World Health Organization Protocol for Population-based Monitoring of HIV Drug Resistance Emerging During Treatment and Related Program Factors at Sentinel Antiretroviral Therapy Clinics

2012 Update
1. Introduction

1.1. Global Expansion of Antiretroviral Treatment

In 2001, the United Nations General Assembly Special Session on HIV/AIDS (UNAIDS) 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 to avoid use of substandard treatment leading to poor outcome and the emergence of HIV drug resistance in populations treated with ART.¹

To support countries in Africa, the Caribbean and Asia that are heavily affected by the HIV/AIDS epidemic, President George W. Bush announced the Emergency Plan for AIDS Relief (PEPFAR) in 2003. The goals of this plan are to treat 2 million patients with highly active antiretroviral treatment (HAART), prevent 7 million new HIV infections and care for 10 million people infected or affected by the epidemic. Combined with support available through WHO’s “3 by 5” strategy and the Global Fund to Fight AIDS, Tuberculosis, and Malaria (GFATM), several million individuals living with HIV/AIDS in resource-limited countries will receive ARV drugs.

The use of ART in developed countries has been associated with the development of HIV drug resistance (HIVDR).² Because of the error-prone nature of HIV replication, its 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 persons in treatment even if appropriate HAART regimens are provided and optimal adherence to therapy is supported.³ For countries using a population-based approach for ART regimen selection, WHO also recommends a standardized population-based approach for ongoing evaluation of the relationship of ART program factors to HIVDR arising during ART.
1.2. HIV epidemic and ART in [Country specific Information]

1.3. Justification for HIVDR monitoring surveys in the first year of ART at sentinel sites

This HIVDR monitoring protocol will focus on a consecutively selected cohort of patients starting ART in each of several representative sentinel sites, and evaluate population level HIVDR prevention 12 months after ART begins, as well as factors that may have affected that outcome during the first year and that could be addressed, if necessary, by changes at the site or programme level. Programmatic achievement of $\geq 70\%$ HIVDR prevention in populations at one year after start of ART is the WHO suggested standard to minimize the emergence of HIVDR, and this standard will be applied for X. This standard represents a consensus of international experts and takes into account the literature demonstrating that well-functioning ART programs can achieve $>70\%$ viral load suppression in resource-limited settings, which is comparable to that achieved in resource-rich settings. Information for this survey is collected from existing medical records regarding basic demographic and clinical information, patients’ scheduled and actual appointments (appointment keeping), ART regimens prescribed, ART pick-ups (drugs dispensed and date of pick-up), changes in regimen (substitutions of ARV drugs within first-line); relevant routinely performed laboratory tests (such as CD4 counts), changes in clinical status, and determination of the patient’s clinical status; specifically, if the individual has died, transferred out, become lost to follow up, stopped ART, been switched to second-line ART, or is still alive and on first-line ART. The participant’s previous ARV experience prior to starting first-line therapy at baseline and endpoint along with adherence as measured by a 30-day visual analogue scale (VAS), at endpoint will be collected with an additional questionnaire.

1.3.1. Justification for duration of the evaluation and measurement intervals

The choice of the first year of therapy for monitoring of program success is based on both practical and theoretical considerations:

Although three to six months of ART is sufficient to evaluate the effectiveness of an ART regimen, it is insufficient to evaluate program success in retaining patients and successfully continuing to deliver drugs effectively to prevent HIVDR emergence.
Losses to follow-up, problems with appointment keeping or drug-pickup, and discontinuities in drug supply may be more likely to occur after the first six months. The evaluation of a full year of a treatment program's functioning yields more information.

Studies have shown that even in resource-limited settings, well-functioning ART programs can achieve $\geq 70\%$ viral load suppression. The existence of a wide variety of studies demonstrating this degree of success at one-year provides a benchmark against which to compare ART program functioning.

Information and baseline measurements on all patients starting ART are available at baseline. Use of a cross-sectional methodology, which would not allow measurement of baseline mutations, the evaluation of previous ARV experience in individuals no longer attending the site, or the evaluation of mutations before switch in persons who have already switched regimens, would be less informative.

1.3.2. Justification for collection of laboratory measurements

Individuals maintaining viral suppression while on therapy are unlikely to develop HIVDR, and for population-based evaluations, groups with evidence of viral suppression can be said to have no "effective" HIVDR, that is, no HIVDR that will adversely reduce the effectiveness of their ARV treatment. For this reason, a minimum-resource estimate of an ART program's success at minimizing HIVDR during the first year of ART can be based on the proportion of the cohort with evidence of HIVDR prevention, evaluated by a viral load measurement 12 months after beginning ART or at time of switch.

HIVDR testing at baseline provides information on resistance transmitted or acquired prior to starting ART. Individuals who have received ARVs as part of Prevention of Mother to Child Transmission (PMTCT) programs, or who have previously taken mono- or dual-therapy, or who have obtained ARV drugs through private or informal settings are eligible for ART, and because transmitted resistance may occur in some areas and affect outcomes, this protocol will evaluate the extent to which baseline mutations may be associated with outcomes during the first year.

HIVDR testing at time of switch to a second line regimen or after 12 months of first-line ART is an incomplete but important measure that will help to characterize the patterns of mutations developing among those with insufficient viral suppression, and may help to generate hypotheses about the effects of previous PMTCT or informally
available ARVs, about the quality or tolerability of one or more drugs in the first line regimen, or about the effects of additional treatment experienced by the patient.

1.4. Case definition and other definitions

- **Baseline**: Baseline is defined as the time of commencement of first-line ART. Baseline specimens should be collected within one month prior to starting ART.

- **Endpoints** defined as the time at which the outcome evaluation is performed, when the individual can be classified with one of these endpoints:
  
  o Still on a first line ART regimen **12 months** after commencement of first-line ART.
  
  o Time of **switch** from a first-line to a second-line ART regimen during the first 12 months after commencement of first-line ART.
  
  o Time of **stop** of the first-line ART regimen during the first 12 months after commencement of first-line ART.
  
  o Time of first classification as **lost to follow-up** during the first 12 months after commencement of first-line ART.
  
  o Time of **death** during the first 12 months after commencement of first-line ART.

- **HIVDR**, for the purposes of HIVDR surveys, is considered low, high, or intermediate drug resistance to one or more NRTIs, NNRTIs, or PIs as defined by the Stanford HIVDR database website.

- **Possible HIVDR**. Specimens from patients still on a first-line ART regimen with a viral load $\geq 1000$ copies/mL and no evidence of HIVDR on genotype testing are classified as having **possible HIVDR**. Patients who have been **lost to follow-up** or who **stopped** ART within the first 12 months of ART, and from whom no specimens are available for classification, are also classified as having **possible HIVDR**. Other patients for whom no endpoint viral load or HIVDR result is available, and who did not transfer out or die during their first 12 months of HIVDR, are also classified as having **possible HIVDR**. This definition is only used for the purpose of the protocol of classifying **endpoints** with regards to outcome results.
- **HIVDR prevention**, for the purposes of HIVDR surveys, is defined as a specimen with an HIV RNA <1000 copies/mL **12 months** after initiating ART. Individuals who have switched to second-line with undetectable virus within the first 12 months are not classified as achieving HIVDR prevention. Such a switch would usually be clinically inappropriate and programmatically premature, so these individuals are classified separately (see below).

- **Undetectable viral load at switch.** Participants whose regimen is switched to second-line ART during their first 12 months of treatment, and whose viral load is measured as <1000 copies/mL just before the switch takes place, are classified as having **undetectable viral load at switch**.

- **Endpoint HIVDR evaluation not performed (censored).** Individuals whose endpoint is **transfer out or death** are not included in the analysis of HIVDR prevention (They are removed from the numerator and denominator before the estimation of HIVDR prevention prevalence in the first year of ART).

- **Viral suppression:** For the purposes of HIVDR prevention monitoring, viral suppression is defined as an HIV RNA <1,000 copies/ml on viral load testing.

- **Twelve-month follow-up visit:** The twelve-month visit is defined as the clinic visit on which all twelve-month routine follow-up procedures are performed. In practice, the visit may take place up to 15 months after ART begins.

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

- **Substitution:** is defined as change from one first-line ARV regimen to another ARV first-line regimen, for example due to adverse events or intolerance. For the purposes of HIVDR Monitoring, the new regimen instituted at time of substitution is recorded in the database and can be analyzed, but this is not an **endpoint event**; the patient will continue to be followed until an **endpoint event**.

- **Transfer out:** “Transfer out” is defined as the transfer of HIV care from the HIVDR monitoring site to another identified ART delivery site for patients who have not stopped first line ART at the time of transfer.

- **Death:** Death refers to the recorded death of a patient for which a date (at least month/year) is recorded within 12 months following the start of first line ART.
Stop: An ART stop for the purposes of HIVDR monitoring is defined as the complete cessation of ART by a patient who has not restarted ART by the time of the 12 month blood draw although he or she remains in care at the site. Stops usually take place because of a patient decision or a decision by the clinical team, and generally reflect either a planned treatment interruption of ART or a decision based on poor adherence. Operationally, “stop” is defined as the endpoint if a patient still attending the clinic has taken no ART in the 30 days before the 12-month blood draw.

On first-line ART at 12 months: A patient is defined as still on first-line ART at 12 months, and therefore eligible to have a residual specimen sent for viral load testing and HIVDR testing from the 12-month blood draw, if he or she has taken an ARV first-line drug within 30 days of the 12-month blood draw and is not described by any of the definitions of the "STOP" endpoint above. The first-line regimen may be the ART regimen on which the patient began ART 12 months before, or an alternative regimen that was started during a substitution.

Loss to follow up: A patient is defined as “lost to follow up” if he or she has not returned to clinic or pharmacy for a scheduled appointment or drug pick-up > 90 days after the missed appointment/drug pick-up and there is no information to classify the patient in one of the other endpoint categories, such as death or transfer out. Patients who have been classified as “lost to follow up” for some period during the first 12 months after beginning ART, but have returned to the clinic for care by the time of the 12-month follow-up appointment should not be classified as “lost to follow up” for purposes of HIVDR monitoring. Such patients will be classifiable as STOP, SWITCH, or "STILL ON FIRST-LINE ART AT 12 MONTHS."

12 month appointment: For clinical purposes, WHO and most other organizations promulgating ART guidelines recommend a thorough clinical evaluation approximately 12 months after ART is initiated. The appointment at which this thorough evaluation is performed may occur between 11 - 15 months after ART has begun. For the purposes of the survey, the 12 month appointment is defined as the appointment at which procedures dictated by the clinic for the 12-month evaluation are performed, as long as the appointment occurs between 11 - 15 months after ART was initiated at the clinic.
On time drug pick up is defined as pick up of ART prior to the time when previously dispensed drugs would have run out if taken according to schedule.

On time appointment keeping is defined as keeping an appointment either prior to, or on the date of the scheduled appointment or no more than seven days following the scheduled appointment. While a surrogate can pick up ART and the pick-up may be classified as on time, a surrogate cannot attend the clinic visit on behalf of a patient. If a surrogate attends a clinic visit on behalf of a patient this is not an on-time appointment; rather this is properly classified as a missed appointment.

2. Objectives

2.1. HIVDR Monitoring objectives

1. At each site, estimate the proportion of the ART site population achieving HIVDR prevention, as measured by viral load suppression, i.e. ≥ 70% viral suppression in populations after one year of first-line ART.

2. Identify specific HIVDR mutations and mutation patterns in populations not achieving prevention of HIVDR on first-line ART.

3. Identify programmatic factors potentially associated with the prevention (or non-prevention) of HIVDR

4. Report and disseminate results and recommendations
   - to support optimal ART program functioning at the sites,
   - to apply lessons learned to other ART program sites for expansion of HIVDR survey,
   - to suggest further studies or evaluations to provide additional information on program factors associated with HIV drug resistance emergence, or methods for optimizing program functioning, and
   - to support planning and decision making to optimize ART effectiveness
2.2. Hypotheses

1. ART programs meeting the following conditions will demonstrate HIVDR prevention in ≥70% of patients 12 months after ART start, with the exception noted below in hypothesis number 2:
   - Appropriate first-line regimens were prescribed at ART start for 100% of participants
   - There were 0 months of ARV drug stock-outs during the survey
   - ≥80% of patients kept 100% of expected clinical appointments in the first 12 months of ART
   - ≥90% of participants picked up all of their drugs on time in the first 12 months of ART
   - < 20% of participants starting ART were lost to follow-up in the first 12 months of ART

2. An ART site with ≥15% of patients beginning first line ART with evidence of intermediate- or high-level resistance [3] associated with resistance to NNRTIs will not demonstrate HIVDR prevention in ≥70% of patients 12 months after ART start even if the conditions described in hypothesis 1 are met.

3. An ART site with ≥15% of patients beginning first line ART with a history of PMTCT will demonstrate HIVDR prevention in ≥70% of patients 12 months after ART start if the conditions described in hypothesis 1 are met.

4. An ART site with ≥15% of patients beginning first line ART with a history of non-PMTCT use of ARVs will demonstrate HIVDR prevention in ≥70% of patients 12 months after ART start if the conditions described in hypothesis 1 are met.

2.3. Intended use of results

Results from HIVDR monitoring at sentinel sites will contribute valuable information to the national HIVDR working group to be used in evidence-based decision making on maintaining the effectiveness of first-line ART and supporting ART program practices associated with HIVDR prevention. Results may also contribute to discussions on the selection of ARV regimens for prophylaxis, including PMTCT.
Results from these surveys will assist the national HIVDR working group to expand to other sentinel sites in the country and design further rounds of HIVDR monitoring.

The HIVDR working group, with support from WHO and CDC, will present results at the regional and global level, which will support discussions and evidence-based decisions on the effectiveness of internationally recommended first- and second-line regimens, discussions on vaccine design, development of microbicides for HIV prevention, and the selection of ARV regimens for prophylaxis, including PMTCT.

3. Procedures/Methods

3.1. Design overview

Prospective survey method. Individuals who meet the criteria defined in Sections 3.3.1 and 3.3.2 are enrolled consecutively based on the ART start date.

At the time of enrolment (baseline), basic patient information will be abstracted from the patient records, including socio-demographic information, regimen prescribed and date of start of ART, CD4 count and WHO clinical staging. Previous ARV exposure (including PMTCT, previous ART) will be extracted from a survey questionnaire. A “special” blood draw will be collected for plasma at enrolment for genotypic HIV drug resistance testing. All plasma specimens at enrolment will be collected on the day of first ART drug pick-up for the patient before the patient has consumed any dose of ART.

A second set of non-laboratory information is collected from medical records abstraction at the end of the survey period. Individuals’ endpoints are extracted: on first line ART at 12 months, switch, LTFU, Stop. The information extracted includes the dates of scheduled and actual appointments, the dates of scheduled and actual pharmacy pick-ups, and changes in regimen. Information about ARV history prior to initiation of first-line ART at the site and the 30-day adherence assessment using the Visual Analogue Scale (VAS) are collected using the endpoint questionnaire; the questionnaire is administered by a provider to those participants who reach the endpoint switch to second-line ART or on first-line ART at 12 months. At the endpoints of switch and on first-line ART at 12 months, a “special” blood draw will be collected for plasma for viral load
quantification and HIVDR testing will be performed on specimens with detectable viral loads.

Additionally, site-specific variables will be collected at the time that the survey starts and at 12 months. These variables include information on drug supply, number of months with drug stock-outs during the survey period, and presence of adherence counselling for individuals starting and on ART, etc.

(See Data section 4 for lists of variables and Appendix 1 for questionnaire)

3.2. HIVDR sentinel monitoring sites

According to the WHO generic protocol, HIVDR sentinel monitoring should focus first on sentinel sites where ART is provided according to national ART prescribing guidelines. After the first year, sentinel monitoring will be expanded to a variety of sites representing the various treatment situations within the country.

3.3. Survey Population

The population for the survey will be HIV infected patients at the selected ART site who satisfy the following inclusion and exclusion criteria.

3.3.1. Participant inclusion criteria

Patients attending the four selected OPCs who meet the following criteria:

1. Individuals who consent following the written informed consent process, and
2. Who are eligible to initiate, and do initiate, an adult first-line ART regimen at a participating site, regardless of age. This includes individuals who have had previous ART elsewhere (including in the private sector) if they are eligible to initiate first-line ART at this site, with the exception noted below under 3.3.2.

3.3.2. Participant exclusion criteria

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

2. Individuals enrolled in a clinical trial or clinical research study (either at the sentinel monitoring site or another location)
3. Individuals who are part of an observational cohort for whom more follow-up
efforts are made than for other ART patients treated at the site (Patients enrolled in an observational cohort for whom no additional follow-up procedures are included may be eligible).

4. Individuals restarting ART, who have previously started and stopped ART at the sentinel survey site.

5. Individuals transferring in from another ART site who are at the time of transfer currently taking a three- or four-drug first-line ART regimen

6. Individuals with HIV-2 or HIV-1/HIV-2 co-infection are excluded from monitoring surveys.

3.4. Sample size at each clinic

The number enrolled at each survey site must be sufficient to allow a reasonable expectation that at least 96 individuals who start therapy at that site will be classifiable at Endpoint (Appendix 2). 96 individuals initially starting ART at the site, plus an additional number calculated for that site, reflecting the numbers who transferred out and those who died among the first 100 patients starting ART in the same quarter in the previous year, will be consecutively enrolled based on the date of ART start. This will provide the estimate of the proportion of adults with HIVDR suppression at 12 months with a 95% confidence interval of +/-10% irrespective of the incidence of viral suppression.

3.5. Detailed enrolment

3.5.1. Monitoring survey procedures

All patients with whom the decision has been made to initiate ART at the sentinel site on or after the identified HIVDR monitoring survey start date will be enrolled consecutively until the effective survey sample size is reached. Consecutive enrolment will be based on the date (and time if recorded) on which the blood draw for genotyping is performed (i.e. the day patients make first ARV drug pick-up). In the last individual counseling session before ART initiation, doctors or nurses at the ART site will ask patients if they want to participate in the survey. Oral consent will be obtained. Patients who consent will follow the survey procedures briefly described in Section 3.1. Individuals enrolled in the evaluation will be assigned HIVDR survey identification
(SID) numbers. This number will be in a format like Country-(Clinic name)-xxxx, such as Country-BH-xxxx for Beverly Hospital. Enrolment 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.

### 3.5.3. (Baseline) data and specimen collection

For each participant:

- Basic socio-demographic information and clinical information will be extracted from the medical record. Date started and information on regimen prescribed will be extracted from the pharmacy record system. See section 4.1.1 for list of variables to be collected (see also Appendix 1). All variables are available from the standardized patient record system at the ART clinic, the HIV CARE/ART CARDS, and the pharmacy record system. A separate questionnaire will be administered about ARV history prior to initiation of first-line ART at the site.

- Information on specimen collection and processing will be recorded to characterize the specimen that will be used for HIVDR testing. (section 4.1.4)

- Each participant will have a “special” blood draw HIVDR on the first ARV dispensing day at the clinic. (See Specimen processing below).

### 3.5.4. Endpoint data and specimen collection

- Patient variables are listed in section 4.1.2 (see also Appendix 1). Data are recorded retrospectively, following the evaluation.

- Specimen tracking variables, for patients for whom specimens are collected at endpoint are listed in section 4.1.4.

- Information about ARV history prior to initiation of first-line ART at the site and the 30-day adherence assessment using the VAS are collected using the endpoint questionnaire; the questionnaire is administered by a provider to those participants who reach the endpoint switch to second-line ART or on first-line ART at 12 months.

- At either the 12 month visit routine lab draw, or at switch, each participant will have “special” blood draw for viral load testing. Specimens with a viral load > 1,000 copies/ml will be further assayed for viral genotypic resistance (see Specimen...
processing below). Patients, who die, transfer out or who are lost to follow up will not be able to have blood specimen collected.

The HIVDR coordinators will calculate the projected endpoint of the survey 12 months from the start of ART date and record this date in the log. All endpoint classifications will be recorded and verified by experts at data analysis. Follow-up procedures between baseline and endpoint will be per routine at the clinics. Viral load and HIVDR testing will be done at the University of the Witwatersrand Genotyping Laboratory.

3.6. Specimen collection, handling, processing, and tracking

3.6.1. Plasma specimens

On the day of first day of the survey, all patients who start ART and who consent to the survey will have a “special” blood drawn for HIVDR (baseline specimen). Five (5) ml of blood will be drawn from each participant into an anticoagulant tube (EDTA) available at all ART sites, which will yield ~2.5 ml of plasma. Blood specimens will be collected following venipuncture procedures currently implemented at the ART sites. Mandatory information will be recorded on the label of the specimen tube including the HIVDR-SID, with the addition of “B” for baseline specimen, and the date/time of blood draw. Specimen variables will be recorded on the specimen manifest and accompany the specimen during its handling, transport, and storage at the collection site, the processing/storage laboratory, and the accredited HIVDR genotyping laboratory. A copy of the final and complete manifest is sent to the country working group and the WHO region and headquarters for quality assurance. All specimen collection, handling and processing will be done following WHO laboratory guidance for specimen collection, handling and processing.

http://www.who.int/hiv/pub/drugresistance/hiv_reslab_strategy.pdf

Sample site operational procedures are described in Appendix 4.

Eligible plasma specimens for HIVDR Monitoring may will be shipped to a WHO accredited laboratory for viral load and genotyping.
3.7. Patient confidentiality

3.7.1. Administration of the oral consent

A brief oral consent process, which requests consent to draw additional blood specimens and abstract non-identifying information will be administered to all patients identified as eligible to start ART and about to make their first ARV drug pick-up. The consent information includes the facts that the laboratory results will be returned to the medical chart for those whose blood is tested. Consent will be asked before ART initiation, i.e. at the last individual counselling session of each patient (Appendix 3).

3.7.2. Return of Laboratory Results to the Medical Chart

Results will be sent back from the WHO accredited laboratory to ART site and to HIVDR monitoring coordinators. Using the log book which includes both patient ID and matching HIVDR-SID, HIVDR monitoring coordinators and clinic coordinators will work together to return the results to each patient chart (Appendix 5). The results will be discussed with the patient by the clinician on the next regularly scheduled visit, and should be able to be forwarded to another clinician or clinical site at the patient's request. Clinicians at the ART site may use the results for patient management purposes if relevant.

3.7.3. Confidentiality

Specimens and laboratory results Aliquots sent for viral load testing and HIVDR testing will be labeled 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 HIVDR coordinators. Results returned to the clinic will also be labeled 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 that will allow medical records abstraction at baseline and endpoint, and laboratory specimens from baseline and endpoint, to receive the same HIVDR-SID for each patient. These will be destroyed before specimens and non-identifying data items are sent to the viral load and HIVDR testing laboratories.
Data held in the national, regional and central HIVDR monitoring databases

No data are collected for the survey except that collected routinely for clinical purposes at the site. Data entered into the national HIVDR database include only the HIVDR-SID, basic sociodemographic and clinical data, current regimen information, data on number of appointments and drug pick-ups scheduled and kept between baseline and endpoint, and specimen tracking information such as dates blood is drawn, the plasma is separated and aliquoted. No personal identifying information on participants will be made available. Any suspected breach of confidentiality will be dealt with immediately.

HIVDR monitoring databases, laptops, and forms. Databases will be password-protected so that only persons working on the HIVDR Monitoring survey will have access. Laptop computers, HIVDR Monitoring questionnaires, supplemental HIVDR Monitoring forms, laboratory specimen tracking forms, logs, and genotype results will be locked in file cabinets or drawers when not being used by monitoring staff. The HIVDR analysis database will be kept in a password-protected computer at NICD.

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. Individual variables from monitoring site – Baseline

- Site
- HIVDR-SID (25 characters max)
- Date ART started
- ART regimen
- Gender
- Date of birth
- Age (calculated field if DOB is entered)
- Residence of patient (District/City/Region)
- Occupation
- Risk factors
- Previous ARV exposure: PMTCT and/or other (including regimen and duration if available)
- Current pregnancy and date of last pregnancy (for female)
- Weight
- On tuberculosis treatment at start of ART (TB regimen)
- CD4 cell count and CD4 date
- WHO stage
- Viral load and viral load date (will be done as part of the survey)
- Date and time of blood draw
- Name or initials of staff member drawing blood
- Baseline sequence for the protease region and the relevant portion of the reverse transcriptase region of the HIV genome [to be uploaded from genotyping lab]
- Data entry person
- Data entry date

4.1.2. Individual variables from monitoring site – Endpoint
- HIVDR-SID (25 characters)
- Date ART started
- ART regimen
- Gender
- Date of birth
- Age (calculated field if DOB is entered)
- Regimen substitution between Baseline and Endpoint (yes/no, if yes regimen and date)
- Previous ARV exposure before ART start: PMTCT and/or other (regimen and duration [obtained from the endpoint questionnaire])
- 30-day proportion of ART doses taken estimate (%) using Visual Assessment Scale at endpoint
- CD4 cell count and CD4 date closest to Endpoint
● Endpoint status (death, transfer out, loss to follow up, stop ART, switch, still on ART at 12 months)
● WHO stage change during the period between Baseline and Endpoint (at Baseline and highest between Baseline and Endpoint)
● New OIs during the period between Baseline and Endpoint (dates and types of OI)
● Endpoint status (death, transfer out, loss to follow up, stop ART, switch, still on ART at 12 months – the status will be verified by experts at data analysis)
● Endpoint date
● Dates of scheduled clinic appointments between ART start and endpoint
● Dates of actual visits to clinic between ART start and endpoint
● Number of appointments scheduled between baseline and endpoint
● Number of appointments kept on time (within 7 days of scheduled appointment) between baseline and endpoint
● Dates of scheduled ARV drug pick-ups
● Dates of actual ARV drug pick-ups
● Number of drug pick-ups scheduled between ART start and endpoint
● Number of drug pick-ups made on time (before drugs previously dispensed would be used up) between ART start and endpoint
● Date and time of blood draw
● Viral load at endpoint
● On tuberculosis treatment during ART (TB regimen and time)
● Current pregnancy and date of last pregnancy (for female)
● Weight at 6 and 12 month (or at Endpoint)
● Endpoint sequence for the protease region and the relevant portion of the reverse transcriptase region of the HIV genome
● Data entry person
● Data entry date
● Name or initials of staff member drawing blood
4.1.3. Clinic (site) variables Record on survey start date + 12 months from start date

- Catchment area and population groups served
- Number of patients started on ART in the past 12 months
- Number of patients planned to be started on ART in the next 12 months
- Number of months in previous 12 months in which one or more first-line ARV drugs were insufficient for patients already on ART
- Number of months in previous 12 months in which one or more first-line ARV drugs were insufficient for patients scheduled to start ART
- Number of ARV stock-outs in previous 12 months
- Method of determining eligibility for ART
- Provider/patient ratio
- Training level for persons who start patients on ART
- Training level for persons who provide routine care during ART
- Location of ARV drug pick-ups (pharmacy in clinic, pharmacy off-site, treatment room in clinic, other (specify))
- Role of staff who dispense ARV drugs (physician, nurse, pharmacist, other (specify))
- Procedures for following up patients who do not return to clinic for ART appointments
- Costs of care (record 0 if no cost)
  - Cost of initial registration at clinic
  - Cost of each appointment
  - Cost of first-line ARV drugs
  - Cost of each routine laboratory test used in ART
  - Cost of special laboratory tests used in ART
- Days of the week; clinic opening and closing times for ART clinical appointments
- Days of the week; pharmacy opening and closing times for ARV drug pick-ups
- Maximum, minimum, and mean distance traveled by patients to clinic; brief description of most commons means of transport
• Longest, shortest, and mean waiting times for routine ART appointment at clinic
• Longest, shortest, and mean waiting times for ART drug pick-ups

4.1.4 Laboratory variables to be recorded on specimen manifests

Mandatory Information to be recorded on the label of specimen tube and aliquot includes the HIVDR survey ID, with the addition of a "B" for the baseline specimen and an "E" for the endpoint specimen, and the date/time of blood draw.

The following information should be recorded on the specimen manifest and accompany the specimen during its handling, transport, and storage at the collection site, the processing/storage laboratory, and the designated HIVDR genotyping laboratory.

**Point of collection**
- HIVDR SID (survey ID) (B if specimen collected at baseline; E if specimen collected at endpoint)
- Date/time of collection on the original EDTA collection tube

**Collection Site**
- Sentinel survey site ID
- Collection site laboratory specimen ID number (if collection site routinely assigned specimen numbers)
- Survey ID number (HIVDR-SID) (B if specimen collected at baseline; E if specimen collected at endpoint)
- Date/time of collection (recorded from label of specimen, see above)
- Volume of liquid specimen collected
- Date specimen sent from the collection site to the processing/storage lab
- Transport procedure description, including packaging and approximate temperature

**Processing/storage laboratory**
- Laboratory ID number (if any additional laboratory number was assigned)
• Survey ID number (HIVDR-SID) (B if specimen collected at baseline; E if specimen collected at endpoint)
• Date and time specimen was received at the processing laboratory
• Condition at time of receipt (frozen, cold, room temperature/ hemolyzed, contaminated, intact, other--describe)
• Date and time of plasma separation
• Volume of plasma aliquots for HIVDR and VL testing
• Number of aliquots (each aliquot is sequentially numbered for example: 1-4, etc.
  volume for each vial
• Date, time, and temperature of initial plasma aliquot refrigeration, if any
• Date, time, and temperature of initial plasma aliquot freeze, if any
• Dates and times of any thaws and dates and times of refreezes of plasma aliquots, if any
• Date and time specimens sent from the processing/storage site to the accredited genotyping laboratory
• Storage conditions for aliquots
• Description of shipping procedure to the genotyping lab, including packaging and approximate temperature

**Designated genotyping laboratory**

• Genotyping Laboratory ID number (if any additional laboratory number was assigned)
• Survey ID number (HIVDR-SID) (B if specimen collected at baseline; E if specimen collected at endpoint)
• Date and time specimen was received at the genotyping laboratory
• Condition at time of receipt (frozen, cold, room temperature/ hemolyzed, contaminated, intact, other--describe)
• Date and time of specimen freeze at genotyping laboratory, if any
• Date and time of any specimen thaws and date and time of any refreezes, if any
• Date of viral load test and viral load assay type
• Date of amplification and sequencing attempted, and assay type
• Amplification success (yes/no)
• Likely reason(s) for amplification failure
• Aliquot number used for viral load/genotyping
• Date preliminary fasta file and interpretation returned to national HIVDR working group
• Date final quality assured fasta file and interpretation returned to national HIVDR working group

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.

4.1.5. Variables from 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 RT region of the pol gene from position 1 through position 990-1200 will be sent as a text file. The entire nucleotide sequence of the protease region of the pol gene, and the nucleotide sequence of the RT region of the pol gene from position 1 through a position between nucleotide 900 and 1200 will be reported electronically from the genotyping laboratory. HIVDR is defined by an estimated level of resistance (low, moderate, or high) based on the Stanford HIV drug resistance algorithm will be reported for each drug and drug class of interest. A chromatogram and a hard copy report will also be sent for each specimen.

Information on specimens for which no sequence is available should 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).
4.2. The HIVDR sentinel monitoring database

A data abstraction form will be created to abstract data from patient records at the ART clinic. The form will be designed to cover all variables listed in 4.1 (and in Appendix 1). Data will be abstracted by HIVDR monitoring coordinators or survey coordinators on a routine basis, e.g. monthly, at baseline, and at endpoint. Data at the clinic level will be obtained separately before the survey starts and after 12 months. The WHO HIV Surveillance and Monitoring database program will be used.

- Checks for legally acceptable values will be built into the data system
- The database must include programming to accept electronic text files representing nucleotide sequences from protease and RT regions of the *pol* gene and generate variables representing amino acids in positions associated with mutations and separate variables with an interpretation for each drug. These variables will not require direct data entry but should be created by the program.
- A monthly or quarterly report for the laboratory and collection site will be automatically generated, noting data entry problems, missing information, and conditions that could jeopardize amplification for genotyping.
- Completed questionnaires and any identifiable individual data will be stored in a locked filing cabinet.
- All data forms will be sent to MOH where HIVDR monitoring coordinators will be responsible for entering data into the database and generating necessary reports. Important data elements such as endpoint classification (e.g., lost to follow-up, regimen switch, regimen substitution) will be checked and verified by experts at country level and WHO before entry and analysis.

4.3. Results/Analyses

A separate analysis will be performed for each ART site. The summary report will then bring together data from all selected ART sites; the level of this report will be the level of the site (e.g., the summary report will report the number and percentage of sites achieving the $\geq 70\%$ HIVDR prevention rate, the percentage of previous ARV experience at each site, etc). Data from patients treated at separate sites will not be combined.
4.3.1. Baseline factors

The following analyses will be performed for the cohort for whom baseline information is available:

- Range of ART start dates
- Basic demographic and clinical descriptions of the sample of patients starting ART, including age, sex, initial CD4 count, initial WHO stage, and any additional factors of interest.
- The proportion with reported previous ARV experience (Subcategories: Overall previous ARV experience, PMTCT, other ARV experience).
  - The overall proportion with any type of previous ARV experience will be calculated using as the numerator individuals with any type of previous ARV exposure and using as the denominator the total number of survey participants at baseline.
  - The proportion of female sentinel survey participants at baseline with PMTCT exposure will be calculated using as the numerator the number of women with PMTCT experience and using as the denominator the total number of women participating in sentinel survey.
  - The proportion of individuals with other ARV experience will be calculated using as the numerator the number of individuals with other ARV experiences and as the denominator the total number of survey participants at baseline.
  - For those for whom drugs or drug classes are available describing the previous ARV experience, classification will be made of "possible baseline HIVDR" to that drug class, whether or not mutations are detected.
  - The proportion of individuals with detectable HIVDR at baseline for each drug class will be calculated using as the numerator individuals with low, intermediate, or high resistance as defined by the Stanford algorithm, at baseline and using as the denominator the total number of survey participants with available sequences at baseline. This proportion is classified as having HIVDR to each relevant drug class at baseline. Appropriate sub-analyses will be performed to calculate the proportion with HIVDR by specific mutation, mutation pattern and by specific ARV drug.
**HIVDR at baseline** may represent transmitted or acquired HIVDR and together with **previous ARV experience** serve to inform the interpretation of viral suppression and HIVDR mutations present 12 months after initiation of first-line ART. Subcategory analyses will be performed to include specific drug class, specific drugs, and specific mutations of interest.

The proportion of the population with **possible HIVDR at baseline** will be calculated using as the numerator the number of individuals with history of any previous ARV experience (PMTCT, informal, other) who have no HIVDR mutations detected (i.e., “wild type” virus) at baseline, and using as the denominator the total number of survey participants at baseline. Given the limitation of HIVDR testing in detecting mutations that are present in lower frequencies, a population with a history of exposure to a specific drug (or drug class in the case of NNRTI) is considered as potentially having archived resistance to that drug or drug class, which in turn may influence patient and programmatic success of viral suppression.

- The proportion of individuals prescribed **appropriate first-line ART** as defined by the HIVDR WG prior to the start of the survey will be calculated using as the numerator the total number of individuals initiating ART who are prescribed an appropriate first-line ART regimen and using as the denominator the total number of individuals initiating ART at baseline.

Univariate analyses will be performed to evaluate associations between relevant demographic factors and the elements described above. Additional analyses will be performed to assess for association between **previous ARV experience** and the major endpoint and HIVDR outcomes described in Section 1.4.

**4.3.2 Endpoint Analysis**

The following analyses will be performed for the cohort who started at baseline:

- Proportion reaching each endpoint (before censoring):
  - Endpoints will include **Still on first-line ART at 12 months**, **Lost to**
follow up, Stop, Switch, Death, Transfer Out

- Proportions in the final analysis data set, reaching endpoints that are included in the final analysis
  - Endpoints will include **Still on first-line ART at 12 months, Lost to follow up, Stop, Switch**
- Basic demographic and clinical descriptions of the sample of patients included in the analysis, including age, sex, initial CD4 count, initial WHO stage, and any additional factors of interest. (Demographic and clinical descriptions of the endpoint analysis dataset should be compared with those of the baseline dataset, and any differences noted in the discussion of the results.)

Endpoint analyses will be performed using data only from participants with endpoints other than **transfer out** and **death**. Percentages calculated will include:

- The proportions of patients who reach various levels of **on-time ARV drug pick-up** will be calculated. Survey participants should be classified as reaching the following levels of on-time drug pick-up: <50%, 50%-59%, 60%-69%, 70%-79%, 80%-89%, 90%-99%, 100% (Subsets of these categories may also be calculated if these do not reflect particular country targets). To classify each participant into one of the **on-time ARV drug pick-up** categories, the proportion of on-time pick-ups should be calculated by using as the numerator the number of on-time drug pick-ups made between baseline and endpoint, and as the denominator the number of expected drug pick-ups between baseline and endpoint (this number should be based on the dates calculated for pick-up based on when previously picked-up drugs would have run out). Dividing the numerator by the denominator and multiplying by 100, the percentage of on-time drug pick-ups made will be obtained. Once the proportion of on-time drug pick-ups is calculated for each participant included in the analysis, the cohort proportion for each **on-time ARV pick-up** level is then easily calculated. For instance, the proportion with 100% on-time drug pick-up is calculated using as the numerator the number of individuals who picked up their drugs on-time 100% of the times between baseline and endpoint, and as the denominator the number with an
HIVDR outcome classification at endpoint (that is, individuals whose endpoints are one of the following: lost to follow-up, on first-line ART at 12 months, stop, or switch. As explained in the introduction of this section, an individual whose endpoint is death or transfer out is not included in the numerator or the denominator).

- The proportions of patients who reach various levels of on-time clinic appointment keeping will be calculated. Survey participants should be classified as reaching the following levels of on-time clinic appointment keeping: <50%, 50%-59%, 60%-69%, 70%-79%, 80%-89%, 90%-99%, 100% (Subsets of these categories may also be calculated if these do not reflect particular country targets). To classify each participant into one of the on-time clinic appointment keeping categories, the proportion of on-time clinic appointment should be calculated by using as the numerator the number of on-time appointments attended between baseline and endpoint and as the denominator the number of expected or scheduled clinic appointments between baseline and endpoint. Dividing the numerator by the denominator and multiplying by 100, the percentage of on-time appointments attended will be obtained. Once the proportion of on-time appointments is calculated for each participant included in the analysis, the cohort proportion for each on-time clinic appointment level is then easily calculated. For instance, the proportion with 100% on-time clinic appointment is calculated using as the numerator the number of individuals who attended clinic appointments on-time 100% of the times between baseline and endpoint, and as the denominator the number with an HIVDR outcome classification at endpoint (that is, individuals whose endpoints are one of the following: lost to follow-up, on first-line ART at 12 months, stop, or switch. As explained in the introduction of this section, an individual whose endpoint is death or transfer out is not included in the numerator or the denominator).

- The proportions of patients reporting various levels of adherence, as reflected by the VAS 30-day adherence question, will be calculated for participants reaching defined endpoints (switch and on first-line ART at 12 months). Participants should be classified as reporting the following levels of adherence: <50%, 50%-59%, 60%-
69%, 70%-79%, 80%-89%, ≥90%. Subsets of these categories may also be calculated if these do not reflect particular country targets. To calculate the proportion of the survey cohort belonging to each level of adherence, the VAS results should be recorded and categorized. Once the levels of adherence have been recorded and categorized for each participant included in the analysis, the cohort proportion for each level is easily calculated. For instance, the proportion with 100% adherence is calculated using as the numerator the number of individuals with 100% adherence and as the denominator the total number of individuals with the endpoint switch or on first-line ART at 12 months. However, HIVDR WG should be aware that previous reports, defining the requirement of ≥95% adherence to prevent HIVDR emergence, were based on studies of patients taking unboosted PI-based ART regimens. Current evidence on regimens consisting of 2 NRTI + 1 NNRTI has demonstrated that 70%-80% adherence is likely to be sufficient to prevent the emergence of resistance [30]).

Univariate analyses assessing the association of age, sex, previous PMTCT, previous ARV experience, appointment keeping and drug pick-up, and adherence as measured by the 30-day VAS with the level of HIVDR prevention and with particular resistance patterns will be performed.

**4.3.3 Endpoint Outcome Measures**

For endpoint outcome measures, patients reaching death and transfers out endpoints are censored from the analyses based on the assumption that these individuals are unlikely to die in the first year due to HIVDR, and that a transfer out, as a true cross-clinic transfer, does not represent a treatment failure. The methods used to estimate the main outcome measures are described below.

- **HIVDR prevention.** The proportion of individuals in the cohort achieving HIVDR prevention and who are on first-line ART at 12 months will be estimated by HIV viral load testing. To calculate the proportion of individuals achieving HIVDR prevention on first-line ART at 12 months the numerator is the number of participants with HIV viral load <1000 copies/ml after 12 months of first-line ART.
The denominator is the total number of individuals in the cohort who initiated ART at baseline minus patients reaching death and transfer out endpoints.

- **Switch.** All switches to second-line ART during the first 12 months of ART are possible premature switches. Sub-classifications are: undetectable viral load at switch, possible HIVDR at switch (no viral load result or no sequence at switch, or detectable viral load with no mutation pattern associated with low, intermediate, or high level resistance), and HIVDR (low, intermediate, or high level resistance to one or more drugs according to the Stanford classification). Switches should be analysed and reported separately from the other endpoints, regardless of the outcome. A participant whose viral load is suppressed and whose regimen was switched cannot be classified as HIVDR prevention in the survey. Although the person may not have HIVDR at the time, his or her ART options have been prematurely limited, and the switch may have been inappropriate.

- **Possible HIVDR.** Participants who are categorized as lost to follow-up, stop, or for whom no specimens are available at endpoint are classified as having possible HIVDR. All these survey participants should be included in the numerator when calculating possible HIVDR (100% of participants reaching these endpoints are categorized as having possible HIVDR). Additional analyses may also be reported in which some proportion of these individuals are categorized differently, but the initial analysis should be based on the assumption of possible HIVDR. Survey participants reaching the endpoints on first-line ART at 12 months or switch with HIV viral loads $\geq 1000$ copies/ml at endpoint, and whose HIV sequence does not contain a combination of mutations producing low, intermediate, or high level drug resistance according to the Stanford classification, should also be included in the possible HIVDR numerator. Individuals for whom no viral load result and/or no sequence are available are also placed in this category. For the overall evaluation of possible HIVDR in the survey, add the numbers of participants classified in the above mentioned categories to determine the possible HIVDR numerator. The denominator should include all participants whose outcome is not dead or transferred out.

- **HIVDR.** The proportion of the survey cohort with HIVDR at the endpoint on first-line ART at 12 months or switch will be calculated first, and then the percentage of
those with HIVDR among all participants with an analyzable endpoint. HIVDR is defined as low, intermediate, or high level resistance to one or more NRTI, NNRTI, or PI as defined by the Stanford scoring system. A separate table breaking down HIVDR to individual drug classes and drugs among the individuals in this category should be produced. Prevalence of HIVDR among the entire cohort should not be calculated or reported.

To calculate the proportion of the population with HIVDR at 12 months the numerator is the number of individuals on first-line ART at 12 months with a viral load \( \geq 1000 \) copies/mL and a combination of mutations producing low, intermediate, or high level HIVDR. The denominator is the number of participants on first-line ART at 12 months.

To calculate the proportion of the population with HIVDR at switch the numerator is the number of individuals who switch and have an HIV RNA \( \geq 1000 \) copies/mL with one or more HIVDR mutations detected on genotypic testing. The denominator is the total number of individuals who switch during the survey period.

To calculate the proportion with HIVDR at endpoint, add the numerators of participants with HIVDR from the categories switch and on first-line ART at 12 months. The denominator should include all participants whose outcome is not dead or transferred out.

Specific drug resistance patterns, and specific mutation patterns of interest, among survey participants not achieving viral suppression at 12 months or at time of switch will be aggregated and described by specific mutation, mutation pattern and by drug and drug class. Mutations will be determined using the Stanford website HIVDR genotyping algorithm, and HIV drug resistance will be defined by low, intermediate, or high resistance to one or more NRTIs, NNRTIs, or PIs.

The HIVDR database developed by WHO will perform and report the results of most of the analyses described above.

The data should be exported to data analysis software such as SAS, SPSS, Stata, Epi-Info, or other statistical packages to perform multivariate analyses to evaluate the associations of factors of interest with the outcomes: HIVDR prevention, possible
HIVDR and its various subcategories, and specific HIVDR patterns.

Basic demographic factors should be included in the initial multivariate analyses. Factors of interest may include: level of baseline CD4 count, initial regimen, HIV-1 subtype, previous ARV experience, baseline resistance pattern, on-time-ARV pickups, on-time-clinic appointment-keeping, VAS score, and other items of interest. Multisite analyses, including ART site as an additional factor, may be required for some multivariate analyses.

5. Ethical consideration

5.1. Review by Institutional Review Board (IRB)

The country will submit the protocol to the X IRB for ethical review and approval. Before implementation the protocol will be reviewed by WHO-Headquarters.

5.2. Risk benefit ratio

Risks for this survey are minimal, as it involves using remnant blood specimens from routinely collected blood. Minimal information (basic non-identifying demographic and clinical information) will be captured for survey purposes from existing medical records. Information derived from the project serves an important public health purpose. Estimates of HIVDR prevention will help the country planners improve ARV delivery programs 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 3). All questions that arise will be addressed. Participants will be clearly informed that their participation in the evaluation 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.

No incentives will be provided for participating in the survey.

List of appendices:
Appendix 1. Patient data collection form for Baseline and Endpoint
Appendix 2. Sample size calculation
Appendix 3. Informed consent
Appendix 4. Site operational procedures
Appendix 5. VL and HIVDR results return form
Appendix 6. Country information summary form
Appendix 1: WHO HIVDR Monitoring Questions-baseline

Date: ____/____/____
Patient HIVDR-SID: _______________________
Doctor/nurse’s name: _______________________

- Before starting ARV at this clinic, have you ever taken ARV?
  1 [ ] Yes  2 [ ] No

- If yes, what is the purpose of your using ARV?
  1 [ ] PMTCT  2 [ ] Self-paid  3 [ ] Other _______________________

- Do you know what regimen you used: ____________________________

- Do you remember how long you used that regimen: _____ months or from
  ___/___/___ to ___/___/___
WHO HIVDR Monitoring Questions-endpoint

Date: ____/____/____
Patient HIVDR-SID: _______________________
Doctor/nurse’s name: _______________________

(to ask patients at 12-month visit or before switch)

- Before starting ARV at this clinic, have you ever taken ARV?
  1 [ ] Yes    2 [ ] No

- If yes, what is the purpose of your using ARV?
  1 [ ] PMTCT    2 [ ] Self-paid    3 [ ] Other _______________________

- Do you know what regimen you used: _____________________________

- Do you remember how long you used that regimen: ___ months or from ___/___/___ to ___/___/___

Please place an “X” on the line below at the point showing your best guess about how much of your current antiretroviral medication you have taken in the past 30 days. 0% means you have taken none of your current antiretroviral medication, 50% means you have taken half your current antiretroviral medication, 100% means you have taken every single dose of your current antiretroviral medication.

<table>
<thead>
<tr>
<th>0%</th>
<th>10%</th>
<th>20%</th>
<th>30%</th>
<th>40%</th>
<th>50%</th>
<th>60%</th>
<th>70%</th>
<th>80%</th>
<th>90%</th>
<th>100%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Estimate percent indicated: ______%
Appendix 2: Sample Size Calculation for each site

The number of participants enrolled at each survey site must be sufficient to allow a reasonable expectation that at least 96 individuals who start ART at that site will be classifiable at endpoint.

The effective sample size is estimated as 96 individuals initially starting ART at the site, plus an additional number calculated for that site reflecting the number of patients who transferred out or died among the first 100 patients starting ART in the same time period during the previous year (i.e., 96 + number of expected deaths in their first year of treatment among 100 people starting ART at the site + the number of anticipated transfers out in their first year of treatment among 100 people starting ART at the site).

An effective survey sample size of 96 patients provides a 95% confidence interval of +/- 10% for the proportion with HIVDR prevention, regardless of the cumulative incidence of viral suppression. The calculations demonstrating that this effective survey sample size will give an estimate of the proportion of individuals with HIVDR prevention at 12 months with a 95% confidence interval +/- 10% is found in Appendix 4. The point estimate is used to classify the proportion of HIVDR prevention among the patients at the site who started ART 12 months previously as being above or below the 70% threshold.

<table>
<thead>
<tr>
<th>Sample size (N) calculation for population-based monitoring of HIVDR prevention at a sentinel site in the first year of ART</th>
</tr>
</thead>
<tbody>
<tr>
<td>M= Number of deaths/100 patients starting ART at the site in the previous year</td>
</tr>
<tr>
<td>T= Number of transfer outs/100 patients starting ART at the site in the previous year</td>
</tr>
<tr>
<td>N= sample size required at start of survey</td>
</tr>
</tbody>
</table>

\[
N = 96 + M + T
\]
Appendix 3. Example Informed Consent Form

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 drug resistance of human immunodeficiency virus (HIV). You are being asked to be part of this survey because you are about to start treatment for HIV at this clinic. We are doing this survey to determine how well the treatment program is functioning at this site and identify any factors that may improve its quality. If you agree to participate, extra tests will be performed on your blood specimens that will be drawn during your first year of treatment. The 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. If you start treatment for HIV (called ART), a small quantity of blood (about 1 table spoon) will be drawn on the day you start ART at this clinic. We will collect blood a second time within a year from the time you started treatment. Your doctors or nurses will let you know when the second blood draw will be made. We will at most collect blood two times for this survey.

2. Your blood will be sent for 2 extra tests. Your first blood collection will be sent for an HIV drug resistance (HIVDR) test. Your second blood collection will be used to do viral load testing. This test is done to see how much HIV is in your blood. An HIVDR test will be done if there is a certain amount of HIV in your blood.

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.

4. 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, sex, a list of your medications, and results of your routine tests, will be collected from your medical chart.

5. A report on the results of the extra 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**

1. There should not be any risks or discomfort in this survey, except for a minor pain or bruise at the site of needle stick which is common with every blood draw.
Benefits:
1. Right now experts don’t think that most people being treated for HIV in need routine viral load testing or HIVDR testing. However, you and your doctors may find the results helpful in talking about treatment plans.
2. 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?

I have read or have someone read to me all the information in the consent above and I agree/disagree to participate in the survey.

Patient’s signature and name _____________________________

Doctor/nurse’s name and signature ___________________________
Appendix 4. HIV Drug Resistance Monitoring Site Operational Procedures.

A. ART SITE NAME:

B. SURVEY TEAM:

<table>
<thead>
<tr>
<th>Name</th>
<th>Position</th>
<th>Responsibilities</th>
</tr>
</thead>
<tbody>
<tr>
<td>ART Clinical Liaison</td>
<td></td>
<td>● Supervise all HIVDR monitoring activities at ART site</td>
</tr>
<tr>
<td></td>
<td></td>
<td>● Liaison between clinic, pharmacy, national laboratory and the Monitoring Coordinator</td>
</tr>
<tr>
<td></td>
<td></td>
<td>● Review eligibility of all enrolled patients at time of filling in survey logbook</td>
</tr>
<tr>
<td></td>
<td></td>
<td>● Reconcile logbook with Enrolment CARDS</td>
</tr>
<tr>
<td></td>
<td></td>
<td>● Maintain the survey logbook of participants and keep all consent forms, Enrolment CARDS and Endpoint CARDS, and questionnaires</td>
</tr>
<tr>
<td></td>
<td></td>
<td>● Maintain confidentiality of all data collection instruments, questionnaires, logbooks, and consent forms</td>
</tr>
<tr>
<td></td>
<td></td>
<td>● Receive individual patient and aggregate clinic monitoring survey results from the Monitoring Coordinator and discuss with clinic staff</td>
</tr>
<tr>
<td>Name</td>
<td>Position</td>
<td>Responsibilities</td>
</tr>
<tr>
<td>--------------------</td>
<td>------------------</td>
<td>------------------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>ART clinic</td>
<td>doctors</td>
<td>• Identify eligible patients following inclusion/exclusion criteria on or after selected survey start date</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Explain survey to patients and obtain informed consent</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Administer baseline and endpoint questionnaires</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Fill out Enrolment CARDS</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Issue HIVDR SID and attach Monitoring Stickers to Patient Care Booklet</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Ensure survey logbook is completed (varies by site)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Send patient to phlebotomy for “special blood draw” after clinical evaluation</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Identify Monitoring patients at 12-month visit and administer Endpoint CARD</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Identify Monitoring patients being switched to second-line ART and administer Endpoint CARD</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Maintain patient charts per routine procedures and record actual clinic visit dates and expected clinic visit dates</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Discuss viral load and genotyping results with patients</td>
</tr>
<tr>
<td>ART clinic</td>
<td>nurses</td>
<td>• Explain survey and answer any questions if patients enquire; direct to clinic doctors if unable to answer questions</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Keep track of patients per routine procedures</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Assist with identifying patients at 12-month visit and switch to second-line ART</td>
</tr>
<tr>
<td>Name</td>
<td>Position</td>
<td>Responsibilities</td>
</tr>
<tr>
<td>---------------------------</td>
<td>----------------------------</td>
<td>--------------------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
</tbody>
</table>
| Pharmacists               |                            | ● Maintain pharmacy records per routine procedures and record actual ARV pick-up dates  
● Administer pill counts to all patients and record this number in PHARMACY RECORD per routine  
● Identify all patients initiating ART and picking up ARV drugs for the first time. Fill out Enrolment CARD and send patient to the clinic during the period of enrolment.  
● Dispense ARV drugs for 1st pick-up ONLY if patient has an Enrolment CARD filled out correctly during the period of enrolment.  
● Collect all Enrolment CARDs after dispensing 1st ARV pick-up in Enrolment CARD Box. |
| ART clinic data clerks    |                            | ● Maintain electronic medical record data per routine procedures (transfer Patient Care Booklet data into electronic medical record)  
● Assist data abstraction team during baseline and endpoint data abstraction.                                                                 |
| ART clinic counselors     |                            | ● Explain survey and answer any questions if patients enquire; direct to clinic doctors if unable to answer questions  
● Keep track of patients per routine procedures                                                                                                                   |
<table>
<thead>
<tr>
<th>Name</th>
<th>Position</th>
<th>Responsibilities</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phlebotomists</td>
<td></td>
<td>- Explain survey and answer any questions if patients enquire; direct to clinic doctors if unable to answer questions</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Draw “special blood” as scheduled for patients presenting with Enrolment CARDS at baseline and patients presenting for endpoint “special blood” draw, and maintain universal safety precautions for all procedures</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Fill out Enrolment CARDS (blood draw date)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Fill out specimen manifest</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Send patient to pharmacy with Enrolment CARD after filling out blood draw date</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Use ONLY EDTA tubes for “special blood draw”</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Label “special blood draw” EDTA tubes with pre-printed labels with sequential HIVDR SID</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Place “special blood draw” EDTA tubes into racks prepared for national lab collection at specified times.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Communicate with Clinical Liaison and national lab technician if there are any pending blood draw at the last national lab pick-up</td>
</tr>
<tr>
<td>Name</td>
<td>Position</td>
<td>Responsibilities</td>
</tr>
<tr>
<td>---------------------------</td>
<td>---------------------------</td>
<td>----------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>National Laboratory</td>
<td>technican</td>
<td>● Receive blood specimen from phlebotomists with specimen manifest at specified</td>
</tr>
<tr>
<td></td>
<td></td>
<td>times.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>● Ensure that there are no pending blood draws at the last national lab pick-up</td>
</tr>
<tr>
<td></td>
<td></td>
<td>● Fill out specimen manifest</td>
</tr>
<tr>
<td></td>
<td></td>
<td>● Package specimen on ice for overnight transport to national lab</td>
</tr>
<tr>
<td></td>
<td></td>
<td>● Ensure specimens are picked-up by courier.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>● Communicate with national lab</td>
</tr>
<tr>
<td>National Laboratory</td>
<td>Liaisons</td>
<td>● Supervise monitoring survey at ART clinic, especially laboratory-related</td>
</tr>
<tr>
<td></td>
<td></td>
<td>procedures of specimen collection, processing, labeling, storage, transport to</td>
</tr>
<tr>
<td></td>
<td></td>
<td>WHO accredited laboratory</td>
</tr>
<tr>
<td></td>
<td></td>
<td>● Assure that there is continuous supply of EDTA tubes and labels at the ART site</td>
</tr>
<tr>
<td></td>
<td></td>
<td>and phlebotomy area (supply and delivery)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>● Assure that there is continuous supply of packaging materials and ice for</td>
</tr>
<tr>
<td></td>
<td></td>
<td>transport to national lab</td>
</tr>
<tr>
<td></td>
<td></td>
<td>● Maintain communication with national lab</td>
</tr>
<tr>
<td>Name</td>
<td>Position</td>
<td>Responsibilities</td>
</tr>
<tr>
<td>----------------------</td>
<td>---------------------------------------</td>
<td>----------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>National Laboratory</td>
<td>National Laboratory Coordinator</td>
<td>● Supervise activities at all sentinel Monitoring sites, especially laboratory procedures</td>
</tr>
<tr>
<td></td>
<td></td>
<td>● Supervise National Laboratory Liaisons</td>
</tr>
<tr>
<td></td>
<td></td>
<td>● Supervise collection, labeling, packaging, transport, processing, and storage of specimen at all sentinel Monitoring sites</td>
</tr>
<tr>
<td></td>
<td></td>
<td>● Maintain communication with sentinel Monitoring sites in regards to specimen being sent to accredited laboratory</td>
</tr>
<tr>
<td></td>
<td></td>
<td>● Assure that there is always an technician available at the national lab to receive and process specimen from sentinel Monitoring sites (even Saturdays if applicable)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>● Supervise delivery of specimen to Genotyping laboratory</td>
</tr>
<tr>
<td></td>
<td></td>
<td>● Communicate with Genotyping laboratory about specimen delivery and receipt</td>
</tr>
<tr>
<td></td>
<td></td>
<td>● Data quality-assurance with genotyping laboratory, regional virologist and Headquarter virologist (with Monitoring Coordinator)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>● Analysis</td>
</tr>
<tr>
<td>Name</td>
<td>Position</td>
<td>Responsibilities</td>
</tr>
<tr>
<td>------------</td>
<td>-------------------------------</td>
<td>----------------------------------------------------------------------------------</td>
</tr>
</tbody>
</table>
|            | Monitoring Coordinator       | ● Supervise monitoring surveys at all sentinel Monitoring sites (with regular site visits as outlined in I. “Supervision” section.  
● Lead data abstraction teams  
● Verify data collection  
● Enter data into WHO/CDC HIVDR database  
● Receive data from genotyping laboratory  
● Data quality-assurance with genotyping laboratory, regional virologist and  
  Headquarters virologist (with national Laboratory Coordinator)  
● Analysis of all Monitoring Survey data  
● Write Monitoring Survey Reports  
● Communicate with X Technical Working Group |
C. INTRODUCTION:

- Three ART delivery sites will pilot HIVDR sentinel monitoring. Sentinel HIVDR monitoring will be performed routinely as a public health function after the pilot year. HIVDR sentinel monitoring is not a research study. Sentinel HIVDR monitoring, in combination with other HIVDR prevention elements, will give the country a solid evidence-base to make public health recommendations and improve programmatic functioning and minimize preventable HIVDR.

- HIVDR sentinel monitoring allows the national HIVDR working group to evaluate drug resistance arising in the treated population in the first year of ART and associated treatment program factors related to the emergence of HIVDR.

- Some drug resistance cannot be avoided, because HIV mutates rapidly and regimens must be taken for life. However, if HIVDR is emerging at an inappropriately high level, it may be associated with program factors that can be adjusted with more resources, better systems, different regimens, or more training. Sentinel monitoring will help the working group plan policies and make programmatic interventions to minimize the emergence of drug resistance at all ART sites throughout the country.

- HIVDR sentinel monitoring collects a minimal amount of information before ART starts and at endpoint: one year after start, or at regimen switch.
D. SURVEY SCHEMATIC:

The schematic above illustrates the possible paths from baseline to outcomes. A cohort of patients is consecutively consented and enrolled into the survey at each site until the required sample size is achieved. Baseline data collection is performed and residual specimens for HIVDR testing are collected.

After ART initiation, patients continue in the survey for up to 15 months until one of the following endpoints is reached: death, transfer out, stop ART with no restart at the time of the 12 month-evaluation date, lost to follow-up, switch to second-line ART, or still on first-line ART at 12 months.

At endpoint, data and specimen collection of viral load and genotypic testing is performed for those whose endpoints are switch and on first-line ART at 12 months; retrospective data collection alone is performed for those whose endpoints are lost to follow-up and stop.

Patients with viral suppression on first-line at 12 months are classified as having achieved HIVDR prevention. Patients who switch to second-line before 12 months with viral
suppression are classified with the outcome suppressed viral load at switch. Patients whose endpoints are switch or on first-line ART at 12 months, who have detectable viral loads with no detectable HIVDR mutations are classified with the outcome possible HIVDR. Patients who are classified as stop or lost to follow-up are also classified with the outcome possible HIVDR. Patients with endpoints switch or on first-line ART at 12 months who have detectable viral loads and low, intermediate, or high HIVDR are classified with the outcome HIVDR.

E. TIME POINT AND OUTCOMES:

Definition of time points

- **Baseline:** Defined as the time of commencement of first-line ART. Baseline specimens should be collected within 1 month prior to starting ART.
- **Endpoint:** Defined as the time at which the outcome evaluation is performed, i.e. when the individual reaches a state or experiences an event that can be classified into one of the following categories:
  - **On first-line ART at 12 months:** A patient is defined as still on first-line ART at 12 months.
  - **Switch:** Defined as the change from a first-line to a second-line ART regimen and is consequent on the failure of first-line therapy, as defined in national ART guidelines.
  - **Stop:** An ART stop for the purposes of HIVDR monitoring is defined as the complete cessation of ART by a patient who has not restarted ART by the time of the 12-month blood draw, although he or she remains in care at the site. Stops usually take place because of a patient decision or a decision by the clinical team. Stops generally reflect either a planned treatment interruption or ART or a decision based on poor adherence. Operationally, ‘stop’ is defined as the endpoint if a patient still attending the clinic has taken no ART in the 30 days before the 12-month blood draw.
  - **Loss to follow-up:** A patient is defined as ‘lost to follow-up’ if he or she has not returned to the clinic or pharmacy for a scheduled appointment or drug pick-up >90 days after the missed appointment/drug pick-up and there is no information
to classify the patient in one of the other endpoint categories, such as ‘death’ or ‘transfer out.’

- **Death**: Death refers to the recorded death of a patient for which a date (at least month and year) is recorded within 12 months following the start of first-line ART.

- **Transfer out**: Defined as the transfer of HIV care from the HIVDR monitoring site to another identified ART delivery site for patients who have not stopped first-line ART at the time of transfer.

- **Substitution**: Substitution for reasons of toxicity or drug-drug interaction is defined as the change from the standard first-line ART regimen to an alternate first-line ART regimen: one drug is substituted for another within the same class. For the purposes of HIVDR monitoring, the new regimen instituted at the time of substitution is recorded in the database and can be analysed, but this is not an endpoint; the patient will continue to be followed until an endpoint event.
**Outcomes at 12 months or earlier endpoint**

The following is how we define HIVDR population outcomes.

<table>
<thead>
<tr>
<th>DEFINITION</th>
<th>OUTCOMES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Suppressed viral load at 12 months</td>
<td>HIVDR Prevention</td>
</tr>
</tbody>
</table>

**Classification of outcomes in those lacking evidence of HIVDR prevention**

<table>
<thead>
<tr>
<th>OUTCOMES</th>
<th>DEFINITION</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIVDR</td>
<td>Detectable viral load plus &quot;resistant&quot; genotype at 12 months</td>
</tr>
<tr>
<td>Possible HIVDR</td>
<td>Detectable viral load plus &quot;susceptible&quot; genotype at 12 months</td>
</tr>
<tr>
<td>Possible HIVDR</td>
<td>Lost to follow-up or stop</td>
</tr>
<tr>
<td>Suppressed viral load at switch of ART</td>
<td>Suppressed viral load at switch of ART</td>
</tr>
<tr>
<td>Censored from outcome analysis. They are removed from the numerator and denominator.</td>
<td>Transfer out, death</td>
</tr>
</tbody>
</table>

- On first-line ART at 12 months and switch are endpoints which can be classified with regards to HIVDR, because viral load and genotyping (if viral load > 1000 copies/ml) are performed on patients reaching these endpoints.

- Lost to follow-up and the stop are endpoints which do not yield specimens for viral load measurement or for HIVDR testing. However, because sentinel surveys assume that individuals who are lost to follow-up or who stop ART have taken some portion of prescribed ART, and a stop of ART can generate HIVDR, especially if adherence was less than 100% beforehand, these individuals are classified as having possible HIVDR.

- **The endpoints transfer out and death are not classifiable with regard to HIVDR without additional data not collected as part of HIVDR prevention surveys. Patients with these endpoints are thus omitted from the analysis.**
F. PROCEDURES:

1. Start date:
   - The survey team will randomly choose a start date. The team will prepare 30 (or 31) tickets and write down 30 (or 31) days of the month chosen to start, then randomly pick one of the tickets. The date on the chosen ticket will be start date and any patients who will start ARV on or after that date at the ART site will be screened and asked for consent prior to their first ART drug pick-up. For example if July is chosen, the dates July 1, July 2...July 31 will be written on 31 tickets. If the “July 10” ticket is drawn, July 10 will be start date for the ART site.

2. Determination of eligibility:
   - Patients are eligible if they are starting their three-drug regimen for the first time at the ART site (that is, they have not previously had a first-line regimen at this site and stopped) and have not transferred in from another ART site on a three-drug regimen. Consecutive eligible patients must all be included in monitoring.

   - **Participant Inclusion Criteria:**
     a. Eligible for ART, as defined by country’s national guidelines.
     b. Patients who initiate an adult ART regimen, on or after the survey start date, regardless of age, unless the national protocol specifies an age restriction to comply with national regulations. This includes individuals who have previous ARV drug experience (e.g., PMTCT, other ARV exposure) if they are eligible to initiate and do initiate ART at the sentinel site, with these exceptions:
        i. Individuals reinitiating ART who have previously started and stopped ART at the sentinel survey site (a three-line regimen for PMTCT administered at the site is not considered an ART start and stop).
        ii. Individuals transferring in from another ART site who are at the time of transfer currently taking a three- or four-drug ART regimen
     c. Patients who are able to give consent for participation in the survey following the informed consent process according to the country’s protocol

   - **Participant exclusion criteria:**
     a. Individuals known to be infected with HIV-2 or individuals known to be infected co-infected with HIV-1 and HIV-2.
b. Individuals enrolled in a clinical trial or clinical research study (either at the sentinel monitoring site or another location).

c. Individuals who are part of an observational cohort for whom more follow-up efforts are made than for other ART patients treated at the site (Patients enrolled in an observational cohort for whom no additional follow-up procedures are eligible for HIVDR monitoring surveys).

3. Informed consent:
   - A brief oral consent process, which requests consent to draw additional blood specimens at baseline and at endpoint and abstract non-identifying information will be administered to all patients identified as eligible to start ART and about to make their first ARV drug pick-up.

4. Consecutive Enrolment:
   - All patients with whom the decision has been made to initiate ART on or after the identified HIVDR monitoring survey start date will be enrolled consecutively until the effective survey sample size is reached (130). This enrolment will occur just before the first ART pick-up. Consecutive enrolment will be based on the date and time on which the patient is issued a HIVDR SID. First drug pick-up on the same date of issuing the HIVDR SID will be verified by reconciling the survey logbook with the Enrolment CARDS.
   - If the patient did not have a first drug pick-up or baseline blood draw on the same day as issuing the HIVDR SID, and returns to the ART clinic for a first drug pick-up, they will be issued a new Enrolment CARD and restart the enrolment process with a NEW HIVDR SID.
   - If the patient did not pick up drug on the same date as the issuing of HIVDR SID, but had a baseline blood draw, but picks up drug at a later date, the date and time of enrolment will be changed to reflect the later pick-up date. (This new enrolment date should be within 90 days of the baseline blood draw.)

5. Enrolment CARD
   - An Enrolment CARD will be issued by the ART clinic doctor or the pharmacist on the day of enrolment/ARV first pick-up. The Enrolment CARD will be crucial in assuring that there is an unbroken chain from informed consent, issuing the HIVDR
SID, administering the baseline questionnaire, “special blood draw”, to first ARV drug pick-up.

- This card should be carried by the participant throughout the day and filled by the doctor, pharmacist and phlebotomists.
- The pharmacist should never dispense 1st pick-up ARV drugs without a properly filled-out Enrolment CARD from the day designated as the start date of the Monitoring Survey at the site.
- The pharmacist will collect the Enrolment CARDS and place them in a collection box after the first ARV pick-up has been completed.
- The Clinical Site Liaison or Clinic doctor should reconcile the Enrolment CARD with the Survey logbook at the end of each day to assure that all patients enrolled in the Survey logbook actually finished the chain and had the “special blood draw” and picked-up drug.
- Enrolment CARDS should not be dispensed after 16:00 as to avoid a patient being enrolled and not being able to complete the process of being consented, having a baseline questionnaire and baseline “special blood draw” in time for the last specimen pick-up from national lab. If an Enrolment CARD is dispensed late in the day, the doctor must communicate with the phlebotomist that a late blood draw may be coming. The phlebotomist can then communicate this information to the national lab technician picking-up the specimen.
- The Enrolment CARD will have fields for: unique number/CDC number, informed consent “yes” “no”, HIVDR SID, date of issuing HIVDR SID, phlebotomy date, ART start date and pharmacy patient number.
- A participant issued an Enrolment CARD will be on the fast-track system and will not join the normal lines for the doctor, the phlebotomy and the pharmacy. The patient should be informed of this fast-track system at the time of issuing the Enrolment CARD. (This fast-track system is to ensure that the participants do not have to wait longer than the usual time that they would have to wait for their routine care.)

6. **Endpoint CARD:**

- The Endpoint CARD will be issued to all participants who present for their 12-month clinical visit. (must include a doctor visit) The Endpoint CARD will ensure that all
participants will have the appropriate tests performed on the 12-month visit for Monitoring purposes.

- The Endpoint CARD includes the HIVDR SID, the date of endpoint questionnaire, and the date of endpoint blood draw.
- The doctor will fill out the HIVDR SID from the Survey Sticker in the Patient Care Booklet. After administering the endpoint questionnaire, the doctor will fill in the date of endpoint questionnaire. After completion of the 12-month clinical visit, the doctor will send the participant to the phlebotomist with the Endpoint CARD.
- The phlebotomist will perform the endpoint blood draw and will fill out the date of endpoint blood draw in the Endpoint CARD. This Endpoint CARD will be collected by the phlebotomist and given to the Clinical Site Liaison at the end of the day.

7. Use of “special” blood draw for HIVDR genotyping:
- Genotyping is performed on a “special” blood draw taken before ART starts and at endpoint. At endpoint, a viral load is also performed because if the viral load is suppressed that is the best indication that there is no effective HIV drug resistance.

8. Questionnaire
- A brief questionnaire is administered prior to therapy to evaluate whether the patient has had any ARV drug experience, through prevention of mother-to-child transmission programs or through use of another person's ARV drugs, private prescribing, or non-medical purchase of ARVs. At endpoint, a question about adherence is also asked.
- The questionnaire should take less than 5 minutes to administer
- The questionnaire should be administered in private, not in a group situation – this would be difficult and time consuming. The questionnaires will be administered by the physician at the initial interaction; stored and labeled as above.
- The questionnaires should be kept in a locked cabinet.

9. HIVDR Monitoring Survey logbook
- An HIVDR Monitoring Survey logbook should be kept on-site that records the patient ART number, CDC number, unique number, sequentially assigned survey identification number (HIVDR SID), DOB, date and time, date of baseline and endpoint blood draw, verification of 1st drug pick-up, 12-month visit date, date of
endpoint, endpoint code, and doctor in charge. The logbook with the patient’s will be kept in a locked cabinet at the site.

- The Survey logbook should be filled out after enrolment of each patient by the Clinical Liaison. (How this will be done will vary from site to site.)
- The Clinical liaison must reconcile the survey logbook with the Enrolment CARDS. Date and time of issuing HIVDR SID, date of baseline blood draw and verification of 1st drug pick-up must be filled out in the Enrolment CARD and the survey logbook for the enrollment of the patient to be final.

10. Specimen Manifest

- Information should be recorded on the specimen manifest and accompany the specimen during its handling, transport, and storage at the collection site, the processing/storage laboratory, and the accredited HIVDR genotyping laboratory. 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.

11. Data abstraction

- WHO trained data abstractors will abstract data directly from the patient’s medical record or download the required minimum dataset from the electronic patient medical record, from pharmacy records, and from the questionnaires. Data abstractors will be sent to the site after enrolment has been completed, then at the end of the study period (15 months after start of enrolment).
- The data abstractor needs to be able to locate and access the Patient Care Booklets, the electronic medical record and PHARMACY RECORD records, and any other information that is necessary for the database. If the information is properly recorded in the electronic medical record, the paper record may not need to be accessed. The data abstractor will also require access to the questionnaire at baseline and endpoint, along with the Enrolment CARDS. Patient care booklets will be labeled with a Monitoring Sticker indicating monitoring participation. Records will be stored and retrieved at each visit in the standard fashion.
12. Return of results

- Each site will receive results on the emergence of HIVDR at the site and associated factors. Additionally, patients being monitored will have their viral load and HIVDR testing results returned to the clinic.
- Results from the WHO-accredited regional genotyping laboratory will be sent back to the ART Clinical Site Liaison by the HIVDR Monitoring Coordinator. The Clinical Site Liaison will use the list to return the result of each patient to his/her medical chart. The Clinical Site Liaison will also discuss with the patient’s doctor about the result and how to inform the patient. All clinical decision-making will be done by ART site doctors.
- Close of survey: when results are available and returned to all participants and all data is entered and verified, the survey may end at the ART site. It is estimated that the duration of survey at the ART site will be 16 months. The Clinical Site Liaison and HIVDR Monitoring Survey Coordinator will destroy the list of participants.

G. PATIENT FLOW: (see flow diagram)

1. Decision to start ART/Prescription:

- The decision is made to start ART based on the CD4 count or WHO clinical stage by the ART site doctors. The ARV prescription will be written by the doctors.
- If the first ARV pick-up will occur on the same day as the ARV prescription, the doctor will begin the assessment for eligibility and informed consent. (Step 3 below)

2. Identification of potential participants:

- The pharmacy will identify patients who come in for their first ART pick-up. (These patients will not be in the PHARMACY RECORD system) These patients will be given an Enrolment CARD which will be carried with the patient throughout the process of enrolment. The pharmacist will write down the patient’s unique ID number or CDC number on this Enrolment CARD. Then the patient will be sent to the ART clinic to be seen by doctors trained for the monitoring survey. (no ARV drug will be dispensed at this point) The patients should pick up their Patient Care Booklet before seeing the doctor.

3. Assessment for Eligibility/Informed Consent:
• Patient from Pharmacy: the patient will present their Enrolment CARD to the doctors trained for monitoring.

• Patient already in clinic: the doctor will issue new Enrolment CARD and fill in the patient’s unique ID number or CDC number.

• The doctors trained for monitoring will assess the patient for eligibility. If eligible, the doctors will explain and ask if the patient wants to participate in the survey. Then oral consent will be obtained for those who wish to participate.

• The completed informed consent forms will be saved in the informed consent box stored in a locked cabinet.

• The doctors will mark in the Enrolment CARD whether the patient has given informed consent for the study or not. (If the patient wishes not to participate in the study, he/she will be sent back to the pharmacy for drug pick-up. The patient will present their Enrolment CARD to the pharmacist which indicates that they wish not to participate in the survey. This Enrolment CARD will be saved for future use.)

4. Assignment of the HIVDR SID number:

• Individuals enrolled in the evaluation will be assigned HIVDR survey identification (HIVDR SID) numbers by the doctors.

• The Monitoring Survey Stickers will have a pre-printed sequential HIVDR SID. The doctor will fill out the ART start date on the stickers.

• The doctors will attach the Monitoring Survey Stickers to the inside of the front cover of the Patient Care Booklets.

• The HIVDR SID will be written onto the Enrolment CARDS along with the date.

5. Survey Logbook

• The doctors will fill in the survey logbook, including the unique number/CDC number, HIVDR SID, date and time of issuing HIVDR SID.

6. Baseline Survey Questionnaire:

• The doctors will administer the baseline survey questionnaire and will write the HIVDR SID onto the questionnaire.

• The doctors will then refer participants to the ART phlebotomist with their Enrolment CARD after the routine appointment is completed and all of patient’s issues and questions have been addressed.
7. **“Special Blood Draw”:**
   - The participant will present their Enrolment CARD to the phlebotomist.
   - Each participant will have a “special blood draw” for genotyping on the first ARV dispensing day at the clinic before actual drug pick-up.
   - After the blood draw, the phlebotomist will fill in the draw date on the Enrolment CARD.
   - The phlebotomist will fill out the specimen manifest with the HIVDR SID and draw date and time. Then the specimen will be sent to national lab along with the specimen manifest.

8. **Initial regimen and drug pick-up:**
   - The phlebotomist will send the participant back to the pharmacy with the Enrolment CARD for first drug pick-up.
   - The pharmacist will only dispense initial ARV drugs to patients with a completed Enrolment CARD:
     - Enrolment CARD marked as “no” for informed consent
     - Enrolment card marked with (phlebotomy date)
   - The Enrolment CARDS will be saved for future use.
   - If a patient adamantly refuses to even go through the enrolment process, they can be marked as refusing informed consent by the pharmacist and dispensed their first ARV pick-up.

9. **Patient Routine ART pick-up:**
   - Follow-up procedures for all patients in the survey will be as routine practice.
   - **On time drug-pick up** is defined as pick-up of ART by the patient or designated surrogate prior to completion of the previous supply. For example: If 30 pills are dispensed and the patient is taking a once daily regimen; on time drug pick-up is defined as on or before day 30. On-time drug pick-up will be captured from the PHARMACY RECORD system. At every drug pick-up, the pharmacist will count pills in hand and record this number into the PHARMACY RECORD system per routine ARV dispensing at the site.
   - **On time appointment keeping** is defined as keeping an appointment within 7 days of the scheduled appointment, or before the scheduled appointment. While a
surrogate can pick-up ART and have it be an on time drug pick-up a surrogate cannot attend the clinic visit on behalf of a patient. If a surrogate attends a clinic visit on behalf of a patient this is not an on-time appointment; rather this is properly classified as a missed appointment. The doctors will record every clinic appointment and next scheduled appointment into the Patient Care Booklet. This information will be subsequently transferred to electronic medical record by the data clerks.

10. Losses to follow-up:
   - Loss to follow-up is defined as failure to pick up ART (appointments) for 90 days after the pill supply would have run out. This information is noted in the Patient Care Booklet, the electronic medical record and PHARMACY RECORD system. No special attention will be paid to LTFU happening in the monitoring cohort above and beyond the routine clinical practice at the sentinel site.

11. Death
   - If a patient dies this information is recorded in the Patient Care Booklet, the electronic medical record and PHARMACY RECORD system. At present, deaths are known only if they occur in hospital with communication to the ART clinic or if the clinic is notified by family members.
   - No special attention will be paid to identifying deaths at the monitoring site above and beyond the routine clinical practice at the sentinel site.

12. Transfers out:
   - If a patient makes a planned transfer to another facility, this is recorded in the Patient Care Booklet, the electronic medical record and PHARMACY RECORD system.
   - No special attention will be paid to identifying transfer out at the monitoring site above and beyond the routine clinical practice at the sentinel site.

13. Stops:
   - If the patient and/or his or her clinician decide that ART should be stopped; stop is recorded in the Patient Care Booklet, the electronic medical record and PHARMACY RECORD system. Note that for monitoring, stop is an endpoint and may be physician directed or patient directed.

14. Substitution:
• If an alternate first-line regimen is started it is recorded in the Patient Care Booklet, the electronic medical record and PHARMACY RECORD system.
• For HIVDR monitoring substitution is **not** an endpoint.

15. **Switch:**

• Most facilities do not have many switches to second-line regimens. If a patient switches from first to second line therapy it is recorded in the Patient Care Booklet, the electronic medical record and PHARMACY RECORD system. For HIVDR monitoring, switch is an endpoint which necessitates data collection, questionnaire administration, and “special blood draw”.

• The “special blood draw” will be sent for viral load testing and genotyping if the viral load is not suppressed to <1000 copies/mL. The “special blood draw” should be done on the day of first pick-up of the new ARV regimen. Even if a viral load was already done by the site to make the decision to switch the patient to second-line, the “special blood draw” should be done for viral load testing.

• The doctors will inform the Clinical Site Liaison of any patients who will be switched to 2nd line during the first 12 months of follow-up. On the day of switch, doctors will ask patients about their adherence to ARV using the endpoint questionnaire.

16. **12-month follow-up**

• An in-depth patient assessment is performed 12 months after the patient starts ART.

• The assessment is recorded in the Patient Care Booklet, the electronic medical record and PHARMACY RECORD system.

• At **11 months** from the start of enrolment at the site, the Monitoring Coordinator will visit the site and collect all the Patient Care Booklets for the monitoring patients. These charts will be placed together in a separate section, separated from other chart so that they can easily be identified when the patients come in for their 12-months visit.

• **On the 12th month visit,** doctors will ask monitoring patients about their adherence to ARV using the endpoint questionnaire. Participants will have their routine blood draw. An additional 5 ml (“special blood draw”) will be drawn for VL and genotyping. Specimen processing (including label, transportation) will be the same as described at baseline.
H. LABORATORY PROTOCOL:

1. Blood draw:
   - Phlebotomists will draw 2 EDTA tubes (FILL TUBES) from each patient (with vaccutainers). The national lab will provide EDTA tubes for use at ART site. The national laboratory liaison must assure continuous supply of EDTA tubes to the phlebotomy area at the ART site. (“Special blood draws” for the Monitoring Survey will ONLY be collected in EDTA tubes. Each tube will be pre-printed with the sequential HIVDR SID. (Pre-printed labels will include 2 labels for the 2 tubes and 1 label for the survey specimen manifest.)
   - After mixing the contents of the tube (inverting 3-4 times to mix with EDTA), all blood tubes will be placed at room temperature vertically in a rack in the phlebotomy room. The phlebotomist will attach the label with the pre-printed sequential HIVDR SID onto the specimen manifest and also fill out the date and time of blood draw.

2. Transport to on-site national laboratory:
   - The national laboratory technician will come to the ART site phlebotomy room to pick up the “special blood draw” specimen at 3 times during the day (11:00, 14:00, and 16:45). The laboratory technician will place the tubes into a plastic box and transport the specimen with the specimen manifest to the on-site the national laboratory.
   - At the 16:45 specimen pick-up, the technician must query the phlebotomist if there are any pending “special blood draws” before leaving.
   - If any Monitoring participant should come for phlebotomy AFTER the last specimen pick-up at 16:45, the blood draw should NOT be performed. (The participant can choose to a) come back on another day for first drug pick-up and baseline blood draw, or b) refuse to participate in the survey and pick up their drugs.

3. Storage at on-site national laboratory:
   - The collected specimen will be placed at 4-8 °C. (NOT placed in FREEZER). A copy of the specimen manifest form is kept at the ART site.

4. Transport to national laboratory:
• The on-site national laboratory will collect all the EDTA tubes collected during the day and package them in a cooler with ice for transport. This specimen will be transported to national lab by courier.

• The courier will pick up the cooler at 17:00 and the specimen manifests, which will be in an envelope addressed to: National laboratory. The specimen will be transported overnight to national lab.

5. Receiving specimen at national laboratory

• When blood specimens arrive at national laboratory in the morning of the next day, staff will check the quality of specimens (refer to specimen manifest variables) and record this in the specimen manifest.

6. Processing of specimen at national laboratory

• The staff will perform plasma separation using centrifuge at 2,000 xg for 10 minutes.

• The plasma will be suctioned out with separate Pasteur pipettes to transfer the plasma into cryotubes, avoiding suctioning of whole blood along with the plasma. This should all be done in a biosafety hood. The Pasteur pipette will be changed after each specimen is completed.

• Plasma will be divided into 2-3 cryotubes (with screw caps) and labeled with appropriate HIVDR SID. (These labels will be scientific-grade labels able to withstand -80 degrees C)

• The cryotubes will be frozen at -80°C immediately after separation.

• The laboratory staff will complete the specimen manifest.

7. Storage of specimen at National laboratory:

• The cryotubes will be stored in the -80°C freezer at the lab. (There are two 80°C freezers available with a backup generator in case of power outages. The laboratory staff will keep track of freezer temperatures as per laboratory protocol.)

8. Transport to WHO-accredited Genotyping Laboratory:

• These frozen specimen will be transported as a batch to the WHO-accredited laboratory special courier, along with the specimen manifest after baseline and endpoint have all been collected.

• The Laboratory Coordinator will email and phone the Genotyping Laboratory to communicate with them about the specimen and to confirm receipt of specimen.
Blood specimen for patients who reach endpoints of on first-line therapy at 12 months will be processed and transported in the same way as baseline specimen. A specimen manifest should be filled out in the same way as the baseline specimen. The phlebotomist should fill out the Endpoint CARD, collect them, and give them to the Site Clinical Liaison at the end of each day.

Blood specimen for patients who switch to second-line therapy will be processed and transported in the same way as baseline specimen. A specimen manifest should be filled out in the same way as baseline specimen. The phlebotomist should inform the national laboratory of the specimen and transport it to the laboratory. The phlebotomist should fill out the Endpoint CARD and give it to the Site Clinical Liaison at the end of the day. The specimen for patients switching to second-line therapy will be stored in the national laboratory freezer until all endpoint specimen have been collected.
I. SUPERVISION by the Monitoring Coordinator:

1. Enrolment Visit:
   - The HIVDR Monitoring Coordinator will be at the ART site before the start of enrolment. On the first day of enrolment the HIVDR Monitoring Coordinator will be at the ART site to make sure that patients are being enrolled correctly. This will be done for the first 3-4 days, and longer if needed.
   - The HIVDR Monitoring Coordinator will complete the site-profile on the first day of enrolment.

2. Mid-point Visit:
   - A supervisory mid-point visit will be conducted by the Monitoring Coordinator at 6 months. During this trip, the Monitoring Coordinator will work with the Clinical and Laboratory Liaisons to make sure patients who switch to second-line ART are being identified and VL and genotyping is being done.

3. Baseline Data Abstraction:
   - The Monitoring Coordinator will supervise the data abstraction teams that will be sent out to the ART site at the end of enrolment.

4. 11-Month Visit:
   - The Monitoring Coordinator will be at the ART site at 11 months after the initiation of the study to review the cohort of starters with the Clinical Site Liaison and prepare the charts for the 12-month visit.

5. 12-Month Visit:
   - On the 12th month visit, the Monitoring Survey Coordinator will be at the ART site to make sure that monitoring patients are being identified, the endpoint questionnaire is being administered, and the “special blood draw” is being performed.

6. Endpoint Data Abstraction:
   - The Monitoring Coordinator will supervise the data abstraction teams that will be sent out to the ART site at the end of the study (15 months).
   - The HIVDR Monitoring Coordinator will complete the site-profile at the completion of the study.

J. IMPORTANT ISSUES TO CONSIDER PRIOR TO START:
- Identifying problems as soon as they arise: Are ineligible patients being identified as eligible? Are the responsible individuals failing to enter information in the log? Is there a problem with the assignment of the SID? Is the questionnaire more time consuming than planned? Are questions unclear? Are patients failing to answer the questions? Are the responses being recorded incorrectly? What will be the mechanisms to identify these and other possible problems as soon as they arrive, and who is responsible for communicating with the HIVDR Monitoring Survey Coordinator and working out a solution? Who contacts WHO and or technical consultants for advice?

- During the pilot, medical records should be scrutinized at least monthly to record the characteristics of any eligible individual for whom information is incomplete or a specimen is not available. A mechanism to record this information, and improve uptake, should be developed.

- During the pilot, 10% of data should be re-abstracted by a person other than the HIVDR Monitoring Survey Coordinator. Who will do this and how often? Who will do the formal comparison, identify problems, recommend needed changes, and report on whether they have been made.
Appendix 5: Laboratory Results Notification Form

EXAMPLE 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 This test was done as part of an evaluation of the antiretroviral therapy programme 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 programme 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 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 programme 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 programme guidelines.

If you have any questions contact (Name and contact information to be filled in by the working group prior to survey begins)].
Flow Diagram of Monitoring Survey (EXAMPLE)

IF 1st drug pick-up on separate day

Pharmacy: pharmacists
1) Identification of 1st ART pick-ups
2) Issue Enrollment CARD
3) Fill out CDC unique number on Enrollment CARD
4) Send to Clinic with Enrollment CARD and Patient Care Booklet

IF 1st drug pick-up on the same day

Clinic: doctors
1) From Pharmacy: Receive patient who presents Enrollment CARD
2) From Clinic: Issue Enrollment CARD and fill out CDC unique number on Enrollment CARD
3) Assess for eligibility (inclusion and exclusion criteria)
4) Explain monitoring survey
5) Administer informed consent
6) Fill out Enrollment CARD (YES/NO for informed consent)
   (if NO send to pharmacy)
7) If YES, issue Monitoring Survey Sticker with sequential HIVDR SID and fill in ART start date onto sticker
8) Attach Survey Sticker onto Patient Care Booklet
9) Fill out Enrollment CARD (HIVDR SID and date of issuing SID)
10) Administer Baseline Questionnaire. Fill out Enrollment card date
11) Survey logbook filled out by Clinical Liaison (unique/CDC number, DOB, date/time of issuing SID)
12) Send to Phlebotomy with Enrollment CARD

Phlebotomy: phlebotomist
1) Receive patient who presents Enrollment CARD
2) Perform special blood draw
3) Fill out Enrollment CARD (blood draw date)
4) Fill out specimen manifest
5) Send to pharmacy with Enrollment CARD

Pharmacy: pharmacist
1) Check Enrollment CARD
2) Dispense 1st ARV drugs if:
   a) Enrollment CARD marked as “no” for informed consent
   b) Enrollment CARD marked with phlebotomy date
3) Fill out Enrollment CARD (ART start date and pharmacy number)
4) Collect all Enrollment CARDS

Clinical Liaison: Reconcile Enrollment CARDS with Survey Logbook

Fast-track

IF wishes NOT to participate in survey
References


4 Koenig SP et al., Scaling up ART treatment programmes in resource-limited settings” the rural Haiti experience. AIDS 2004; 18 Suppl 3:S21-S25

5 Coetzee D et al., Outcomes after two years of providing antiretroviral treatment in Khayelitsha, South Africa. AIDS 2004; 18:887-895.


8 Jordan MR et al. World Health Organization surveys to monitor HIV drug resistance prevention and associated factors in sentinel antiretroviral treatment sites. Antivir Ther 13 S: 2:15-23