World Health Organization Protocol for Surveillance of Transmitted HIV Drug Resistance:

2012 update
1. Survey overview

The purpose of this survey is to classify the prevalence of transmitted HIV drug resistance (TDR) among individuals recently infected with HIV and not exposed to antiretrovirals (ARVs) in specified geographic areas. TDR survey results inform national and global public health planners on the selection of future first- and second-line ART regimens and current Prevention of Mother to Child Transmission (PMTCT) and pre- and post-exposure prophylactic regimens (PreP and PEP).

WHO has developed a minimum-resource TDR survey method which classifies TDR in specific populations in a defined geographic region [1]. Survey inclusion criteria maximize inclusion of individuals likely to have been recently infected and not exposed to ARVs, thus maximizing the likelihood that any observed resistance has been transmitted and minimizing inclusion of individuals with acquired HIV drug resistance (HIVDR). This minimum-resource method uses small sample sizes (N≤47) and truncated sequential sampling to provide prevalence classifications of low (<5%); moderate (5-15%); high (>15%) TDR to each drug and drug class [2]. TDR is defined for each drug and drug class by using the WHO surveillance drug resistance mutations list [3].

The TDR survey may be embedded within HIV sero-surveys to minimize costs and human resources. For example, many countries with high HIV prevalence perform HIV sero-surveillance in populations of women attending antenatal clinics (ANC). TDR surveys may leverage this routine sero-surveillance and assess TDR in this population. TDR surveys may be embedded in HIV sero-surveys being performed in other suitable groups, including commercial sex workers (CSW), injecting drug users (IDU), and men who have sex with men (MSM) provided appropriate epidemiological criteria are developed to maximize the inclusion of members of the target population who are likely to be recently infected. This approach may be particularly suited for countries with concentrated HIV epidemics or low prevalence generalized epidemics.

Alternatively, TDR surveys may be performed at sites where HIV diagnostic testing is routinely performed and where data and specimen quality are suitable. For example, TDR surveys may be performed at Voluntary Counselling and Testing (VCT) sites, Prevention of Mother to Child Transmission (PMTCT) sites or Sexually
Transmitted Infection (STI) clinics.

TDR surveys are always performed in a defined geographic region and ideally assess TDR among one population risk group: ANC attendees, VCT attendees, MSM, IDU, etc. To achieve this, multiple sites of the same type within the defined geographic region are often combined and contribute data to one survey. A geographic region may be a city or a larger health planning unit such as a district or province. In geographic regions where one site type does not yield sufficient numbers for surveillance, HIV diagnostic site types (ANC, VCT, etc) and different population risk groups (pregnant women, VCT attendees, etc) may be combined to yield the necessary sample size to perform the survey. However, when sites types and populations are combined it is important for countries and public health planners to carefully consider the appropriate public health actions resulting from data generated from populations representing mixed HIV risk groups.

In order to minimize the inclusion of chronically HIV infected individuals (>3 years), eligibility is restricted to specimens from persons < 22 years of age (maximum <25 years of age), and to primigravid females (when leveraging HIV sero-surveys conducted among women attending ANC sites or when sampling women attending ANC sites) when this information is available. Other eligibility criteria may be applied whenever appropriate information is routinely available such as CD4 counts, previous ARV experience, known duration of infection, and sign/symptoms of advanced disease.

Specimen types used in this survey include plasma, serum, or dried blood spots. HIV genotyping is performed at WHO accredited genotyping laboratories and TDR is defined by the presence of one or more mutations per the WHO surveillance drug resistance mutations list [3].

2. Background

2.1 Global Expansion of Antiretroviral Treatment

In 2001, the United Nations General Assembly Special Session on HIV/AIDS recommended that ARV drugs be made available in resource-limited countries to address the disparity between rich and poor countries regarding access to ART. Following this recommendation, WHO elaborated public health guidelines to support and facilitate the
implementation of ART in resource-limited areas. In an effort to initiate as many eligible individuals as quickly as possible and maintain them successfully on ART, the global strategy calls for a simplified and standardized population-based approach to treatment and the use of science-based evidence to support treatment protocols in order to avoid use of substandard treatment associated with poor treatment outcomes and the emergence of HIVDR [4].

The basis of ART scale-up is one potent first-line regimen and one alternate, both consisting of one non-nucleoside reverse transcriptase inhibitor (NNRTI) supported by two nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs), and one second-line regimen, depending on 2 NRTIs and a protease inhibitor (PI) such as ritonavir whose efficacy is "boosted" (enhanced) by another PI, ritonavir, in low doses [5]. An estimated 8 million people living with HIV were receiving ART in low and middle income countries in 2011[6].

**2.2 HIV Drug Resistance**

HIV-1 is a pseudo-diploid RNA virus characterized by a very rapid and error prone replication, a high mutation rate in the presence of drug selective pressure, viral recombination, and the need for lifelong treatment [7]. Because of these characteristics some degree of HIVDR is anticipated to occur among persons on treatment even if appropriate ART regimens are provided and optimal adherence to therapy is supported. HIVDR refers to the ability of HIV to continue replicating in the presence of one or more drugs which usually suppress its replication. Drug-resistant HIV can replicate in the presence of the relevant drugs; most commonly, mutations are observed in HIV reverse transcriptase (RT) and protease (PR). Emergence of drug resistant HIV subpopulations can significantly limit the ability of ARVs to suppress replication. Once resistant strains emerge and replicate, they can persist indefinitely either in circulating virus or archived in memory T cells as proviral DNA [7].

Acquired HIVDR occurs when resistance mutations are selected for by drug selective pressure in individuals receiving ART. Transmitted HIVDR occurs when previously uninfected individuals are infected with drug-resistant virus.
2.3 HIV Drug Resistance Testing

There are two established methods available for HIVDR testing: genotypic and phenotypic testing. In this survey, genotypic HIVDR testing will be employed. Genotypic testing evaluates the amino acid sequence of HIV's *RT* and *PR* enzymes and identifies changes (or mutations) which are associated with drug resistance. Not all mutations conferring resistance emerge as a result of exposure to ARVs. Some mutations are polymorphic and occur naturally at low levels in the absence of drug selective pressure. To support analysis of TDR surveys, WHO developed a list of HIV-1 mutations applicable to all HIV-1 subtypes. Mutations appearing on the list are not seen, or occur at a very low level, among all HIV-1 subtypes in the absence of ARV drug selective pressure and are therefore suitable for studies of TDR. Details of the principles underlying the selection of the mutations, and the recommended list, were published in January 2007 [8], and updated in 2009 [3].

2.4 The WHO HIV Drug Resistance surveillance and Monitoring Strategy

A population-based approach to ART scale-up requires a population-based strategy to assess and prevent the emergence and transmission of HIVDR. Responding to countries concerns about the emergence and potential impact of HIVDR on treatment success, WHO has developed a global HIVDR prevention and assessment strategy designed to be integrated into routine national HIV prevention and monitoring activities [9]. The WHO strategy consists of three main assessment elements:

The strategy's foundation is the monitoring of HIVDR Early Warning Indicators (EWIs) at all clinics, or large numbers of representative clinics [9]. EWIs assess prescribing practices, rates of retention and loss to follow-up, population level adherence to ART, drug supply continuity, and virological suppression, which have been shown to be major predictors of HIV disease progression, HIVDR and death [10-18]. EWI results provide clinic and program managers with data about how their clinics perform compared to international targets aimed at minimizing emergence of HIVDR [9].

WHO surveys of acquired HIVDR are performed at sentinel clinics and supplement EWI data by describing prevalence and patterns of HIVDR in adult and
paediatric populations experiencing ART failure [19].

WHO surveys of transmitted HIVDR (TDR) classify TDR as low (<5%), high (>15%) or moderate (5-15%) in populations likely to have been recently infected [1].

Results of EWIs, TDR and acquired HIVDR surveys provide a comprehensive picture of ART program success in minimizing HIVDR emergence; results are linked to specific public health actions designed to improve quality of care and support selection of current and future ART regimens.

3. Survey Objectives

3.1 Objective

The objective of the TDR survey is to classify the prevalence of TDR as low (<5%), moderate (5-15%), or high (>15%) for each drug and drug class, in populations recently infected with HIV and not exposed to ARVs, or in one or more subgroups of that population, in a defined geographic area. The target population of this survey is newly infected individuals. Because identification of this population is challenging, epidemiologic criteria are used to maximize selection of recently-infected individuals not exposed to ARVs, thus maximizing the likelihood that observed HIVDR is transmitted and minimizing the inclusion of individuals with acquired HIVDR.

3.2 Justification and Intended Use of Results

The TDR survey method outlined in this protocol uses minimum resources and provides information to alert ART programs to potential threats that TDR could cause for first-line ART regimen effectiveness. Restricting the surveys to geographic areas and specific populations will allow an “early warning” that TDR is emerging without diverting resources from more important diagnostic and clinical priorities. Using a small number of sentinel HIV survey specimens to classify TDR prevalence into one of three categories, rather than initiating full-scale representative surveillance, will provide sufficient information for public health action as precise prevalence estimates are unlikely to lead to different recommendations.
Because of the sample size required, the TDR survey method is inexpensive compared to full-scale surveillance. Additionally, if the TDR survey is combined with existing HIV sero-surveys, the use of data and remnant specimens collected for existing sero-surveys leverages the sampling procedures already in place to ensure a reasonably representative sample of HIV infected persons in a geographic area, and precludes the need for training in data and specimen collection. Conducting surveillance of TDR in the context of routine HIV diagnostic testing can also use relatively representative sampling methods and minimize resources, though more effort is required to put in place procedures for data abstraction, specimen collection, and confidentiality.

TDR survey results alert planners, clinicians, and programme staff if additional measures should be considered to prevent unnecessary emergence of HIVDR at ART sites, to strengthen HIV transmission prevention efforts among ART patients, and to evaluate whether initial ART regimens and pre- and post-exposure prophylactic regimens will continue to be effective at the time of the survey and in the future. Additionally, if HIVDR testing is available in a country, TDR survey results may support deliberations on whether HIVDR testing prior to ART initiation is desirable in certain areas or for specific populations.

4. Survey Definitions, Design and Procedures

4.1 Overview of survey design and procedures:

The TDR survey is cross sectional and uses data and specimens collected from 47 consecutive, eligible, newly diagnosed HIV-positive individuals. Operationally this means collection of 70 specimens to ensure amplification of at least 47 specimens to permit TDR classification. Specimens for TDR surveys may be collected as part of sentinel sero-surveys, during behavioural surveys among high-risk groups, or at sites where HIV diagnostics services are routinely offered. In higher HIV prevalence settings, the survey population may include participants of HIV sero-surveys or antenatal clinic attendees. However, in concentrated and low-prevalence epidemic settings, it may only be possible to conduct TDR surveys among select high-risk group populations in whom
HIV prevalence is significantly elevated. Specimens should be collected within a maximum period of 12 months, to ensure that results reflect the current epidemic in the selected geographic area. HIV genotyping is conducted at WHO-accredited HIVDR genotyping laboratories, and results are analyzed using the WHO surveillance drug resistance mutations list [3].

4.2 Survey Location

4.2.1. Location:
This survey is suitable for implementation in countries provided sufficient eligible specimen numbers are available in each proposed geographic area.

4.2.2. Location:
TDR surveys are not designed to provide representative data of an entire country; rather surveys are designed to classify TDR in specific geographic areas and within specific populations within a country. Geographic areas are preferably defined as cities (urban area); however, if the required sample size cannot be reached in a city, a geographic area may be defined by the next large health planning unit (district or province) within the country. When choosing which geographic area to assess, countries should prioritize areas where ART has been widely available (>30% of eligible individuals receiving ART) for ≥3 years.

Different geographic areas within a country require separate TDR surveys, because treatment conditions differ, populations are different and transport and other factors affecting adherence are different. Therefore, specimens from different geographic areas should never be combined.

4.3 Survey Setting
The TDR survey is a cross-sectional survey. Ideally it uses data and remnant specimens from HIV sentinel surveys (e.g. ANC site). Alternatively, TDR surveys may be performed in sites where HIV diagnostic testing is routinely offered (e.g. VCT, STI, PMTCT) and may target general populations or specific high risk groups (MSM, CSW,
IDU). The TDR survey uses a minimum-resource method that should not require the establishment of complicated or a costly survey infrastructure. Thus, implementation of TDR surveys should make use of existing staff and logistics to collect the necessary eligible specimens for analysis.

There are two types of established HIV-related behavioural survey. For TDR surveys conducted as a supplement to an existing HIV sentinel sero-survey, the source of the survey population for specimens is the diagnostic site participating in the HIV sero-survey, most commonly an antenatal clinic (ANC). In many countries with concentrated HIV epidemics, it is not feasible to conduct TDR surveys as a supplement to an HIV sero-survey because the number of HIV infections detected in ANC or other sentinel sero-survey sites are often insufficient to reach the required sample size within a reasonable time period (≤12 months). In such settings, TDR surveys can be conducted as a supplement to an existing HIV-related behavioural survey that is focused on a specific high-risk population.

If it is not feasible to implement the TDR survey as a supplement to an existing representative survey, the TDR surveys can be implemented using specimens collected for HIV diagnosis among clients accessing services at sites offering HIV diagnostic services. The focus population of these surveys may be the general clients at these sites (e.g. VCT, STI, PMTCT) or specific high risk populations who may be accessing HIV testing services at these sites (MSM, CSW, IDU). The selection of a particular type of HIV diagnostic setting for the implementation TDR surveillance requires careful review of data from previous years to ensure that:

- The general client or high-risk group accessing services at the selected sites are representative of the population that is the focus of the TDR survey
- A sufficient number of eligible specimens can be successfully collected within a maximum period of 12 months. If collection of the necessary number of eligible specimens from the focus population is expected to take > 12 months, additional steps may be taken to ensure adequate numbers (see Section 5.3). If after evaluation of these additional steps, it is determined that the minimum sample size cannot be achieved in a maximum period of 12 months, a TDR survey should not be pursued in that setting and/or geographic area.
4.3.1 Survey Settings: Criteria for Sero-surveys

If HIV sero-surveys are proposed as the source for TDR survey specimens, the following conditions must be met:

- HIV sero-survey methodology is already in use and at least one round of surveys has already been conducted with results demonstrating feasibility of TDR surveillance.
- HIV sero-surveys are conducted using specimens collected using unlinked anonymous specimens (unless a consented process is proposed).
- Information is routinely collected and reported in the surveys on age or age group, and on the number of previous pregnancies in female participants.
- One of these two criteria:
  - In each geographic area, 70 HIV positive specimens were identified in the previous survey from individuals < 25 years of age, and, in the case of female participants, from women with no previous pregnancy.
  - OR
    During the next planned sero-survey, arrangements can be made, if necessary, to extend the duration of the survey until a sufficient number of eligible specimens have been collected and/or to increase the number of sites in the geographic area from which specimens are obtained.

4.3.2 Criteria for TDR surveys performed at sites routinely performing HIV testing

If the TDR survey is not performed as part of a HIV sero-survey, TDR survey site types must be carefully selected to minimize the possibility that individuals included will have been infected for $\geq 3$ years or that participants included in the survey will have been previously diagnosed and may have used ARVs. Thus, settings where HIV testing is routinely offered and conducted among all clients can be considered for TDR surveys. These settings include:

- Antenatal clinics (ANC)
- HIV Voluntary Testing and Counselling (VCT) Centres
Sexually Transmitted Infection (STI) Clinics
Prevention of Mother to Child Transmission (PMTCT) Clinics

If one of these site types is selected for a TDR survey, criteria for selection of appropriate sites are listed below. Sites should meet all 3 listed criteria:

- Every eligible person attending the selected sites during the period of the survey will have an equal chance of being included in the survey
- All individuals attending the site receive HIV testing routinely; or HIV testing uptake among individuals attending the site is >95%; or specimens drawn from all individuals at the site for other purposes are routinely tested for HIV as part of an unlinked anonymous sero-survey
- Persons diagnosed with HIV at the sites selected are representative of either
  - all HIV-infected persons in the geographic areas or
  - of HIV-infected individuals in a subgroup population of interest (that is the focus of the TDR survey) in the geographic area

Based on previously collected information, 70 specimens from individuals newly diagnosed with HIV meeting the eligibility criteria will be available within a maximum of 12 months from all participating sites offering the same services in the specified geographic area.

Ideally, it should be possible to administer a brief TDR survey-specific questionnaire to ensure that specimens are collected from those who are truly eligible and representative of the focus population for the survey.

In order to ensure that the necessary number of TDR survey specimens is obtained within a maximum of 12 months, it is preferable to collect specimens from all or as many sites of the same site type as possible within a designated geographic area. Therefore, this means that the Total N=70 specimens come from multiple sites of the same type in the same geographic area.
4.3.3 Types of Sites or Settings NOT appropriate for TDR Surveys

There are certain types of settings where HIV diagnostic testing is offered which are not appropriate sites to conduct TDR surveillance, irrespective of the local HIV epidemiology. These include:

- **Hospitals/inpatient medical facilities**: Many individuals diagnosed with HIV during hospitalization often have symptoms of stage 3 or 4 HIV disease. This inpatient population is not likely to reflect those who have been recently infected with HIV, and is thus not appropriate group for the TDR surveys.

- **Tuberculosis (TB) Clinics**: Routine HIV testing for TB patients is now commonplace in many countries. However, the majority of individuals with TB who are diagnosed with HIV already have stage 3 or 4 HIV disease. Therefore, this population is also not likely to represent those who have been recently infected with HIV and thus TB clinics are not an appropriate HIV diagnostic site to be used for TDR surveillance.

- **Primary Health Clinics/General Health Clinic**: While some proportion of individuals accessing primary health services may be healthy/asymptomatic, the majority of clients at these clinics are accessing health services because of an acute or chronic health-related symptom or illness. If HIV testing is not routinely performed or offered for all individuals who visit primary health clinics, those who are diagnosed with HIV are often symptomatic and do not represent those who have been recently infected. Furthermore, in most epidemic settings, the number of individuals diagnosed with HIV in primary health clinics within a 12-month period is not likely to be sufficient to successfully conduct the TDR survey.

- **Blood Banks**: Most blood banks routinely screen donor blood for HIV infection. However, many blood banks exclude individuals who report recent high-risk behaviour or exposure. Therefore, those who are accepted as blood donors may be a biased population, and may not reflect the populations who have most recently been infected with HIV.

- **VCT sites physically located and associated with hospitals or clinics**: These sites may not be appropriate because a large percentage of testing done on patients may
be secondary to clinical suspicion of HIV/AIDS.

4.4. Survey Eligibility

Because most individuals newly infected with HIV are not diagnosed during the period of recent infection, WHO recommends that TDR surveys target newly diagnosed groups in which a relatively high proportion of recently infected persons never exposed to ARVs are likely to appear.

The following participant inclusion and exclusion criteria are designed to maximize the likelihood of collecting survey specimens from recently infected HIV-positive individuals with no prior ARV exposure. In all sites, enrolment is limited to HIV-positive specimens collected from adults who are younger than a specified age limit. In the antenatal population, enrolment is restricted to women in their first pregnancy to limit the time for potential risk exposures and to minimize the possibility of prior exposure to PMTCT ARV prophylaxis. For surveys focused on a particular high-risk group population, enrolment is limited to specimens collected from individuals whose first risk-defining event occurred in the past 3 years. For example, if a survey is focused on IDUs, the individual’s initial use of injecting drugs occurred within the last 3 years. Irrespective of the survey population, individuals who have previously tested positive for HIV, those with prior known exposure to ARVs for any duration, those who have had an AIDS defining illness, and those who are eligible to start ART are excluded from TDR surveys.

Specific caveats for surveys focused on high-risk group populations:

Before focusing a survey on a specific group, the country should assess whether the individuals likely to transmit HIV to that group have substantial access or exposure to ART.

Additionally, it is critical to understand the epidemiologic characteristics of the high-risk group population that will be the focus of the TDR survey within the specified geographic area. This includes gathering information on factors such as the age at which most members of this group initiate the specific high-risk behaviour (i.e. at what age do most CSW in the area initiate sex work, or at what age do most IDU in the area begin injecting drugs), the type and frequency of access the particular high-risk group has to
HIV diagnostic services in the area, and the geographic locations of the specific high-risk group within that region. This information is essential to inform the decision of whether or not a representative TDR survey focused on that specific high-risk group can be successfully conducted in the selected geographic area, and which HIV diagnostic sites are appropriate to include in such a survey. It may additionally reveal the potential biases of a TDR survey focused on the specific high-risk group in that geographic area, which are important to consider when interpreting survey results.

4.4.1 Required Minimal TDR Survey INCLUSION Criteria: Applicable in ALL TDR Surveys

The following INCLUSION criteria are applicable to TDR surveys conducted in ANY site type and among ANY population.

- **Adult:** The minimum age of when an individual is considered an adult varies from country to country. In many settings, the lower age limit of adulthood is 18 years, while in other countries it may be range from 14-16 years. The lower age limit for enrolment of adults in the survey should be determined by the country, based on what is considered standard and acceptable in the local setting.

- **Age:** In all surveys, only individuals < 25 years old should ever be considered eligible. However, it is preferable to limit the age to individuals < 22 years, to minimize risk exposure period and maximize the likelihood of enrolling only individuals who have been recently infected and who have no previous ARV exposures. The maximum age of survey participants should be based on the local HIV epidemic and data regarding age of sexual debut in the geographic area of the survey. For example, if data indicate that most recently infected individuals in the geographic area and population of interest are between 16-20 years of age, then only those < 21 years should be considered eligible.

- **HIV serostatus:** Specimen is confirmed HIV positive per country protocol
4.4.2 Required TDR Survey EXCLUSION Criteria: Applicable in ALL TDR Surveys

- Age > 25 years
- HIV negative serostatus

4.4.3 TDR Survey EXCLUSION Criteria: Applicable when available

- Known previous positive HIV test
- Prior exposure to antiretroviral drugs
- Any previous or present WHO HIV Stage III or IV infection
- Current eligibility to initiate ART
- CD4 cell count < 500 cells/mm³
- If female, previous pregnancy

4.4.4 Eligibility Criteria: Applicable to TDR Surveys embedded in ANC sero-surveys

Specific additional inclusion criteria apply to TDR surveys focused on particular populations.

4.4.4.1 Antenatal Care (ANC) Clients

For TDR surveys conducted at ANC sites, or are focused on ANC populations survey inclusion criteria are as follow:

- **Antenatal clinic attendee**: A pregnant woman who is accessing services at the clinic for pre-natal care.
- **Gravidity**: Enrolment is limited to women who are in their first pregnancy (no previous pregnancy)
- **Age**: < 25 years old. However, it is preferable to limit sample to individuals age < 22 years, when feasible

Exclusion criteria as per 4.4.3 when applicable
4.4.4.2 General Clients at VCT Sites

Eligibility criteria for TDR surveillance in VCT sites offering routine HIV testing

- **VCT Client**: A person who is voluntarily accessing HIV testing and counselling services at the sites. If possible, enrolment should be restricted to walk-in clients. This would limit the enrolment of VCT clients who have been referred to HIV testing services because of Provider Initiated Testing and Counselling (PITC) policies because of clinical symptoms that are suspicious for HIV disease.

- **Gravidity**: Among women, enrolment is limited to those who have never been pregnant. If a woman is accessing VCT services as part of antenatal screening, she is eligible for survey inclusion only if she is a primigravida.

- **Age** < 25 years old. However, it is preferable to limit sample to individuals age < 22 years, when feasible

For TDR surveys focusing on “general VCT clients,” survey exclusion criteria are (if available):

- Gravidity: Women who have had a previous pregnancy
- Known previous positive HIV test
- Prior exposure to ARVs
- Age > 25 years old

4.4.4.3 General Clients at STI Clinic

For TDR surveys focused on “general STI clinic clients” (individuals assessing clinical services at an STI clinic); additional survey inclusion criteria are:

- **Sex**: Male and female clients can be included in the survey.
- **Gravidity**: Among women, enrolment is limited to those who have never been pregnant.
- **Type of STI Client**: Enrolment should be restricted to clients who were diagnosed with their first STI < 3 years ago. This criterion helps to increase the likelihood that the individual client’s period of higher risk exposure to STIs and HIV was limited to the last 3 years. This information may not be routinely available at all
STI sites. Thus, the administration of a special TDR survey questionnaire is recommended to capture this important information.

For TDR surveys focusing on “general STI clients,” survey exclusion criteria are (if available):

- **Gravidity:** Women who have had a previous pregnancy
- **Known previous positive HIV test**
- **Prior exposure to ARVs**
- **Age > 25 years old**

### 4.4.4.4 Female sex workers

- **Sex worker:** Person who engages in sex work or the exchange of sex for money. This may include “direct” or “formal” sex workers, who are sometime included in registries and often found in brothels, as well as “indirect” or “casual” sex worker, who do not engage in sex work full time and unlikely to be included in registries. Sex workers may be recruited from STI or VCT sites.
- **Duration of sex work:** Female commercial sex worker who has been performing sex work ≤ 3 years.
- **Sex:** Biological females
- **Gravidity:** Enrolment should be limited to those who have never been pregnant. However, knowledge of the local epidemiology of sex work and HIV should be taken into account when making this decision, particularly in concentrated and low-prevalence epidemic settings **Age < 25 years**

Exclusion criteria as per 4.4.3 when applicable

### 4.4.4.5 Male commercial sex workers

- **Commercial sex worker:** Person who engages in sex work or the exchange of sex for money. This may include “direct” or “formal” sex workers, who are sometime
included in registries and often found in brothels, as well as “indirect” or “casual” sex worker, who do not engage in sex work full time and unlikely to be included in registries.

- **Duration of sex work**: Male commercial sex worker who has been performing sex work for no more than 3 years.
- **Sex**: Biological males
- **Age < 25 years**

Exclusion criteria as per 4.4.3 when applicable

4.4.4.6 People who use drugs (injection drugs)

- **Injecting drug user**: An individual who has injected drugs any time in the last 3 years.
- **Sex**: Both men and women can be included in the survey.
- **Gravidity**: Among women, enrolment should be limited to those who have never been pregnant. In many settings, the male sexual partners of female IDUs are also IDUs, suggesting that these women are at increased risk for HIV through contaminated needles as well as sexual transmission. Additionally, some female IDUs also engage in commercial sex work, placing them at increased risk for HIV through sexual transmission.
- **Age < 25 years**
- **Exclusion criteria when available**
- **No drug use**
- **Initiation of risk behaviour 3 years prior to survey**

4.4.4.7 Men who have sex with men

- **MSM**: Any man who has sex with another man, regardless of sexual orientation or gender identity, not considering the fact that he may also be having sex with women.
- **MSM status**: MSM who began having sex with other men < 3 years ago.
- **Sex**: Biological men
- Age < 25 years
- Exclusion criteria as per 4.4.3 when applicable

### 4.4.5 Additional TDR Survey Eligibility Criteria: For Situations where Special Laboratory Testing is conducted

In certain situations, such as in prospective cohorts/studies, specimens may be specially evaluated for recent HIV infection using specialized laboratory methods, or detailed information may be available on previous negative HIV tests. In these special situations, the following additional inclusion criteria apply to all TDR survey populations:

- Regardless of the person’s age, the specimen shows evidence of recent HIV infection, using a validated laboratory method for determination of recent infection in an individual. *The specific test used should be discussed and approved by the country in consultation with laboratory specialists and WHO-HQ HIVDR team.*

and/or

- Regardless of the person’s age or pregnancy status, the participant fulfils all three of the following criteria:
  - A documented negative HIV test < three years before the current specimen was collected
  - No documented positive HIV test prior to that performed on the remnant specimen used in the TDR survey
  - No previous exposure to ARV drugs

and/or

- Regardless of age or gender, documented CD >500 cells/mm\(^3\) at time of the TDR survey and no previous exposure to ARVs.

### 4.5 Enrolment

As mentioned above, TDR surveillance relies on the collection of specimens from
consecutive eligible individuals in each defined survey population and setting. Details of consecutive enrolment for each type of survey setting are discussed below.

4.5.1 TDR Surveillance Conducted as a supplement to HIV Sero-surveys using ANC site

Consecutive sampling is traditionally used to select the eligible participants whose specimens will be tested for HIV in an HIV sentinel sero-surveys. For example, in ANC HIV sero-surveys, the first eligible pregnant woman, and each subsequent eligible pregnant woman attending the clinic during the sero-survey period thereafter is included in the sero-surveys until the desired number is achieved. In such a setting, specimens from all participants deemed eligible for the sentinel survey should be initially included in the TDR survey.

For the TDR survey, an additional sampling procedure will be superimposed on the HIV sentinel sero-survey. After specimens are unlinked, those from participants meeting the additional TDR survey inclusion criteria should be identified in some way such as a coloured label or a distinctive ink mark on the label, and a notation made on the survey form or portion of the form sent to the HIV testing laboratory. In all sites, it is important to record the date and time of blood draw in order to identify the consecutive order of the specimens. The HIV testing laboratory will further process and screen specimens, and only those specimens identified as HIV seropositive and meeting eligibility criteria for TDR will be sent to a WHO HIVDR accredited laboratory for genotyping.

Data routinely collected on individuals for the purposes of the HIV sero-survey will be the source of data abstracted for the TDR survey. In these settings, the country should determine which of the routinely collected HIV sentinel survey variables will be abstracted for each of the TDR survey participants. Data should be abstracted for each TDR survey participant using a standardized form.

4.5.2 TDR Surveillance at Sites that offer HIV diagnostic services

For TDR surveillance that cannot be conducted as a supplement to HIV sero-surveys, conducted at sites that offer HIV diagnostic services (such as VCT sites, ANC
clinics, STI clinics, PMTCT, etc), the procedure for enrolment may differ in each country or geographic area. In each type of setting, potential participants who meet the inclusion criteria are consecutively selected, and specimens are drawn for HIV testing. In some settings, it may be necessary to obtain informed consent from eligible participants before enrolment in the TDR survey.

Country should determine the necessary data/variables of interest that will be collected for all TDR survey participants. Data routinely collected for HIV testing purposes, such as data collected for STI clinic registers or VCT site registers, may be used to abstract data for the TDR survey. If all data related to participant inclusion and exclusion criteria (at least Exclusion Criteria 1-3) are not routinely collected at the site, then a brief additional TDR survey standardized questionnaire should be instituted for the duration of the survey to assess client eligibility and obtain participant data for TDR Survey. In all sites and surveys, it is important to record date and time of blood draw in order to identify the consecutive order of the specimens. The HIV testing laboratory will further process and screen specimens, and only those specimens identified as HIV seropositive and meeting TDR Survey eligibility criteria will be sent to the WHO HIVDR accredited laboratory for genotyping.

**4.6 Survey Timeline**

A suggested timeline for the planning, site selection, protocol development, implementation, and analysis of the TDR survey at the country level is listed below. Timelines should be modified as necessary for the national protocol.

**Month 1-3:**
- Initial discussions among HIVDR Working group with HIV surveillance staff to evaluate whether sero-surveys or behavioural surveys meet criteria for TDR surveys, and if not, to evaluate potentially appropriate sites
- Identification of initial geographic area(s) and sites (and population subgroups if applicable) for TDR surveillance
- Identification of needed resources
- Identification of TDR surveillance coordinator and, if considered necessary, an
HIVDR surveillance subgroup in-country

- Coordination with Ministry of Health, National AIDS committee, and national/regional WHO and GAP representatives (or equivalents from other organizations)
- Identification of WHO-accredited HIVDR genotyping laboratory
- Identification of processing labs within country where specimens will be aliquoted or spotted (if necessary), stored, and shipped

**Month 2-4:**

- Pre-implementation site visits or meetings with behavioural survey team
- Eligibility determination and abstraction procedures developed
- Procedures for specimen handling and transport developed in detail
- Procedures for data collection and recording developed in detail, including specimen tracking system
- Coordination with HIV sentinel sero-survey data management planned or site-based data abstraction from routine records planned
- Procedures for confidentiality assurance developed
- Site-based Standard Operating Procedures (SOPs) written
- Development of national protocol
- Clearance of protocol through Ethics Committee Review/international IRB as appropriate
- Training implemented for specimen handling, processing, and shipping
- Training implemented for specimen tracking database and data capture
- National database put into place and tested
- TDR survey data manager identified
- “Test” specimens handled, processed and transported to genotyping lab to evaluate planned system

**Month 5:**

- Specimen collection and transport begins
• Specimen tracking data entry begins
• Capture of participant data begins
• Weekly evaluations of system functioning by HIVDR Survey Coordinator
• Weekly telephone conferences with consultative committee
• Problems addressed and system changed if necessary
• Feedback from processing lab to sites and HIVDR Survey Coordinator regarding success of specimen transport, general appearance of specimens, as soon as specimens arrive -- information recorded in specimen tracking system
• If specimens have been sent to genotyping lab, feedback from genotyping lab to processing lab and HIVDR Survey Coordinator regarding success of specimen transport, general appearance of specimens, as soon as specimens arrive -- information recorded in specimen tracking system. This bullet may not be relevant until a later period in many countries.
• If data are entered locally on laptops, data exported to central database

Month 6-14:
• Specimen collection, transport and tracking + feedback from processing lab (and genotyping lab if applicable) continues
• Capture of data continues or begins
• Bi-weekly or monthly evaluations of system functioning by HIVDR Survey Coordinator
• Monthly telephone conferences with HIVDR-WG, or as needed to address problems
• If specimens have been sent to genotyping lab, information on success of extraction, amplification, and genotyping, problems identified to HIVDR Survey Coordinator, HIVDR-WG, and processing lab – information recorded in specimen tracking system
• If specimens have been sent to genotyping lab, genotyping results captured in local database and merged with relevant HIV sentinel sero-survey or behavioural survey variables, or variables abstracted from HIV diagnostic sites
• If data are entered locally on laptops, data exported monthly to central database
Month 13-18

- Data analysis completed
- Evaluation of first TDR survey completed, including suggestions for modifications of procedures for subsequent surveys
- Report to HIVDR-WG
- Report on HIVDR findings disseminated to policy-makers, ART programme staff, clinicians, community groups
- Evaluation of whether additional surveys are required, and if so, when. Higher priority should be given to HIVDR early warning indicators and surveys of HIVDR developing during ART and associated factors if resources are scarce and if transmitted resistance to relevant drug classes and drugs are < 5%.

5. Sampling Strategy

5.1 Sampling strategy

To determine whether the prevalence of HIVDR associated mutations can is classified as low (<5%), moderate (5-15%), or high (>15%) in a defined geographic area, the maximum number of specimens from HIV positive eligible persons which will be genotyped is 47. However, up to 50-70 specimens should be sent for genotyping to ensure that amplification and genotyping is successful for 47 specimens. Therefore, the national survey coordinators should plan to collect 50-70 specimens from eligible individuals within a maximum period of 12 months.

The sample size for TDR surveys is based on truncated sequential sampling (Annex 3). Sequential sampling is an alternative approach in which the maximum sample size is fixed in advance, but the required sample number may in fact be smaller than the maximum number. Prevalence is categorized and sample size is determined on the basis of results. That is, observations are collected individually or in small batches and, after each observation or batch of observations, the data are examined to see whether a decision about HIVDR prevalence classification may be made from the accumulated
data. If a decision can be made, sampling may stop before the maximum numbers of specimens have been genotyped. Sequential sampling makes classifications based on filling in a simple form called a sampling and classification plan (See Section 8.1 figure 1). Countries should decide whether or not genotyping should be attempted on all specimens at the same time, or whether a staged approach will be used. Often it is cheaper to batch all the specimens and genotype them at once, in which case the staged approach is not relevant.

5.2 Determining the projected number of eligible TDR survey specimens in different survey settings

5.2.1 Sentinel sero-survey or behavioural survey

For TDR surveys that are conducted as a supplement to an existing sentinel sero-survey or behavioural survey, the results of previous years’ surveys should be used to determine the projected number of eligible TDR survey specimens.

In HIV sero-surveys or behavioural surveys, the ability to obtain 50-70 eligible HIV positive specimens depends upon several factors:

- the sample size of the HIV survey in the geographic area
- the proportion of women < 22 years of age with no previous pregnancy (if relevant) enrolled in the previous survey in the area and the
- proportion of men < 22 years of age (if relevant) and the HIV prevalence among participants in the age group of interest
- HIV prevalence among target population in the area

**Example:** In a geographic area with total ANC sero-survey sample of 3,000 planned for the next sentinel sero-survey, and a 40% proportion of pregnant women aged ≤ 22 years and in their first pregnancy in the previous survey, the number of women in the TDR survey target group is projected as 1,200 (40% X 3000). An HIV prevalence of 3% in the previous survey gives a projected 36 eligible TDR survey specimens for the TDR survey (.03 X 1200); a prevalence of 5%: 60 eligible specimens (.05 X 1200); and a 10% HIV prevalence: 120 specimens (.10 X 1200).
5.2.2 HIV Diagnostic Site

For TDR surveys that are conducted at sites that offer HIV diagnostic services, site records (at the proposed VCT, STI clinic or ANC clinic sites) should be reviewed to evaluate whether a minimum of 50 eligible HIV positive specimens can be collected within the proposed survey time period. Review of site records should reveal the proportion of newly diagnosed individuals who meet all of the pre-defined survey eligibility criteria who received their first positive HIV diagnosis for the same time period in the previous year. If records provide additional eligibility criteria, they should be applied. The minimum criteria that should be evaluated to determine whether the proposed sites will yield a sufficient survey sample size are:

- The total number of individuals who were tested at the diagnostic sites during the previous year
- The proportion of individuals tested who meet all survey eligibility criteria
- HIV prevalence among the survey target population in the area. If specific vulnerable groups are the focus of the planned survey, the calculations should be restricted to HIV diagnoses among that group.

**Example:** For TDR surveys that are planned among general VCT clients at VCT sites in District A, there were 3,000 individuals tested in a comparable (6-9 months) time period during the previous year. Of those 3,000 individuals, 30% met all of the TDR survey eligibility criteria (age, sex, gravidity, asymptomatic) during that period, indicating 900 (30% X 3000) projected potential participants in the TDR survey target group. An HIV prevalence of 3% in that group gives a projected 27 eligible TDR survey specimens for the TDR survey (.03 X 900); a prevalence of 5%: 45 eligible specimens (.05 X 900); and a 10% HIV prevalence: 90 specimens (.10 X 900).

5.3 Special considerations: options to achieve the required TDR survey sample size if anticipated enrolment is low

As a rule, surveillance of TDR should not be conducted in areas where the
minimum sample size cannot be achieved. Proper survey planning at the time of site selection should prevent situations where an adequate number of eligible specimens have not been collected during the proposed survey period. If anticipated enrolment in the chosen sites within the geographic will not achieve the required 70 specimen (or at least 60), the following steps may be taken to achieve the necessary sample size.

**Step 1:** Combine multiple sites of the same type within geographic area and/or extend survey to 12 months

IF sample size is not reached:

**Step 2:** Combine multiple sites of different types (ANC/VCT/STI) within geographic area and/or extend survey to 12 months

IF sample size is not reached:

**Step 3:** Extend geographic area to include next largest health planning unit combining multiple sites of the same type, and/or extend survey to 12 months

IF sample size is not reached:

**Step 4:** Extend geographic area to include next largest health planning unit combining multiple sites of different types (ANC/VCT/STI), and/or extend survey to 12 months

5.3.1 Special considerations: options to achieve the required TDR survey sample size if enrolment is very slow

In the event that adequate enrolment has not been achieved after following the above planning steps, survey coordinators may consider one of the two following options. Irrespective of these options, a TDR survey should not be performed for >12 months.
5.3.1.1 Option 1: Extend the sampling period beyond the originally scheduled time period

If the TDR survey is being done in conjunction with an HIV sero-survey, since HIV sentinel sero-survey guidelines recommend oversampling of the individuals < 25 years of age, and include an option for extending the sero-survey to target this group, it may be possible to restrict the sample to this age group for the additional time period and to utilize the HIV testing results for the sentinel sero-survey. In diagnostic sites, the time period for a survey may also be extended. However, surveillance should NOT be performed for > 12 months.

5.3.1.2 Option 2: Staged genotyping of specimens

If at least 34 eligible specimens have been collected, and 12 months have elapsed, these 34 specimens should be sent to the accredited HIVDR laboratory for genotyping. Specimen collection should be continued while waiting to see if a classification of TDR prevalence can be made based on the results of these 34 specimens. If no classification can be made based on the initial specimen shipment, additional specimens collected while awaiting results should be sent for genotyping. Additional specimen collection should be stopped. If the total number of specimens is still insufficient for classification, no estimate of TDR prevalence classification can be made, and this should be reported as the final result of the survey.

6. Data Collection and Data Flow

Data collected for the TDR surveys include clinical and demographic variables collected or abstracted at the time of survey enrolment, specimen tracking data collected at the time of the blood draw and at the processing laboratory, and finally nucleotide sequence data from the accredited HIVDR laboratory. This section describes the methods of data collection, the required variables for each component of the survey, and flow of data from survey sites to a central site for data management and analysis.
6.1 Methods of Data collection

For TDR surveys conducted as a supplement to an existing HIV sentinel or behavioural sero-survey, the form used for data collection for the sero-survey will also be used for the TDR surveys, along with a supplemental specimen information form. Client variables that will be eventually abstracted to the TDR survey database will be the same as those already on the standard HIV sentinel sero-survey form or behavioural survey form. One or more data items may be added to the survey forms for the purpose of a TDR survey, if approved by the national surveillance program, the sero-survey coordinator, and other partners; otherwise, the forms will be used unchanged. However, modifications to the sero-survey form should not be permitted if these changes could jeopardize the survey process. If variables that are required for TDR surveys are not and cannot be included on the sero-survey form, the TDR surveillance should not be performed as part of the sero-survey. Surveys using unlinked anonymous testing use a survey code rather than name or another personal identifier to link socio-demographic data with laboratory data; the same code will be used for the TDR survey.

For TDR surveys performed at HIV testing/diagnostic sites, variables should be abstracted from routine records onto a standardized TDR survey data collection form/questionnaire. Additional variables, including those listed as optional variables in Table 1B below as well as those added in the country-specific protocol, should also be abstracted using this standardized data collection form, which should be administered at the time of survey enrolment. However, if the required variables are not in the routine records, and cannot be added to a questionnaire without jeopardizing service provision, the TDR survey should not be performed at the site.

A supplemental TDR survey specimen tracking form will be used during the blood draw and subsequently at the specimen processing laboratory, but will include no identifying information about the client. If arrangements cannot be made for the required laboratory variables to be recorded at the blood draw site or the processing laboratory, the TDR survey should not be performed at the site or sites associated with that laboratory.
6.2 Variables

6.2.1 Demographic and clinical variables (obtained at survey enrolment site)

Variables collected in HIV sentinel sero-surveys, behavioural surveys and HIV diagnostic sites often include age or age group, sex, education level and occupation, residence, HIV "risk group" or testing category, and gravidity and parity. If possible, all these variables, and any other relevant sociodemographic data collected, such as duration of stay at the present residence, should be captured for the TDR survey.

The absolute minimum required demographic/clinical variables at TDR site are: age, sex, and gravidity (for females). While these are the minimum required variables for the survey, the collection of any additional information that allows further classification of eligibility will improve the survey. When available, the inclusion of additional variables related to HIV clinic status, previous exposure to ARV drugs, and results of other laboratory tests should be recorded for the TDR survey. The required and optional variables to be obtained or abstracted at the specimen collection/survey enrollment site are listed below in Table 1A and Table 1B respectively.

6.2.2 Variables collected at blood draw (Obtained at specimen collection site)

The specimen survey code, the specimen collection facility and the date and time of specimen collection are required to be recorded at blood draw. Recording of date and time of blood draw is critical when there is more than one site in a geographic area for the survey. Required variables from the specimen collection site are:

- Survey specimen code
- Specimen collection facility
- Date of specimen collection
- Date of birth or age at blood draw
- Number of previous pregnancies (gravidity, include current pregnancy)
- Mark or code indicating potential eligibility of specimen for TDR surveillance
- Date of specimen collection
- Time of specimen collection

Optional variables are as follows:
6.2.3. Variables collected in the processing or HIV testing laboratory.

Variables to be collected in the laboratory are those which will allow evaluation of conditions that affect the probability of amplification and genotyping. The minimum required variables to be recorded include: specimen condition, the date of freezing of serum and the temperature of the freezer, the volume available and the dates of any subsequent thaws and refreezes. The initials of the person recording the information and the date recorded should also be recorded.

**Required laboratory variables:**

- Date specimen was received in processing laboratory
- Condition of specimen
- Date HIV was confirmed
- Volume of remnant serum/plasma aliquoted, or number of DBS, for HIVDR genotyping
- Date serum/plasma specimen was frozen and temperature of freezer
- Number of subsequent thaws and re-freezes
- Date specimen was sent to the genotyping lab
• Date form filled out, and initials of staff completing it

If possible, the following variables should be recorded: date and time of specimen receipt in processing laboratory, date and time of centrifugation and separation (for serum), condition of specimen (after separation for serum; at time of receipt in lab for DBS), volume of remnant serum aliquoted for HIVDR genotyping, date and time of freezing of serum; temperature at which serum is frozen; date and time of any subsequent thaws and refreezes, time and conditions under which specimen was sent to genotyping lab.

**Optional laboratory variables**

• Time (in addition to date) specimen was received in processing laboratory
• Date and time of centrifugation/separation of serum
• Time (in addition to date) of specimen freeze
• Date and time specimen was thawed (for each thaw)
• Date and time specimen was re-frozen (for each subsequent freeze)

Because this information will only be recorded for eligible HIV positive specimens sent for genotyping, it is suggested that “batch” information be recorded in a laboratory log and subsequently transferred to TDR survey supplemental laboratory form, for the relatively few specimens that prove to be eligible for TDR surveillance.

**6.2.4 Variables from the genotyping laboratory**

For each specimen, the date received in the genotyping lab, the condition of the specimen, and, if applicable, the date of amplification and genotyping should be noted. For each specimen, it should be noted whether amplification was attempted and whether it was successful, and an additional variable noting reasons if amplification was not attempted. For specimens that are genotyped, 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 900-1200 should be reported electronically from the genotyping laboratory.
6.3 Data Flow

Generally, survey data forms are forwarded to a central site and entered into a database. Two approaches are recommended for data flow. Approach A, listed below, is only suitable for TDR surveys that are conducted as a supplement to an existing sero-survey/behavioural survey. Approach B is suitable both for TDR surveys conducted as part of an existing sero-survey as well for those conducted at HIV diagnostic sites.

6.3.1 Data Flow from Survey Site, Approach A

This approach is suitable only for TDR surveys conducted as a supplement to an existing sero-survey.

Sociodemographic and serologic data are collected on separate sections of a perforated form. The sociodemographic data section is completed by clinic staff at the survey site, and the serologic data section is completed by a laboratory technician at the HIV testing laboratory. In the case of a sero-survey, both sections are forwarded to the surveillance programme’s national data manager who then matches the two sections by the unique survey code. A TDR survey supplemental specimen information data form, identified with the same survey code, is also completed by a laboratory technician at the HIV testing laboratory for eligible TDR survey specimens only, and sent along with the serologic data section to the national HIV surveillance data manager, who then forwards a copy of the sociodemographic data and the TDR survey specimen form to the HIVDR survey coordinator.

6.3.2 Data Flow from survey site, Approach B

This approach is suitable for a TDR survey conducted as a supplement to existing sero-surveys OR for TDR surveys at HIV diagnostic sites.

Both sociodemographic and serologic data are collected on a single form. After the socioeconomic information has been recorded on the form at the site, the form is then sent with the blood specimen to the HIV testing laboratory.
In the case of a sero-survey, the laboratory technician who tests the specimens records the HIV and other test results on the form and sends the completed form to the sero-survey manager. The TDR survey supplemental specimen information data form, identified with the same survey code, is also completed by a laboratory technician for eligible TDR survey specimens only, and sent along with a copy of the sociodemographic information form to HIVDR survey data manager.

**In the case of TDR surveys conducted at an HIV diagnostic site**, the forms for specimens that are not confirmed as HIV positive are destroyed, and only laboratory results are sent back to the survey site. Forms for eligible TDR survey specimens that are confirmed as HIV positive are sent to the HIVDR survey data manager along with the TDR survey supplemental specimen information data form, which is identified with the same survey code.

### 6.3.3 Data Flow from Specimen Processing Laboratory

At the specimen processing laboratory, which is frequently also the HIV testing laboratory, information on specimen batches, with dates and times of receipt in HIV testing lab, centrifugation, separation, and all processes taking place before HIV testing should be recorded in a routine log. For HIV positive eligible specimens, this information should be transferred to the supplemental TDR survey laboratory form that is then sent on to the HIVDR survey data manager and the accredited genotyping laboratory.

At the processing laboratory, a separate log should be kept listing the coded identifier and the date of freezing for each aliquoted eligible HIV positive specimen (serum, plasma, or DBS) identified as HIV positive and subsequently stored for the TDR survey. Although DBS should be stored at room temperature, if they are refrigerated or frozen this information should be recorded. In this log, any additional relevant information such as thaws and re-freezes should be recorded. The log should also include the date specimens are sent for genotyping.

When specimens are sent to the genotyping laboratory, a specimen manifest should be included listing each specimen’s coded survey identifier, and the volume of serum or number of DBS for each participant. A copy of the manifest should be kept at the HIV testing laboratory. A separate copy of the specimen manifest should be sent
every month to the HIVDR data manager, to be compared against the specimen information subsequently received at the genotyping lab.

6.3.3 Data Flow from the genotyping laboratory

Data from the genotyping laboratory should, if possible, be encrypted and sent electronically to the HIVDR data manager. Data should be sent from the genotyping laboratory for every specimen received, including specimens for which no sequence is available. The survey coded identifier and the date of specimen receipt in the genotyping lab should be recorded for all specimens. 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 should be sent as a text file, preferably in FASTA format (headed by the survey code preceded by “<”, with the nucleotide sequence beginning on the next line). A chromatogram and a hard copy report should 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). At the same time as the accredited HIVDR genotyping laboratory returns results to the country, it must send FASTA files to WHO headquarters for quality assurance.

7. Laboratory Issues

Detailed specimen handling and processing guidance can be found at http://www.who.int/hiv/pub/drugresistance/hiv_reslab_strategy.pdf. All HIVDR testing must be performed at a WHO designated laboratory for HIVDR surveillance.

8. Data management and analysis

8.1 The TDR survey data analysis

TDR survey data analysis is performed using the WHO HIV drug resistance data
base tool which generates TDR survey reports. The database application is available from WHO.

8.2. Data management and data entry

Data entry should take place at the level of the TDR survey management. The TDR survey data manager will receive data and forms from survey sites, from the processing laboratory, and from the genotyping laboratory. As described in section 6.3, the forms received by the HIVDR data manager include:

- Survey clinical/demographic form from the survey site (which may be the sero-survey form or a special TDR survey form for surveys conducted at diagnostic sites)
- TDR survey specimen supplemental information form from the processing laboratory
- Specimen manifest form from processing laboratory: link specimen codes with TDR survey ID
- FASTA files from genotyping laboratory

Sociodemographic data and data on HIV positive specimens should be entered into the HIVDR database application database, using a plan consistent with local data flow. Before data can be analysed for the TDR, the sociodemographic data for the must undergo cleaning and verification. The final data entry of the relevant sociodemographic data for the TDR database should take place after this process is complete, and an additional eligibility check should take place at that time. Any specimen which no longer meets TDR age, gravidity, or other eligibility criteria after the final cleaning should be removed from the HIVDR database application.

Similarly, data from the TDR specimen supplemental form should be cleaned and checked, and then entered into the database before genotyping results are received. The HIV testing specimen code, from the specimen manifest, should also be checked against the sero-survey database, or the records at participating HIV diagnostic sites to confirm that all specimens sent for genotyping were eligible; and that all eligible specimens were
sent for genotyping. After checking, the specimen code should also be entered into the database, thereby allowing a link between the specimen code and the TDR survey ID. The specimen manifests should be destroyed after need for the data has ended.

Data should be entered from each form twice (“double data entry”) to check for accurate entry. Data from the supplemental TDR laboratory form should be entered as the forms come in, to prevent a backlog and catch data collection errors. At least 10 forms should be manually validated (that is, data should be re-entered to see if abstraction was accurate from paper records) from each TDR form. Completed data forms must be stored in a locked filing cabinet with access only by TDR survey staff.

The HIVDR data manager should clean and verify the genotyping data as soon as they arrive. Problems with amplification or with insufficient specimen volumes should be noted and should lead to prompt action to correct the problem. These data, in the form of FASTA files, can be imported directly into the WHO HIVDR database application. The database will then generate analysis variables for each specimen from the sequence, including the amino acid coded for at each relevant position in protease or RT associated with HIVDR, and an interpretation of whether there is a resistance mutation on the WHO list of transmitted resistance mutations for each drug class.

8.3 Descriptive analyses

After data cleaning, the HIVDR data manager should work with the HIV data manager or appropriate counterpart to produce descriptive tables of the age distribution of the persons sampled for the survey in each site, and sub-tables of the number of eligible clients for TDR surveillance at each site, the number for whom specimens were sent for genotyping, the number of amplifications attempted, and the number of successful amplifications.

8.4 Quality Assurance of Survey Sequences

Before survey analysis is performed, sequences must undergo quality assurance as described in the WHO laboratory guidance document available at: http://www.who.int/hiv/pub/drugresistance/hiv_reslab_strategy.pdf and sequences must
be sent to WHO headquarters for quality assurance. Any queries will be resolved between the WHO virologist and a virologist from the sequencing laboratory in collaboration with the country performing the TDR survey. Laboratories may resolve some problems by re-interpretation, by confirming a close epidemiologic relationship between individuals with closely associated sequences, or by re-sequencing specimens. Chromatograms of sequences with mixtures occurring at resistance-related positions in RT and protease are also re-examined to confirm the presence of a resistance mutation within the mixture. Unacceptable sequences may not be included in the analysis.

8.5 Analysis of sequences and mutations

After sequence queries have been addressed, the sequences are evaluated one at a time, ordered consecutively according to the date and time of blood draw, by using the WHO HIVDR database application. If a major resistance mutation is seen in a sequence, eligibility criteria for the individual concerned, and for the other individuals included in the survey, should be re-examined. In previous surveys, major mutations have sometimes been associated with the inclusion of ineligible participants [21]. If there is evidence that participants are ineligible, they should be excluded from the analysis. If insufficient information is available to confirm eligibility, the potential for inclusion of ineligible participants must be included in the discussion section of the report. If a mutation is seen that may indicate TDR, the HIVDR survey coordinator should also review the regimens used in the country.

8.6 Classification of HIVDR prevalence

Figure 1 shows the recommended sampling plan for HIV TDR surveys.
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<td>22</td>
<td>ND</td>
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<td>24</td>
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Genotyping of specimens may stop when a classification of HIVDR prevalence has been made on the basis of this chart.

A classification of HIVDR prevalence is made when, among the sample number genotyped (see the SNG column), the running total of specimens found with HIVDR (in the RT column on the same horizontal line) is less than the lower limit specified in the LL column to the left of the RT column, or greater than the upper limit specified in the UL column to the right of the RT column.

When one of these conditions occurs, HIVDR prevalence can be classified either as <5% (if the RT is less than the LL number on the same horizontal line) or >15% (if the RT is greater than the UL number). If neither of these conditions has occurred after the 47th specimen has been genotyped, prevalence is classified as ≥5% and ≤15%.

A separate “sampling and classification plan” (see Figure 1) must be filled in for each ARV drug class of interest. Classifications are made based on whether, among the number of specimens genotyped, the number with HIVDR is less than the number specified in the Lower Limit column on the same line, or > than the number specified in the Upper Limit column on the same line. If so, a classification of prevalence to the particular drug or drug class can be made. The analysis ends as soon as the running total of sequences with one or more relevant mutations is greater than an upper limit, or less than a lower limit, specified by the method for that number of sequences. The derivation of the lower and upper limits for each number of sequences has been described in detail [20]. Categorization of HIVDR prevalence as below or above each of the two thresholds is performed separately for Protease inhibitors (PIs), nucleoside/ nucleotide reverse transcriptase inhibitors (NRTIs), and non-nucleoside reverse transcriptase inhibitors (NNRTIs); if prevalence to a class is categorized as > 5%, prevalence is categorized for each drug in the class. If no sequences with relevant resistance mutations appear among the first 34 specimens, prevalence can be classified as < 5% because the lower limit is 1. A categorization of > 15% can be based on a few as the first 14 specimens if the number of sequences with relevant resistance mutations among them is higher than the upper limit of 5. If prevalence cannot be classified using these minimum numbers of sequences, the analysis continues until a classification can be made. If after 47 sequences
are evaluated the number is neither below the lower limit (2) or above the upper limit (8), prevalence is between 5-15%.

Analysis can first be limited to the first 14 specimens:

If >5 specimens with HIVDR are found when 14-24 specimens have been genotyped, then the HIVDR prevalence to that drug class is classified as “high prevalence” (>15%).

If this is not the case, then analysis should continue until the 34th specimen:

If >6 specimens with HIVDR are found when 25-34 specimens have been genotyped, then the HIVDR prevalence to that drug class is classified as “high prevalence” (>15%)

If no specimen with one or more known mutations associated with HIVDR has been found after the 34th specimen is genotyped, the prevalence is classified as “low prevalence” (<5%).

If HIVDR prevalence cannot be classified on the basis of the initial 34 specimens, additional specimens must be analysed. The running total of specimens with HIVDR (RT column) among the sample number genotyped (SNG column) should be compared to the numbers in the LL and UL columns on the same horizontal line until the maximum of 47 specimens has been analysed.

If the number of specimens with HIVDR in the RT column on the same horizontal line as the number genotyped in the SNG column is below the lower limit then sampling stops and the population is classified as low prevalence.

If the number of specimens exhibiting HIV drug resistance found in the survey sample (labelled running total) is above the upper limit then sampling stops and the population is classified as high prevalence.

If the maximum survey sample size (47) is reached without limit being crossed then sampling stops and the population is classified as moderate prevalence (≥5% and ≤15%).
8.7 Interpretation of findings

Because results from HIVDR surveys are intended to trigger specific public health actions, WHO recommends that policy makers critically review survey results and implement appropriate interventions, which differ according to the detected level of transmitted or acquired drug resistance.

8.7.1 Potential biases and limitations applicable to any survey result

Irrespective of the classification of transmitted HIVDR prevalence in the survey, several potential biases and limitations to the survey findings must be taken into account. These include:

TDR surveys classify prevalence of transmitted HIVDR based on a small number of specimens. Using specimens from HIV sentinel sero-surveys are one of the best ways of obtaining specimens to evaluate TDR in countries where most individuals are not diagnosed until years after infection. However, TDR surveillance will not evaluate whether specific groups of persons are at higher risk of transmitted HIVDR than other groups, unless separate surveys are performed in sites serving separate risk groups, and sufficient numbers of specimens are collected for a separate survey of each risk group.

No method can easily capture a representative sample for TDR in geographic areas where most individuals infected with HIV are not diagnosed during recent infection, especially if those who are diagnosed during that period differ from those who are not. HIVDR prevalence in persons < 25 years of age and females with no previous pregnancy is a reasonable surrogate measure for transmitted HIVDR, but if many persons in this group happen to have been infected years before, and ART has only become widespread in the last year, transmitted resistance could be underestimated. Use of previous HIV testing history to determine recent infection may create a bias because persons who test frequently may differ from those who do not.

8.7.2 Low prevalence classification (< 5%) for transmitted HIVDR

If TDR is classified as low (<5%) in a specific geographic area, no changes in current ART guidelines (PMTCT, ART, PEP, PrEP) are warranted; national programmes
should make plans to repeat the survey in two years in the same geographic area.

8.7.3 *Moderate prevalence classification (> 5%, <15%) for transmitted HIVDR*

If TDR is classified as moderate (5-15%) for the NNRTI drug class in a specific geographic area, extra quality assurance of laboratory and epidemiological data should be performed to ensure accuracy of survey results. If no sequence quality assurance issues are noted and appropriate survey inclusion/exclusion criteria were followed, surveillance of TDR should be immediately repeated in the same geographical area to confirm the results and expanded to additional areas.

A moderate TDR classification alerts programme planners to transmission of significant levels of HIVDR. National HIV programmes should react to by reviewing potential sources of HIVDR transmission in the area surveyed, through assessment of: clinic factors favouring HIVDR emergence (EWIs); rates of VL suppression 12 months after ART initiation at representative ART sites in the area of survey (WHO surveys of acquired HIVDR); performance of HIV prevention programmes to minimize HIVDR transmission; coverage of HIV testing services (high coverage of HIV testing increases awareness of HIV status and reduce the risk of unintended HIVDR transmission).

Before changing ART policy based on TDR survey results, policy-makers should conduct representative surveillance of HIVDR in populations initiating ART. The prevalence of HIVDR in population initiating ART will inform policy-makers when/if they should consider: 1) changing first-line regimens (from NNRTI-based to PI-based regimen) at the population level, 2) introducing baseline genotyping test at individual level to guide therapy (where feasible); or 3) intensifying VL monitoring (e.g. during first 12 months following ART initiation).

If a survey conducted among pregnant women at antenatal care (ANC) sites in a specific geographic area shows moderate level of TDR, and results are confirmed by repeated surveys, policy-makers should consider implementing full scale national surveillance in a representative sample of all HIV-infected pregnant women. Policy implications include: switching from NNRTI-based to PI-based PMTCT or performing resistance genotyping in all HIV-infected pregnant women to guide the decision on which PMTCT regimen will be most effective. Since policy decisions should take into account
cost-effectiveness analysis, WHO is exploring the possibility to develop specific
guidance to help HIV/ART policy makers to interpret survey results using an economic
d lens. When full-scale surveillance of HIVDR in HIV-infected pregnant women is not
feasible, sub-analyses of HIVDR prevalence in women initiating ART may be
considered.

8.7.4 High prevalence classification (>15%) for transmitted HIVDR

If high level (>15%) of NNRTI-TDR are detected, the same actions listed for
moderate HIVDR should be taken. In this case, however, WHO does not recommend
repeating the survey in the same area to confirm the results because chance of gross
misclassification (low as high or high as low) is <5/10,000 surveys, but instead
recommends immediately performing full-scale national surveillance of HIVDR in
populations initiating ART in order to inform first-line ART policy. In order to inform
selection of PMTCT regimen, full-scale surveillance of HIVDR among HIV-infected
pregnant women should be conducted. These surveys will provide point prevalence
estimate of HIVDR and will trigger public health actions based on cost-effectiveness
thresholds. When full-scale surveillance of HIVDR in HIV-infected pregnant women is
not feasible, sub-analyses of HIVDR prevalence in women initiating ART may be
considered.

8.8. Dissemination of results

An annual HIVDR report for the country should be produced which should
include TDR survey results and recommendations for action based on the results. The
report should be sent to policy makers, clinicians, community groups, laboratorians,
people living with HIV, including survey participants, sites participating in the TDR
survey, clinics involved in ART, and other interested parties. Care should be taken in
presenting the data to emphasize that TDR survey results represent the sites (and
subgroups, if applicable) surveyed, not the country. Data from more than one TDR
survey cannot be aggregated, but must be presented separately. Prior to writing and
dissemination of report, all sequence data should be shared with WHO HQ for
quality assurance and support in analysis/interpretation.
9. ETHICAL ISSUES

9.1 Confidentiality and Consent

As the TDR survey is generally conducted either as a supplement to HIV sero/behavioural surveys, or at sites where HIV diagnostic testing is routinely performed, the TDR survey will use the procedures to protect confidentiality are already in place at these sites/surveys. For example, if an unlinked anonymous HIV sero-survey is being performed, the procedures will extend to the TDR using this protocol. The unique survey code number assigned to specimens for the purpose of the HIV sentinel sero-survey will be used to label TDR survey specimens sent for genotyping. HIV diagnostic sites using the unlinked anonymous process should follow the same procedures. For any TDR survey, neither the name of the participant nor other personal identifying information will appear on the supplemental laboratory specimen information form or the genotyping results. No personal identifying information of participants will be made available to WHO, CDC or other survey collaborators. Any suspected breach of confidentiality must be dealt with immediately. The National AIDS Committee or equivalent, and the National HIVDR-WG, and all partners involved in ethics review must be notified promptly of these events.

The following principles will be followed to protect TDR survey participant anonymity:

- **Collect only required data.** Data should be restricted only to selected sociodemographic data elements, eligibility determination, and specimen tracking information. These should not be detailed enough to identify an individual.

- **Make no permanent record of sero-survey participation.** A copy of the survey code should not be kept in the medical chart or the client’s health card. Names or sociodemographic data should not be included in laboratory logbooks.

- **Do not link databases to other databases containing identifying information.** Unlinked anonymous HIV sero-surveys with a TDR survey component should not be linked to any other research, clinical, or laboratory database with additional identifying information.
Protect databases, laptops, and forms. Databases must be password-protected so that only persons working on TDR surveys have access. Laptop computers, sero-survey forms, supplemental TDR survey laboratory specimen tracking forms, logs, and genotype results should be locked in file cabinets or drawers when not being used by surveillance staff. The HIVDR analysis database should, if possible, be held on a stand-alone computer or a secure server.

Restrict access to HIV test results, genotype results, and clinical records. Data management staff should ensure that HIV test results and genotype results are not available to staff who abstracted sociodemographic data and collected serum aliquots, or to sero-survey clinic staff. Sero-survey and TDR survey staff members who work with the databases should not have access to patient records.

Encrypt electronic data before sending it. Data should be encrypted before sending them electronically. If possible, send data through a secure data network.

9.2 Consent

In areas where additional information is sought from TDR participants or where additional blood is drawn, informed consent may be required for enrolment in the TDR survey. In some countries, National HIVDR-WGs and/or ethics committees have decided that consent should be required and results returned even where these conditions do not apply. An oral consent process is recommended.

9.3 Protocol ethical review.

If possible, expedited review should be requested. The TDR survey has been conceived as a survey that should be conducted as an amendment to unlinked anonymous HIV sentinel sero-survey protocols or behavioural survey protocols, or as a survey that will use similar methodology at an HIV diagnostic site. HIV sentinel sero-surveys and HIV behavioural surveys are considered public health evaluations and have been awarded non-research status from international and national ethics review boards in many participating countries. TDR survey protocols in resource-limited countries have also received non-research determinations.
10. GLOSSARY

Survey Definitions

**HIV drug resistance**: For the purposes of TDR surveillance, HIVDR is considered the presence of one or more TDR mutation(s) as per the WHO surveillance drug resistance mutations list [3].

**Consecutive eligible specimens**: Eligible specimens are selected for genotyping consecutively; that is, in the order in which the original blood draws or finger sticks were performed (not the order in which they were received in the laboratory or the order in which HIV testing was performed).

**Focal epidemic**: HIV prevalence is consistently > 1% in pregnant women in one or more specific geographic areas in a country, but not in the country as a whole.

**Concentrated epidemic**: HIV prevalence is consistently > 5% in at least one defined subpopulation and is < 1% in pregnant women in urban areas.

**Generalized epidemic**: HIV prevalence is consistently > 1% in pregnant women.

**Unlinked anonymous HIV testing (without informed consent)**: HIV testing of portions of specimens collected for other routine purposes, such as syphilis testing, or specimens collected for diagnostic purposes which are subsequently unlinked from identifying data. Specimens are unlinked by ensuring that they are labelled with a code that does not allow identification of the individual. No personally identifying information is included in the information obtained. No informed consent or counselling is required, and results are not returned.
11. References


