,nonDTG}$). Additional adjustments must be made using the anticipated proportion receiving ART who receive a viral load test by regimen type ($q_{VT,DTG}$ and $q_{VT,nonDTG}$) and the anticipated proportion tested for viral load who are also have viral non-suppression by regimen type ($q_{VNS,DTG}$ and $q_{VNS,nonDTG}$). The anticipated eligible population size can be approximated by

$$N_{DTG} \approx N^h_{ART,DTG} * q_{VT,DTG} * q_{VNS,DTG} * prop_{complete} \text{ and}$$

$$N_{nonDTG} \approx N^h_{ART,nonDTG} * q_{VT,nonDTG} * q_{VNS,nonDTG} * prop_{complete}.$$

Countries should exercise caution when using these approximations, since they are meant to provide an upper bound on the eligible population size and rely on the assumption of there not being any large programmatic shifts that could drastically alter, and specifically increase, the number of people who have viral non-suppression, undergo viral load testing or are receiving ART for the current survey period. If the approximations are suspected to be poor, countries may want to use an inflation factor. Finally, if historical data are available over a longer time period (e.g. 1 year), divide by a factor to obtain the values for a three-month period. For example, divide by four for data collected over a year-long period.

A6.1.2 Calculating standard sample sizes

If the anticipated number of eligible case specimens from people taking DTG-containing regimens is large (such as more than 300 000 DTG case specimens), the required sample sizes for estimating either the prevalence of acquired HIV drug

resistance to DTG among people taking DTG-containing regimens or the prevalence of overall acquired HIV drug resistance among people receiving any regimen follow from the parameters specified in Table 2, using the following formula:

$$n_i = \left\lceil \frac{1.96^2 * p_i * (1-p_i)}{L_i^2} \right\rceil,$$

where $i{=}DTG$ indicates people receiving DTG-containing regimens and $i{=}overall$ indicates people receiving any regimen. n_i is the required sample size for group i, p_i is the expected prevalence of resistance among those with viral non-suppression for group i (acquired HIV drug resistance if $i{=}$overall and acquired HIV drug resistance to DTG if $i{=}$DTG), L_i is the desired absolute precision for group i and $\lceil \cdot \rceil$ is the ceiling function and rounds the inner value up to the nearest integer. The sample size formula is obtained by inverting the 95% Wald confidence interval using the normal approximation.

Using the assumptions from Table 2, the required sample size for estimating the prevalence of overall acquired HIV drug resistance is 267 ($n_{overall}{=}267$) and for estimating the prevalence of acquired HIV drug resistance to DTG among people taking DTG-containing regimens is 325 ($n_{DTG}{=}325$). Since the overall prevalence of acquired HIV drug resistance will be estimated using a combination of the 325 DTG specimens sampled and additional specimens sampled from people receiving non-DTG-containing regimens, a required sample size for non-DTG eligible case specimens, n_{nonDTG}, must also be determined (see subsection A6.1.4).

A6.1.3 Calculating country-specific sample sizes

For many countries, the anticipated number of eligible case specimens may be small. In this case, the target level of precision can be obtained with smaller required sample sizes by applying a finite population correction in the design. The sample size formula is thus adjusted as:

$$n_i = \left\lceil \frac{N_i * 1.96^2 * p_i * (1 - p_i)}{L_i^2 * N_i + 1.96^2 * p_i * (1 - p_i)} \right\rceil$$

where terms are defined as in subsection A6.1.2, with the additional term, N_i, defined as the anticipated number of eligible case specimens for group i, for $i{=}DTG$ or overall. Obtaining approximations for N_{DTG} and N_{nonDTG} is described in subsection A6.1.1, and $N_{overall}{=}N_{DTG} + N_{nonDTG}$.

A6.1.4 Required sample sizes from people taking non-DTG-containing regimens and total sample sizes

To determine the required sample sizes for non-DTG eligible case specimens, we must estimate the anticipated proportion of eligible case specimens that are from people receiving non-DTG-containing regimens, denoted by $prop^h_{nonDTG}$. We will approximate this using the historical data described in subsection A6.1.1.

$$prop^h_{nonDTG} \approx \frac{N^h_{VNS,nonDTG}}{N^h_{VNS,DTG} + N^h_{VNS,nonDTG}} \left(\text{or } \frac{N^h_{VT,nonDTG}}{N^h_{VT,DTG} + N^h_{VT,nonDTG}}, \text{ or } \frac{N^h_{ART,nonDTG}}{N^h_{ART,DTG} + N^h_{ART,nonDTG}} \right).$$

The non-DTG required sample size will be given by $n_{nonDTG}{=}n_{overall} * prop^h_{nonDTG}$. The total required sample size will be $n_{DTG}+ n_{nonDTG}$ and will lie somewhere between the DTG sample size ($n_{DTG}{=}325$) and the sum of the DTG and overall sample sizes ($n_{DTG}+ n_{overall}{=}592$), depending on the value of $prop^h_{nonDTG}$.

The above sample size calculations are automated in the WHO online sample size calculator (available at https://worldhealthorg.shinyapps.io/ADR_LabBasedMethod_2/), in which countries can enter their relevant historical data and finite population correction preference and obtain all required sample sizes as outputs.

A6.2   Inflating sample size for design effect and genotyping failure

The required sample sizes above, n_{DTG}, $n_{overall}$ and n_{nonDTG}, must undergo two inflations. The first inflation is for increased variance due to sampling of clinics (clusters) within laboratories. The sample size is inflated to offset this increased variance by incorporating the design effect (DE). For this activity, we will set DE = 1.5, which corresponds to anticipating

that the variance of our estimates when using cluster sampling will be 50% greater than the variance when using simple random sampling of individuals. Stata code to support the calculation of the actual design effect, and corresponding intracluster correlation, during the analysis phase is provided in subsection A6.8, and future protocols may update the design effect based on these estimates.

The second inflation accounts for the fact that not all sampled specimens will successfully amplify. Sample sizes must therefore account for the genotyping failure rate, g, and ensure that there are sufficient case specimens after the samples undergo genotyping amplification.

The target sample sizes, denoted by m_{DTG} and m_{nonDTG}, are the required sample sizes inflated by the design effect, DE, and by a factor of $(1 - g)$, where g is the genotyping failure rate. WHO recommends setting DE=1.5 and g=30%, with the target sample sizes given by:

$$N_{DTG} \approx N_{ART,DTG}^{h} * q_{VT,DTG} * q_{VNS,DTG} * prop_{complete} \text{ and}$$

$$N_{nonDTG} \approx N_{ART,nonDTG}^{h} * q_{VT,nonDTG} * q_{VNS,nonDTG} * prop_{complete}.$$

A total target sample size of $m_{DTG} + m_{nonDTG}$ will be necessary. This is also automatically calculated and reported in the WHO online sample size calculator (available at https://worldhealthorg.shinyapps.io/ADR_LabBasedMethod_2/).

A6.3   Determining the number of clinics to sample

The total number of clinics to be sampled, c, is chosen during the first design phase. From previous acquired HIV drug resistance survey experiences, c is recommended to be between 20 and 40 (1). Although sampling more clinics is always preferred from a statistical perspective, it comes at increased costs. Enough clinics should be sampled to ensure that at least two clinics are sampled per laboratory, c_j≥2 for all laboratories that serve two or more clinics. This is to enable direct estimation of within-laboratory variation for the acquired HIV drug resistance prevalence estimates.

When determining the number of clinics to be sampled, decision-making may be aided by using available clinic-level historical data on viral non-suppression, viral load testing or ART enrolment, similar to the approach in subsection A6.1.1.

In the ideal situation, countries have access to historical viral non-suppression data for each ART clinic. With these data, countries can calculate the median size of clinics: that is, the median number of individuals per clinic, in a recent three-month period, who were receiving ART, underwent viral testing and had viral non-suppression, obtained for DTG-containing and non-DTG-containing regimens. We use $M_{VNS,DTG}^{h}$ and $M_{VNS,nonDTG}^{h}$ to denote these quantities. The recommended minimum number of clinics to be sampled is given by:

$$c = \left\lceil \max\left\{ \frac{m_{DTG}}{M_{VNS,DTG}^{h} * prop_{complete}}, \frac{m_{nonDTG}}{M_{VNS,nonDTG}^{h} * prop_{complete}} \right\} \right\rceil,$$

where m_{DTG} and m_{nonDTG} are the DTG and non-DTG target sample sizes and $prop_{complete}$ is the anticipated proportion of individuals with all required survey variables. For a more conservative approach, we recommend using the 25th percentile clinic size rather than the median clinic size.

If historical clinic-level viral non-suppression data are not available, a country may use corresponding information on the median number of individuals per clinic, in a recent three-month period, who were receiving ART and underwent viral testing ($M_{VT,DTG}^{h}$ and $M_{VT,nonDTG}^{h}$). As in subsection A6.1.1, an additional adjustment must be made using the anticipated proportion of people receiving viral load tests who also have viral non-suppression ($q_{VNS,DTG}$ and $q_{VNS,nonDTG}$). The recommended minimum number of clinics to be sampled is given by:

$$c = \left\lceil \max\left\{ \frac{m_{DTG}}{M_{VT,DTG}^{h} * q_{VNS,DTG} * prop_{complete}}, \frac{m_{nonDTG}}{M_{VT,nonDTG}^{h} * q_{VNS,nonDTG} * prop_{complete}} \right\} \right\rceil.$$

If clinic-level viral non-suppression and viral load testing data are not available, a country may use corresponding historical information on the median number of people per clinic, in a recent three-month period, who were receiving ART ($M_{ART,DTG}^{h}$

and $M^h_{ART,nonDTG}$). Additional adjustments must be made using the anticipated proportion receiving ART who receive a viral load test ($q_{VT,DTG}$ and $q_{VT,nonDTG}$) and the anticipated proportion virally tested who also had viral non-suppression ($q_{VNS,DTG}$ and $q_{VNS,nonDTG}$). The recommended minimum number of clinics to be sampled is given by:

$$c = \left\lceil \max\left\{ \frac{m_{DTG}}{M^h_{ART,DTG} * q_{VT,DTG} * q_{VNS,DTG} * prop_{complete}}, \frac{m_{nonDTG}}{M^h_{ART,nonDTG} * q_{VT,DTG} * q_{VNS,nonDTG} * prop_{complete}} \right\} \right\rceil.$$

If this historical data are available over a length of time that differs from three months, simply assume that people are uniformly distributed over that length of time and divide by a factor to obtain the values for a three-month period. For example, divide by four for data collected over a year-long period.

In some situations, there may be no access to any clinic-level data at the design stage. When this is the case, choosing the number of clinics to be sampled can be aided by the national-level historical data provided in subsection A6.1.1. The total number of clinics in the country, C, must also be provided. The recommended minimum number of clinics to be sampled is given by:

$$c = \left\lceil \max\left\{ \frac{C * m_{DTG}}{N_{DTG}}, \frac{C * m_{nonDTG}}{N_{nonDTG}} \right\} \right\rceil,$$

where N_{DTG} and N_{nonDTG} are the anticipated number of eligible case specimens among people receiving DTG-containing regimens and people receiving non-DTG-containing regimens, estimated as in subsection A6.1.1. This approach uses a simpler method based on the mean clinic size, in contrast to the above methods that used a median approach. The median approach requires clinic-level data that may be difficult to obtain, but it is more robust against extreme scenarios in which the sizes of clinics may vary substantially within a country. All approaches for determining the minimum recommended number of clinics to sample have been automated in the WHO online sample size calculator (available at https://worldhealthorg.shinyapps.io/ADR_LabBasedMethod_2/).

A6.4   Allocating the number of clinics to sample across laboratories

The last step of the first phase of design is deciding how to allocate the number of clinics for sampling, c, across laboratories. The sampled clinics from each viral load laboratory will receive training and supervision to ensure completion of viral load requisition forms for specimens sent to the laboratories for viral load testing. Before the study period begins, the total number of clinics to be sampled will be allocated across laboratories, proportional to the number of clinics served by the laboratory.

If a laboratory j has a total of C_j clinics that send in samples to the laboratory for viral load testing, then c_j clinics will be sampled from the laboratory, with c_j / C_j set to be approximately the same for all laboratories. This can be calculated using $c_j = c * C_j / C$, rounded to the nearest integer. For each viral load laboratory, the c_j clinics will be sampled from the C_j¬ total clinics using simple random sampling without replacement. The allocation of clinics to sample across laboratories is automatically calculated in the WHO online sample size calculator (available at https://worldhealthorg.shinyapps.io/ADR_LabBasedMethod_2/).

A6.5   Interventions and specimen collection for sampled clinics

Before and during the study period, each sampled clinic, k, should receive intensive interventions to ensure that all viral load results are sent to the laboratory with completed requisition forms as described in subsection 5.4. Each laboratory j will then report $N^c_{i,j}$, the total number of eligible case specimens during the study period from people receiving regimen i (DTG-containing or non-DTG-containing) from the sampled clinics, with $N^c_{i,j} = \sum_{k=1}^{c_j} N_{i,j,k},$, where c_j is the number of clinics sampled from the jth laboratory.

A6.6   Example: sample size calculations and allocation across strata using the formulas detailed in this annex

Annex 7 provides an example of the methods described in Section 5 using the online WHO sample size calculator. Here we compute these same values by hand using the formulas detailed in this annex.

A6.6.1 Determining the required sample sizes, overall and by DTG and non-DTG eligible case specimens

Suppose a country has three laboratories and 100 clinics. Laboratory 1 serves 30 clinics, laboratory 2 serves 50 clinics and laboratory 3 serves 20 clinics. The anticipated proportion of people with all required survey variables is estimated to be 0.8 after intervention ($prop_{complete}$=0.8). Further, suppose the country has national-level historical data on viral load testing from a recent three-month period. In that period, the number of people who were receiving an ART regimen and who underwent viral load testing was 13 500 for people receiving DTG-containing regimens and 8500 for people receiving non-DTG-containing regimens ($N^h_{VT,DTG}$=13 500 and $N^h_{VT,nonDTG}$=8500). In addition, historical data suggest that the anticipated proportion of those receiving viral load tests who also had viral non-suppression is 0.3 for both people receiving DTG-containing and people receiving non-DTG-containing regimens ($q_{VNS,DTG}$=$q_{VNS,nonDTG}$=0.3).

First, the anticipated eligible population sizes are estimated, where

$$N_{DTG} \approx N^h_{VT,DTG} * q_{VNS,DTG} * prop_{complete} = 13\ 500 * 0.3 * 0.8 = 3240 \text{ and}$$

$$N_{nonDTG} \approx N^h_{VT,nonDTG} * q_{VNS,nonDTG} * prop_{complete} = 8500 * 0.3 * 0.8 = 2040.$$

These population sizes are quite small; thus, the finite population correction is used when calculating the required sample sizes for the DTG estimate and for the overall estimate. Using the assumptions summarized in Table 2 (the expected prevalence of drug-specific resistance is 3.5% for people taking DTG and 50% overall; the desired absolute precision is ±2% for people taking DTG and ±6% overall) and the sample size formula for small populations described in subsection A6.1.3, the required sample size for people receiving DTG-containing ART is 295 and the required sample size for people receiving ART (regardless of the regimen) is 254:

$$n_{DTG} = 295 \text{ and } n_{overall} = 254.$$

Next, the required sample size for non-DTG eligible case specimens is calculated. As described in subsection A6.1.4, the anticipated proportion of eligible case specimens that are from people receiving non-DTG-containing regimens is given by:

$$prop^h_{nonDTG} \approx \frac{N^h_{VT,nonDTG}}{N^h_{VT,DTG} + N^h_{VT,nonDTG}} = \frac{8500}{13\ 500 + 8500} = 0.386.$$

The required sample size for DTG eligible case specimens remains n_DTG=295, and the required sample size for non-DTG eligible case specimens will be n_{nonDTG}=$n_{overall}$ * $prop_{nonDTG}$=254*0.386=99. All sample sizes are rounded up to the nearest whole number. This gives a total required sample size of 295+99=394.

A6.6.2 Determining the target sample sizes

The DTG and non-DTG required sample sizes must then be inflated to account for the design effect (set to 1.5) and a 30% genotyping failure rate. Using the steps in subsection A6.2 and rounding up to the nearest integer, where

$$m_{DTG} = \frac{n_{DTG}*1.5}{(1-0.3)} = \frac{295*1.5}{0.7} = 633 \text{ and}$$

$$m_{nonDTG} = \frac{n_{nonDTG}*1.5}{(1-0.3)} = \frac{99*1.5}{0.7} = 213.$$

A6.6.3 Determining the number of clinics to sample

Suppose the country also has clinic-level historical data on viral load testing. The median number of individuals per clinic, in a recent three-month period, who were receiving an ART regimen and who underwent viral load testing, was found to be 135 for those on DTG-containing regimens and 85 for those on non-DTG-containing regimens ($M^h_{VT,DTG}$=135 and $M^h_{VT,nonDTG}$=85). The recommended minimum number of clinics to be sampled is:

$$c = \left\lceil \max\left\{ \frac{m_{DTG}}{M^h_{VT,DTG} * q_{VNS,DTG} * prop_{complete}}, \frac{m_{nonDTG}}{M^h_{VT,nonDTG} * q_{VNS,nonDTG} * prop_{complete}} \right\} \right\rceil$$

$$= \left\lceil \max\left\{ \frac{633}{135 * 0.3 * 0.8}, \frac{213}{85 * 0.3 * 0.8} \right\} \right\rceil$$

$$= \lceil \max\{19.5, 10.4\} \rceil$$

$$= 20$$

Due to budget constraints, the country chooses to sample the recommended minimum number of 20 clinics.

A6.6.4 Allocating number of clinics to sample across laboratories

Finally, using the steps described in subsection A6.4, the clinics to be sampled are allocated across laboratories, proportional to each laboratory's share of the overall number of clinics. Recall that this country has three laboratories and 100 clinics, with laboratory 1 serving 30 clinics, laboratory 2 serving 50 clinics and laboratory 3 serving 20 clinics.

Laboratory 1 accounts for 30/100 = 30% of the total clinics in the country, laboratory 2 accounts for 50% and laboratory 3 accounts for 20%. Since the total number of sampled clinics will be allocated across laboratories, proportional to the number of clinics feeding into each laboratory, the number of clinics sampled from laboratory 1, c_1, should be 30% of the total number of sampled clinics: c_1= 0.3 * 20 = 6. For laboratory 2, c_2=0.5 * 20 = 10, and for laboratory 3 c_3=0.2 * 20 = 4. Note that each laboratory has at least 2 clinics sampled to allow for direct estimation of within-laboratory variation of prevalence estimates.

6.6.5 Allocating the sample size to each sampled clinic

After the survey period ends and all necessary viral load testing is complete, there will be information on the total number of eligible case specimens for both DTG-containing and non-DTG-containing regimens. In addition, the distribution of these specimens across clinics will be known.

For this scenario, there are 800 eligible case specimens from people receiving DTG-containing regimens (N^c_{DTG}=800) and 400 eligible case specimens from people receiving non-DTG-containing regimens (N^c_{nonDTG}=400). Further, for clinic 1 of laboratory 1, there are 19 DTG case specimens and 7 non-DTG case specimens. Since clinic 1 of laboratory 1 accounts for 19/800=0.024 of the total DTG case specimens and 7/400=0.018 of the total non-DTG case specimens, the target sample sizes allocated to the clinic, rounded up, are given by:

$$m_{DTG,1,1} = m_{DTG}\left(\frac{N_{DTG,1,1}}{N^c_{DTG}}\right) = 633\left(\frac{19}{800}\right) \approx 16 \text{ and}$$

$$m_{nonDTG,1,1} = m_{nonDTG}\left(\frac{N_{nonDTG,1,1}}{N^c_{nonDTG}}\right) = 213\left(\frac{7}{400}\right) \approx 4.$$

Sixteen DTG case specimens and 4 non-DTG case specimens are allocated to clinic 1 of laboratory 1, and these specimens are sampled using systematic sampling. Table A6.2 provides the remaining data for the example as well as the allocation of case specimens across laboratories and clinics for the remaining clinics.

Table A6.2. Sample sizes of eligible case specimens allocated across clinics

Laboratory, j	Sampled clinic ID, k	Number of DTG eligible case specimens, $N_{DTG,j,k}$	Number of non-DTG eligible case specimens, $N_{nonDTG,j,k}$	Target DTG sample size, $m_{DTG,j,k}$	Target non-DTG sample size, $m_{nonDTG,j,k}$
1	1	19	7	(19/800)*633 = 16	(7/400)*213 = 4
	2	27	28	(27/800)*633 = 22	(28/400)*213 = 15
	3	41	22	(41/800)*633 = 33	(22/400)*213 = 12
	4	65	7	(65/800)*633 = 52	(7/400)*213 = 4
	5	15	37	(15/800)*633 = 12	(37/400)*213 = 20
	6	65	37	(65/800)*633 = 52	(37/400)*213 = 20
2	1	68	5	(68/800)*633 = 54	(5/400)*213 = 3
	2	48	33	(48/800)*633 = 38	(33/400)*213 = 18
	3	45	18	(45/800)*633 = 36	(18/400)*213 = 10
	4	4	22	(4/800)*633 = 4	(22/400)*213 = 12
	5	15	22	(15/800)*633 = 12	(22/400)*213 = 12
	6	13	9	(13/800)*633 = 11	(9/400)*213 = 5
	7	50	30	(50/800)*633 = 40	(30/400)*213 = 16
	8	28	7	(28/800)*633 = 23	(7/400)*213 = 4
	9	55	16	(55/800)*633 = 44	(16/400)*213 = 9
	10	36	33	(36/800)*633 = 29	(33/400)*213 = 18
3	1	52	38	(52/800)*633 = 42	(38/400)*213 = 21
	2	71	9	(71/800)*633 = 57	(9/400)*213 = 5
	3	27	17	(27/800)*633 = 22	(17/400)*213 = 10
	4	56	3	(56/800)*633 = 45	(3/400)*213 = 2
Total		800	400	644[a]	220[b]

[a] Slightly larger than the target sample size of 633, due to rounding up for clinic-specific sample sizes.
[b] Slightly larger than the target sample size of 213, due to rounding up for clinic-specific sample sizes.

A6.7   Analysis

The subsection describes the statistical details for each of the five outcomes. Stata code is provided to automate this analysis.

A6.7.1 Notation

We use the following notation throughout this subsection.

i = Subscript for regimen, where i = DTG, non-DTG or overall.

j = Subscript for viral load laboratory. $j=1,\ldots,J$, where J is the total number of viral load laboratories.

k = Subscript for clinic. $k=1,\ldots,c_j$, where c_j is the total number of clinics sampled for laboratory j.

l = Subscript for individual case specimens.

a = Subscript for membership in the subgroup of interest.

$Y_{DTG,j,k,l}$ = binary outcome of acquired HIV drug resistance among all eligible case specimens l from people receiving DTG-containing regimens in laboratory j, clinic k.

$p_{overall}$ = estimated prevalence of acquired HIV drug resistance among all eligible case specimens.

p_{DTG} = estimated prevalence of acquired HIV drug resistance among eligible case specimens from people receiving DTG-containing regimens.

$p_{DTG,j}$ = estimated prevalence of acquired HIV drug resistance among eligible case specimens from people receiving DTG regimens from laboratory j.

$X_{DTG,j,k,l}$ = binary outcome of acquired HIV drug resistance to DTG among eligible case specimens from people taking DTG-containing regimens in laboratory j, clinic k.

p^*_{DTG} = estimated prevalence of acquired HIV drug resistance to DTG among eligible case specimens from people receiving DTG regimens.

$Y_{DTG,j,k,l}$ = binary outcome of acquired HIV drug resistance among all eligible case specimens l from people receiving non-DTG-containing regimens in laboratory j, clinic k.

p_{nonDTG} = estimated prevalence of acquired HIV drug resistance among eligible case specimens from people receiving non-DTG regimens.

$p_{nonDTG,j}$ = estimated prevalence of acquired HIV drug resistance among eligible case specimens from people receiving non-DTG regimens from laboratory j.

$q_{overall}$ = prevalence of viral load suppression among all viral load tests with the required variables recorded.

q_{DTG} = prevalence of viral load suppression among all viral load tests with the required variables recorded among people receiving DTG regimens.

q_{nonDTG} = prevalence of viral load suppression among all viral load tests with the required variables recorded among people receiving non-DTG regimens.

C_j = total number of clinics served by laboratory j.

c = total number of clinics sampled.

c_j = number of clinics sampled from laboratory j.

$N_{overall}$ = total number of eligible case specimens.

N_h = total number of eligible case specimens in stratum h.

N_{DTG} = total number of eligible case specimens from people receiving DTG regimens.

N_{nonDTG} = total number of eligible case specimens from people receiving non-DTG regimens.

$N_{DTG,j,k}$ = total number of eligible case specimens from people taking DTG-containing regimens in laboratory j, clinic k.

$N_{nonDTG,j,k}$ = total number of eligible case specimens from people taking non-DTG regimens in laboratory j, clinic k.

$m^*_{DTG,j}$ = observed number of sampled individuals taking DTG-containing regimens with acquired HIV drug resistance results, in laboratory j.

$m^*_{nonDTG,j}$ = observed number of sampled individuals taking non-DTG-containing regimens with acquired HIV drug resistance results, in laboratory j.

m^*_h = observed number of sampled individuals with acquired HIV drug resistance results, in stratum h.

$T_{DTG,j}$ = total number of people taking a DTG-containing regimen who received a viral load test (regardless of the results) and have all required variables from each laboratory, j, during the survey period.

$T_{nonDTG,j}$ = total number of people taking a non-DTG-containing regimen who received a viral load test (regardless of the results) and have all required variables from each laboratory, j, during the survey period.

$T^{VS}_{DTG,j}$ = total number of people taking a DTG-containing regimen who received a viral load test, had viral suppression and have all required variables from each laboratory, j, during the survey period.

$T^{VS}_{nonDTG,j}$ = total number of people taking a non-DTG-containing regimen who received a viral load test, had viral suppression and have all required variables from each laboratory, j, during the survey period.

$T_{i,a,j}$ = total number of people receiving regimen i (DTG or non-DTG) and subgroup a (for example, a=female) tested and with required variables for each laboratory, j, that were tested during the survey period.

$T^{VS}_{i,a,j}$ = total number of people receiving regimen i (DTG or non-DTG) and subgroup a (for example, a=female) tested and with required variables for each laboratory, j, that were tested and had viral suppression during the survey period.

A6.7.2 Calculation of survey sampling weights

Survey sampling weights can be calculated by taking the reciprocal of the probability of a case specimen being selected. For the survey design, sampling unfolds in two stages.

> The first stage involves stratification by viral load laboratory, with clinics as the primary sampling units. Suppose there are J laboratories, each denoted by $j,j=1,…,J$. In each laboratory j, c_j clinics out of a total of C_j are sampled without replacement.

> The second stage involves stratification by regimen, with case specimens as the secondary sampling units. Regimen i refers to either DTG-containing regimens or non-DTG-containing regimens. For each regimen i, $m^*_{i,j,k}$ case specimens ultimately contribute to the observed sample size out of a total of $N_{i,j,k}$ eligible case specimens from sampled clinic k of laboratory j.

The probability of a specimen being sampled is given by:

P(specimen l on regimen i, from clinic k in laboratory j, is selected)

$=P$(clinic k in laboratory j is selected)

$\qquad$ * P(specimen l in regimen i is selected | clinic k in laboratory j is selected)

$$= \frac{c_j}{C_j} \times \frac{m^*_{i,j,k}}{N_{i,j,k}}$$

The survey sampling weight for a case specimen l on regimen i from clinic k in laboratory j is the inverse of the probability of selection:

$$w_{i,j,k,l} = \frac{C_j}{c_j} \frac{N_{i,j,k}}{m^*_{i,j,k}}.$$

A6.7.3 Analysis for outcomes 1 and 2

Outcome 1 is defined as: prevalence of acquired HIV drug resistance among individuals with viral non-suppression and receiving ART, regardless of the ART regimen.

Outcome 2 is defined as: prevalence of acquired HIV drug resistance to DTG among individuals with viral non-suppression receiving ART and who are taking a DTG-containing regimen.

Prevalence and variance of DTG-specific resistance among people receiving DTG-containing regimens

Prevalence

An unbiased estimate of HIV drug resistance to DTG among eligible case specimens from individuals receiving DTG regimens can be obtained by using a ratio estimator for estimating the prevalence. A ratio estimator is used because the total number of eligible case specimens for all clinics in the population is unknown. Only the number of eligible case specimens for the clinics that are included in the sample is known.

Subsection A6.7.2 shows that the sampling weight for each case specimen is $w_{i,j,k,l} = \frac{C_j \, N_{i,j,k}}{c_j \, m^*_{i,j,k}}$. Let $X_{DTG,j,k,l}$ denote the binary

outcome variable for DTG-specific resistance among people taking a DTG-containing regimen, equal to 1 if patient l in clinic k laboratory j has DTG-specific resistance and 0 if not. Similarly, let $\hat{p}^*_{DTG,j,k}$ be the estimated prevalence of HIV drug resistance to DTG among eligible case specimens receiving DTG-containing regimens in clinic k of laboratory j. The prevalence estimate is given by

$$\hat{p}^*_{DTG} = \frac{\sum_{j=1}^{J} \sum_{k=1}^{c_j} \sum_{l=1}^{m^*_{DTG,j,k}} w_{DTG,j,k,l} X_{DTG,j,k,l}}{\sum_{j=1}^{J} \sum_{k=1}^{c_j} \sum_{l=1}^{m^*_{DTG,j,k}} w_{DTG,j,k,l}}$$

$$= \frac{\sum_{j=1}^{J} \frac{C_j}{c_j} \sum_{k=1}^{c_j} \frac{N_{DTG,j,k}}{m^*_{DTG,j,k}} \sum_{l=1}^{m^*_{DTG,j,k}} X_{DTG,j,k,l}}{\sum_{j=1}^{J} \frac{C_j}{c_j} \sum_{k=1}^{c_j} N_{DTG,j,k}}$$ by plugging in the values for the weights

$$= \frac{\sum_{j=1}^{J} \frac{C_j}{c_j} \sum_{k=1}^{c_j} N_{DTG,j,k} \, \hat{p}^*_{DTG,j,k}}{\sum_{j=1}^{J} \frac{C_j}{c_j} \sum_{k=1}^{c_j} N_{DTG,j,k}}$$

Variance

The variance formula is obtained by linearizing the ratio estimator for stratified two-stage cluster samples. For simplicity, let $\hat{p}^*_{DTG} = \frac{\hat{z}}{\hat{v}}$, where $\hat{z} = \sum_{j=1}^{J} \frac{C_j}{c_j} \sum_{k=1}^{c_j} \frac{N_{DTG,j,k}}{m^*_{DTG,j,k}} \sum_{l=1}^{m^*_{DTG,j,k}} X_{DTG,j,k,l}$ and $\hat{v} = \sum_{j=1}^{J} \frac{C_j}{c_j} \sum_{k=1}^{c_j} N_{DTG,j,k}$.

The linearized variance estimator is given by

$$\widehat{Var}(\hat{p}^*_{DTG}) = \frac{1}{\hat{v}^2} \left(\widehat{Var}(\hat{z}) + \hat{p}^{*2}_{DTG} \widehat{Var}(\hat{v}) - 2\hat{p}^*_{DTG} \widehat{Cov}(\hat{z}, \hat{v}) \right),$$

where $\widehat{Var}(\hat{v}), \widehat{Var}(\hat{z})$, and $\widehat{Cov}(\hat{z}, \hat{v})$ can be estimated by the corresponding sample variances and covariances, which are also obtained through linearization and incorporate the finite population corrections at both stages. For example, for $\widehat{Var}(\hat{z})$, the sample estimate is given by:

$$\widehat{Var}(\hat{z}) = \sum_{j=1}^{J} \left(1 - \frac{c_j}{C_j}\right) \frac{c_j}{c_j - 1} \sum_{k=1}^{c_j} \left(X_{DTG,j,k} - \frac{X_{DTG,j}}{c_j} \right)^2$$
$$+ \sum_{j=1}^{J} \left(\frac{c_j}{C_j}\right) \sum_{k=1}^{c_j} \left(1 - \frac{m^*_{DTG,j,k}}{N_{DTG,j,k}}\right) \frac{m^*_{DTG,j,k}}{m^*_{DTG,j,k} - 1} \sum_{l=1}^{m^*_{DTG,j,k}} \left(X_{DTG,j,k,l} - \frac{X_{DTG,j,k}}{m^*_{DTG,j,k}} \right)^2,$$

where

$$\hat{X}_{DTG,j,k,l} = \frac{N_{DTG,j,k}}{m^*_{DTG,j,k}} X_{DTG,j,k,l}$$ is the weighted total number of people in clinic k with DTG-specific resistance that are represented by person l in clinic k laboratory j;

$$X_{DTG,j,k} = \frac{1}{m^*_{DTG,j,k}} \sum_{l=1}^{m^*_{DTG,j,k}} \hat{X}_{DTG,j,k,l}$$ is the weighted total represented by clinic k in laboratory j; and

$$X_{DTG,j} = \frac{C_j}{c_j} \sum_{k=1}^{c_j} X_{DTG,j,k}$$ is the weighted total represented by laboratory j.

The estimates for $\widehat{Var}(\hat{z})$ and $\widehat{Cov}(\hat{z}, \hat{v})$ are similarly obtained.

Prevalence and variance of any resistance stratified by DTG- and non-DTG-containing regimens

Prevalence

An unbiased estimate of the prevalence of any HIV drug resistance among eligible case specimens, stratified by DTG and non-DTG regimens, is similar to the DTG-specific resistance estimate. Let $Y_{i,j,k,l}$ denote the binary outcome variable for any drug resistance among people receiving regimen i, equal to 1 if person l in clinic k laboratory j has drug resistance and 0 if not; and let $p_{DTG,j,k}$ be the estimated prevalence of any HIV drug resistance among eligible case specimens on DTG-containing regimens in clinic k of laboratory j.

$$\hat{p}_{DTG} = \frac{\sum_{j=1}^{J} \frac{C_j}{c_j} \sum_{k=1}^{c_j} \frac{N_{DTG,j,k}}{m^*_{DTG,j,k}} \sum_{l=1}^{m^*_{DTG,j,k}} Y_{DTG,j,k,l}}{\sum_{j=1}^{J} \frac{C_j}{c_j} \sum_{k=1}^{c_j} N_{DTG,j,k}} = \frac{\sum_{j=1}^{J} \frac{C_j}{c_j} \sum_{k=1}^{c_j} N_{DTG,j,k} \, \hat{p}_{DTG,j,k}}{\sum_{j=1}^{J} \frac{C_j}{c_j} \sum_{k=1}^{c_j} N_{DTG,j,k}}$$

$$\hat{p}_{nonDTG} = \frac{\sum_{j=1}^{J} \frac{C_j}{c_j} \sum_{k=1}^{c_j} \frac{N_{nonDTG,j,k}}{m^*_{nonDTG,j,k}} \sum_{l=1}^{m^*_{nonDTG,j,k}} Y_{nonDTG,j,k,l}}{\sum_{j=1}^{J} \frac{C_j}{c_j} \sum_{k=1}^{c_j} N_{nonDTG,j,k}} = \frac{\sum_{j=1}^{J} \frac{C_j}{c_j} \sum_{k=1}^{c_j} N_{nonDTG,j,k} \, \hat{p}_{nonDTG,j,k}}{\sum_{j=1}^{J} \frac{C_j}{c_j} \sum_{k=1}^{c_j} N_{nonDTG,j,k}}$$

Variance

The variance formulas for both estimates are obtained in the same way as for the DTG-specific resistance estimate, obtained by linearizing the ratio estimator. For regimen i (i=DTG or non-DTG), let $\hat{p}_i = \frac{\hat{z}}{\hat{v}}$ where

$$\hat{z} = \sum_{j=1}^{J} \frac{C_j}{c_j} \sum_{k=1}^{c_j} N_{i,j,k} \, \hat{p}_{i,j,k} \text{ and } \hat{v} = \sum_{j=1}^{J} \frac{C_j}{c_j} \sum_{k=1}^{c_j} N_{i,j,k}.$$

The linearized variance estimator is given by $\widehat{Var}(\hat{p}_i) = \frac{1}{\hat{v}^2} \left(\hat{p}_i^2 \widehat{Var}(\hat{v}) + \widehat{Var}(\hat{z}) - 2\hat{p}_i \widehat{Cov}(\hat{z}, \hat{v}) \right)$,

where $\widehat{Var}(\hat{v}), \widehat{Var}(\hat{z})$, and $\widehat{Cov}(\hat{z}, \hat{v})$ can be estimated by the corresponding sample variances and covariances, which are also obtained through linearization and incorporate the finite population corrections at both stages.

Prevalence and variance of any resistance overall

Prevalence

An unbiased estimate for HIV drug resistance prevalence among all eligible case specimens is of the same form as the DTG- and non-DTG-specific estimates, but it uses data from all sampled specimens from both DTG-containing and non-DTG-containing regimens. To simplify notation, let I denote the set (DTG, non-DTG). The overall prevalence estimate for any drug resistance is given by:

$$\hat{p}_{overall} = \frac{\sum_{j=1}^{J} \frac{C_j}{c_j} \sum_{k=1}^{c_j} \sum_{i\in I} \frac{N_{i,j,k}}{m^*_{i,j,k}} \sum_{l=1}^{m^*_{j,k,l}} Y_{i,j,k,l}}{\sum_{j=1}^{J} \frac{C_j}{c_j} \sum_{k=1}^{c_j} \sum_{i\in I} N_{i,j,k}} = \frac{\sum_{j=1}^{J} \frac{C_j}{c_j} \sum_{k=1}^{c_j} \sum_{i\in I} N_{i,j,k} \hat{p}_{i,j,k}}{\sum_{j=1}^{J} \frac{C_j}{c_j} \sum_{k=1}^{c_j} \sum_{i\in I} N_{i,j,k}}$$

Variance

The variance formula is obtained by linearizing the ratio estimator. Let $\hat{p}_{overall} = \frac{\hat{z}}{\hat{v}}$,

where $\hat{z} = \sum_{j=1}^{J} \frac{C_j}{c_j} \sum_{k=1}^{c_j} \sum_{i \in I} N_{i,j,k} \hat{p}_{i,j,k}$ and $\sum_{j=1}^{J} \frac{C_j}{c_j} \sum_{k=1}^{c_j} \sum_{i \in I} N_{i,j,k}$.

The linearized variance estimator is given by $\widehat{Var}(\hat{p}_{overall}) = \frac{1}{\hat{v}^2}\left(\hat{p}_{overall}^2 \widehat{Var}(\hat{v}) + \widehat{Var}(\hat{z}) - 2\hat{p}_{overall}\widehat{Cov}(\hat{z},\hat{v})\right)$,

where $\widehat{Var}(\hat{v}), \widehat{Var}(\hat{z})$, and $\widehat{Cov}(\hat{z},\hat{v})$ can be estimated by the corresponding sample variances and covariances, which are also obtained through linearization and incorporate the finite population corrections at both stages of sampling.

A6.7.4 Analysis for outcome 3

Outcome 3 is defined as: prevalence of acquired HIV drug resistance among individuals with viral non-suppression and receiving ART, stratified by other key variables.

Subgroup analysis can be performed to estimate the prevalence of acquired HIV drug resistance in subgroups determined by other key variables such as age, gender, ART regimen, ART line, previous ART regimen, pregnancy status and breastfeeding status. Each of these analyses will involve a complete-case analysis that excludes missing data. Those in the subpopulation and not missing the variable of interest are used to calculate the prevalence estimate, and all individuals are used to calculate the standard errors. This allows the randomness of the subgroup size in the sample to be incorporated into variance estimation.

Note that the methods in this section pertain to subgroups where membership is not defined by a stratifying variable. For subgroup analysis in which membership in the subgroup is defined by a stratifying variable, such as viral load laboratory or regimen (DTG, non-DTG, overall), then analysis can proceed as in outcome 1, restricting analysis to the subgroup of interest and using the outcome relevant to the type of resistance desired.

Prevalence and variance for subgroup estimation

Prevalence

Prevalence estimates for the subgroup of interest involve a ratio estimator: the denominator is the number of sampled individuals belonging to the subgroup, and the numerator is the number of sampled individuals belonging to the subgroup with acquired HIV drug resistance.

Recall that the strata are defined by regimen (DTG or non-DTG) and by laboratory, so there are $H=2J$ strata in total, each defined by a specific regimen group and laboratory. These strata can be labelled by $h, h=1,\ldots,H$. Suppose the subgroup of interest is denoted by a, with membership in the subgroup labelled through an indicator variable, $I_{i,j,k,l}(a)$, equal to 1 if individual l on regimen i, in clinic k of laboratory j belongs in the subgroup, and equal to 0 otherwise. The estimated prevalence of drug resistance among those within the subgroup is:

$$\hat{p}_a = \frac{\sum_{j=1}^{J} \frac{C_j}{c_j} \sum_{k=1}^{c_j} \sum_{i \in I} \frac{N_{i,j,k}}{m_{i,j,k}^*} \sum_{l=1}^{m_{j,k,l}^*} I_{i,j,k,l}(a) Y_{i,j,k,l}}{\sum_{j=1}^{J} \frac{C_j}{c_j} \sum_{k=1}^{c_j} \sum_{i \in I} N_{i,j,k} I_{i,j,k,l}(a)},$$

where $Y_{i,j,k,l}$ binary outcome variable for acquired HIV drug resistance for case specimen l on regimen i, in clinic k of laboratory j.

Variance

Variance estimation for the subgroup prevalence is approximated using a linearized variance estimator. The denominator in the prevalence estimate is not fixed because the number of sampled individuals belonging to the subgroup is random and depends on the specific sample that is chosen. To account for this additional sample-to-sample variability, linearized variance estimation incorporates all observations but multiplies the weights of those not in the subgroup by 0. Note that the validity of linearization depends on having a sufficiently large size of the subgroup in the sample. If the subgroup sample size is too small, composite or model-based estimators may be considered, or it may be decided not to conduct the subgroup analysis.

For simplicity, let $\hat{p}_a = \frac{\hat{z}}{\hat{v}}$, where

$$\hat{z} = \sum_{j=1}^{J} \frac{C_j}{c_j} \sum_{k=1}^{c_j} \sum_{i \in I} \frac{N_{i,j,k}}{m_{i,j,k}^*} \sum_{i=1}^{m_{j,k,t}^*} I_{i,j,k,l}(a) \, Y_{i,j,k,l} \quad \text{and} \quad \hat{v} = \sum_{j=1}^{J} \frac{C_j}{c_j} \sum_{k=1}^{c_j} \sum_{i \in I} N_{i,j,k} \, I_{i,j,k,l}(a)$$

The linearized variance estimator is given by

$$\widehat{Var}(\hat{p}_a) = \frac{1}{\hat{v}^2}\left(\hat{p}_a{}^2 \widehat{Var}(\hat{v}) + \widehat{Var}(\hat{z}) - 2\hat{p}_a \widehat{Cov}(\hat{z}, \hat{v})\right),$$

where $\widehat{Var}(\hat{v}), \widehat{Var}(\hat{z}),$ and $\widehat{Cov}(\hat{z}, \hat{v})$ can be estimated by the corresponding sample variances and covariances, which are also obtained through linearization and incorporate the finite population correction at both stages of sampling.

These methods are given for overall prevalence and variance estimates of resistance among a subgroup of interest. For subgroup estimates restricted to a certain regimen or laboratory, simply restrict the data first, then apply these estimation and inference methods.

A6.7.5 Analysis for outcomes 4 and 5

Outcome 4 is defined as: prevalence of viral load suppression (viral load <1000 copies/mL) among individuals receiving ART.

These estimates will be based on information collected during the analysis stage: (1) $T_{i,j,}$ the total number of people receiving regimen i tested and with required variables from sampled clinics in laboratory j during the study period and (2) $T_{i,j}^{VS}$ the number of such people who also had viral suppression.

The prevalence of viral load suppression among people receiving DTG-containing-regimens for whom all required variables were reported, q_{DTG}, is estimated as:

$$\widehat{q_{DTG}} = \frac{\sum_{j=1}^{J} \frac{C_j}{c_j} T_{DTG,j}^{VS}}{\sum_{j=1}^{J} \frac{C_j}{c_j} T_{DTG,j}}$$

Similarly, for non-DTG and overall, the formulas are:

$$\widehat{{}_{non}DTG} = \frac{\sum_{j=1}^{J} \frac{C_j}{c_j} T_{nonDTG,j}^{VS}}{\sum_{j=1}^{J} \frac{C_j}{c_j} T_{nonDTG,j}} \quad \text{and} \quad \widehat{q_{overall}} = \frac{\sum_{j=1}^{J} \frac{C_j}{c_j} (T_{DTG,j}^{VS} + T_{nonDTG}^{VS})}{\sum_{j=1}^{J} \frac{C_j}{c_j} (T_{DTG,j} + T_{nonDT})}$$

Because the survey population is restricted to eligible case specimens during the study period, the prevalence of viral load suppression in these groups can be reported with complete certainty for the clinics sampled. However, uncertainty in both the numerator and denominator must be accounted for when extending these results to the population of interest because we do not have any information from the C_j-c_j clinics that were not sampled.

The variance formulas are obtained by linearizing the ratio estimator for stratified cluster samples. For the estimate among people receiving DTG-containing-regimens, let $\widehat{q_{DTG}} = \frac{\hat{z}}{\hat{v}}$, where $\hat{z} = \sum_{j=1}^{J} \frac{C_j}{c_j} T_{DTG,j}^{VS}$ and $\hat{v} = \sum_{j=1}^{J} \frac{C_j}{c_j} \cdot T_{DTG,j}$. The variance estimator is

$$\widehat{Var}(\widehat{q_{DTG}}) = \frac{1}{\hat{v}^2}\left(\widehat{Var}(\hat{z}) + \widehat{q_{DTG}}{}^2 \widehat{Var}(\hat{v}) - 2\widehat{q_{DTG}} \widehat{Cov}(\hat{z}, \hat{v})\right),$$

where $\widehat{Var}(\hat{v}), \widehat{Var}(\hat{z}),$ and $\widehat{Cov}(\hat{z}, \hat{v})$ can be estimated by the corresponding sample variances and covariances, which are also obtained through linearization and incorporate the finite population corrections at both stages. Let $T_{DTG,j,k}$ denote the total number of people receiving regimen i tested and with required variables from clinic k in laboratory j during the study period, and let $T_{DTG,j,k}^{VS}$ be the corresponding number who also have viral suppression. $\widehat{Var}(\hat{v}), \widehat{Var}(\hat{z}),$ and $\widehat{Cov}(\hat{z}, \hat{v})$ are given by:

- $\widehat{Var}(\hat{z}) = \sum_{j=1}^{J} \left(1 - \frac{c_j}{C_j}\right) \frac{c_j}{c_j - 1} \sum_{k=1}^{c_j} \left(T_{DTG,j,k}^{VS} - \frac{T_{DTG,j}^{VS}}{c_j}\right)^2$

- $\widehat{Var}(\hat{v}) = \sum_{j=1}^{J} \left(1 - \frac{c_j}{C_j}\right) \frac{c_j}{c_j - 1} \sum_{k=1}^{c_j} \left(T_{DTG,j,k} - \frac{T_{DTG,j}}{c_j}\right)^2$

- $\widehat{Cov}(\hat{z}, \hat{v}) = \sum_{j=1}^{J} \left(1 - \frac{c_j}{C_j}\right) \frac{c_j}{c_j - 1} \sum_{k=1}^{c_j} \left(T_{DTG,j,k}^{VS} - \frac{T_{DTG,j}^{VS}}{c_j}\right)\left(T_{DTG,j,k} - \frac{T_{DTG,j}}{c_j}\right).$

The variance estimates for $q_{\widehat{nonDTG}}$ and $q_{\widehat{overall}}$ are similarly obtained.

Outcome 5 is defined as: prevalence of viral load suppression (viral load <1000 copies/mL) among individuals receiving ART, stratified by other key variables.

We will use the same approach as described for outcome 4 above to estimate the prevalence of viral load suppression in subgroup a, with information provided on: (1) $T_{i,a,j}$, the total number of people receiving regimen i tested and with required variables in subgroup a from laboratory j during the study period and (2) $T^{VS}_{i,a,j}$, the number of those tested in that subgroup who also had viral suppression. We will use these quantities to estimate:

$$\widehat{q_{DTG,a}} = \frac{\sum_{j=1}^{J} \frac{C_j}{c_j} \cdot T^{VS}_{DTG,a,j}}{\sum_{j=1}^{J} \frac{C_j}{c_j} \cdot T_{DTG,a,j}} \quad \text{for people in subgroup a receiving DTG-containing regimens;}$$

$$\widehat{q_{DTG,a}} = \frac{\sum_{j=1}^{J} \frac{C_j}{c_j} \cdot T^{VS}_{nonDTG,a,j}}{\sum_{j=1}^{J} \frac{C_j}{c_j} \, T_{nonDTG,a,j}} \quad \text{for people in subgroup a receiving non-DTG-containing regimens; or}$$

$$\widehat{q_a} = \frac{\sum_{j=1}^{J} \frac{C_j}{c_j} \cdot (T^{VS}_{DTG,a,j} + T^{VS}_{nonDTG,a,j})}{\sum_{j=1}^{J} \frac{C_j}{c_j} \, (T_{DTG,a,j} + T_{nonDTG,a,j})} \quad \text{for overall viral load suppression in subgroup a.}$$

Similar to outcome 4, these estimates will have some uncertainty because not all clinics are sampled. The variance for $\widehat{q_{DTG,a}}$ is given by:

$$\widehat{Var}(\widehat{q_{DTG,a}}) = \frac{1}{\hat{v}^2}\left(\widehat{Var}(\hat{z}) + \hat{p}^{*2}_{DTG}\widehat{Var}(\hat{v}) - 2\hat{p}^{*}_{DTG}\widehat{Cov}(\hat{z},\hat{v})\right),$$

where $\widehat{q_{DTG,a}} = \frac{\hat{z}}{\hat{v}}$ and

- $\widehat{Var}(\hat{z}) = \sum_{j=1}^{J}\left(1 - \frac{c_j}{C_j}\right)\frac{c_j}{c_j - 1}\sum_{k=1}^{c_j}\left(T^{VS}_{DTG,a,j,k} - \frac{T^{VS}_{DTG,a,j}}{c_j}\right)^2$

- $\widehat{Var}(\hat{v}) = \sum_{j=1}^{J}\left(1 - \frac{c_j}{C_j}\right)\frac{c_j}{c_j - 1}\sum_{k=1}^{c_j}\left(T_{DTG,a,j,k} - \frac{T_{DTG,a,j}}{c_j}\right)^2$

- $\widehat{Cov}(\hat{z},\hat{v}) = \sum_{j=1}^{J}\left(1 - \frac{c_j}{C_j}\right)\frac{c_j}{c_j - 1}\sum_{k=1}^{c_j}\left(T^{VS}_{DTG,a,j,k} - \frac{T^{VS}_{DTG,a,j}}{c_j}\right)\left(T_{DTG,j,k} - \frac{T_{DTG,a,j}}{c_j}\right)$.

The variance estimates for $q_{\widehat{nonDTG,a}}$ and $q_{\widehat{overall,a}}$ are similarly obtained.

A6.8 Stata code for the alternative method described in Section 5

User-friendly instructions are provided below for data analysis in Stata. To use the code, data must follow the format described in subsection 4.3, with patient-level and site-level information following the configuration of the Excel data upload template and HIV drug resistance sequences in FASTA file format. Sequence identification numbers and eligible case specimen identifiers must be identical and follow WHO convention.

In Stata, estimation and inference can be implemented using the svy package. The default variance estimation used is linearization (based on a first-order Taylor series linear approximation). All variance computations include finite population corrections. Alternative statistical packages can be used to perform data analysis as long as they properly adjust for survey weights, clustering and stratification (if necessary). All statistical packages are expected to yield identical point estimates, but not all statistical packages are expected to yield identical standard error estimates and confidence intervals. Statistical

packages that do not allow users to specify the finite population correction at each stage of sampling will overestimate the standard error, especially in countries with small eligible populations.

Subsections A6.8.1–A6.8.6 provide Stata code for processing and combining the two data sets, and subsections A6.8.7–A6.8.10 provide code for analysing the survey outcomes. All code displayed can be found in a downloadable Stata do-file that can run all pre-processing and analysis instructions at once.

A6.8.1 Import viral load laboratory and patient-level data into Stata

Begin by importing viral load laboratory and patient-level data from the Excel data capture tool.

1. To start, one may choose to create a do-file so that commands can be saved and then run. Click on the notepad icon corresponding to "New Do-file Editor" on the top-left corner of the Stata viewer, then save the do-file that is created.

2. Clear any previous output and set the working directory to the directory containing the data files. For example, if the directory is "C:/Documents", run the following code:

```
clear
```

```
cd "C:/Documents"
```

3. Import each sheet of the Excel data capture tool file, storing the first row as headers, changing header names to uppercase and saving each sheet as its own .dta file using uppercase letters. Given the Excel file name "patient_data_A2.xlsx", run:

```
import excel using "patient_data_A2.xlsx", describe
```

```
forvalues sheet=1/`=r(N_worksheet)' {
```

```
    local sheetname = r(worksheet_`sheet')
```

```
    import excel using patient_data_A2, sheet("`sheetname'") firstrow case(upper)
```

```
    local sheetname = upper(subinstr("`sheetname'", " ", "_", .))
```

```
    save "`sheetname'", replace
```

```
    clear
```

```
}
```

The Excel file should contain six sheets titled: (1) survey information, (2) VL lab information, (3) ART clinic information, (4) configuration, (5) survey participants and (6) participant treatments.

A6.8.2 Import HIV drug resistance data into Stata

1. Import the HIV drug resistance data, storing the first row as headers and changing all header names to uppercase. Given the file name "FASTA_A2.xlsx", run:

```
import excel using "FASTA_A2.xlsx", sheet("ResistanceSummary") firstrow case(upper)
```

The resistance data file should be an Excel file containing one sheet entitled "ResistanceSummary".

2. Rename SEQUENCENAME as PARTICIPANTID. Drop all cells without a subject ID. Drop all unnecessary variables. Replace NAs as missing.

```
rename SEQUENCENAME PARTICIPANTID
```

```
drop if missing(PARTICIPANTID)
```

```
drop *SCORE ALGORITHM* STRAIN GENES PI* NRTI* NNRTI* INSTI*
```

```
destring, ignore("NA") replace
```

3. For each of the resistance level variables, reclassify the variable as a binary resistance indicator, with levels 1-2 corresponding to susceptible (no HIV drug resistance), and levels 3-5 corresponding to HIV drug resistance. Rename resistance type variables.

```
ds *LEVEL

local plist = r(varlist)

foreach i of local plist {

    replace `i' = 0 if `i' < 3 & !missing(`i')

    replace `i' = 1 if `i' >= 3 & !missing(`i')

}

rename *LEVEL *_RES
```

4. Generate variables of DTG-specific resistance, any boosted PI resistance, any NRTI resistance, any NNRTI resistance, any INI resistance and any acquired HIV drug resistance, according to the definitions in subsection 4.3.

```
gen DTG_ADR = DTG_RES

egen ANY_PI = rowmax(ATVR_RES DRVR_RES LPVR_RES)

egen ANY_NRTI = rowmax(ABC_RES AZT_RES D4T_R DDI_RES FTC_RES TDF_RES)

egen ANY_NNRTI = rowmax(EFV_RES NVP_RES)

egen ANY_INI = rowmax(BIC_RES DTG_RES EVG_RES RAL_RES)

egen ANY_ADR = rowmax(ANY_PI ANY_NRTI ANY_NNRTI ANY_INI)
```

5. Save the modified HIV drug resistance data as a .dta file. In this example, we save the data as "RESISTANCE_SUMMARY.dta".

```
save RESISTANCE_SUMMARY, replace
```

A6.8.3 Prepare clinic-level data

1. Remove the previous data set, then load the clinic-level data, stored as "ART_CLINIC_INFORMATION.dta", and rename the variables for total DTG and non-DTG case specimens.

```
clear

use ART_CLINIC_INFORMATION.dta

rename NELIGIBLEDTGCASESPECIMENS NDTG

rename NELIGIBLENONDTGCASESPECIMENS NNONDTG
```

2. Save the modified data as a .dta file. In this example, we save the data as "ART_CLINIC_INFORMATION.dta".

```
save ART_CLINIC_INFORMATION, replace
```

A6.8.4 Prepare patient-level data on ARV drug treatment regimen

1. Remove the previous data set and then load the treatment regimen data, stored as "PARTICIPANT_TREATMENTS.dta".

```
clear

use PARTICIPANT_TREATMENTS.dta
```

2. Exclude observations missing a subject ID or corresponding to past ART. Drop unnecessary variables and rename ARV drug types so that all variable names begin with a letter.

```
drop if missing(PARTICIPANTID) | upper(CURRENTARTYN) == "N"
```

```
drop OTHERARVDRUG CURRENTARTYN
```

```
replace ARVDRUG = "ARV_" + ARVDRUG
```

3. Generate an indicator variable of DTG-based ART, equal to 1 if a person is taking a DTG-containing regimen and 0 if a person is taking a non-DTG-containing regimen.

```
gen TEMP_DTG = cond(inlist(ARVDRUG, "ARV_DTG", "ARV_TLD", "ARV_JUL"), 1, 0)
```

```
by PARTICIPANTID, sort: egen DTG = max(TEMP_DTG)
```

```
drop TEMP_DTG
```

4. Reformat the ARVDRUG variable so that each ARV drug type is created as a new binary variable, set to 1 if the person's regimen includes the drug and 0 if not.

```
gen ON = 1
```

```
reshape wide ON, i(PARTICIPANTID) j(ARVDRUG) string
```

```
rename ON* *
```

5. Save the modified data as a .dta file. In this example, we save the data as "PARTICIPANT_TREATMENTS.dta".

```
save PARTICIPANT_TREATMENTS, replace
```

A6.8.5 Prepare patient-level data on other variables

1. Remove the previous dataset and then load the patient-level data, stored as "SURVEY_PARTICIPANTS.dta". Drop observations with missing subject ID or with children and adolescents.

```
clear
```
```
use SURVEY_PARTICIPANTS.dta
```
```
drop if missing(PARTICIPANTID) | substr(PARTICIPANTID, 23, 1) != "a"
```

2. Generate a variable named "VLLABCODE" that corresponds to the viral load laboratory for each person.

```
gen VLLABCODE = substr(PARTICIPANTID, 14, 3)
```

3. Recode unknown values as missing.

```
recode DATE* (9999 = .)
```
```
recode AGE (-9 = .)
```
```
foreach var of varlist PRIORART BREASTFEEDINGSTATUS PREGNANCYSTATUS CURRENTART {
```
```
    replace `var' = "." if `var' == "UNK"
```
```
}
```

4. Save the modified data as a .dta file. In this example, we save the data as "SURVEY_PARTICIPANTS.dta".

```
save SURVEY_PARTICIPANTS.dta, replace
```

A6.8.6 Merge all data sets

1. Use a many-to-one merge to merge the viral load laboratory data on total case specimens.

```
merge m:1 VLLABCODE using VL_LAB_INFORMATION, keepusing(NARTCLINICS) keep(match) nogenerate
```

2. Use a many-to-one merge to merge the clinic-level data on total case specimens and clinic-level metadata.

```
merge m:1 CLINICCODE using ART_CLINIC_INFORMATION, keepusing(NDTG NNONDTG) keep(match) nogenerate
```

3. Merge in the treatment regimen data by subject ID.

```
merge 1:1 PARTICIPANTID using PARTICIPANT_TREATMENTS, keep(match) nogenerate
```

4. Merge in the HIV drug resistance data by subject ID.

```
merge 1:1 PARTICIPANTID using RESISTANCE_SUMMARY, keep(match) nogenerate
```

5. Save the combined and reorganized data as a .dta file. In this example, we save the data as "ALL_DATA.dta".

```
save ALL_DATA.dta, replace
```

A6.8.7 Create survey weights and other necessary variables and declare survey design

1. Remove the previous dataset and load in the combined data.

```
clear
```

```
use ALL_DATA.dta
```

2. Create the first level of stratification by laboratory.

```
egen STRATA1 = group(VLLABCODE)
```

3. Create the second level of stratification: DTG versus non-DTG regimen.

```
egen STRATA2 = group(DTG)
```

4. Generate the variable for total DTG and non-DTG case specimens per sampled clinic. This corresponds to the second-stage populations.

```
gen CLINIC_POP = cond(DTG == 1, NDTG, NNONDTG)
```

5. Generate the first stage sampling weight, calculated as the total number of clinics per laboratory, divided by the sampled number of clinics per laboratory.

```
by VLLABCODE, sort: gen WEIGHT1 = NARTCLINICS/_N
```

6. Generate the second stage sampling weight, calculated as the total number of eligible case specimens per regimen and clinic, divided by the number sampled.

```
by CLINICCODE DTG, sort: gen WEIGHT2 = CLINIC_POP/_N
```

7. Generate the sampling weight as the product of the first- and second-stage sampling weights.

```
gen WEIGHTS = WEIGHT1 * WEIGHT2
```

8. Set the stratified two-stage clustered survey design with finite population correction. If there exists a stratum or multiple strata with only one unit sampled, sampling errors cannot be estimated for all strata independently, and Stata will report a missing standard error. We recommend addressing this by setting the standard errors for single-unit strata to be the average of the standard errors for other strata. This is represented by the single unit (scaled) term in the code below.

```
svyset CLINICCODE [pweight = WEIGHTS], strata(STRATA1) fpc(NARTCLINICS) || _n, strata(STRATA2) fpc(CLINIC_
    POP) singleunit(scaled)
```

A6.8.8 Analysis for outcomes 1 and 2

1. Obtain estimates and confidence intervals for the prevalence of overall drug resistance among everyone. In the output, the point estimate, standard error and 95% confidence interval of interest are located in the row labelled "1".

```
svy: proportion ANY_ADR
```

This command gives confidence intervals expressed on the logit scale. For Wald confidence intervals, simply add citype(wald)to the end:

```
svy: proportion ANY_ADR, citype(wald)
```

The design effect can also be obtained:

```
estat effects
```

The estimated ICC can be reported as well:

```
svyset CLINICCODE, weight(WEIGHT1) strata(STRATA1) fpc(NARTCLINICS) || _n, weight(WEIGHT2) strata(STRATA2) fpc(CLINIC_POP) singleunit(scaled)
```

```
svy: melogit ANY_ADR || CLINICCODE:
```

```
estat icc
```

2. Obtain estimates and confidence intervals for the prevalence of overall drug resistance among people receiving DTG regimens.

```
svy, subpop(if DTG==1): proportion ANY_ADR
```

3. Obtain estimates and confidence intervals for the prevalence of overall drug resistance among individuals receiving non-DTG regimens.

```
svy, subpop(if DTG==0): proportion ANY_ADR
```

4. Obtain estimates and confidence intervals for the prevalence of DTG-specific drug resistance among individuals receiving DTG-containing regimens.

```
svy, subpop(if DTG==1): proportion DTG_ADR
```

A6.8.9 Analysis for outcome 3

Some examples of subgroup analysis are given below.

1. Obtain prevalence and variance estimates of overall drug resistance among men.

```
svy, subpop(if GENDER == "M"): proportion ANY_ADR
```

2. Obtain prevalence and variance estimates of any NRTI drug resistance among individuals receiving DTG regimens.

```
svy, subpop(if DTG==1): proportion ANY_NRTI
```

A6.8.10 Analysis for outcome 4

1. Remove the previous dataset and load in the clinic-level data.

```
clear
```

```
use ART_CLINIC_INFORMATION.dta
```

2. Use a many-to-one merge to merge the viral load laboratory data on number of ART clinics per laboratory.

```
merge m:1 VLLABCODE using VL_LAB_INFORMATION, keepusing(NARTCLINICS) keep(match) nogenerate
```

3. Generate the sampling weights, calculated as the total number of clinics per laboratory, divided by the sampled number of clinics per laboratory.

```
by VLLABCODE, sort: gen WEIGHTS = NARTCLINICS/_N
```

4. Set the survey design with finite population correction. Set the standard errors for single-unit strata to be the average of the standard errors for other strata.

```
svyset _n [pw = WEIGHT1], strata(VLLABCODE) fpc(NARTCLINICS) singleunit(scaled)
```

5. Obtain estimates and confidence intervals for the prevalence of viral load suppression among individuals receiving DTG-based ART.

```
ratio TDTGVS / TDTG
```

6. Obtain estimates and confidence intervals for the prevalence of viral load suppression among individuals receiving non-DTG-based ART.

```
ratio TNONDTGVS / TNONDTG
```

7. Obtain estimates and confidence intervals for the prevalence of viral load suppression among all individuals receiving ART.

```
gen TOTALVS = TDTGVS + TNONDTGVS
```

```
gen TOTAL = TDTG + TNONDTG
```

```
ratio TOTALVS/TOTAL
```

A6.8.10 Analysis for outcome 5

Some examples of subgroup analysis are given below.

1. Obtain estimates and confidence intervals for the prevalence of viral load suppression among men receiving DTG-based ART.

```
ratio TDTGVSMEN / TDTGMEN
```

2. Obtain estimates and confidence intervals for the prevalence of viral load suppression among individuals 0–9 years old.

```
gen TOTALVSAGE09 = TDTGVSAGE09 + TNONDTGVSAGE09
```

```
gen TOTALAGE09 = TDTGAGE09 + TNONDTGAGE09
```

```
ratio TOTALVSAGE09 / TOTALAGE09
```

Reference

1. Surveillance of HIV drug resistance in adults receiving ART (acquired HIV drug resistance). Geneva: World Health Organization; 2014 (https://www.who.int/hiv/pub/drugresistance/acquired_drugresistance/en, accessed 21 December 2020).

ANNEX 7. EXAMPLE: SAMPLE SIZE CALCULATIONS AND ALLOCATION ACROSS STRATA USING THE METHODS DESCRIBED IN SECTION 5

This annex provides an example of how to calculate the sample size of eligible case specimens and allocate it across strata using the WHO online sample size calculator (available at https://worldhealthorg.shinyapps.io/ADR_LabBasedMethod_2/) for the alternative approach described in Section 5.

A7.1 Example: the first design phase to determining the required sample sizes and number of clinics to sample

At this phase, countries engaging in this version of acquired HIV drug resistance surveillance should have all the information needed to: (1) budget for a proposal, (2) begin planning and (3) sample clinics that will undergo more intensive interventions to aid the completion of laboratory requisition forms. This annex provides examples of the steps for the first phase of design, described in subsections 5.3.1 to 5.3.4.

Inputs

Suppose a country has three laboratories, with 100 clinics distributed among the laboratories as indicated in Table A7.1. In the online sample size calculator, the user can begin by specifying the number of laboratories and then entering the number of clinics served by each laboratory (see Fig. A7.1). For clarity, the user can also input the laboratory names in the input table to make it easier to track the laboratory sample allocation.

Table A7.1. **Example of the number of clinics served by each viral load testing laboratory**

	Laboratory 1	Laboratory 2	Laboratory 3	Total
Number of clinics served, C_j	30	50	20	100

Fig. A7.1. **Online sample size calculator: example input number of clinics served by each viral load testing laboratory**

Next, the user can fill in the available national-level historical data (Fig. A7.2). After specifying the anticipated proportion of people (adults or children and adolescents) with all required survey variables, the user clicks on the type of historical data available (numbers with viral non-suppression, viral load testing or receiving ART) and fills in the number of people in the specified category among those on DTG-containing and non-DTG-containing regimens, along with any adjustments necessary for the approximations. Fig. A7.2 specifies viral load testing data. These data are used to calculate the proportion of eligible case specimens from people receiving DTG- and non-DTG-containing regimens and can also be used for the finite population correction, if desired.

The anticipated number of DTG and non-DTG eligible case specimens is shown below the historical outputs, and these numbers can guide whether or not the user wishes to apply the finite population correction to reduce the required sample sizes. The finite population correction is not necessary if the anticipated number of eligible case specimens is large (such as more than 300 000 DTG-eligible case specimens). In this example, the anticipated number of eligible case specimens remains fairly small (3240 DTG and 2040 non-DTG), and thus the finite population correction is used (Fig. A7.2).

Fig. A7.2. Online sample size calculator: example input national-level historical data of adults receiving ART and finite population correction

The next inputs for the first design phase involve historical clinic-level data (Fig. A7.3). If historical data on the median number of people per clinic is available, the user selects "yes", then specifies the type of the data and inputs the median sizes as well as any necessary adjustment proportions into the highlighted boxes. If this data is not available, the user clicks "no," and the minimum number of clinics will be based on the mean clinic sizes, estimated using the national-level historical data previously specified. The minimum number of clinics recommended to be sampled is shown in red below the inputs. For this example, median clinic sizes for viral load testing are available, and a minimum number of 20 clinics is recommended.

Finally, the user enters the actual number of clinics to be sampled for the survey (Fig. A7.3). This number should be at least this minimum recommended value and is ideally larger, if the budget allows. After this information is entered, the user clicks "Submit".

Fig. A7.3. Online sample size calculator: example input clinic-level historical data of adults receiving ART and number of clinics to be sampled

Output

WHO's online sample size calculator automatically calculates all of the details for sample size calculations. The user can then click on the "Output: Sample Sizes" tab on the side panel. On this page (Fig. A5.4), the required sample sizes for DTG and overall are reported and are equal to n_{DTG} = 295 and $n_{overall}$ = 254 in this example. The online calculator also calculates the proportion of the eligible case specimens that are from non-DTG eligible case specimens (prop$_{nonDTG}$ = 0.39) as well as the required sample size for people taking non-DTG-containing regimens (n_{nonDTG} = 99). Therefore, the total required sample size for this example is $n_{DTG} + n_{nonDTG}$ =394. In addition, the output displays the target sample sizes, in which the required sample sizes have been inflated for the design effect of 1.5 and a 30% genotyping failure rate. The final target sample sizes are m_{DTG} = 633, m_{nonDTG} = 213 and the total: $m_{DTG} + m_{nonDTG}$ =846.

Fig. A7.4. Online sample size calculator: example output of sample size of eligible case specimens from adults receiving DTG-containing ART, non-DTG-containing ART and the total sample size

Next, the user can click on the "Output: Allocation Across Laboratories" button (Fig. A7.5). On this page, the number of clinics to be sampled has been proportionally distributed across the laboratories. For example, laboratory 1 will need to sample 6 clinics ($c_{Lab1} = 6$) because it serves 30% of the total clinics in the country, so it should also contribute 30% of the clinics to the sample. Note that the total number of clinics to sample may differ slightly from those reported on the inputs tab due to rounding. Countries may choose to adjust the number of clinics sampled depending on feasibility.

Fig. A7.5. Online sample size calculator: example allocation of clinics to sample across viral load testing laboratories
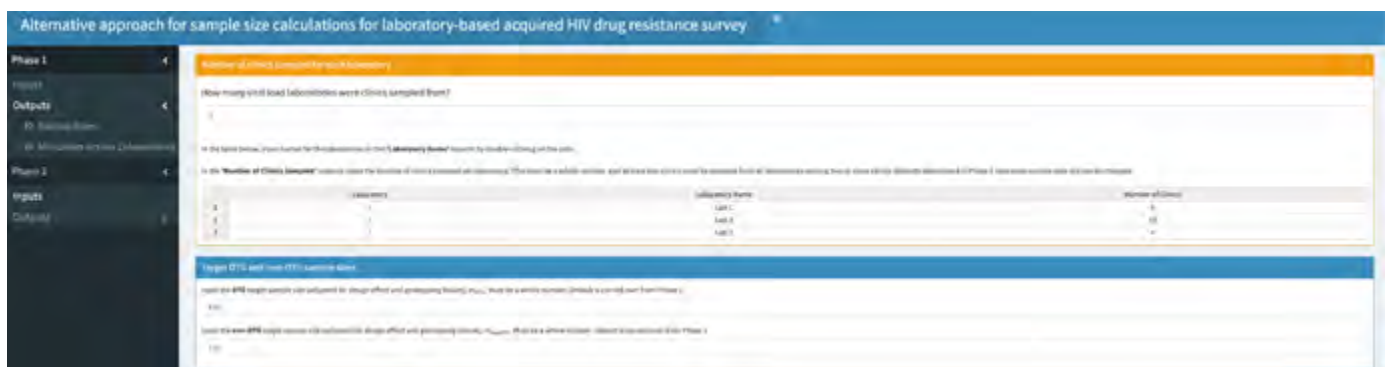
A7.2   Example: the second design phase to allocate the sample size of eligible case specimens to each sampled clinic

Inputs

In the second design phase, the user can navigate to the "Phase 2: Inputs" tab of the WHO online sample size calculator (available at https://worldhealthorg.shinyapps.io/ADR_LabBasedMethod_2/) for the alternative approach described in Section 5 used for the first design phase and begin by inputting the number of laboratories from which clinics were sampled, the number of clinics sampled from each of these laboratories and the target DTG and non-DTG sample sizes (Fig. A7.6). The default values will be those determined from the first design phase inputs. The user can input the laboratory names in the input table to make it easier to track the laboratory sample allocation.

Next, the user can fill in the total number of DTG and non-DTG eligible case specimens from each of the sampled clinics (Fig. A7.7). For clarity, the user can input the clinic names in the input table to make it easier to track the clinic sample allocation. After this information is entered, the user clicks "Submit".

Fig. A7.6. Online sample size calculator: example input number of clinics sampled from each viral load testing laboratory

Fig. A7.7. Online sample size calculator: example input number of adult DTG and non-DTG case specimens, by clinic

Output

The WHO online calculator automatically allocates the number of eligible case specimens that should be sampled from each clinic to meet the target sample sizes. To view this, the user can click on the "Output: Allocation Across Clinics" tab (Fig. A7.8). On this page, the number of eligible case specimens to be sampled has been proportionally distributed across the clinics. For example, laboratory 1 clinic 1 will need to sample 16 DTG eligible case specimens and 4 non-DTG eligible case specimens ($m_{DTG,Lab1,Clinic1} = 16$ and $m_{nonDTG,Lab1,Clinic1} = 4$). Note that, due to rounding, the total number of case specimens to sample may differ slightly from those reported on the sample size output tab. For example, the target DTG sample size for this example will be 643, in contrast to the 633 calculated in the first design phase.

The examples above are for adults receiving ART. For countries implementing surveillance of acquired HIV drug resistance among children and adolescents, analogous sampling to that described in subsections 5.1 to 5.5 (Fig. A7.1 to A7.8.) must be completed.

Fig. A7.8. Online sample size calculator: example allocation of DTG and non-DTG case specimens for adults, by clinic

ANNEX 8. BUDGET CONSIDERATIONS

Below is an example budget for countries implementing the survey and that have data availability >80%. In this example, there are 40 000 remnant viral load specimens with available data from adults and 7000 remnant viral load specimens with available data from children and adolescents. The majority of remnant specimens with data from adults (80%) are from adults receiving DTG-containing regimens and the remainder, 20%, are from adults receiving PI/r or NNRTI-based regimens. Children and adolescents are receiving a mix of regimens, and only overall acquired HIV drug resistance is being estimated in this population. In this example, the required sample size for remnant specimens from adults taking DTG-based regimens is 322. The target sample size from adults taking DTG-based ART is 461 (adjusted for genotyping failure). The required sample size of remnant specimens from adults taking non-DTG-containing regimens is 54, and the adjusted target sample size is 78. Thus, the total survey sample sized is 376 (adjusted for genotyping failures is 539).

The overall sample size for children and adolescents is 257 and adjusted for genotyping failure is 368.

Number of viral load testing laboratories	4				**Total**
Sample size	539 adults and 368 children and adolescents				
Protocol development and training	**Number of staff members**	**Transport costs**	**Per diem payment**	**Number of nights**	
Training of laboratory staff (1 day)	8 (2 per laboratory)	200	150	2	4 000
Production of protocol and training materials					10 000
Survey coordination	**Number of staff**	**Cost per month**	**Number of months**		
National coordinator	1	1 000	6		6 000
Data manager	1	800	4		3 200
Viral load laboratory survey coordinator and viral load laboratory data manager (per laboratory)	2	800	4		6 400
Laboratory (genotyping for reverse transcriptase, protease and integrase; costs including labour)	**Number of specimens (adults and children and adolescents combined)**	**Per unit**			
Genotyping (low end)	907	70			63 490
Genotyping (WHO HIVResNet median)	907	150			136 050
Genotyping (high end)	907	350			317 450
Shipment of specimens to a WHO-designated laboratory (outside the country)					5 000
Technical support					

Consultant and protocol development, data analysis and report writing and flight (US$ 550 for 20 days and daily per diem USD$ 200 for 7 days); international flight USD$ 3000					15 400
Statistical consultant – support statistical analysis (USD$ 550 per day for 12 days)					6 600
Report production, printing and distribution					
Report production and distribution					10 000
Workshop to discuss policy implications and actions (15 outside participants, 15 local)					10 000
			Total (US$ 70 per test)		**140 090**
			Total (US$ 150 per test)		**212 650**
			Total (US$ 350 per test)		**394 050**

Countries implementing the alternative method outlined in Section 5 because they have viral load testing coverage ≥60% but data availability <80% must account for additional costs. Specifically, countries must have in place local methods to support the completion of viral load requisition forms to ensure that all mandatory required data are available on viral load requisition forms during the designated three-month survey period. Countries using this alternative method must sample a minimum of 20 clinics (at least two within each laboratory catchment area). To this end, it is recommended that countries budget at least USD$ 60 000 for supporting viral load requisition forms. This amount permits two people at USD$ 500 per month at each of 20 clinics to work to support the activity for three months.

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