

Draft Guidelines for Surveillance of HIV Drug Resistance

Executive Summary

Introduction

The implementation of highly active antiretroviral therapy (HAART) in developed countries has led to a dramatic drop in the mortality rate of AIDS patients. However, until recently, few people from countries with limited resources had access to life-preserving but expensive antiretroviral (ARV) drugs. WHO is currently launching the *3 by 5 Initiative*, that plans to expand treatment access programmes in resource-limited settings in order to provide by December 2005, 3 million persons with ARVs. Due to the complexity of the issues, the open-ended duration of HIV treatment and the need to begin programmes quickly to treat millions of individuals, fears have been raised that drug resistance could develop quickly in countries with limited resources, spreading rapidly and quickly rendering anti-HIV drugs useless. The history of treatment programmes in developed countries suggest that some degree of resistance will inevitably develop, given the necessity of lifelong treatment for HIV. The consequences of drug resistance include treatment failure, increased direct and indirect health costs, transmission of resistant HIV strains to treatment-naïve subjects and the need to develop new anti-HIV drugs. The extent of these fears may be greater than is warranted. However, while uncertainties remain about the future dimension of the resistance problem and the impact it will have on our ability to effectively treat HIV, there are clear warning signs that resistance could become a major public health problem. It is still difficult to quantify this threat since, until now, it was difficult to collect reliable, standardized and comparable data on the prevalence of transmitted HIV drug resistance transmission at a global level. Data on the prevalence of resistance among untreated subjects ranged broadly from 5% in some studies up to 27%. Recent data from 17 European countries showed that 10% of the untreated patients studied carry primary HIV drug resistance. In resource-limited countries where HIV treatment has been available, studies of untreated persons have been even smaller and less representative, but generally indicate a low prevalence of mutations (from 0-6%) directly associated with resistance.

To better understand the impact that the increased ARV availability in developing countries may have on the emergence and rise of HIV drug resistance, WHO intends to collect reliable and updated information on the prevalence of HIV resistant strains as ARVs become widely available. Three major public health questions need to be answered:

- What is the level of resistance to ARVs in circulating HIV strains?
- How is HIV drug resistance prevalence changing over time in different areas?
- Is increased treatment availability causing a rise in HIV resistance?

Despite the fact that HIV drug resistance does not rise rapidly and that improvements in quality and adherence to treatment programmes can even lead to a decline in transmitted resistance, minimizing drug resistance is important in all countries.

The *Guidelines for Surveillance of HIV Drug Resistance* provide information for developing a system to monitor drug resistance in newly diagnosed, untreated HIV subjects. The document will address important aspects of a good quality surveillance system like sampling, data collection, laboratory testing, data management and analysis, quality control and ethical issues. The estimates may aid in evaluating the initial standard regimens used in the country and the success of expanded access treatment programmes. Finally, they will stimulate discussions on whether pre-treatment drug resistance testing or screening should be considered.

Populations to be considered in HIV Drug Resistance Surveillance and Monitoring

The feasibility and utility of estimating and tracking changes in the prevalence of HIV drug resistance differs in different populations. Different target groups may be sampled to estimate prevalence in these populations.

The WHO Global Resistance Network recommends that HIV drug resistance surveillance should focus on individuals newly diagnosed with HIV in most countries where treatment access is being expanded. Estimates of resistance prevalence in this population will not allow a direct estimation of resistance transmission during a specified time period, but can facilitate tracking of trends. Use of target groups in which a relatively high proportion of recently infected persons are likely to appear may allow trends in transmitted resistance to be tracked over time, providing the sample size is large and providing the proportion of the recently infected is stable from year to year. Such target groups include pregnant women, particularly those in their first pregnancy, and persons under 21 presenting at VCT clinics.

In most resource-limited countries specific sampling of recently infected persons alone for resistance surveillance is not practical and is not recommended.

HIV drug resistance surveillance in persons about to start treatment may not detect mutations in the strains originally infecting these persons, and would not provide comparable prevalences from year to year.

Although routine surveillance of resistance in persons being treated for HIV is generally impractical, HIV drug resistance monitoring may play a part in treatment programme monitoring, if clinical outcomes are being monitored after a specified treatment duration in a cohort beginning treatment at a certain time. Monitoring of programme factors associated with HIV drug resistance, including the proportion of patients started on standard regimens, the regularity of drug supplies, and patient adherence, may help in interpretation of drug resistance analyses. Special studies to evaluate certain aspects of resistance associated with HIV subtypes and treatment regimens may also be useful.

HIV Drug Resistance Threshold Assessment Surveys

Threshold assessment surveys are performed to assess whether transmitted drug resistant HIV is sufficiently prevalent in the country to indicate a need for sentinel surveillance. These surveys also allow evaluation and refinement of sentinel surveillance methods. Sites are chosen not for representativeness, but because they offer diagnostic testing to groups or because they are located in areas where transmitted HIV drug resistance may appear first.

It is suggested that the threshold resistance prevalence be set at > 5%. This is an arbitrary figure chosen because in countries where HIV treatment began first, studies indicated that the prevalence of transmitted resistance remained at <4% for many years before increasing.

Threshold assessment survey sample numbers are chosen so that the >5% threshold is triggered by the finding of one person whose HIV strain contains major mutations associated with resistance. The minimum sample number is 52, but it is suggested that the sample be expanded to at least 70 to ensure that 52 eligible samples will be transported successfully and have virus amplified to resistance genotyping.

If possible, restrict the survey sample only to individuals likely to have been infected recently with HIV - for instance, persons under a certain age, or young women tested during their first

pregnancy. The exclusion of persons with AIDS-defining signs or symptoms may increase the likelihood that those included may be relatively recently infected.

If it is not possible to restrict the sample to a certain subgroup, then planners may wish to increase the numbers included, based on information obtained on previous new diagnoses. That is, if 30% of the sample is not likely to have been recently infected -- if for instance 30% of persons diagnosed at the site in the previous year had AIDS at the time of diagnosis -- then, if possible, the sample number should be expanded (in this case, to as many as 100) to increase the likelihood of including at least 70 recently infected persons and at least 52 usable samples.

Resistance threshold sampling may be performed in more than one site, provided that HIV diagnosis is offered to the same target group, or mix of target groups, at all sites, and that there is no reason to believe that the risk of transmitted drug resistance differs among the sites. If planners wish to target two different groups or site types, separate full samples should be chosen from each.

Sentinel Surveillance

The move to sentinel surveillance of HIV drug resistance may take place as soon as one HIV strain containing major mutations associated with resistance is found in a resistance threshold survey site for the two successive years, if it is confirmed that it was not exposed to ARV drugs in either year. In that site, if an approved protocol for expansion to routine surveillance is in place, the sample number may be increased in the second year to allow an estimate of the prevalence of transmitted resistance in that site. Alternatively, sentinel surveillance may begin in the following year.

Appropriate site selection is important. Sites should already have been **assessed for their ability** and willingness to incorporate **HIV drug resistance surveillance** into their **routine activities**. Major activities include **specimen and data collection**. **Training, resource, and staffing needs** should be thoroughly discussed and planned. A **site coordinator** should be appointed from site staff.

The **sample** size for sentinel surveillance is dependent on the number of individuals diagnosed in the selected sites. The sample size should be chosen to allow detection of a prevalence of 5% with 95% confidence intervals between 4%-6% and a power of 80%. Successive samples of the selected size should also have a 95% chance of detecting a rise in prevalence from 5% to 10%. **Sequential selection** of HIV positive persons newly diagnosed at the site, in the order in which they appear for HIV testing, is the most practical method of obtaining an unbiased sample.

Persons eligible to be included in the sample include persons who are **newly diagnosed with HIV** for whom there is **no evidence of previous exposure to antiretroviral drugs**. Other criteria such as age or residency may also be applied. If informed consent is included in the methodology, the person must be **able to consent** or belong to a group for whom consent is waived.

Target Group and Site Selection

Target groups are those 'targeted' because they share common characteristics, are accessible and are likely to provide useful estimates of resistance. Sites are HIV testing centres or clinical institutions where persons in the target groups present themselves for routine HIV testing. Target groups to represent the population of persons newly diagnosed with HIV and the subset of recently infected persons, are found among groups already targeted for HIV testing.

For HIV drug resistance threshold surveys, sites should be chosen where transmitted resistance is most likely to be found first. The group doesn't need to be representative of new HIV diagnoses in the country or area (although the sample must be selected to be representative of all those newly diagnosed at the survey site).

For sentinel surveillance, sites should be chosen where individuals being tested are likely to be representative of their target groups, and to produce a collective sample that represents persons newly diagnosed with HIV in the country or the geographic area. With the exception of representativeness, comparability and the desirability of integrating HIV drug resistance surveillance with general HIV surveillance, the characteristics for target groups and sites are the same for resistance threshold surveys and sentinel surveillance.

For sentinel surveillance, target groups and the target sites through which they are reached should ideally have the following characteristics:

- **Representativeness.** The target group need not be representative of the population of the country as a whole, but should be representative of the population newly diagnosed with HIV, or an identifiable subgroup within that population.
- **Inclusion of a relatively high proportion of recently infected persons.** The group should have a reasonable chance of including a high proportion of recently infected persons among the newly diagnosed. This can be achieved by including a high proportion of adolescence or young adults, or pregnant women tested during their first pregnancy. Sites in which most individuals receive HIV testing for reasons other than illnesses associated with AIDS will include more recently infected persons than clinical sites where testing is performed because of AIDS-related illnesses like tuberculosis.
- **High numbers of HIV-positive specimens among persons tested.** If possible, groups and sites targeted should include relatively high numbers of individuals with HIV, to ensure large enough sample numbers to justify the use of resources.
- **Ability to collect required information** as part of the routine functioning, in order to:
 - Ensure that only one specimen per person is included
 - Exclude specimens from persons with previous HIV diagnoses
 - Exclude specimens from persons with previous exposure to HIV drugs
- **Comparability from year to year.** To evaluate trends in HIV drug resistance, comparable proportions of target groups representing newly diagnosed persons with HIV should be included annually, or information must be available to evaluate changes in inclusion criteria that could be associated with apparent changes in HIV drug resistance prevalence. Either sites should diagnose comparable groups (in terms of relevant characteristics such as age, gender, and HIV exposure risk) year after year, or data on such characteristics should be available.
- **Integration with routine HIV surveillance.** As far as possible, site selection and data collection for HIV drug resistance surveillance should be integrated with routine HIV surveillance.

In countries with low level or concentrated HIV epidemics, persons tested at **Voluntary Counselling and Testing (VCT) clinics are the target group** most likely to be representative and VCT clinics are likely to provide sufficient numbers of samples. Sexually transmitted infection (STI) clinics are a second possibility, but individuals receiving HIV testing there may be less represented than those tested at VCT. Clinics where illnesses associated with HIV, such as TB, are a third possibility, but individuals receiving HIV testing in these venues may be both unrepresentative and less likely to be recently infected.

In countries with generalized HIV epidemics, persons tested at **VCT clinics and pregnant women** are the two target groups likely to be representative and to provide sufficient

numbers of samples. Because sufficient numbers of specimens will be more generally available in a generalized epidemic, measures to increase the proportion of recently infected persons, such as restriction of the sample to a young age group or a first pregnancy, may be taken. In some circumstances, blood donors and potential military recruits may also be considered for surveillance in generalized epidemics.

Basic Demographic and Clinical Data Collection

Routine HIV drug resistance surveillance methodology should utilize as far as possible data collected during the routine functioning of existing HIV surveillance systems, and routine diagnostic and clinical services.

For collection of additional information, systems should be set up that can operate routinely without requiring large resources and without interfering with routine functioning of other systems. Detailed collection of information should not be seen as part of routine surveillance, but should be reserved for special studies to evaluate hypotheses generated by surveillance.

Basic demographic and clinical data to be collected for resistance surveillance include **age group, gender, date of HIV diagnosis and date of specimen collection. A unique participant number** must be assigned and recorded. **Data to exclude persons with a history of previous HIV diagnosis or ARV drug use** should be collected if possible, but for the minimum dataset an "unknown" category is included for sites in which this information cannot be routinely collected. An **expanded dataset**, to be collected if at all possible, would include definite information on previous diagnostic and treatment history, birthdate or exact age at blood draw, and area of residence. Other data, such as dates and results of previous HIV tests and clinical data, are desirable if they can be captured easily.

Data collection can be implemented either through routine HIV surveillance processes, or through special survey methods, or a combination. **Routine surveillance methodology attempts to utilize the HIV diagnostic and clinical systems already in place, and to utilize data collected during the routine functioning of these systems.** These are supplemented as necessary. **Survey methodology utilizes special procedures to collect data especially for the purpose of the survey.** Both methods have strengths and weaknesses that will be discussed in this section. **Both are feasible strategies** and the choice depends on local circumstances.

If data are to be captured from the HIV surveillance system, a copy of the HIV surveillance form or the capture of required items from the HIV database may be used to provide information for the HIV drug resistance surveillance database. It may be possible to add minimal additional items needed for HIV drug resistance surveillance to the routine HIV surveillance system. Alternatively, the form may be supplemented if necessary by interview or medical records review.

If special data collection methods to accompany HIV drug resistance testing are designed, data may be collected by interview at the time of blood draw and entered onto the HIV drug resistance surveillance form, and supplemented with information from the routine surveillance system or medical records review.

To develop data collection methods, planners should first evaluate routine information collection in potential sites, and note procedures during which additional data could be collected. An attempt should be made to develop a uniform data collection method compatible with the least burden on site staff, minimal additional resources, and a reasonable means for continual monitoring of data quality.

Specimen Collection

Specimen collection can be implemented either using routine surveillance methodology or survey methodology, or a combination. Both methods have advantages and limitations that will be discussed in this section.

Routine surveillance methodology attempts to utilize as far as possible the HIV diagnostic and clinical systems already in place and to utilize data and specimens collected for other purposes. These are supplemented as necessary.

If the routine surveillance method is chosen at least one ml of serum will be aliquoted from each eligible HIV diagnostic specimen. Specimens will be sent for HIV drug resistance testing only if the initial HIV test proves reactive.

If an additional blood draw takes place routinely for every newly diagnosed person either immediately for HIV test confirmation, or not more than one month after HIV diagnosis for clinical purposes, a dried blood spot may be collected at that time, or plasma or serum may be aliquoted after separation. A dried plasma spot may also be made after separation.

In most settings the collection of HIV diagnostic sera is preferred. Dried blood spots, which can be stored and transported at room temperature, should be considered when diagnostic sera are not available for all newly diagnosed persons, or when quick separation and freezing of specimens, or transport of specimens on dry ice, cannot be performed.

Survey methodology utilizes special procedures to collect specimens especially for the purpose of the survey. If the survey method is chosen for specimen collection, a special blood draw will take place after an eligible individual is determined to be HIV seropositive. The specimen to be sent for HIV resistance genotyping may be a tube of blood, a dried blood spot, a dried plasma spot, plasma, or serum.

Plasma is most frequently used for HIV resistance genotyping when a dedicated blood draw is performed for this purpose. In most settings where a special blood draw for HIV drug resistance surveillance is performed plasma will be preferred. If the routine clinical specimen is drawn in a tube from which serum is separated, serum may be used instead. Dried blood spots should be considered when quick separation and freezing of specimens, or transport of specimens on dry ice, cannot be performed. If quick separation is possible but freezing or transport of frozen specimens is not possible, dried plasma spots may be considered.

The HIV Drug Resistance Testing Laboratory

HIV resistance genotyping is being implemented as a routine laboratory test by an increasing number of diagnostic and service laboratories. However, the technology is still complex, expensive and demands a specific laboratory infrastructure. The equipment, staff training for test performance and results interpretation, as well as for ongoing quality control of the entire test procedure, are more demanding than for many routine tests.

The laboratory set-up needed to perform HIV-1 drug resistance genotyping includes the availability of appropriate logistical procedures and the capacity to perform diagnostic PCR based assays. In addition, the DNA sequencing equipment needed for HIV-1 drug resistance genotyping is highly specialised and uses laser technology to detect the DNA fragments. This equipment is sensitive to fluctuations in power and to power failure. DNA sequence analysis machinery is generally expensive, as are the assay reagents. Given the number of laboratory steps and manipulations needed to generate a genotypic profile from a clinical sample, the quality of the final result is highly dependent on that of each of the steps in between. Therefore it is essential

that laboratory staff performing genotyping are well trained and that regular quality control monitoring by means of external proficiency panels as well as by daily positive and negative (run) controls are implemented from the start.

Implementation of HIV-1 drug resistance genotyping for the purposes of surveillance should only be considered by laboratories in which all of the above points can be addressed. HIV drug resistance genotyping is still a demanding laboratory test, sensitive to variation in the end result. Furthermore, highly sensitive and expensive equipment is needed to perform the test. Computing support is required. Implementation of HIV-1 drug resistance genotyping should only be considered by laboratories where a sufficiently high number of clinical samples require genotyping analysis annually to ensure ongoing laboratory experience in the performance of the test and analysis of the results.

Data Management

Surveillance of HIV drug resistance requires plans, procedures and infrastructures for the collection, transfer, integration and analysis of data. These data management systems complement project design and implementation, laboratory processing and testing, and statistical analysis.

The goals of data management in a surveillance system include:

- Ensuring collection of appropriate, complete and accurate data
- Minimizing the labour and resources required for data entry and data capture
- Organizing the data in a form suitable for analysis
- Producing reports for dissemination
- Ensuring the confidentiality and the protection of privacy
- Monitoring, evaluating and trouble-shooting data system operations

The data management system for HIV drug resistance surveillance should whenever possible utilize data collection systems already in place for HIV surveillance and clinical care. However, the data system must also include HIV drug resistance results and link these data with other participant information.

Data management tasks will involve a variety of persons and institutions, including:

- Sites where participants access HIV testing and other services, where data management activities will be directed by a resistance data manager at each site
- HIV testing and processing laboratories, where additional specimen tracking information must be recorded, where additional clinical information may be generated and where data from sites may be collated under the direction of a laboratory data manager
- The HIV drug resistance testing laboratory, where HIV drug resistance testing will be generated
- A national data centre, headed by a national resistance data coordinator, who will provide training, coordinate data management activities at all sites, facilitate linkage of datasets, ensure data security, work with statisticians and epidemiologists to analyze the data, and produce reports

In many countries, additional national or international organizations will assist the national data centre, including universities, other partner institutions, the World Health Organization, or the WHO HIVResNet.

Data for HIV drug resistance surveillance includes:

- Data on individual participants and specimens, collected or captured at the specimen collection site, at other sites where participants access services, or from related surveillance systems, and

- Laboratory data: HIV drug resistance testing results (genetic sequencing data), and possibly other assay results (such as CD4 counts, viral loads, or tests for recent infection)

HIV Drug Resistance Data and Reports

It is suggested that HIV drug resistance genotyping data be transferred electronically from the resistance genotyping laboratory to the national data centre in the form of a nucleotide sequence text file or files. Data for use directly in analyses and clinical reports will generally be derived from the nucleotide sequence national data centre. All analyses of cumulative results must begin with original sequence data rather than a set of summary mutation lists. Analyses should be updated using the latest information on HIV drug resistance. Before programmes are run, each sequence should be evaluated for inclusion in the analysis based on its quality.

A file of amino acid mutations found in an individual HIV strain can be generated by translating the nucleotide sequence and comparing it to a standard reference amino acid sequence. It is suggested that the HXB2 sequence, which is available on the Los Alamos database and has been widely used in past studies, be used for comparison. Additional computer programmes can be applied to this file to produce updated lists of mutations associated with resistance, clinical interpretations and HIV subtype specifications. These programmes will be available and regularly updated on the WHO HIV Drug Resistance website.

The HDRST national data centre should produce annual reports, including overall prevalence of HIV drug resistance and prevalence of resistance to specific drugs and drug classes, subtype prevalences, prevalence of key "indicator" mutations, and estimates of trends.

The HIV Drug Resistance Surveillance Team

The HIV Drug Resistance Surveillance Team (HDRST) should work with the National AIDS Committee to **assess the utility and feasibility of integrating HIV drug resistance surveillance into plans for expanded treatment access and, if the case, ensure the integration.** The team should include experts from all necessary fields, including **virology, epidemiology, data management, clinical medicine, laboratory logistics and quality assurance, and programme management and evaluation.**

The team should **assess the capacity** in the country and **identify specific needs** after deciding on the populations to be included in routine surveillance, target groups and sites to be involved. The development of **partnerships** with institutions with relevant expertise is important, as are plans for training and the transfer of relevant technology and skills to the country.

Initially, the team should assess whether the prevalence of transmitted HIV drug resistance in the country may have reached a trigger level of > 5%, indicating a potential need for full scale sentinel surveillance. Discussion of these activities and the surveillance of HIV drug resistance in various populations is found in subsequent chapters.

The HDRST national data centre should produce annual reports, including overall prevalence of HIV drug resistance and prevalence of resistance to specific drugs and drug classes, subtype prevalences, prevalence of key "indicator" mutations and estimates of trends.

Ethical Considerations and Protocol Development

The initial HIV drug resistance surveillance protocol, whether it describes routine surveillance methodology or research methodology, should be developed with consideration of relevant ethical principles and submitted to **review by appropriate ethics committees.** Relevant principles include **autonomy and self-determination, beneficence, and justice.**

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The piloting of new surveillance methodology, including methodology for HIV drug resistance surveillance is generally considered research and requires informed consent. In certain circumstances, the HIV Drug Resistance Surveillance Team may request ethics committees to grant a non-research determination based on the plan to incorporate the methodology into routine HIV surveillance.

Ethical considerations also include procedures for maintaining confidentiality, including limited access to and timely destruction of lists linking results to individuals, exclusion of identifying information from databases, the use of encryption, aggregate reporting, and methods to monitor and correct breaches in confidentiality.

Informed consent documents should include information about the project staff, purposes, and procedures, potential risks and benefits, the participant's rights, and confidentiality measures. A waiver of informed consent may also be requested if the HIV diagnostic blood draw is used for resistance surveillance rather than a special blood draw; and if demographic and clinical data are obtained from routine surveillance databases or medical records rather than interview; and if local and national ethical guidelines allow such a request. In some countries waivers of informed consent for HIV drug resistance surveillance have been granted based on minimal risk: protection of the rights and welfare of participants; the impossibility of surveillance in selected sites without a waiver of informed consent; and the provision of results and appropriate information to participants.

The vulnerability of certain populations should be considered in protocol development. In some countries, the potential benefit to some vulnerable populations of HIV drug resistance testing has led to their inclusion in HIV drug resistance surveillance.

Protocol development includes a project summary, a list of investigators, background information, project justification and uses for results, project design, objectives and hypotheses, methods (populations, informed consent and confidentiality, laboratory procedures, data collection and management, analytic plan), training, monitoring and evaluation.