World Health Organization Protocol for Cross Sectional Surveillance of Acquired HIV Drug Resistance in Populations Failing First-line Antiretroviral Therapy
Protocol Summary

1 Introduction

1.1 Overview

In 2001, the United Nations General Assembly Special Session on HIV/AIDS (UNGASS) recommended that antiretroviral (ARV) drugs should be made available in resource-limited countries to address the disparity between rich and poor countries regarding access to antiretroviral therapy (ART). Following this recommendation, the World Health Organization (WHO) elaborated public health guidelines to support and facilitate the implementation of ART in resource-limited settings. Key components of the guidelines include 1) Standardization and simplification of ARV regimens; and 2) Use of science-based evidence to support treatment protocols and avoidance of substandard treatment leading to poor outcome and emergence of HIV drug resistance (HIVDR).1

In 2003, WHO and the Joint United Nations Programme on HIV/AIDS (UNAIDS) created the 3x5 initiative which established the goal of placing 3 million eligible HIV positive patients on ART by 2005.2 To support countries in Africa, the Caribbean and Asia that are heavily affected by the HIV/AIDS epidemic, the President’s Emergency Plan for AIDS Relief (PEPFAR) was launched in 2003. The goals of this plan were to treat 2 million patients with ART and prevent 7 million new HIV infections over the following 5 years. By 2010, 6.6 million adults and children in low and middle-income countries were receiving ART,3 and in June 2010 UNGASS set a goal of initiating 15 million individuals worldwide on ART by 2015.4

The use of ART in developed countries has been associated with the development of HIVDR.5 Because of the error-prone nature of HIV reverse transcriptase, the virus’ high mutation rate in the presence of drug selective pressure, and because of the need for lifelong treatment, it is anticipated that some degree of HIVDR will occur among populations on treatment even when appropriate ART regimens are provided and optimal adherence to therapy is supported.6 ART scale up has been successful using a public health approach including use of standardized protocols and simplified patient monitoring. In countries using a population-based model of ART delivery, population based tools for assessing emergence, transmission and prevention of HIVDR are required.7,8
In response to concerns regarding emergence and transmission of HIVDR, the WHO in collaboration with the United States Centers for Disease Control and Prevention (US-CDC) and WHO HIVResNet (an advisory body of experts from over 50 institutions) developed a global strategy for the assessment and prevention of HIVDR.\textsuperscript{7,8} The foundation of WHO's HIVDR prevention and assessment strategy is the routine monitoring of HIVDR Early Warning Indicators (EWIs) at all ART clinics, or a large number of representative clinics. HIVDR EWIs assess factors known to create situations favourable to the emergence of HIVDR. Data are abstracted from routine medical and pharmacy records and EWI monitoring is supported by standardized guidance and data abstraction tools. Monitored annually, HIVDR EWIs provide clinic specific information which is used to make clinic and program level adjustments to optimize patient care, thus minimizing factors that may contribute to the emergence of HIVDR. HIVDR EWIs are supplemented by results from surveys of transmitted and acquired drug resistance.

Transmitted HIVDR occurs when a previously uninfected individual is infected with a drug resistant HIV strain. The WHO recommends surveys of transmitted HIVDR to classify prevalence of HIVDR in recently infected populations in specific geographic regions. Acquired HIVDR occurs when individuals develop mutations while on ART often due to treatment interruptions, poor adherence, inadequate drug concentrations or the use of suboptimal drug regimens. Surveys of acquired HIVDR assess the emergence of HIVDR and associated clinic and programme factors in sentinel ART clinics within countries.

As of July 2011, 53 surveys of acquired HIVDR have been implemented. Notably implementation of current surveys of acquired HIVDR has been challenging because the survey's prospective method requires a relatively large number of patients (N~130) and 12-15 months of follow-up before endpoint classifications can be made. While the prospective survey has provided comprehensive results including genotypes prior to ART initiation and has identified predictors of acquired HIVDR at sentinel ART clinics, implementation challenges have limited its widespread use and have made assessment of acquired HIVDR, at large numbers of ART clinics representative of national ART programmes, difficult. These challenges have led to descriptions of HIVDR which are unlikely to represent the true picture of HIVDR in populations failing ART.
To address these limitations, WHO in collaboration with the US-CDC, PEPFAR, and WHO HIVResNet developed a simpler retrospective cohort survey to assess acquired HIVDR at clinics providing ART in resource limited settings. The new survey method is designed to have a maximum specimen collection period of 3 months and should be easier to implement in large numbers of sentinel ART clinics thus providing a more representative description of acquired HIVDR within countries scaling up ART. Furthermore, because it is anticipated that WHO HIVDR EWIs will be routinely monitored at ART clinics implementing the retrospective cohort survey, links between EWIs and HIVDR will be strengthened.

1.2 Brief overview of the survey method

This protocol outlines a retrospective cohort survey method for assessing acquired HIVDR. The goals of this survey are to:

- Classify the proportion of adult patients with virological failure 12-15 and 24-36 months after initiation of first-line ART
- Classify the proportion of paediatric patients with virological failure ≥ 12 months after initiation of first-line ART
- Describe detected HIVDR and describe differences in population level virological suppression observed in sentinel ART clinics which represent different models of care delivery

The survey uses a two stage sampling strategy. The primary sample will identify sentinel ART clinics within a national ART programme where the survey will be conducted. Lot Quality Assurance Sampling (LQAS) will determine the secondary sample to assess HIVDR among a small consecutive sample of patients attending the sentinel clinics. This survey is designed such that future rounds of survey implementation are performed by repeating the primary sampling method to identify a new sample of sentinel ART clinics. At each sentinel ART clinic, a secondary sample of patients will be obtained defined by the duration of time since first-line ART initiation.

At sentinel adult ART clinics, two samples are obtained:

1.) Adults 12-15 months after ART initiation
2.) Adults 24-36 months after ART initiation
   At sentinel paediatric ART clinics, one sample will be obtained:
   1.) Paediatric patients ≥ 12 after ART initiation
      Operationally, at sentinel clinics sampling will occur simultaneously and any one
      individual patient will never be in both samples.
      Patients included in the survey will have dried blood spot (DBS) specimens collected for
      viral load testing. Specimens with a viral load ≥ 1000 copies/mL will be genotyped to determine
      HIVDR.

      Several characteristics of the LQAS method make it appropriate to use in this survey. These
      characteristics include simplicity of its sampling method and low cost due to small sample
      sizes. The basis of LQAS theory is the identification and counting of ‘defects’ which in this
      survey are patients with virological failure during the defined time period after initiation of first-
      line ART. Using LQAS, the proportion of survey participants with virological failure will be
      classified as high or low. Since ART clinics will be stratified into models of care delivery,
      identification of clinics classified as having a high proportion of patients failing to achieve
      virological suppression both necessitate investigation as well as support prioritization of
      programmatic optimization efforts. At the national level, the retrospective survey will yield a
      cumulative dataset of detected HIVDR which may be used by public health planners to guide the
      choice of current and future ART regimens.

1.3 Justification for assessment of virological failure at 12-15 months and 24-36 months in adult patients and greater than 12 months in paediatric patients

   According to the 2010 WHO HIV treatment guidelines, viral load testing is not an
   essential part of ART delivery. However when resources permit, viral load testing should be used
   either in a targeted or routine manner to detect virological failure in patients receiving ART. In
   many clinical trials and observational research cohorts, rates of virological failure 12-15 months
   after ART initiation have been used as a marker of ART success; additionally, many countries
   have implemented or are planning to implement routine viral load testing of patients 12 months
   after ART initiation.

   An important goal in the optimization of population based ART delivery is the
   minimization of population level virological failure. In the context of this survey, suppression of
HIV viral load is the same as *HIVDR prevention*. For the purposes of this survey, *virological failure* is defined as viral loads \( \geq 1000 \) copies/mL at 12-15 and 24-36 months after initiation of *first-line ART* in adults and \( \geq 12 \) months after initiation of *first-line ART* in paediatric patients.

Assessment of population level *virological failure* in adults 24-36 after initiation of *first-line ART* investigates whether population level *viral suppression* has been maintained for periods greater than one year and provides an opportunity to characterize *detected HIVDR* in patients who may have been failing ART for \( > 12 \) months. Adults with *detected HIVDR* 24-36 months after initiation may have different mutations or mutation patterns (specifically an accumulation of thymidine analogue mutations) than adult populations found to be failing *first-line ART* 12-15 months after initiation. In settings where blood is collected routinely at 12 months for viral load testing in adult populations, this survey method could be adapted to use remnant specimens from a 12 month blood draw for HIVDR testing.

In this survey, paediatric populations on ART for \( \geq 12 \) months will be surveyed. There are several reasons for choosing this timeframe. First, after the introduction of the Treatment 2.0 strategy by the WHO, decentralization of services has been advanced as a strategy to increase access to care and treatment.\(^3\) In general, paediatric ART clinic populations are smaller than adult ART clinic populations and decentralization of services will further decrease the individual ART clinic paediatric populations available to being sampled. Additionally, the UNAIDS Strategic Plan for 2011-2015 set a goal of eliminating vertical transmission of HIV,\(^10\) which will significantly decrease paediatric HIV incidence rates. Therefore due to ongoing decentralization of service and anticipated decreases in pediatric infection, a longer timeframe was chosen to increase the paediatric population which will be sampled.

### 1.4 Use of Dried Blood Spot Specimens (DBS) for viral load testing

As a component of the WHO HIVDR Laboratory Strategy, DBS specimens are advocated as an alternative to plasma specimens for HIVDR surveillance in resource limited settings. Historically, WHO has not recommended the use of DBS for HIVDR testing in populations receiving ART due to overall lower population level viral loads which may result in failure to amplify virus. However, recent studies investigating DBS under various simulated field conditions have demonstrated promising outcomes.\(^{11,12,13,14}\) Two studies have reported a lower limit of detection of viral load 2000-4000 copies/mL while others have demonstrated
amplification of HIV from DBS specimens with viral loads of <1000 copies/mL as well as from DBS specimens with viral loads ranging from 290-72000 copies/mL\textsuperscript{15,16}. These studies and additional unpublished data generated by WHO accredited HIVDR genotyping laboratories support the use of DBS with a lower limit of detection of HIV viral load of 1000 copies/mL. Therefore, due to recent advances in viral load and genotyping from DBS, the retrospective survey of acquired HIVDR will use DBS and define detectable viral load as ≥ 1000 copies/mL. WHO provides guidance for the optimal collection, processing and storage of DBS for HIVDR genotyping which must be strictly followed when implementing this survey. For guidance on appropriate collection, processing, storage, shipping and handling of DBS refer to: http://www.who.int/hiv/topics/drugresistance/dbs_protocol.pdf.

1.5 Definitions

Adult and paediatric patient: The decision as to whether a patient is classified as an adult or paediatric patient will be based on ART clinic categorization criteria. For the purposes of this survey, if the patient is categorized at the ART clinic as an adult, the survey will categorize the patient as an adult. Similarly, if a patient is categorized at the ART clinic as a paediatric patient, the survey will classify the patient as a paediatric patient.

Class boundary: One input of the LQAS method which describes acceptable upper and lower limits of virological failure for the adult and paediatric surveys based on review of the medical literature and expert opinion. In this survey, class boundaries were determined by WHO, PEPFAR and US-CDC in consultation with experts from WHO HIVResNet.

Consecutive sample: In the context of this protocol, a consecutive sample refers to the manner in which individuals will be considered for enrolment into the study. Once the survey starts at a sentinel ART clinic, every consecutive patient at the sentinel clinic will be considered for enrolment.

Consumer Probability of Error (CPE): In this protocol, CPE is a level of acceptable error decided by expert opinion, which represents the acceptable risk that LQAS classifies the prevalence of virological failure as low when it is, in fact, high.

Detected HIVDR: Defined in cases where mutation patterns to one or more nucleotide reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs) or protease inhibitors (PIs) are found on HIVDR testing which are defined by the
Stanford HIVDR algorithm\textsuperscript{17} and categorized as conferring low, intermediate or high drug resistance.

**Dried Blood Spot (DBS):** A specimen type used for measurement of viral load and HIVDR testing in this survey. DBS specimens are easily prepared in the field either by finger stick or heel stick. In order to maximize successful viral load and HIVDR testing from DBS specimens, DBS collection, processing and storage must follow the procedures as outlined in the WHO Manual for HIV Drug Resistance Testing using Dried Blood Spots Specimens.\textsuperscript{18}

**First-line ART at 12-15 months:** An adult patient is defined as on first-line ART at 12-15 months if he or she was classified by the ART clinic using national/international guidance as having been on a first-line ART regimen at the last clinic/pharmacy visit immediately prior to the clinic visit falling within the survey period. The first-line regimen may be the ART regimen which the patient initiated 12-15 months before, or it may be an alternative first-line regimen that was started as a substitution. Patients classified by the ART clinic (following standard national ART programme guidance) as having stopped ART at the clinic/pharmacy visit immediately prior to the clinic visit falling during the survey period are excluded from this survey.

**First-line ART at 24-36 months:** An adult patient is defined as on first-line ART at 24-36 months if he or she was classified by the ART clinic (following standard national/international guidance) as having been on a first-line ART regimen at the last clinic/pharmacy visit immediately prior to the clinic visit falling within the survey period. The first-line regimen may be the ART regimen which the patient initiated 24-36 months before, or it may be an alternative first-line regimen that was started as a substitution. Patients classified by the ART clinic using standard national ART programme guidance as having stopped ART at the clinic/pharmacy visit immediately prior to the clinic visit falling during the survey period are excluded from this survey.

**First line ART at ≥ 12 months:** A paediatric patient is defined as on first-line ART at ≥12 months if he or she was classified by the ART clinic (following standard national/international guidance) as having been on a first-line ART regimen at the last clinic/pharmacy visit immediately prior to the clinic visit falling within the survey period. The first-line regimen may be the ART regimen which the patient initiated ≥12 months before, or it may be an alternative first-line regimen that was started as a substitution. Patients classified by the ART clinic using standard national ART programme guidance as having stopped ART at the
clinic/pharmacy visit immediately prior to the clinic visit falling during the survey period are excluded from this survey.

**HIVDR laboratory:** For the purposes of this protocol, HIVDR laboratory refers to a laboratory accredited by the WHO for the purposes of HIVDR testing (and viral load testing).

**HIVDR prevention:** Defined in cases where the survey specimen has a viral load of <1000 copies/mL.

**In-transit:** Individuals are classified as in-transit if they receive routine ART care at another ART clinic but due to travel pick up ART at the sentinel ART clinic being sampled in this survey. Individuals who are classified as in-transit are excluded from this survey.

**Lot Quality Assurance Sampling (LQAS):** In its application to this survey, LQAS is a statistical method used to categorize a clinic as having high or low proportion of patients who are failing to achieve *viral suppression* by analyzing a *consecutive sample* of patients. Parameters of LQAS include the clinic size, upper and lower *class boundaries of virological failure*, and acceptable levels of classification error. The LQAS method produces a decision rule (d) and sample size (n) which is used for classification. In this case, d and n will be used to categorize a clinic as having high or low proportion of patients who are failing to achieve *viral suppression*. See Appendix 1 for a more detailed description of the LQAS method.

**Possible HIVDR:** Defined in cases where survey specimens from patients on a first-line ART regimen have a viral load ≥1000 copies/mL and no detected HIVDR.

**Provider Probability of Error (PPE):** In this context, PPE is a level of acceptable error decided by expert opinion which represents the acceptable risk that LQAS classifies the prevalence of *virological failure* as high when it is, in fact, low.

**Stop:** An ART stop for the purposes of this survey is defined as the complete cessation of ART by a patient who has not restarted ART by the time of the clinic visit immediately preceding the *survey clinic visit*. Stops usually take place because of a patient decision or a decision by the clinical team, and generally reflect either a planned treatment interruption or a decision based on poor adherence.

**Substitution:** Defined as a change from one first-line ART regimen to another first-line ART regimen (intra-class substitution), for example due to adverse events or toxicity.

**Survey clinic visit:** For the purposes of this survey, the survey clinic visit is defined as the ART clinic visit at which the patient is considered for inclusion. For adult populations this is
either 12-15 months or 24-36 months after initiation of ART and for paediatric populations this is ≥ 12 months after initiation of ART.

Survey start date: The survey start date is the date on which an individual sentinel ART clinic starts enrolling patients into the survey. Once the ART sentinel clinic has started evaluating patients for enrolment, every consecutive patient who presents for care at that ART sentinel clinic will be considered for enrolment.

Switch: Switch is defined as a change in regimen from a first-line to a second-line ART regimen.

Viral suppression: For the purposes of this survey viral suppression is defined as an HIV viral load of <1000 copies/mL.

Virological failure: For the purposes of this survey virological failure is defined as an HIV viral load ≥ 1000 copies/mL.

2. Objectives

2.1. HIVDR monitoring objectives

- Describe HIVDR in adult and paediatric patients who have failed to achieve a viral load <1000 copies/mL at 12-15 months and 24-36 after initiation of ART for adult populations and ≥ 12 months after initiation of ART for paediatric populations at sentinel ART clinics
- Describe emergence or changes in patterns of HIVDR observed at different time periods
- Classify the proportion of adult patients failing first-line ART at 12-15 months after initiation of ART at each adult sentinel ART clinic
- Classify the proportion of adult patients failing first-line ART at 24-36 months after initiation of ART at each adult sentinel ART clinic
- Classify the proportion of paediatric patients failing first-line ART at ≥12 months after initiation of ART at each paediatric sentinel ART clinic
- Provide a description of how different models of care delivery within a national ART program perform in achieving population level viral load suppression in patients receiving ART
2.2. Intended use of results

Results from HIVDR monitoring at sentinel clinics will contribute data for evidence-based decision making on maintaining the effectiveness of first-line ART and supporting ART programme practices associated with HIVDR prevention. Routine surveillance of acquired HIVDR will create a cumulative HIVDR dataset (See Figure 1). This cumulative dataset will be useful for countries transitioning from stavudine (d4T) to tenofovir (TDF) or zidovudine (ZDV/AZT) or otherwise introducing TDF or ZDV into their recommended ART regimens, as different population level patterns of HIVDR are likely to emerge than have previously been observed. Unlike some HIVDR studies, this survey will classify patients with virological failure and no detected HIVDR as having possible HIVDR.

Figure 1 – Role of Cumulative Survey Datasets in Assessment of HIVDR and Overall Programmatic Status

Each individual sentinel clinic’s results as well as accumulated data over time will provide a population-level description of acquired HIVDR and provide information on how well sentinel clinics, chosen to represent different models of care delivery, achieve population-level virological suppression. Routine implementation of this survey over time will ensure timeliness of datasets.
By classifying population level virological failure above or below predefined levels at sentinel clinics, this survey will identify clinics and models of care delivery which may benefit from further investigation into why higher levels of virological failure are observed. Given that HIVDR EWIs are monitored routinely at all clinics implementing this retrospective survey, investigation of HIVDR EWIs may identify associated clinical or programme factors, which can be adjusted to optimize population level ART delivery. The ecological associations observed between HIVDR EWIs and results of this survey may support identification of factors which require additional investigation or optimization. Because sentinel ART clinics included in the survey represent models of ART delivery within a country, the identification of programmatic factors which may require adjustment at a sentinel clinic may signal that other clinics of the same type may require further investigation or adjustment.

The types of additional investigations at sentinel ART clinics which may evolve from this survey include case series or case control studies designed to identify and characterize patient and/or programmatic factors associated with virological success or failure.

3. Procedures/Methods

3.1. Design overview

Sampling will occur at two levels. The primary sample is the sample of ART clinics chosen to represent different models of ART delivery and the secondary sample consists of eligible adults or paediatric patients sampled from within the chosen sentinel ART clinics.

A stratified sampling method will be used to determine which ART clinics to include in the survey (primary sample). Once enrolment begins, a consecutive sample (secondary sample) of eligible patients will have DBS specimens obtained for viral load testing. Minimal demographic data will be abstracted from patient medical records at time of enrolment. If the patient's viral load is $\geq 1000$ copies/mL, a genotype will be performed. Using the LQAS method described in Appendix 1, the proportion of adult patients with virological failure at 12-15 and 24-36 months after ART initiation and the proportion of paediatric patients with virological failure at $\geq 12$ months after ART initiation will be classified above or below defined levels. The adult cohorts may be sampled at the same time at the same sentinel clinic, however the same
patient should not be sampled more than once (See Figure 2 for an illustration of the retrospective design).

**Figure 2:** Retrospective Study Design for Cohort 12-15 Months After ART Initiation

This diagram depicts the retrospective study design of the survey of adult patients 12-15 months after initiation of ART (the adult 24-36 month survey and paediatric ≥ 12 month survey are not depicted here but are analogous in design except for the time period after ART initiation). At the survey start date (noted as ‘START’ on this diagram), adult patients will be identified who have been on ART for 12-15 months. Patients A-E are sampled. Patient F started ART within the specified time period but died. Patient G, H and I all started ART within the specified time, but were lost to follow up, had an ART stop or transferred out, respectively. Thus, patients F to I are excluded from the survey.

### 3.2. Selection of sentinel ART clinics

Sentinel ART clinics will be selected for this survey by a stratified sampling method. Refer to section 3.4 for information about the sampling method used to select sentinel ART clinics.
3.3. Selection of individuals included in the survey at sentinel ART clinics

Inclusion and exclusion criteria will be used to determine eligibility for enrolment in the survey. Secondary sampling within the ART clinic will be performed using LQAS. Refer to section 3.4 for more information about survey inclusion and exclusion criteria, the secondary sampling method and LQAS.

3.4 Detailed enrolment

3.4.1. Primary sampling method – Selection of sentinel ART clinics

A stratified sampling method is used to determine which ART clinics will serve as sentinel monitoring clinics (clinics where the survey is performed). Stratification is based on fixed (location: urban or rural) and program (non-random) effects (degree of specialization: Hospital, Health Centre or Clinic and supporting organization: NGO/Mission or MoH.). The first step in the selection of sentinel ART clinics is the creation of an organogram which describes the fixed and program effects present in the country national ART programme (Figure 3). The organogram is created such that all fixed and programmatic effects have been described. This creates categories of models of care delivery within the national ART programme (designated as shaded boxes in Figure 3). Next, ART clinics within the national ART programme are categorized based on their fixed and program effects. In order to create a sample of ART clinics to be included in the survey, one clinic from each model of care delivery category should be selected (Figure 3). The survey will only include main clinics and excludes outreach and integrated management of adulthood illnesses (IMAI) sites.
**Figure 3: Stratification of ART Clinics and Sampling Strategy: Country**

ART clinics within the national ART program are categorized based on fixed and programmatic factors which represent distinguishing features of the clinic. In this organogram, a fixed factor is clinic geographic location (urban versus rural) and programmatic factors are the level of specialization and sponsoring/supporting organizations (MoH versus Mission/NGO). The models of care delivery are indicated by shaded boxes.

The total number of clinics which will serve as sentinel adult ART clinics is dependent on the number of models of care delivery categories and resources available for survey implementation. According to the organogram in Figure 3, there are 9 models of care delivery. One adult ART clinic from each model of care delivery is chosen. According to the resources available for the survey, 15 sentinel ART clinics will be surveyed. Additional clinics chosen to
be included in the survey after one clinic from each model of care delivery has been included are identified based on the distribution of clinics between all of the models of care delivery. This calculation is described in Appendix 2. Sentinel clinics chosen to be sampled in this survey are found in Table 1.

**Table 1. Sentinel ART clinics sampled in retrospective survey**

<table>
<thead>
<tr>
<th>Type of ART clinic</th>
<th>Name of ART Clinic Sampled</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urban, Hospital, MoH</td>
<td></td>
</tr>
<tr>
<td>Urban, Hospital, Mission/NGO</td>
<td></td>
</tr>
<tr>
<td>Urban, Health Centre, MoH</td>
<td></td>
</tr>
<tr>
<td>Urban, Clinic, MoH</td>
<td></td>
</tr>
<tr>
<td>Rural, Hospital, MoH</td>
<td></td>
</tr>
<tr>
<td>Rural, Hospital, Mission/NGO</td>
<td></td>
</tr>
<tr>
<td>Rural, Health Centre, MoH</td>
<td></td>
</tr>
<tr>
<td>Rural, Health Centre, Mission/NGO</td>
<td></td>
</tr>
<tr>
<td>Rural, Clinic, MoH</td>
<td></td>
</tr>
</tbody>
</table>

**3.4.2 Secondary sampling method – Selection of patients at sentinel ART clinics**

The required within-clinic sample size is calculated in order to classify the prevalence of virological failure amongst those on ART using a two-class LQAS classifier. The LQAS sampling and classification plan is created by examination of cumulative hypergeometric probabilities for values of n (the sample size) and d (the decision value) that meet standards for classification error at different levels of prevalence. Class boundaries are levels of prevalence that define unacceptable and acceptable proportions of population level virological failure for the purposes of this survey. WHO, PEPFAR, US-CDC in consultation with experts from WHO HIV ResNet have agreed upon the upper and lower class boundaries of virological failure for the adult and paediatric surveys, based on review of the medical literature and expert opinion. Class boundaries were chosen to be aspirational as to mobilize increased resources and effort to improve outcomes, but also to be realistic as to what can be achieved if specific barriers are to be overcome.
The following class boundaries will be used as inputs into the LQAS model:

<table>
<thead>
<tr>
<th>Sample population</th>
<th>Upper class boundary (proportion of virological failure)</th>
<th>Lower class boundary (proportion of virological failure)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adults 12-15 months after initiation of ART</td>
<td>30%</td>
<td>15%</td>
</tr>
<tr>
<td>Adults 24-36 months after initiation of ART</td>
<td>35%</td>
<td>20%</td>
</tr>
<tr>
<td>Paediatric patients ≥ 12 months after initiation of ART</td>
<td>35%</td>
<td>20%</td>
</tr>
</tbody>
</table>

**Figure 4 – Overview of the Application of LQAS Theory to Categorization of ART Clinics**

The process begins with determining the estimated population size (clinic size), PPE, CPE and the predetermined class boundaries for high and low prevalence of viral suppression. These parameters are entered into the LQAS tool to yield n and d. A consecutive sample of specimens is collected from the clinic. Then using n and d, a classification can be made. All samples with a viral load of ≥1000 copies/mL will have genotype testing performed.
In order to calculate \( n \) and \( d \), there must be an estimation of the population size. The following steps describe the procedure for calculating the estimated population size.

1. Estimation of population size for adult populations at 12-15 months after ART initiation:
   After review of historical patient visit logs, an estimate of the three month enrolment and the twelve month retention rate is made. The estimated population size is calculated by multiplying the estimated three month enrolment and the twelve month retention rate.
   
   \[
   \text{Estimated population size}_{12-15 \text{ months}} = (3 \text{ month enrolment}) \times (12 \text{ month retention rate})
   \]

2. Estimation of population size for adult populations at 24-36 months after ART initiation:
   After review of historical patient visit logs, an estimate of the 12 month enrolment and 24 month retention rate is made. The estimated population size is calculated by multiplying the estimated 12 month enrolment by the 24 month retention rate.
   
   \[
   \text{Estimated population size}_{24-36 \text{ months}} = (12 \text{ month enrolment}) \times (24 \text{ month retention rate})
   \]

3. Estimation of population size for paediatric populations \( \geq 12 \) months after initiation of ART:
   After review of historical patient visit logs, the estimated population size for the paediatric population \( \geq 12 \) months consists of all paediatric patients who initiated ART \( \geq 12 \) months from the start of the survey.

   These estimates of population size will then be used in an online calculator tool to determine \( n \) and \( d \) \( ^{20} \) (http://www.brixtonhealth.com/hyperLQAS.html). By using the predefined class boundaries, the estimated population size, CPE and PPE, the LQAS model will calculate \( n \) and \( d \) values (See Appendix 1 for an example using the online LQAS calculator). Separate \( n \) and \( d \) values will be calculated for each sentinel ART clinic (See Appendix 3 for examples of estimating population size and corresponding \( n \) and \( d \) values).

Since viral load testing will likely not occur in real time, in practice, \( n \) number of patients will be sampled consecutively and after viral load testing is performed \( d \) will be used to assess if the sentinel clinic can be classified as having a high or low proportion of patients with failure to attain viral load suppression. In addition, \( \sim 10\% \) more patients above \( n \) will be collected during enrolment in order to account for failure to amplify specimens or specimen loss. Further discussion between the country conducting the survey and the HIVDR genotyping laboratory can
help facilitate agreement on a percentage of additional patients to enrol in order to account for anticipated rates of failure to amplify specimens.

3.4.3. Participant inclusion criteria

Patients attending the ART sentinel clinics are eligible if they meet the following criteria:

1. Adults who have been on first-line ART for 12-15 months OR
2. Adults who have been on first-line ART for 24-36 months OR
3. Paediatric patients who have been on first-line ART for ≥ 12 months AND
4. Individuals who consent following the informed consent (oral) process

Criteria 1 through 3 represent populations which will be sampled separately.

3.4.4. Participant exclusion criteria

Patients who meet any of the following criteria are excluded from the survey:

1. Individuals who are on second-line ART (has had a switch in ART)
2. Individuals classified as having stopped ART at the time of their last attended clinic/pharmacy visit prior to their survey clinic visit
3. Individuals who are classified as transfer-in from another ART clinic on ART
4. Individuals with HIV-2 or HIV-1/HIV-2 co-infection, if information available
5. Adults patients who have been on first line ART for < 12 months, 16-23 months or > 36 months
6. Paediatric patients who have been on first line ART for < 12 months
7. Individuals in-transit

3.4.5 Enrolment

At the time of survey start, patients will be assessed for inclusion in the survey as they attend the sentinel ART clinic for routine care. Assessment for inclusion in the study is performed in a consecutive manner. That is, once the clinic starts recruiting patients, every eligible consecutive patient must be considered for enrolment. If a patient satisfies the inclusion criteria and is not excluded by the exclusion criteria, he/she may be enrolled in the survey. Once a patient is deemed a candidate to be included in the survey, informed consent is administered. The consent for this survey is oral. An example of consent for this survey is provided in
Appendix 4 along with a patient information sheet in Appendix 5. After consent, a unique de-identified HIVDR survey identifier (HIVDR-SID) will be assigned to the patient. Appendix 6 describes how HIVDR-SID numbers are assigned. Clinical data will be abstracted from patients’ medical/pharmacy record by ART clinic staff at the time of consent and specimen collection. Clinical data will be limited to age, gender, date of ART start and ART regimen. These data will be recorded in a log book (Appendix 7). The log book will be kept at the clinic in a secure, locked location. Data will be abstracted by clinic staff from this log book at the time of DBS collection but abstracted data will never include identifying information (See Appendix 8 for abstraction form). On the date of enrolment, DBS will be collected. Protocols for collection of the DBS, handling, processing and tracking are described in 3.6. See Figure 5 for an overview of standard procedures at ART sentinel clinics.

**Figure 5: Overview of Standard Procedures at Sentinel ART Clinics**

![Diagram of standard procedures]

After the survey start, a *consecutive sample* of patients from the ART sentinel clinic are enrolled using inclusion and exclusion criteria. Those that fulfil the enrolment criteria and not excluded by the exclusion criteria will be enrolled. Basic demographic data and informed consent will be collected. Those that accept enrolment will have DBS collected. DBS will have viral load testing performed. Using the LQAS method, the proportion of patients with virologic failure will be classified as having a low or high. All specimens with a viral load of \( \geq 1000 \) copies/mL will genotyped to determine HIVDR.
3.5 Specimen collection, handling, processing, and tracking

3.5.1. Specimen collection

Sentinel ART clinics will collect DBS specimen according to the WHO Guidance for DBS specimen collection and handling for HIV drug resistance testing. DBS will be collected either via finger stick or heel stick, dried and stored appropriately\(^\text{18}\) (http://www.who.int/hiv/topics/drugresistance/dbs_protocol.pdf). (See Figure 6 for more information regarding DBS specimen transport chain to HIVDR reference laboratory which is further described in the above WHO Guidance document).

Countries using plasma specimens for this survey may refer to the WHO website for recommendation on processing of plasma specimens\(^\text{21}\) (http://www.who.int/hiv/pub/drugresistance/hiv_reslab_strategy.pdf).

**Figure 6:** Transportation Chain of DBS specimen from collection site to HIVDR laboratory

- **Dried Blood Spot (DBS) specimen collected using finger stick or heel stick**
  - **Does collection site have -20°C facilities?**
  - **Yes**
    - Collection Site
    - DBS specimen stored at -20°C until shipped to HIVDR laboratory for viral load and genotype testing. Specimen should be frozen as soon as possible after collection
  - **No**
    - Transport DBS specimen, at ambient temperature, to intermediate facility for storage. Specimen must be frozen within 14 days of collection
    - Intermediate facility
    - DBS specimen to be stored at -20°C before transport to HIVDR laboratory
    - DBS specimen thawed and shipped to HIVDR laboratory
    - DBS arrives at HIVDR laboratory where viral load and genotype testing

---
3.6.2. Laboratory used for DBS

DBS specimens will be processed, packaged, stored, and shipped according to WHO guidance to laboratories accredited by the WHO for viral load and HIVDR testing from DBS.

3.7. Patient confidentiality

3.7.1. Administration of informed consent

An informed consent process, which requests consent to collect DBS and abstract non-identifying information, will be administered to all patients identified as eligible to participate in the survey. The consent information will include the fact that the laboratory results will be returned to the medical chart for individuals participating in the study whose blood has viral load testing and, if appropriate, genotype testing performed. Further information about the consent process can be found in Section 5.3.

3.7.2. Return of laboratory results to the medical chart

All results of viral load testing and HIVDR will be sent back from the HIVDR laboratory to the ART clinic via a standardized form (see Appendix 9). Using the log book which includes both patients’ unique identification numbers and their corresponding identifying information, the results of viral load testing and HIVDR will be returned to each patient chart. Once results are available, they will be discussed with the patient by the clinician at the next regularly scheduled visit, and should be able to be forwarded to another clinician or ART clinic at a patient's request. Clinicians at the ART clinic may use the results for patient management purposes, if relevant. A national contact will be designated who will be available to discuss HIVDR results with clinicians.

3.7.3. Confidentiality

Specimens and laboratory results DBS sent for viral load testing and HIVDR genotyping will be labelled only with the HIVDR-SID before being sent to any laboratories. Neither the name of the participant nor other personal identifying information will appear on the results that are returned to the ART clinic. Results returned to the clinic will also be labelled only
with the HIVDR-SID; the logbook will be used only at the clinic level to link the results to the appropriate individuals so that results can be returned to the appropriate medical charts.

**Logs** Paper or electronic logs will be kept on-site which will allow for return of viral load testing and resistance testing to participants’ medical records. These data will be kept secured by lock and key, or, in the case of electronic logs, will be password protected.

**Data sent to HIVDR laboratory, country, region and WHO** Any data from the survey regarding viral load suppression or HIVDR will include only the HIVDR-SID, basic sociodemographic and clinical data, current regimen information, and specimen tracking information. No personal identifying information on participants will be made available to the HIVDR laboratory, the referral laboratory, country ministry of health, the WHO national, or regional office or headquarters, or other project collaborators. Any suspected breach of confidentiality will be dealt with immediately by the appropriate authorities. HIVDR genotypes will be sent simultaneously from the WHO accredited laboratory in the form of a FASTA file to the country and WHO headquarters for quality assurance. Quality assurance evaluation will be performed according to WHO guidance.

**HIVDR monitoring databases, laptops, and forms.** Databases will be password-protected so that only persons working on this survey will have access. Laptop computers, HIVDR forms, laboratory specimen tracking forms, logs, and genotype results will be locked in file cabinets or drawers when not being used by survey staff.

**Transfer of electronic data.** Data will be encrypted before sending them electronically.

### 4. Data management and analysis

#### 4.1. Data and Data Flow

**4.1.1. Patient variables**

Patient variables which will be collected include patient age, gender, ART regimen, start date of ART. History of previous ARV exposure is recommended by WHO to be abstracted from routine medical records, when feasible. (See Table 2) As this variable is not available in routine medical records in the country, it will be not analyzed in this survey. Upon enrolment, the patient will also be assigned an HIVDR-SID number as described in section 3.5.1.
Table 2: Patient Variables

<table>
<thead>
<tr>
<th>Patient Variable</th>
<th>Justification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient Age</td>
<td>Basic demographic information</td>
</tr>
<tr>
<td>Patient Gender</td>
<td>Basic demographic information</td>
</tr>
<tr>
<td>Start date of ART</td>
<td>Ensure patient meets inclusion criteria</td>
</tr>
<tr>
<td>Current ART regimen</td>
<td>Interpretation of detected HIVDR, if any</td>
</tr>
</tbody>
</table>

4.1.2. Variables collected by the genotyping laboratory

For each specimen successfully amplified and genotyped, the date of genotyping will be reported. The entire nucleotide sequence of the protease region of the pol gene, and the nucleotide sequence of the reverse transcriptase region of the pol gene from position 1 through position 990-1200 will be sent as a FASTA file. An interpretation of the observed level of resistance (low, intermediate, or high) based on the Stanford HIV drug resistance algorithm\(^{17}\) ([http://sierra2.stanford.edu/sierra/servlet/JSierra](http://sierra2.stanford.edu/sierra/servlet/JSierra)) will be reported for each drug and drug class of interest. As noted below, a set of variables will be recorded on a laboratory log which will assure collection of important data during the laboratory testing process.

Information on specimens for which no sequence is available will include whether amplification was attempted and whether it failed, and any reason for lack of attempt to extract and amplify (e.g., specimen lost, insufficient volume, specimen contaminated or grossly haemolysed).

4.1.3 Laboratory variables to be recorded on DBS specimen manifests

Point of collection
- HIVDR SID (survey ID)
- Record if finger stick or heel stick specimen used for DBS collection
- Date of collection of DBS specimen
- Number of dried blood spots made
- Duration of drying (hours) of DBS prior to packing
- Date of packing of DBS specimen
If -20°C or below storage is available at site of collection:

- Date of freezing
- Date brought back to ambient temperature
- Date of changing of desiccant bags
- Date of transport to HIVDR laboratory

If -20°C or below facility is NOT available at site of collection:

- Date specimen shipped from clinic to intermediate facility
- Date of specimen arrival at site for freezing at -20°C
- Packaging status of specimen (packing intact? At ambient temperature? Condensation present?) on arrival to site for freezing
- Date of freezing to -20°C or below at intermediate facility
- Date of shipment of DBS specimen to HIVDR laboratory

**HIVDR laboratory**

- Packaging status of specimen (packing intact? At ambient temperature? Condensation present?) at time of sending specimen to HIVDR laboratory
- Date of arrival of DBS specimen to HIVDR reference laboratory
- Number of desiccant bags which were pink on arrival to HIVDR reference laboratory
- Date of freeze, if applicable
- Date of genotyping

Copies of specimen manifests are sent along with specimens from point of collection to the **HIVDR laboratory** (Appendix 10). A copy of the final and complete manifest is sent to the country working group and the WHO headquarters for quality assurance.

### 4.2. Results/Analyses

A cumulative HIVDR data set with FASTA files from all specimens with *detected HIVDR* will be reported to the national level as well as the WHO headquarters. Sentinel ART
clinics will be classified as having a low or high proportion of patients with failure to achieve viral load suppression using the LQAS method. Data sets will describe both the classification of individual clinics as having high or low proportions of patients with virological failure as well as detected HIVDR patterns. In addition, although not aggregated, individual clinic data will draw a national picture of detected HIVDR patterns. In combination with EWIs, survey results which categorize clinics as having high or low proportions of patients with virological failure will enable ecological comparisons to be made between virological failure and programmatic components which may require optimization.

Further survey rounds will be conducted using the stratified sampling method described in the Section 3.5.

4.2.1. Analysis of Data

At the sentinel ART clinic level, the following analyses will be performed:

- Demographic description of participants including age, gender, range of dates of starting ART and ART regimens
- Description of detected HIVDR: low, intermediate or high level resistance to one or more NRTI, NNRTI or PI by the Stanford scoring system. HIVDR to be described by mutation, mutation pattern and by drug and drug class
- Classification of the proportion of adults failing first-line ART 12-15 months after initiation
- Classification of the proportion of adults failing first-line ART at 24-36 months after initiation
- Classification of the proportion of paediatric patients failing first-line ART at ≥ 12 months after initiation
- Description of the ability of different models of care delivery within a program to achieve population level viral load suppression in patients receiving ART
5. Ethical consideration

5.1. Review by Institutional Review Board (IRB)

The protocol will be submitted to the Research committee. Before implementation, the adapted protocol will be reviewed by WHO-Headquarters. A country information sheet will also be submitted with the protocol (Appendix 11). This country information sheet will compile important information regarding key contact information of WHO/CDC coordinators, survey personnel, ART sentinel clinic coordinators and the HIVDR laboratory.

5.2. Risk benefit ratio

Risks for this survey are minimal and mainly related to the collection of the specimen. If a DBS specimen is collected by finger stick or heel stick, the risks include pain at the lancet puncture site, infection and bleeding. Minimal information (basic non-identifying demographic and clinical information) will be abstracted for survey purposes from existing medical/pharmacy records. Identifying information which can link the patient’s viral load and HIVDR results is only available at the clinic level and is used for the purposes of returning patient’s viral load and HIVDR results to their patient chart. Information derived from the project serves an important public health purpose. Estimates of virological failure will help the country planners improve ARV delivery program to minimize the emergence of HIVDR and maximize the population benefit of first-line ART regimens. There is a clear population benefit from HIVDR testing and minimal risk to all participating individuals.

5.3. Informed consent

Following explanation of the survey, participants will be given an information sheet to read or, if necessary, it will be read to the participant (Appendix 4). All questions that arise will be addressed. Participants will be clearly informed that their participation in the survey is strictly voluntary, and that they can withdraw at any time and give no reason for withdrawal. All participants will also be informed that withdrawing from HIVDR monitoring will not affect the quality of services they receive from the clinic. All participants must state that they understand and agree to all of the items contained in the information sheet in order to enrol in the evaluation.
For the purpose of this survey, patients will be classified as an adult or paediatric patient according to whether they categorized as an adult or pediatric patient at the ART clinic they attend. However, for the purposes of the informed consent process, country specific laws and regulations must be followed regarding the definition of a minor and the requirements of informed consent for minors.

No incentives will be provided for participating in the survey.
Appendix 1 – Background of Lot Quality Assurance Sampling (LQAS) theory

Overview of sequential sampling and LQAS

LQAS is one of a set of methods known collectively as sequential sampling techniques. Sequential sampling (also known as sequential analysis) is an approach to data-analysis in which the sample size is not fixed in advance. Instead, observations are collected individually and, after each observation has been made, the accumulated data are examined to see whether or not a classification or decision can be made. Sequential sampling combines data-collection and data-analysis into a single process or sampling plan. Sequential sampling approaches can considerably reduce both the sample size requirements and the data-processing overheads of a survey compared to classical methods.

It should be noted that sequential sampling also works with data that are collected in small batches or as a single large batch. In the latter case, sequential sampling is similar to hypothesis testing in classical statistics. This protocol outlines a procedure which will use data collected as a single batch because this simplifies the management of both specimen and data.

Sequential sampling methods are best used in situations where the classification of the prevalence of a condition into categories or classes (e.g. “high” and “low” prevalence) provides sufficient information on which to base decisions to take specific actions. The approach has been adopted in many disease control programs and in this case is applied to the prevalence of acquired HIV drug resistance.

In its simplest and most frequently used form sequential sampling is used to make binary (i.e. two-class) classifications but the technique can be extended to accommodate more granular (i.e. three or more classes) classifications. The application described in this protocol requires only binary classifications.

LQAS method and its application to this protocol

LQAS is widely used in manufacturing industry to judge the quality of a lot (batch) of items. In the industrial context, LQAS is used to identify lots that are likely to contain an
unacceptably large number of defective items. In the public health context, LQAS is used to identify populations with low levels of service coverage or high prevalence of disease.\textsuperscript{22,23,24}

LQAS produces data that are easy to analyse. Data analysis is performed as data are collected and consists of counting the number of defects (e.g. unvaccinated children or cases of the condition of interest) in the sample and checking whether this exceeds a pre-determined number.

LQAS data are collected and analyzed using a sampling plan that specifies a maximum sample size ($n$) and the maximum number of defects allowed in the sample ($d$). Sampling plans are developed by specifying a classification system (e.g. the levels of prevalence that define high and low prevalence situations) and acceptable probabilities of classification error. In this protocol, a different sampling plan will be created for each adult survey at 12-15 and 24-36 months for each sentinel ART clinic and for the paediatric survey at $\geq 12$ months with clinic specific $n$ and $d$ values. In other words, for an adult sentinel ART clinic there will be 2 sets of $n$ and $d$ values – one set for patients 12-15 months after initiation of ART and one set for patients 24-36 months after initiation of ART. Suitable values for $n$ and $d$ are usually found by performing an exhaustive search of cumulative binomial probabilities for combinations of $n$ and $d$ that provide acceptable levels of error while minimising the value of $n$. Different probability distributions or computer-based simulation using empirical distributions may also be used to develop sampling plans. In the case of small populations (i.e. where the population is smaller than a few thousand individuals as in the application discussed in this document) cumulative hypergeometric probabilities are used as this accounts for the effects of sampling without replacement which are important in small populations. See Figures 1 and 2 for further descriptions of $n$ and $d$.

It should be noted that there is no requirement that data are analyzed sequentially (i.e. on a specimen-by-specimen basis). It is common practice to consider small batches of observations sequentially. It is also common practice to collect a complete sample of $n$ observations before applying the decision rules of the sampling plan.
Figure 1 – A LQAS Sampling Plan Expressed as a Plot of the Cumulative Number of Failures to Suppress Against the Sampling Size

In this diagram, the cumulative number of failures is plotted on the y axis and the number sampled is plotted on the x axis. The values for d and n are generated using the LQAS online tool, in combination with other input parameters. If the number of patients with a viral load of $\geq 1000$ copies/mL exceeds d at any point, then the clinic will be classified with high prevalence of virological failure. If, once n number of patients are collected, d number of patients with virological failure is not reached, then the clinic will be classified with low prevalence of failure to achieve virological failure.
In this example $d=7$ and $n=30$ and circles represent individuals sampled in the survey. As patients are enrolled, some are identified as having virological failure. If $d$ is not exceeded by $n$, as depicted in the left panel, then the ART clinic site is classified as low prevalence of virological failure. If the number of failures exceed $d$ before $n$ is reached, then the ART clinic site is classified as high prevalence of virological failure.

In general, the LQAS method produces $n$ and $d$ values which would determine decision points in the enrolment process. However, since viral load testing will likely not occur in real time, in practice, $n$ number of patients will be sampled consecutively and after viral load testing is performed $d$ will be used to assess if the sentinel clinic can be classified as having a high or low proportion of patients with virological failure.

The parameters of LQAS are class boundaries, acceptable levels of error which consist of the consumer probability of error (CPE) and provider probability of error (PPE), and population size. Class boundaries are levels of prevalence that define high and low prevalence situations which are predetermined by expert opinion. Consumer probability of error (CPE) represents the risk the survey classifies the proportion of patients with virological failure as low when it is really high. Provider probability of error (PPE) represents the risk that the survey classifies the proportion of patients with virological failure as high when it is really low. For all three sampling groups (adults patients 12-15 and 24-36 months after initiation of ART and paediatric patients greater than 12 months after initiation of ART), CPE and PPE have been fixed at 0.05 and 0.1, respectively. This reflects concern for classifying a clinic as having an
acceptable rate of *virological failure* when the true rate is actually unacceptable. See appendix 3 for information about how to calculate population size. These parameters are then entered into the online LQAS sampling plan calculator (http://www.brixtonhealth.com/hyperLQAS.html). It should be noted that although this tool requires internet access for initial use, it can be downloaded so that internet access is not required thereafter.

**Figure 3:** LQAS Online Sampling Plan Calculator

<table>
<thead>
<tr>
<th>Population size</th>
<th>Sample size</th>
<th>Decision rule</th>
</tr>
</thead>
<tbody>
<tr>
<td>120</td>
<td>45</td>
<td>9</td>
</tr>
</tbody>
</table>

**Figure 3:** This figure is a representation of a result after entering the LQAS parameters into the online calculator for a cohort of adults 12-15 months after initiation of ART at a sentinel ART clinic. The estimated population size is the calculated as described in Appendix 3 and in this case is 120. The fields “upper threshold and lower threshold” are the predetermined class boundaries defined in Section 3.4. The field “maximum tolerable & alpha error” is the CPE which is defined in all cases to be 0.05. The field “maximum tolerable & beta error” is the PPE which is defined in all cases as 0.1. In this example, the online tool shows a required sample size of 45 (n) and decision rule (d) of 9. The actual alpha error in this example is 0.0482 and the actual beta error is 0.0748.
Appendix 2 – Description of how additional ART clinics are chosen beyond one from each model of care delivery

<table>
<thead>
<tr>
<th>Type of ART clinic site</th>
<th>Column 1</th>
<th>Column 2</th>
<th>Column 3</th>
<th>Column 4</th>
<th>Column 5</th>
<th>Column 6</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of ART clinics within that category</td>
<td>Total number of ART clinics in ART program</td>
<td>Proportion of clinics represented by this ART clinic type</td>
<td>Number of clinics which can be sampled over minimum</td>
<td>Number of sentinel clinics sites which should be sampled in category</td>
<td></td>
</tr>
<tr>
<td>Urban, Hospital, MoHS</td>
<td>23</td>
<td>62</td>
<td>23/62=0.37</td>
<td>5</td>
<td>5*0.37=1.85~2</td>
<td></td>
</tr>
<tr>
<td>Urban, Hospital, Mission/NGO</td>
<td>1</td>
<td>62</td>
<td>1/62=0.02</td>
<td>5</td>
<td>5*0.02=0.1</td>
<td></td>
</tr>
<tr>
<td>Urban, Health Centre, MoH</td>
<td>4</td>
<td>62</td>
<td>4/62=0.06</td>
<td>5</td>
<td>5*0.06=0.3</td>
<td></td>
</tr>
<tr>
<td>Urban, Clinic, MoH</td>
<td>3</td>
<td>62</td>
<td>3/62=0.05</td>
<td>5</td>
<td>5*0.05=0.25</td>
<td></td>
</tr>
<tr>
<td>Rural, Hospital, MoH</td>
<td>5</td>
<td>62</td>
<td>5/62=0.08</td>
<td>5</td>
<td>5*0.08=0.4</td>
<td></td>
</tr>
<tr>
<td>Rural, Hospital, Mission/NGO</td>
<td>5</td>
<td>62</td>
<td>5/62=0.08</td>
<td>5</td>
<td>5*0.08=0.4</td>
<td></td>
</tr>
<tr>
<td>Rural, Health Centre, MoH</td>
<td>7</td>
<td>62</td>
<td>7/62=0.11</td>
<td>5</td>
<td>5*0.11=0.55~1</td>
<td></td>
</tr>
<tr>
<td>Rural, Health Centre, Mission/NGO</td>
<td>3</td>
<td>62</td>
<td>3/62=0.05</td>
<td>5</td>
<td>5*0.05=0.25</td>
<td></td>
</tr>
<tr>
<td>Rural, Clinic, MoH</td>
<td>11</td>
<td>62</td>
<td>11/62=0.18</td>
<td>5</td>
<td>5*0.18=0.9~1</td>
<td></td>
</tr>
</tbody>
</table>
Appendix 3 – Sample population size calculation, $n$ and $d$

Examples for population estimation and correlating $n$ and $d$ values in adult patients in the 12-15 month cohort

<table>
<thead>
<tr>
<th>3 month enrolment</th>
<th>12 month retention rate</th>
<th>Calculated population size</th>
<th>$n$</th>
<th>$d$</th>
</tr>
</thead>
<tbody>
<tr>
<td>500</td>
<td>90%</td>
<td>450</td>
<td>60</td>
<td>12</td>
</tr>
<tr>
<td>250</td>
<td>70%</td>
<td>175</td>
<td>50</td>
<td>10</td>
</tr>
<tr>
<td>100</td>
<td>80%</td>
<td>80</td>
<td>40</td>
<td>8</td>
</tr>
<tr>
<td>50</td>
<td>80%</td>
<td>40</td>
<td>26</td>
<td>5</td>
</tr>
<tr>
<td>25</td>
<td>80%</td>
<td>20</td>
<td>17</td>
<td>3</td>
</tr>
</tbody>
</table>

Examples for population estimation and correlating $n$ and $d$ values in adult patients in the 24-36 month cohort

<table>
<thead>
<tr>
<th>12 month enrolment</th>
<th>24 month retention rate</th>
<th>Calculated population size</th>
<th>$n$</th>
<th>$d$</th>
</tr>
</thead>
<tbody>
<tr>
<td>200</td>
<td>80%</td>
<td>160</td>
<td>52</td>
<td>13</td>
</tr>
<tr>
<td>100</td>
<td>80%</td>
<td>80</td>
<td>41</td>
<td>10</td>
</tr>
<tr>
<td>50</td>
<td>90%</td>
<td>45</td>
<td>29</td>
<td>7</td>
</tr>
<tr>
<td>25</td>
<td>80%</td>
<td>20</td>
<td>27</td>
<td>4</td>
</tr>
</tbody>
</table>
Appendix 4: Informed oral consent form for HIV drug resistance surveillance survey

Hello, my name is (MD or nurse’s name), and I am a (MD/nurse) working at this (name) clinic.

**Purpose of the survey:**
We would like to invite you to participate in a survey which looks at the amount of human immunodeficiency virus (HIV) in your blood (viral load) and if there is any resistance of your HIV to the antiretroviral medications (ARVs) you are currently taking. You are being asked to be part of this survey because you have been on treatment for a specific time period at this clinic. We are doing this survey in order to determine better ways we can monitor for HIV drug resistance (HIVDR) that can affect how well HIV treatment works for you and other patients at this clinic. If you agree to participate, a dried blood spot (DBS) specimen will be collected and will be used for checking for levels of virus and drug resistance in your blood. A DBS requires a finger stick with a lancet so that a drop of blood can be dried onto a special kind of paper. After testing, results will be sent to your medical record and discussed with you. The information will be used to improve HIV treatment programs at this clinic and in the country as a whole.

**Procedures:**
If you agree to participate in this survey, this is what will happen:
1. You tell your provider that you agree to be part of the survey. We will perform a finger stick (to obtain about 5 drops of blood; this may be repeated if the first finger stick was not successful). We will drop your blood onto the special filter paper for testing. The finger stick will be performed by holding the ‘ring’ finger of the non-dominant hand and making a prick with a lancet to the cleaned area (disinfected with alcohol wipes) of the finger tip (lateral side of finger tip).
2. Your blood will be sent for 2 tests. The DBS specimen will be sent for a test to measure the level of HIV in your blood and, if it is present, if there is any HIVDR.
3. The results will be sent to your medical chart. Your doctors will discuss the results with you and use them for your treatment and care at the clinic if valid.

No identifying information about you (such as your name, address, telephone numbers) will be collected for this survey. Some routine information, such as your age, gender, a list of your medications, start date of HIV treatment will be collected from your medical chart.

A report on the results of these tests for patients being treated at this clinic may be published. No identifying information about you or any other patient will appear in the group report.

**Risks/Discomforts**
There should not be any risks or discomfort in this survey, except for a minor pain at the site of finger stick.

**Benefits:**
These tests will help estimate the rates of HIV drug resistance in the patients at this clinic. However, you and your doctors may find the results helpful in talking about treatment plans.
The results of both tests in this survey will help us learn more about HIV in this area (specific names) now and how well treatment is working for patients at this clinic. The results may help doctors choose the best HIV treatments for people in our country and may help the treatment program at this clinic work better.

Your rights
Your participation in this survey is completely voluntary. You can decide not to be in the survey at all. You will still receive all the services routinely available at this clinic. You may leave this survey at any time without any impact on your treatment and care. You will be given a copy of this form to take with you. You may ask any questions about this survey or this consent form now or in the future. If you have questions about this survey, you may contact __________ at the following phone number ______________. You may also call this person if you have questions or concerns about your rights as a subject in this research survey.

Protecting your privacy
Your name, telephone number and address will not be recorded in any forms or reports that come from this survey. The survey will only report group results. Your name will also not be used on either the blood collection tubes or the blood test results. A survey code number will be used instead of your name. All the information that we collect will be kept confidential and anonymous.

Do you agree to participate in the survey?
Appendix 5: Research Subject Information Sheet

Hello, my name is (MD or nurse’s name), and I am a (MD/nurse) working at this (name) clinic.

Purpose:
We would like to invite you to participate in a survey which looks at the amount of human immunodeficiency (HIV) virus in your blood and, if there is any, if there is drug resistance of the HIV. Testing of HIV for resistance to the medications that you take to treat your HIV is not done for everyone in the country but has the potential to help doctors choose the best medicine for you and other patients. We are doing this study to learn different ways to monitor for the development of HIV drug resistance. For this survey, we will be using blood obtained by a finger stick dropped onto a special piece of filter paper; this is called a dried blood spot. These dried blood spots will then be sent to a laboratory to see if you have detectable virus in your blood, and if so, we will do drug resistance testing.

What should you know about your participation in this research study?
Your participation in this study is voluntary; you will not be paid for donating your specimen. Whatever you decide, it will not affect your regular care at your clinic or any other treatment site.

What procedures will be done to you as part of this study?
We will perform a finger stick (to obtain about 5 drops of blood; this may be repeated if the first finger prick was not successful). We will drop your blood onto the special filter paper for testing. The finger stick will be performed by holding the ‘ring’ finger of the non-dominant hand and making a prick with a lancet to the cleaned area (disinfected with alcohol wipes) of the finger tip (lateral side of finger tip).

The blood samples will be transported to a drug resistance laboratory (may add specific details) to determine the number of HIV viruses that you have in your blood. If you have more than 1000 copies of virus per one ml in your blood your blood will also checked to see if your virus has any detectable drug resistance. Your current ARV drug regimen, age, gender and some information about your medical history will be collected for this study. However, no information that can directly identify you will be available to anyone at the laboratory.
To ensure the confidentiality of your information, we will not record your name or address on any form or result. You will only be identified by study ID for which only clinical staff working on this study will use to identify you and your results. The electronic records linking your study ID and name will be kept with the study coordinator at the clinics.

**What are the possible benefits?**

HIV-1 drug resistance testing results may be beneficial to you and your doctor in talking about treatment plans. The results of the survey will be returned to you and your health care providers at your clinic to aid in your treatment. It may also help us better understand how to monitor for HIVDR in HIV treatment programmes such as the one in your country.

**What are the possible risks and discomforts?**

There should not be any risks or discomfort in this survey, except for a minor pain or bruise at the site of the finger stick in your finger which is common in every blood draw.

For more information about risks and side effects, ask the investigator or nurse.

**Your rights**

Your participation in this survey is completely voluntary. You can decide not to be in the survey at all. You will still receive all the services routinely available at this clinic. You may leave this survey at any time without any impact on your treatment and care. You will be given a copy of this form to take with you. You may ask any questions about this survey or this consent form now or in the future. If you have questions about this survey, you may contact __________ at the following phone number __________. You may also call this person if you have questions or concerns about your rights as a subject in this research survey.

**Protecting your privacy**

Your name, telephone number and address will not be recorded in any forms or reports that come from this survey. The survey will only report group results. Your name will also not be used on the dried blood spot paper, blood collection tubes and blood test results. A survey code number will be used instead of your name. All the information that we collect will be kept confidential and anonymous.

Do you agree to participate in the survey?
Appendix 6 – Procedure and description of assignment of HIVDR survey identification number (HIVDR-SID)

Individuals enrolled in the survey will be assigned a HIVDR survey identification (HIVDR-SID) number. This number will be in a format as follows: country abbreviation-Clinic name-unique patient number. For instance, if this survey was taking place in the country at Central Hospital, patient’s HIVDR-SID would look like COU-CH-0001 (COU is the abbreviation for Country, CH the abbreviation for Central hospital and 0001 is the unique patient number at that site). Countries may adapt this HIVDR-SID system in a way that is straightforward and helpful for that country program. Enrollment will be noted in a logbook containing date of blood draw, date of ART start, patient ID (unique at each ART site), and HIVDR-SID.
Appendix 7: HIVDR monitoring collection site logbook

ART SITE NAME: ______________________________
SURVEY START DATE: __/__/____
ANTICIPATED DATE OF SURVEY COMPLETION: __/__/____

<table>
<thead>
<tr>
<th>ART unique number</th>
<th>HIVDR SID</th>
<th>Age</th>
<th>Gender (Circle)</th>
<th>Date of ART initiation</th>
<th>Current ART regimen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Male</td>
<td><strong>/</strong>/_____</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Female</td>
<td><strong>/</strong>/_____</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Male</td>
<td><strong>/</strong>/_____</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Female</td>
<td><strong>/</strong>/_____</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Male</td>
<td><strong>/</strong>/_____</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Female</td>
<td><strong>/</strong>/_____</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Male</td>
<td><strong>/</strong>/_____</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Female</td>
<td><strong>/</strong>/_____</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Male</td>
<td><strong>/</strong>/_____</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Female</td>
<td><strong>/</strong>/_____</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Male</td>
<td><strong>/</strong>/_____</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Female</td>
<td><strong>/</strong>/_____</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Male</td>
<td><strong>/</strong>/_____</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Female</td>
<td><strong>/</strong>/_____</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Male</td>
<td><strong>/</strong>/_____</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Female</td>
<td><strong>/</strong>/_____</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Male</td>
<td><strong>/</strong>/_____</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Female</td>
<td><strong>/</strong>/_____</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Male</td>
<td><strong>/</strong>/_____</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Female</td>
<td><strong>/</strong>/_____</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Male</td>
<td><strong>/</strong>/_____</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Female</td>
<td><strong>/</strong>/_____</td>
<td></td>
</tr>
</tbody>
</table>
Appendix 8: HIV Drug Resistance Survey Data Abstraction Form

Site______________________        Data abstractor ____________________________

1. Enrollment date (dd/mm/yyyy).      ___/___/____

2. HIVDR monitoring identification number .............................................Label

3. Gender (circle one)    Male / Female

4. Age (years) ______ (unknown = 97)

5. Current ART regimen prescribed:


6. Date of initiation of current ART regimen at this ART clinic (dd/mm/yyyy) ___/___/____
Appendix 9: Laboratory Results Notification Form

The following is an example of laboratory result notification form for ART sites participating in HIVDR monitoring surveys.

**Viral Load results**

Viral load testing was done on specimens collected on (MM/DD/YYYY) for HIVDR-SID (XXXXX). This test was done as part of an evaluation of the antiretroviral therapy program at your clinic.

The result is: (XXXX copies/mL).

This result may reflect the status of your patient at the date the blood was drawn. This result is provided for information only, and should not be used to make clinical decisions as such decisions should not be based on one viral load test. Any modification to the patient care or treatment should be based on an evaluation of your patient's current condition, and on ART program guidelines.

If you have any questions contact [local, regional, or national tertiary care physician or National AIDS Program]

**HIV drug resistance test result**

HIV drug resistance test was done on specimens collected on (MM/DD/YYYY) for HIVDR-SID (XXXXXX). This result may give you some information about the status of your patient at the date the blood was drawn. This test was done as part of an assessment of the antiretroviral therapy program at your clinic.

The following mutations were identified: (list of mutations and significance).

Presence of known mutations associated with drug resistance may cause or contribute to adverse antiretroviral therapy outcome, but some patients who have some of these mutations have still experienced satisfactory treatment outcomes on regimens that included drugs to which the test indicates resistance.

This result is provided for information only, and should not be used to make clinical decisions. Any modification to the patient care or treatment should be based on an evaluation of your patient's current condition and on ART program guidelines.

If you have any questions contact (Name and contact information to be filled in by the working group prior to survey begins)].
Appendix 10 - Sample format for HIVDR monitoring survey specimen manifests

HIVDR Monitoring Specimen Manifest

Copies of specimen manifests are sent along with specimen to each laboratory along the way from the point of collection to the HIVDR testing laboratory. A copy of the final and complete manifest is sent to the country working group and to the WHO region and headquarters for quality assurance.

<table>
<thead>
<tr>
<th>SITE OF COLLECTION OF DBS</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIVDR Survey ID (SID)</td>
</tr>
<tr>
<td>Date of Collection</td>
</tr>
<tr>
<td>ART Clinic</td>
</tr>
<tr>
<td>Type of DBS specimen</td>
</tr>
<tr>
<td>Number of Blood Spots Made</td>
</tr>
<tr>
<td>Date Specimen Shipped From Site of Collection</td>
</tr>
</tbody>
</table>

If -20°C or Below Facilities are Available at Site of Collection of DBS

| Date of Freezing of DBS | ___/___/____ |
| Date DBS Brought Back to Ambient Temperature | ___/___/____ |
| Date of Changing Desiccant Bags | ___/___/____ |
| **Date of Transport to HIVDR Laboratory** | ___ / ___ / ______ |
| **If -20°C or below facilities are NOT available at the site of collection – Intermediate Facility** |
| **Date Specimen Shipped from Clinic to Intermediate Facility** | ___ / ___ / ______ |
| **Date of Specimen Arrival at Site for Freezing** | ___ / ___ / ______ |
| **Packaging Status of Specimen on arrival (check if yes)** | Packing intact? | Arrival at ambient temperature? | Any Desiccant bags pink? | Condensation present? |
| **Date of freezing to -20°C or below** | ___ / ___ / ______ |
| **Date of Shipment of DBS to HIVDR Laboratory** | ___ / ___ / ______ |

**HIVDR LABORATORY**

<p>| <strong>HIVDR Survey ID (SID)</strong> | XXX-<strong>-</strong> |
| <strong>Date of receiving</strong> | ___ / ___ / ______ |
| <strong>Condition of specimen</strong> | Packing intact? | Arrival at ambient temperature? | Any Desiccant bags pink? | Condensation present? |
| <strong>If other, describe</strong> |
| <strong>Date of freezing, if applicable</strong> | ___ / ___ / ______ |
| <strong>Date of amplification attempted</strong> | ___ / ___ / ______ |
| <strong>Amplification success</strong> | Yes | No |
| <strong>If no, reason for failure?</strong> |</p>
<table>
<thead>
<tr>
<th>Date of sequencing attempted</th>
</tr>
</thead>
</table>
| Sequencing success? | Yes ☐  
|                           | No ☐  
| If no, reason for failure? |  
| Date preliminary FASTA file and interpretation returned to national HIVDR working group and WHO headquarters | ___ / ___ / ______  
| Date final quality assured FASTA file and interpretation returned to national HIVDR working group and WHO headquarters | ___ / ___ / ______  

Appendix 11: Country Information Summary Form

The following list should be used for country information summary forms in countries receiving financial support for surveys to monitor HIVDR prevention at sentinel ART sites from the United States Centers for Disease Control and Prevention (US CDC) and/or World Health Organization (WHO).

1. Country Name:

2. Principle Investigator(s) (name, title, organization, email, office telephone number, mobile telephone number):

3. Ministry of Health or National AIDS Committee Designated Coordinator (name, title, organization, email, office telephone number, mobile telephone number):

4a. WHO contact in country (Name, email contact, telephone numbers):

4b. CDC contact in country (Name, userid, telephone numbers):

5. Sites selected (site name and location, site liaison name)
   A.
   B.
   C.
   D.

   Please include stratification plan for ART clinics (organogram) on a separate page (See Figure 3 for more information regarding organogram)

6. Laboratory(ies) coordinating specimen handling, transport within country, storage, and shipment to genotyping laboratory:
7. Name and contact details of specimen handling and processing coordinator:

8. Genotyping (HIV drug resistance testing) laboratory (Name, address):

9. Collaborating genotyping laboratories, if any:

10. Additional institutions and organizations collaborating in HIVDR surveys:

11. Date of in-country Institutional Review Board (IRB) or Ethics Committee (EC) approval:

12. Federal Wide Assurance number of IRB or EC:

Attachments (check if attached):
1. Variable list □
2. Data abstraction form, if any □
3. Tracking log format □
4. Consent information sheet □
5. IRB or EC approval letter □
References


14 Garrido C, Zahonero N, Corral A, Arredondo M, Soriano V, de Mendoza C. Correlation between human immunodeficiency virus type 1 (HIV-1) RNA measurements obtained with dried blood spots and


20 “LQAS Sampling Plan Calculator.” Available at http://www.brixtonhealth.com/hyperLQAS.html


24 Myatt M, Mai NP, Quynh NQ et al. “Using lot quality assurance sampling (LQAS) and area sampling to identify priority intervention areas for trachoma control activities- experiences from Vietnam.” Bull World Health Organ. 2005; 83: 756-63.