

## **GUIDELINES FOR SURVEILLANCE OF HIV DRUG RESISTANCE**

<b>BACKGROUND AND INTRODUCTION</b>	<b>7</b>
<b>THE HIV DRUG RESISTANCE SURVEILLANCE TEAM</b>	<b>11</b>
The HIV Drug Resistance Surveillance Team (HDRST) .....	12
HDRST Purposes.....	12
HDRST Activities .....	13
Capacity and Needs Assessment .....	13
Partnerships .....	14
Training, technology transfer and support .....	14
Assessment of the "HIV Drug Resistance Threshold" .....	15
Steps in planning the HIV drug resistance threshold assessment survey.....	15
Planning sentinel surveillance of HIV drug resistance.....	16
<b>ETHICAL CONSIDERATIONS AND PROTOCOL DEVELOPMENT</b>	<b>18</b>
Ethical Considerations .....	19
Informed consent .....	20
Vulnerable Populations.....	20
Confidentiality .....	21
Protocol Development .....	21
<b>POPULATIONS TO BE CONSIDERED IN HIV DRUG RESISTANCE SURVEILLANCE AND MONITORING</b>	<b>23</b>
Persons newly diagnosed with HIV and not previously exposed to HIV drugs.....	24
Use of estimates.....	24
Advantages .....	24
Limitations .....	24
Recommendations for inclusion in HIV drug resistance surveillance .....	24
Potential target groups.....	25
Persons recently infected and not previously exposed to HIV drugs.....	25
Use of estimates.....	25
Advantages .....	25
Limitations .....	25
Recommendations for inclusion in resistance surveillance.....	25

## DRAFT GUIDELINES FOR SURVEILLANCE OF HIV DRUG RESISTANCE

Target groups.....	26
Persons about to begin treatment for HIV and not previously exposed to antiretroviral drugs	26
Uses of estimates .....	26
Advantages .....	26
Limitations .....	26
Recommendations for inclusion in resistance surveillance.....	26
Persons receiving treatment with anti-HIV drugs.....	26
Uses of prevalence estimates .....	26
Advantages .....	27
Limitations .....	27
Recommendations for inclusion in surveillance systems.....	27
HIV drug resistance monitoring in persons receiving treatment for HIV .....	27
Monitoring of programme issues associated with HIV drug resistance .....	28
Special studies.....	28
<b>HIV DRUG RESISTANCE THRESHOLD ASSESSMENT SURVEYS</b>	<b>30</b>
Planning and Implementing Resistance Threshold Surveys .....	31
Assessment of potential sites.....	31
Threshold survey site coordinators.....	31
HIV drug resistance threshold survey sampling design .....	31
Sampling method.....	32
Number to be sampled.....	32
Resistance threshold assessment site selection .....	33
Resistance threshold assessment site characteristics .....	33
Implementation .....	34
Successive Resistance Threshold Surveys .....	34
Interpretation of Results .....	35
Use of Results .....	35
<b>SENTINEL SURVEILLANCE</b>	<b>36</b>
Initiating Sentinel Surveillance .....	37
Numbers of sites and areas to be included .....	37
Assessment of potential sites.....	37
Sentinel resistance surveillance site coordinators .....	38
Sampling and participation .....	38
Sample size .....	38
Sampling strategy .....	38
Eligibility criteria .....	38
<b>TARGET GROUP AND SITE SELECTION</b>	<b>40</b>
Target Group Criteria .....	42
Inclusion of a reasonable proportion of recently infected persons .....	42
High numbers of HIV-positive specimens among persons tested.....	42
Availability of required data .....	42
Routine data collection.....	42

## DRAFT GUIDELINES FOR SURVEILLANCE OF HIV DRUG RESISTANCE

Additional criteria for resistance surveillance only .....	42
Representativeness .....	42
Integration with HIV surveillance .....	42
Comparability from year to year.....	42
Potential target groups/site categories .....	43
Individuals tested at voluntary counselling and testing centres (VCT).....	43
Pregnant women .....	43
Blood donors .....	43
Persons being screened for potential military recruitment.....	44
Persons newly diagnosed with sexually transmitted infections (STI).....	44
Persons newly diagnosed with illnesses associated with HIV, such as tuberculosis .....	44
Individuals tested at other sites .....	45
Persons whose HIV is diagnosed at central HIV diagnostic laboratories .....	45
Target group selection.....	45
Target groups in countries with low-level or concentrated epidemics .....	45
Target groups in countries with generalized HIV epidemics .....	46
<b>BASIC DEMOGRAPHIC AND CLINICAL DATA COLLECTION</b> .....	<b>47</b>
Demographic and basic clinical data items to be collected for ARV resistance surveillance .....	48
Minimum dataset .....	48
Expanded dataset .....	48
Capturing data from HIV surveillance or serosurvey systems .....	48
Advantages .....	48
Limitations .....	49
Special data collection for resistance surveillance .....	49
Advantages .....	49
Limitations .....	49
Developing the data collection system .....	49
<b>DATA MANAGEMENT</b> .....	<b>51</b>
Data Management in HIV Drug Resistance Surveillance .....	52
The National HIV drug resistance surveillance data centre .....	52
Overview of the data centre .....	52
The National HIV Drug Resistance Data Coordinator .....	52
Data Security.....	53
Eligibility determination .....	53
The participant Identifier .....	54
Clinical reports.....	54
Data management at HIV diagnostic and clinical sites .....	54
Determining eligibility at the site.....	54
Consent .....	55
HIV drug resistance participant identifiers (RPID) .....	55
Specimen labels and specimen tracking data .....	55

## DRAFT GUIDELINES FOR SURVEILLANCE OF HIV DRUG RESISTANCE

Demographic, clinical, and interview data collected at the site.....	55
Data transfer from the site and the processing laboratory to the data centre.....	55
Retention of records at participating sites.....	55
Participant registry.....	56
Site data quality assurance.....	56
Specimen manifests.....	56
Data management in the HIV diagnostic or processing laboratory.....	56
Specimen tracking.....	56
Eligibility determination in the diagnostic laboratory.....	56
The RPID.....	57
Specimen manifests.....	57
Data management at the HIV Drug Resistance Testing Laboratory.....	57
HIV drug resistance data transmission.....	57
Development and implementation of the data management system.....	58
Global sharing of HIV drug resistance data.....	58
<b>HIV DRUG RESISTANCE DATA AND REPORTS</b>	<b>59</b>
Electronic transfer of sequence information from the HIV resistance genotyping lab.....	60
Deriving data from the nucleotide sequence.....	60
Sequence quality checking.....	60
Amino acid differences from a standard reference sequence.....	60
Drug resistance mutations.....	60
Drug resistance interpretations.....	61
Sequence subtype.....	61
HIV drug resistance analyses.....	61
Recommended Reporting of HIV Drug Resistance Surveillance Results.....	61
Global analyses of HIV drug resistance.....	61
<b>SPECIMEN COLLECTION</b>	<b>62</b>
Collecting specimens at HIV diagnosis.....	63
Potential specimen types.....	63
Advantages and limitations of specimen collection at diagnosis.....	63
Advantages.....	63
Limitations of specimen collection at diagnosis.....	63
Collecting specimens for resistance genotyping after HIV diagnosis.....	64
Potential specimen types.....	64
Advantages of a blood draw after HIV diagnosis.....	64
Limitations of a blood draw after HIV diagnosis.....	64
Specimen types for HIV Drug Resistance Surveillance.....	65

## DRAFT GUIDELINES FOR SURVEILLANCE OF HIV DRUG RESISTANCE

Sera .....	65
Advantages of sera .....	65
Limitations of sera .....	65
Plasma .....	65
Advantages of Plasma .....	66
Limitations .....	66
Dried Plasma Spots .....	66
Advantages .....	66
Limitations .....	66
Dried blood spots .....	67
Advantages of DBS .....	67
Limitations of DBS .....	67
<b>THE HIV DRUG RESISTANCE TESTING LABORATORY</b> .....	<b>69</b>
HIV Drug Resistance Testing Procedures .....	70
Specimen Collection .....	70
The HIV drug resistance specimen collection laboratory services manual.....	70
Genotyping Procedures.....	71
The HIV drug resistance genotyping laboratory services manual.....	71
HIV Drug Resistance Surveillance Specimen repository .....	71
Available Methodologies .....	71
Commercial sequencing kits.....	71
HIV Viroseq Kit (Abbott Diagnostics), developed by Applera: .....	71
HIV-1 TrueGene kit (Bayer Diagnostics): .....	71
'Home Brew' sequencing methods: .....	72
Choice of resistance testing methodology.....	72
Internal and External Quality Control.....	72
Internal Quality Control .....	72
External Quality Control .....	72
Training.....	73
Transport regulations .....	73
HIV Drug Resistance Genotyping Data.....	73
DNA sequence trace data .....	73
Edited assemblage.....	73
Nucleotide sequence text file.....	73
Storage of data in the HIV drug resistance testing laboratory .....	74
Quality assurance .....	74

**APPENDIX I: SAMPLE SIZE CALCULATIONS FOR SENTINEL SURVEILLANCE**

Estimation in each area or site.....	75
Estimation between consecutive surveys .....	75
Selection of the prevalence required to be detected .....	76
Precision for estimating the effect size .....	76
Fig 1.....	77

**APPENDIX II: THE HIV DRUG RESISTANCE MUTATION LIST FOR ANALYSIS OF SURVEILLANCE DATA .....**

78

## BACKGROUND AND 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. In fact, the World Health Organization conservatively estimates that in 2002, some 6 million people in resource-limited settings are in need of life-sustaining ARV therapy.

The introduction of innovative and affordable ARV combinations in conjunction with the U.N. ARV access programmes should scale up implementation and reach an increasing number of patients where the epidemic impact is the greatest. Unfortunately, most of the 36 million people in the developing world currently living with HIV/AIDS do not yet have access to potentially life-saving treatment programmes. In the wake of the International AIDS Conference in Durban in 2000 and the United Nations General Assembly Special Session on HIV/AIDS (UNGASS) in 2001, the resolve of the international community to address this appalling disparity between treated and untreated, between rich and poor, is stronger than ever before. The key tenets of this approach are:

- 1) Scaling up of ARV treatment programmes to meet the needs of people living with HIV/AIDS in resource-limited settings;
- 2) Standardization and simplification of ARV regimens to support the efficient implementation of treatment programmes;
- 3) Ensuring that ARV treatment programmes are based on the best scientific evidence, in order to avoid the use of substandard treatment protocols which compromise the treatment outcome of individual clients and create the potential for emergence of drug resistant virus.

The World Health Organization has elaborated new therapeutic guidelines (Scaling up Anti-Retro Viral Therapy in Resource Limited settings: Guidelines for a Public Health Approach) to help countries

with limited resources starting ARV therapy. The guidelines provide advice for beginning and maintaining ARV regimens appropriately. In order to minimize costs and logistical problems WHO suggests that countries select a single first and a limited number of second line regimens for large scale use, recognizing that individuals who cannot tolerate or fail the first and second line regimens would be referred for individualized care by specialist physicians.

Regimens may need to be changed because of either treatment failure or toxicity. Treatment failure is frequently related to drug resistance. Standard multi-drug, and/or multi-class regimens are used to minimize drug resistance in all drug treatment programmes, and limited-resource countries following WHO guidelines will have the advantage of beginning treatment programmes with such regimens.

The history of treatment programmes in richer countries suggest that some degree of resistance will inevitably develop, given the necessity of lifelong treatment for HIV. Implementing measures to delay and minimize the development of resistance for each person in treatment is an important goal; maintaining the prevalence of resistance at zero is not realistic.

Although in richer countries it is recommended that drug resistance testing be performed routinely at evidence of treatment failure, the high cost and degree of complexity of HIV drug resistance testing makes this impossible where resources are limited. To ensure that drug resistance arising during the first regimen will not contribute to failure of the second regimen, WHO recommends that the full regimen be changed from a first to a second line combination regimen in treatment failures in the absence of specific information on ARV resistance.

Increasing concerns about HIV drug resistance make the monitoring of resistance to ARV on a population basis a high priority. However, no large representative population studies have been carried out to estimate the prevalence of

## DRAFT GUIDELINES FOR SURVEILLANCE OF HIV DRUG RESISTANCE

resistance among treated patients with HIV with the exception of the US Cost and Service Utilization study, which, in 1999, examined persons who had been in treatment for HIV in 1996.

A systematic random sample was performed and results were weighted to represent approximately 130,000 of the 210,000 adults in HIV care in 1996 who had survived until 1999 and whose HIV viral loads were not suppressed below 500 copies/ $\mu$ l. The prevalence of drug resistant virus in this population was 78%. When individuals whose viral loads indicated suppression were included in the denominator, the prevalence was 51%. There is no evidence that this high level of resistance, however, led to comparably high levels of resistance transmitted to newly infected individuals.

Until very recently, no representative surveillance systems have existed to evaluate the prevalence of transmitted drug resistance systematically. Special studies, most of which were small and/or unrepresentative, indicate that during the early 1990s, despite widespread monotherapy in treated patients, the prevalences of transmitted HIV resistant to one or more antiretroviral agents were below 5% in most developed countries.

In the late 1990s and early in this century, reported rates among the recently infected generally ranged between 10 - 25% in Europe and North America. The most recent reports suggest contradictory or varying trends, with some suggesting little change, others a continuing increase, and in others a decrease in the prevalence of transmitted resistance over time. Representative surveillance systems are beginning to be put in place in Europe, Canada, and the U.S which should provide better estimates.

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.

Because of the complexity and 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 extent of these fears may be greater than is warranted. HIV infection and most viral infections differ from many bacterial infections like gonorrhoea, in which drug resistance spreads rapidly and renders common drugs useless on a population basis. Also, HIV treatment that would now be considered substandard, and that was guaranteed to produce drug resistance, was administered to *all* individuals treated for HIV in richer countries for many years. All regimens were insufficient, because they consisted of only one or two ARV drugs. However, in none of these countries has any anti-HIV drug been rendered useless on a population basis, or removed from standard regimens, because of drug resistance.

For instance, in the early years of HIV treatment in richer countries, monotherapy with zidovudine led to development of resistance in the HIV harboured by most individuals in treatment at that time. Evidence of transmitted resistance to zidovudine definitely exists, but zidovudine remains commonly used, effective, component of basic regimens for individuals starting HIV treatment in these countries. Even where prevalence of resistance has reached the level of 15%-20% among the recently infected, this has not led to an irreversible "spread" of resistant virus or to the need to abandon standard initial regimens. In some European countries and some US cities, levels of drug resistance in persons recently infected with HIV appear to have declined from those seen a few years previously.

Despite the fact that drug resistance does not spread rapidly in HIV, and that improvements in treatment programmes can lead to declines in transmitted resistance, minimizing drug resistance on a programmatic level is important in all countries. Methods for limiting resistance, which include starting HIV treatment at the appropriate time, using standard and appropriate regimens, ensuring the drug supply, supporting adherence, and regular clinical monitoring of patients, are also



## DRAFT GUIDELINES FOR SURVEILLANCE OF HIV DRUG RESISTANCE

methods which ensure an effective programme overall.

On an individual level, for individuals in treatment who have achieved a satisfactory response with their first regimen and have incorporated the regimen successfully into their daily lives, it is preferable to maintain the effectiveness of this regimen as long as possible. In countries where the number of available drugs is limited, efforts to limit the development of resistance during treatment are especially important.

Transmitted drug resistance is also a concern, although it may not spread rapidly. For individuals just beginning treatment, HAART regimens may be suppressive despite some degree of transmitted resistance, but it is obviously preferable to use a regimen to which the virus is fully susceptible.

Although combination chemotherapy has helped to stabilize progression and rates of HIV-1 subtype B infections in many western countries, worldwide epidemics with group M (non-B, A through J) and O subtypes are expanding. HIV-2 has also been found in limited areas. So far as is known, standard ARV regimens used in the treatment of HIV-1 subtype B are effective against all group M subtypes. HIV-1 type O and HIV-2 are naturally resistant to non-nucleoside reverse transcriptase inhibitors.

The epicentres of HIV-1 infection are currently in Africa (71% of new infections with subtypes A, A/E, A/G, C, D, G, O) and Southeast Asia (17% of new infections are subtypes A/E and C). In many cases, new epidemics are due to circulating recombinant forms (CRFs) that are composed of viruses of different subtypes. Particularly troublesome are escalating rates of subtype C infections (50% of new HIV-1 infections), as well as the shift of the pandemic towards densely populated regions of China, India, and the countries of the former USSR. With globalization and immigration, over 40% of new infections in Europe are currently thought to be of non-B subtype origin.

Increasing concerns about HIV drug resistance make the monitoring of resistance on a population basis a high

priority. Many factors influence the development and spread of resistance. The prevalence of HIV drug resistance among the recently infected is an outcome indicator for interventions to minimize ARV resistance among persons in treatment.

This document provides guidelines for developing a system to monitor drug resistance in persons newly diagnosed with HIV, and the subset of recently infected individuals. In countries where access to treatment is being expanded. The estimates obtained will support planners and clinicians in selecting the appropriate regimens for prevention of vertical transmission and post-exposure prophylaxis. The estimates may also aid in evaluating the initial standard regimens used in the country, and the success of expanded access treatment programmes. Finally, they will inform discussions on whether pre-treatment drug resistance testing or screening should be considered.

The use of drug resistance monitoring can be an important part of treatment programme evaluation, but should not be instituted unless more basic treatment monitoring is already in place.

HIV drug resistance surveillance and monitoring systems provide HIV drug resistance information for the following purposes:

- To support public health bodies in targeting education and prevention programmes to address increasing rates or high prevalence of drug resistance
- To support rational use of antiretroviral drugs by treatment programme planners and individual clinicians
- To support the development and revision of treatment guidelines
- To provide a resource for addressing important questions on HIV drug resistance patterns and spread related to HIV genetic diversity.

This document describes the creation of a national team to oversee HIV drug resistance surveillance, initial capacity and needs assessment, initial planning and

## DRAFT GUIDELINES FOR SURVEILLANCE OF HIV DRUG RESISTANCE

implementation, ethical issues, training, and monitoring and evaluation.

Surveillance and monitoring of drug resistance is likely to become an important part of expanded access to HIV treatment, by contributing to the evaluation of the efficacy of regimens and programmes, and providing important public health information.

## 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**. 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 HDRST should collaborate with the National AIDS Committee to ensure that resistance surveillance plans are integrated into the overall plan to provide expanded access to HIV treatment. 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 included. 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 HIV Drug Resistance Surveillance Team (HDRST)**

### **HDRST Purposes**

The purposes of the HDRST include:

- 1) to work with the National AIDS Committee to consider the specific public health uses of HIV drug resistance surveillance in the country, and to assess feasibility of surveillance;
- 2) to develop an appropriate time line for resistance surveillance activities, in coordination with other important implementation plans such as expanding HIV treatment;
- 3) to assess the country's capacity for HIV drug resistance surveillance, to decide on the populations and groups to be targeted, and to identify additional resources and activities needed;
- 4) to perform HIV drug resistance threshold surveys to assess when the frequency of resistance in persons newly diagnosed with HIV has reached the 5% threshold indicating a need for resistance surveillance;
- 5) to implement, when appropriate, HIV drug resistance surveillance;
- 6) to collaborate with the National AIDS Committee and the national treatment programme; to explore the feasibility of treatment programme monitoring by adding a resistance monitoring component to other year-end programme monitoring activities;
- 7) after routine surveillance is established, to consider implementing other special studies for in-depth evaluation of certain aspects of drug resistance within the country;
- 8) to insure implementation of all activities in accordance with international ethical standards designed to promote the well-being and health of individuals and communities;
- 9) to insure the dissemination of results in order to promote and support the public health of the country.

The HIV Drug Resistance Surveillance Team (HDRST) should function in collaboration with the National AIDS Programme.

Experience in HIV surveillance, in clinical treatment of HIV and ARV drugs, in virology, and in surveillance of antimicrobial resistance, preferably HIV drug resistance surveillance, are important qualities for the national coordination team. Professionals with these qualities may be found in various centres of expertise, including the Ministry of Health and the public health system, universities, institutes of research, National Reference Laboratories, and clinical & treatment centers. A core group to coordinate HIV drug resistance surveillance should be selected. If possible, the HDRST should include not only the national AIDS Manager, but liaisons from national HIV surveillance and HIV treatment programme groups.

The HDRST should ideally include at least one person from each of the following fields:

- Epidemiology and data management (with experience in surveillance, sampling, design of data collection instruments, data analysis).
- Electronic data management.
- Virology (with a connection to the National HIV Reference Laboratory, or a laboratory institute recognized as a centre of expertise in matters relating to HIV.) If HIV genotyping is performed in country, the individual should have expertise and experience in relevant techniques.
- Clinical medicine (with experience in HIV treatment).
- Programme management and evaluation (with experience in management and programme evaluation of HIV surveillance and laboratory-based surveys, including logistics, training, data collection, monitoring and evaluation).
- Laboratory management (with expertise in quality assurance and proficiency testing).

The World Health Organization Global HIV Resistance Surveillance Network (WHO HIV ResNet) can provide technical assistance as needed.

## HDRST Activities

The HDRST should work with the National AIDS committee to assess the desirability and feasibility of HIV drug resistance surveillance, and how it can be coordinated with other HIV prevention, treatment, control, and surveillance activities. The committee may decide to delay the initiation of surveillance until treatment programmes are better established, or to perform the capacity assessment and a threshold resistance survey before planning further activities.

The HDRST should coordinate the following activities:

- Assessment of capacity and needs;
- Establishment of partnerships;
- Development and implementation of the resistance threshold survey protocol;
- Development and implementation of the surveillance protocol;
- Submission of the protocols to the appropriate ethics committee;
- Development of guidelines, protocols, and procedures handbooks;
- Training;
- Supervision;
- Data collection;
- Specimen collection and transport;
- Laboratory processing;
- HIV drug resistance testing, if this is performed-in country;
- Collaboration with the resistance testing laboratory, if this is performed outside the country;
- Collation of HIV drug resistance results and epidemiological data;
- Analysis of results;
- Dissemination of results;
- Monitoring and evaluation of all the processes above;
- Maintenance of confidentiality and high ethical standards in all stages of drug resistance surveillance.

## Capacity and Needs Assessment

Standardized questionnaires should be developed to collect the following information and any additional information needed to assess the country's capacity for HIV drug resistance surveillance.

(1) Identification, of country-level laboratory facilities:

- Where HIV diagnostic testing is performed
- Where PCR is performed (viral load)
- Where genotyping is performed for the purpose of HIV drug resistance testing

(2) Identification of sites where > 50 new HIV diagnoses are made yearly and the population groups diagnosed at these sites;

(3) Assessment of data currently collected at sites identified in (2) and the methods for collecting them:

- On standardized forms
- In non-standardized records
- On laboratory forms submitted with HIV diagnostic specimens
- In electronic databases

(4) Assessment, at each site, of the proportion of persons who return to receive their positive HIV test result (and/or other services) no more than three months after the diagnostic blood draw (to assess whether a representative sample of newly diagnosed individuals could be identified using a blood draw for resistance surveillance soon after diagnosis);

(5) Assessment, at each site, of the capacity to collect additional essential information, at diagnosis or on a return visit;

(6) Assessment, at each site, of processing and transport of HIV diagnostic specimens (specimen type; where are specimens centrifuged and separated; how much time between blood draw, separation, and arrival in the diagnostic lab) (See Specimen Collection section);

(7) Assessment, at each site, of the capacity to administer consent and draw an additional blood specimen on the first return visit of a newly diagnosed person with HIV

## DRAFT GUIDELINES FOR SURVEILLANCE OF HIV DRUG RESISTANCE

(or within three months of the diagnostic blood draw)

(8) Assessment of personnel and training needs

(9) Assessment of other resources needed

As well as assessing capacity, the HDRST should work with the National AIDS Committee to specify the purposes that HIV drug resistance surveillance would serve in the plan for expanded treatment access. The public health or programmatic actions that would be triggered by finding a specified prevalence of resistance, or specific trends, in specified populations should be clearly articulated.

The team should decide the populations on which surveillance and monitoring will be focused, and the target groups and sites which could be used to represent them. A clear understanding is needed of the uses, limitations, and feasibility of estimating prevalences in various populations, and the groups that can be targeted to represent these populations. Populations, target groups, and site selection are discussed in the next chapters.

With the National AIDS Committee and the national treatment programme, the HDRST may then decide to seek or utilize resources to perform the resistance threshold assessment survey to ascertain whether the prevalence of transmitted HIV drug resistance has reached the "threshold" of  $\geq 5\%$ . When the threshold is reached, the team should consider implementing more extensive sentinel surveillance programme to evaluate trends in transmitted resistance.

The team is also likely to be questioned about plans for monitoring drug resistance among persons in treatment. Resistance testing as part of initial programme monitoring in specific sites may be appropriate, and special studies may be helpful after treatment access has been expanded.

The team should use the results of the assessment to specify resources required for additional data collection and recording, confidentiality protection, specimen collection, specimen processing and

transport, resistance testing, data analysis, and results dissemination.

### Partnerships

The HDRST should explore partnerships with institutions with experience in surveys, surveillance, research, HIV treatment, laboratory diagnosis, and HIV drug resistance. Potential partners include WHO HIV ResNet, NGOs, academic institutions, and national and international public health organizations. These institutions may be able to support the work of the committee in development of data collection methods and databases, sampling and survey methodology, training materials, and resource allocation.

If laboratory facilities and expertise for resistance testing do not exist within the country, a partnership should also be explored with an external laboratory with appropriate expertise. WHO HIV ResNet can facilitate such partnerships. A list of appropriate resistance genotyping laboratories involved in WHO HIV ResNet and willing to provide assistance will be posted on the WHO HIV website. Collaboration with an external laboratory may include plans for training and technology transfer to one or more laboratories in-country when appropriate.

### Training, technology transfer and support

**Enhancement of research and evaluation skills among the coordinating team and local staff is important; as well as training in implementation of the protocol. General enhancement of skills including epidemiology and laboratory techniques will support local researchers in designing, evaluating, analysing data, and taking appropriate public health actions in future projects.**

In participating HIV diagnostic sites, staff and their supervisors must receive training about the purposes of the project and the potential uses of the data, as well as specific techniques of participant enrolment, data collection, and confidentiality. Site staff who are not directly involved in the project

## DRAFT GUIDELINES FOR SURVEILLANCE OF HIV DRUG RESISTANCE

should receive a basic orientation in the aims and importance of the project.

The data collection system may include capture of data collected at the HIV diagnostic blood draw or in the routine counselling encounter when the HIV result is discussed with the newly diagnosed person. It may also include records abstraction. Lastly, it may include an interview. Existing staff at participating sites, or new data collection staff working for the resistance team, must be trained in appropriate techniques.

Selected staff should receive training in specimen collection, including blood draws and, if necessary, the preparation of dried blood spots (DBS).

Optimal specimen processing and handling before resistance testing is crucial for resistance testing to be successful. Prompt centrifugation and separation of specimens, prompt freezing and transport of sera or plasma, and prevention of contamination, are critical for the project. Laboratory and other staff who routinely deal with HIV diagnostic specimens must understand the necessity of handling specimens for resistance testing appropriately.

Appropriate training in biosafety is important for all staff, both at testing sites and in laboratories, who will be handling or processing specimens.

Training in laboratory techniques for genotypic resistance testing should also be planned if this testing is to take place in-country and additional training is needed.

Training in quality assurance methods for the project, both internal and external, should take place for the resistance testing laboratory staff.

### **Assessment of the “HIV Drug Resistance Threshold”**

The resistance threshold survey allows the HDRST to assess whether HIV drug resistance transmission affects at least 5% of persons newly diagnosed with HIV in a selected site or area. If resistance frequency has not reached this level in a newly diagnosed group at risk for HIV drug resistance transmission, beginning sentinel

surveillance is likely to expend resources unnecessarily. Threshold surveys should continue regularly to assess whether the threshold has been reached.

The resistance threshold of 5% was chosen arbitrarily. It is based only on observational studies that indicate that resistance may remain at 4% or below among the newly diagnosed for many years after drug treatment is instituted in a country, even if such treatment is not optimal. Because of competing priorities in many countries, WHO HIVResNet recommends that routine resistance surveillance be delayed until this threshold is reached.

The “resistance threshold survey” requires fewer resources than full scale surveillance, because its purpose is to detect only whether the 5% level has been reached rather than providing an estimate of resistance prevalence.

Performing one or more resistance threshold surveys supports the HDRST in deciding whether to move forward with sentinel surveillance. It also allows piloting of methods that will be used for sentinel surveillance. Resistance threshold surveys may be conducted annually, or at more widely spaced intervals, until there is an indication that the threshold has been reached. If resources permit annual surveys, these will help to pilot surveillance methodology thoroughly, to develop expertise among the team and the staff, and to evaluate and improve procedures in preparation for sentinel surveillance.

### **Steps in planning the HIV drug resistance threshold assessment survey**

Planning includes:

- (1) Evaluation of potential sites and selection of sites (See Target Group and Site Selection section)
- (2) Development of the sampling plan
- (3) Development of data collection plan and instruments
- (4) Development of specimen processing and transport plan
- (5) Development of drug resistance genotypic testing plan

## DRAFT GUIDELINES FOR SURVEILLANCE OF HIV DRUG RESISTANCE

- (6) Development of training plan
- (7) Development of data management plan
- (8) Development of analysis plan
- (9) Development of data dissemination plan
- (10) Development of monitoring and evaluation plan
- (11) Collation of plans into protocol
- (12) Submission of protocol to ethics committee and alteration of protocol as required
- (13) Development of detailed procedures manuals (sampling, data collection, specimen handling, processing and transport; drug resistance testing (if this will be performed in-country))
- (14) Implementation of protocol

**Resistance threshold surveys should be conducted at one or more sentinel sites serving groups or areas where HIV treatment has been available to the greatest extent, and for the longest duration.**

**The resistance threshold survey is not meant to be representative; the purpose is to look for transmitted resistance where it is most likely to be seen first.**

If one strain of HIV with major mutations associated with resistance is found in a drug-naïve person newly diagnosed with HIV in two successive annual threshold surveys, this will indicate that the “threshold” of 5% may have been reached either in an individual site or among a target group in a specific area.

The methodology used should be that which could be incorporated into a surveillance system in a number of sites, without burdensome resource requirements or extra staff. Research methods require resources that would not be sustainable on a large scale should not be used in the surveys. Extra blood draws, special courier systems for transport, and collection of data using interviews or special instruments should not be included unless they could be incorporated into the routine work of a

larger number of sites without a “research level” of expenditure.

Resistance threshold surveys should initially be conducted for two years in succession, so that lessons learned in the evaluation in the first year can be applied, and the methodology altered accordingly, in the second year. If there are no HIV strains carrying major mutations associated with resistance found in either year, surveys should then be performed every one to three years.

If the availability of HIV drug treatment differs substantially in different areas, the team should focus surveys in treatment programme areas first, and implement sentinel surveillance in those areas when the threshold is reached. At that time or when appropriate, threshold surveys can be initiated in other areas.

More details on the development and implementation of resistance threshold surveys can be found in HIV Drug Resistance Threshold Assessment Surveys section.

### **Planning sentinel surveillance of HIV drug resistance**

The surveillance of drug resistance in newly diagnosed persons with HIV should not be initiated until there is an indication that the level of transmitted resistance in the country may be at or above 5%, but planning should take place before that point. Threshold resistance surveys can provide the opportunity for testing methodology and evaluating potential sentinel surveillance sites.

Planning for sentinel surveillance is comprised of the same steps as threshold survey planning (see previous section). Most of the methodology tested in the resistance threshold survey(s) can be utilized in sentinel surveillance, including data collection instruments, specimen processing and transport plans, databases, and procedures manuals. Sections of the protocol can also be included in the surveillance protocol (or a combined protocol can be submitted). The sampling strategy will differ, and numbers to be tested will be larger, since an estimate of



## DRAFT GUIDELINES FOR SURVEILLANCE OF HIV DRUG RESISTANCE

prevalence is the purpose of surveillance. The data dissemination plans may incorporate reporting of the prevalence of resistance into general HIV surveillance reports.

**If resistance threshold surveys have been performed for more than two years before the expansion to sentinel surveillance, the team may wish to perform the needs and capacity assessment a second time. Additional sites and changes in laboratory capacity may be noted in a second assessment.**

If resistance genotyping was performed with an international partner rather than in-

country, the HDRST may wish to work with WHO HIV ResNet to assess whether to plan the transfer of resistance genotyping technology to the country for routine surveillance. However, resistance genotyping should not be considered for transfer to an in-country laboratory until basic HIV diagnostic services have been made available, and until there is sufficient need for resistance genotyping of a large number of clinical samples within the country to maintain laboratory expertise.

More details on sentinel surveillance of resistance are found in Sentinel Surveillance section.

## ETHICAL CONSIDERATIONS AND PROTOCOL DEVELOPMENT

The HIV drug resistance surveillance protocol 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**.

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, and monitoring and evaluation.

## Ethical Considerations

*Conduct of surveys and surveillance, like that of research, is to be guided by internationally recognized principles of human rights, including the Nuremberg Code and the World Medical Association's Declaration of Helsinki. The application of these principles to the development of protocols is discussed at length in key documents.*

Generally the piloting of new methodology, including surveillance methodology, is considered research and requires full review by an ethics committee in the country or area, and also at the participating site if required. Under some circumstances the HDRST may decide to request a non-research determination for HIV drug resistance surveillance, and a waiver of informed consent from the ethics committee.

A blood draw specifically for the HIV drug resistance genotyping for surveillance purposes and/or an interview to obtain information for resistance surveillance, are procedures that will generally mark the project as "research" rather than routine surveillance. Such procedures generally require informed consent. If these procedures become the standard of care for everyone in the area or the country, ethics committees may agree that the information may be captured without informed consent as part of routine surveillance.

In some countries utilization of a remnant HIV diagnostic specimen for resistance testing, and obtaining of information through the routine HIV surveillance database or medical records, have allowed a non-research determination. Similarly, the use for resistance surveillance of a specimen drawn for other clinical purposes, such as a viral load determination, has been categorized as surveillance rather than research. Finally, the capturing of resistance genotyping results ordered for clinical purposes has been considered surveillance rather than research. In each country, the particular public health uses of the information will be considered by the

appropriate ethics committee to make a determination.

Prior approval by the relevant ethics committees is important regardless of whether the project is ultimately labelled as research or routine surveillance. A full protocol is likely to be required even for a surveillance submission.

The principles of research and public health surveillance ethics are similar, though specific details differ. Ethical considerations are important during the development of the protocol, regardless of whether the project will ultimately be considered research or routine surveillance.

The basic principles are:

1. Respect for persons
  - Fostering of autonomy and self-determination
  - Protection of vulnerable groups
  - Informed consent for research; informed participation for surveillance
2. Beneficence
  - Fostering physical, mental and social well-being
  - Reduction of risks to a minimum
  - Protection of participants
3. Justice
  - Distribution of risk and benefit
  - Equitable recruitment - each potential participant should have the same chance of being recruited
  - Special protection for vulnerable groups

Issues to be considered in developing an HIV resistance surveillance protocol include the groups and populations concerned, whether a special blood draw will be performed, whether additional information will be sought on participants, and whether results will be returned (and if so, the time frame in which they will be available). Whether or not an informed consent process is considered necessary, both staff and HIV-seropositive persons at participating sites will need appropriate information about HIV drug resistance and resistance testing. If

## DRAFT GUIDELINES FOR SURVEILLANCE OF HIV DRUG RESISTANCE

results are returned, plans to inform that they may harbour drug resistant strains of HIV should include appropriate counselling.

### **Informed consent**

Unless a determination has been received from the appropriate ethics committee that informed consent is not necessary, informed consent should be obtained prior to collection of a specimen for HIV resistance surveillance.

Informed consent documents should include:

1. a listing of the project staff,
2. a brief description of the project and the procedures to be performed,
3. a description of the potential risks and benefits,
4. a description of a participant's rights, including:
  - a. the right to receive any services offered routinely at the site, regardless of whether consent is given for study participation
  - b. the right to refuse to participate in the study or part of the study at any time after initially consenting
  - c. the right to ask for additional information, and the person to contact for such information
  - d. the right to be take away a copy of the form and any accompanying information
  - e. a mechanism to contact study staff or ethics committee staff if harm results or may have resulted from the study
5. a description of the mechanisms for protection of confidentiality.

Additional requirements of national or local regulations or guidelines should also be included.

Generic consent forms for a research study utilizing a special blood draw, and for a project utilizing a portion of the HIV diagnostic specimens should be included. Examples of consent forms created for HIV drug resistance studies and surveys are included on the WHO HIV website. Also included is an oral consent information sheet (which is read to participants who

cannot read) used in a project in which written consent has been waived.

Experience in routine surveillance, rather than special studies, for the determination of HIV drug resistance prevalence in a population is limited. In one large country project in which HIV diagnostic specimens are used and information is collected from HIV surveillance databases only, informed consent has been waived by national and local ethics committees on these grounds:

1. Surveillance of HIV drug resistance meets the criterion of minimum risk because there is no additional blood draw or data collection
2. The project does not adversely affect the rights and welfare of the participants
3. Pertinent information will be provided to participants after participation
4. The project could not be conducted with an informed consent component in many clinical and HIV testing sites, given that the clinical or counselling encounter for an HIV test should focus on the HIV test, risk behaviours, and the person's clinical concerns.

In this project, the general HIV test consent includes a line stating that HIV drug resistance testing will be performed on the diagnostic specimen if the HIV test proves to be positive. Results are available within 6 weeks and each participant is informed at post-test counselling that the results may be sent at that time or later to any clinician chosen by the participant.

### **Vulnerable Populations**

It should be noted that HIV drug resistance surveillance differs from many research studies in that participants are not subjected to experimental procedures. The inclusion of some vulnerable populations excluded from many research projects, such as pregnant women, may be actively beneficial.

Ethics committees reviewing some projects have specifically recommended that some vulnerable populations, including prisoners and persons not mentally competent to give

## DRAFT GUIDELINES FOR SURVEILLANCE OF HIV DRUG RESISTANCE

consent as well as pregnant women, should have an equal chance of participation in HIV drug resistance surveillance because of the potential clinical benefit of the results.

### **Confidentiality**

Maintaining confidentiality is an important consideration in the development of the protocol and procedures. In HIV drug resistance surveillance systems it is suggested that databases be designed so that stored results cannot be linked to the names of participants. Local staff can maintain local databases, which will often be code-based, to allow for retrieval of additional information and return of results (if returning results is part of the protocol). Often measures to maintain confidentiality will be well-established as part of HIV/AIDS surveillance, and these measures can be extended to HIV drug resistance surveillance.

Key elements include:

- Limited access to, and security of, any lists allowing HIV results to be linked to individuals (for the purposes of returning results and retrieving relevant minimum data from HIV surveillance databases)
- Destruction of such lists after results are returned and relevant data are retrieved
- Exclusion of identifying information in project databases
- The use of high level encryption processes for any data transfer
- Inclusion only of aggregate, non-identifying information in reports
- Ongoing monitoring and evaluation of confidentiality procedures
- Mechanisms for reporting, minimizing harm from, and immediate correction of errors leading to, any breach of confidentiality.

### **Protocol Development**

In addition to ethics committee submission requirements, protocols should be developed to conform to national regulations, international standards and

recommendations, and institutional operational guidelines.

The protocol for the resistance threshold assessment may be developed separately from the protocol for full scale sentinel surveillance, or one protocol encompassing both may be developed and submitted for ethics committee review. If a combined protocol is written, extensive amendments to the protocol may be necessary after the methodology is tested in the threshold survey. However, the submission and approval of a combined protocol will facilitate an immediate move to sentinel surveillance when indicated by the threshold survey.

The protocol should be developed not only as a document to outline the steps in threshold surveys and sentinel surveillance, but also to educate staff at participating sites and the health community about the public health uses and limitations of HIV drug resistance surveillance. Attention must be paid to how the project contributes to expansion of HIV treatment in the country.

The protocol should include the following sections:

1. Project overview - a general summary of the purposes and procedures for the project
2. List of investigators and sites [to be amended yearly as necessary]
3. Background - The background should include a discussion of HIV drug resistance surveillance globally, including how results have been used, and the reasons for initiating surveillance in the country or area concerned
4. Justification for the project
5. Potential uses for the results
6. Project design
7. Objectives
8. Hypotheses
9. General analytic approach
10. Methods: general
  - How study design or surveillance system addresses hypotheses and meets objectives

## DRAFT GUIDELINES FOR SURVEILLANCE OF HIV DRUG RESISTANCE

- Timeline
11. Methods: Population
    - Case definitions
    - Eligibility criteria/exclusion criteria
    - Vulnerable populations
    - Sampling and enrolment
    - Risks, anticipated benefits, and risk/benefit ratio
  12. Methods:  
Implementation/documentation of informed consent
  13. Methods: Confidentiality
  14. Methods: Laboratory component
    - Specimen collection
    - Transport to local processing laboratory
    - Roles of local laboratory coordinators
    - Specimen processing
    - Specimen storage (if relevant)
    - Determination of specimen eligibility
    - Transport to HIV drug resistance genotyping laboratory
    - Amplification
    - Sequencing
    - Role of HIV drug resistance genotyping laboratory coordinator
    - Quality assurance
  15. Methods: Data collection, management, and processing
    - Data for participant eligibility determination
    - Data collection
      - Participant data
      - Specimen tracking data
      - Instruments
    - Data management
      - Data entry
      - Data entry quality assurance
      - Information management and analysis software
      - Data transfer
    - Data storage and disposition
    - Return of results to participants
  16. Methods (analysis)
    - Statistical and analytic methods
    - Reporting, notification, and reporting of aggregate results
  17. Training
  18. Monitoring and evaluation
- Protocols should be reviewed and approved by national and local ethics committees. Representation of the communities to be included in HIV drug resistance surveillance should be part of the review process.

## **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 prevalences 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.

**Persons newly diagnosed with HIV and not previously exposed to HIV drugs**

**Use of estimates**

The prevalence of drug resistance in target groups of newly diagnosed persons, in any particular year, may be used to represent the prevalence among persons diagnosed that year who could present themselves to a treatment setting during that year or subsequently.

This prevalence estimate could help to evaluate the standard first regimen(s) in use on a programme level. New evidence of high levels of specific transmitted mutations would be of interest to programme planners or clinicians considering individual regimen choices if more than one first regimen is standard.

In some regions of the world, if the proportion of resistance reaches a specified level (e.g., 5% or 10%), routine drug resistance testing may be recommended for all persons newly diagnosed with HIV or all persons beginning HIV treatment.

Resistance in this population does not reflect current transmission of resistant HIV, because many newly diagnosed persons have been infected many years prior to diagnosis. However, if the proportion of recently infected persons within this group remains the same from year to year and is sufficiently large, trends may indicate whether transmission is increasing, decreasing, or remaining stable. Surveillance in newly diagnosed persons in which few recently infected persons are included in annual samples would be unlikely to support accurate estimation of trends.

**Advantages**

Target groups representing the newly diagnosed population are generally accessible. The total numbers of newly diagnosed individuals are often known, so that numbers required for surveillance can be easily calculated.

Resistance prevalence in this group does not represent recently transmitted resistance, but this group is more representative of all

the new patients likely to be evaluated for treatment by a clinician in a particular year. There is increasing evidence that some important transmitted mutations remain detectable for several years after infection, so that some additional information may be gained by evaluating all newly diagnosed persons rather than only the recently infected. The circulating viruses in newly diagnosed persons who are not recently infected may not reflect more resistant viruses with which some were infected originally, and which may still be archived and able to affect treatment adversely. Focusing surveillance on mutations that remain detectable years after infection may be helpful.

**Limitations**

HIV drug resistance prevalence in this population does not reflect transmitted resistance within a certain time period. A direct estimate of the incidence of transmitted resistance could be obtained by restricting the focus to recently infected persons.

Proportions of recently infected persons, persons with relatively recent established infections, and persons infected many years previously, may change from year to year in this population or in the target groups tested in a surveillance system. These changing proportions could affect apparent trends in resistance. Collection of information to evaluate potential changes in these proportions will aid the analysis. In sites where accessibility of HIV testing changes substantially from year to year, changes in prevalence could reflect differences in groups being tested rather than trends in transmission.

**Recommendations for inclusion in HIV drug resistance surveillance**

Persons newly diagnosed with HIV should be a major focus for an initial HIV drug resistance surveillance system in most countries. Target groups representing this population are accessible and estimates obtained can be used for public health purposes. Information to allow comparability of estimates made from year-to-year should be collected.



Methods to differentiate the subgroup of the recently infected from newly diagnosed persons with established infection should be used if possible, but require additional resources. If possible, sample sizes should be calculated using the likely proportion of persons with recent infection among the newly diagnosed in the sites selected.

### **Potential target groups**

Target groups to represent this population include those found in centres where large numbers of new HIV diagnoses are made. Target groups that include a large proportion of recently infected individuals are preferable if it is planned to track trends in resistance transmission by comparing prevalences in the newly diagnosed groups for different years. For estimating transmission trends, targeting individuals having HIV tests in voluntary counselling and testing centres (VCT) may include relatively more recently infected persons than other groups. In countries with a generalized epidemic, pregnant women, especially those in their first pregnancy, may also include more recently infected persons than other groups. Newly diagnosed individuals under the age of 21 may also be more likely to be recently infected.

### **Persons recently infected and not previously exposed to HIV drugs**

#### **Use of estimates**

Annual monitoring of target groups representative of persons recently infected with HIV provides the best obtainable estimate of trends in transmission of drug-resistant HIV.

In some regions of the world, if the proportion of resistance in this population reaches a specified level (e.g., 5% or 10%), routine drug resistance testing may be recommended for all persons known or assumed to be recently infected with HIV who are newly diagnosed or who are beginning HIV treatment. This may not be practical in resource-limited countries, and planners should decide whether a certain level of resistance to key drugs in the standard first regimen(s) would trigger a particular public health action.

### **Advantages**

If resistant virus is being transmitted in the country or the area, mutations associated with resistance are likely to be seen in HIV amplified from newly infected individuals. If new drugs are being used to treat HIV, specimens from the recently infected are the best indicator of whether mutations associated with new drugs are being transmitted.

### **Limitations**

This population is frequently inaccessible. In many countries, the majority of individuals with HIV are not diagnosed until late in their clinical course, when disease develops. Even when this is not the case, identification of recent infected persons may not be possible because of the difficulties in obtaining information or laboratory results indicating recent infection. If information on HIV seroconversion or on seroconversion illness is available, it may be available only for small numbers of recently infected persons who are unrepresentative.

The prevalence estimate among persons with recent infection who are tested may not reflect transmission of resistant HIV in countries where most individuals are not diagnosed until they have clinical symptoms of AIDS. In such countries, the majority of recently infected persons will not be diagnosed during the period of recent infection.

### **Recommendations for inclusion in resistance surveillance**

In countries where information can be obtained to identify sufficient representative numbers of recently infected individuals, this population should be a major focus for surveillance. In most limited-resource countries, trends in groups representing the newly diagnosed population as a whole, which will include some recently infected persons, will be used as a surrogate for trends in transmission of resistance. In other countries, laboratory tests may be used to identify appropriate specimens.

### **Target groups**

In many countries, groups of newly diagnosed individuals likely to contain a high proportion of recently infected persons (see

Target Group and Site Selection section) will be used if recently infected persons cannot be identified. If previous HIV testing information is recorded in VCT centres, blood donation centres, or clinical centres, persons with a known negative HIV test result  $\leq$  one year before a positive HIV test may be targeted. Clinical centres where symptoms of seroconversion illness or other evidence of recent infection is recorded may also be used to locate groups. If the less sensitive EIA test can be applied to HIV diagnostic specimens to identify recent infection, target specimens can be identified using the STARHS algorithm.

**Persons about to begin treatment for HIV and not previously exposed to antiretroviral drugs**

**Uses of estimates**

If a true estimate of the prevalence of resistance among patients about to begin treatment could be made, it would be of use to clinicians whose patients were beginning treatment in the same year and to programme planners considering initial regimens on a programme level. However, the limitations in the interpretation of results obtainable make useful estimates unlikely.

**Advantages**

Patients about to begin HIV treatment are identifiable and accessible, and clinician interest in the results would make specimens easy to obtain.

**Limitations**

Because of site-based, clinician-based, and patient-based variation in decisions about if and when treatment should begin, estimates of prevalence among target groups from this population would generally represent only the patients at the particular sites included. Although a result for an individual patient might be useful to that patient's clinician, prevalence estimates from a particular group and year could not be generalized.

Under current guidelines, individuals beginning drug treatment for HIV in developing countries are likely to have AIDS or advanced HIV disease. Because the time from infection is lengthy, these individuals are unlikely to have circulating virus

reflecting mutations which may be archived with the original infecting virus. In countries in which treatment programmes have started only recently, they are also the least likely to be infected with virus from people receiving treatment.

The make-up of this population will reflect changes in access to care and changing guidelines about when to begin treatment. Prevalence estimates could not be usable to estimate trends.

**Recommendations for inclusion in resistance surveillance**

Because of the difficulty of interpreting information from groups about to begin treatment, they should not be included in a surveillance system for HIV drug resistance. Initial surveillance should focus on target groups newly diagnosed with HIV rather than on the persons about to begin treatment. In sites in which all individuals beginning treatment routinely receive drug resistance testing for clinical reasons, it is suggested that the information be collected and special studies be designed to examine the effect on regimen choice and outcome.

**Persons receiving treatment with anti-HIV drugs**

**Uses of prevalence estimates**

In countries where resistance testing is not routinely available after failure of the first HIV treatment regimen, HIV drug resistance prevalence estimates in persons failing the first standard could be useful in evaluating potential second regimens on a programmatic level.

Because of variation in HIV subtypes, subgroups, and other factors in different geographic regions, country- or area-specific studies on the frequency of specific mutations arising from various regimens could be important.

The prevalence of resistance obtained in a representative sample of treated individuals in a country or area would provide an estimate for the potential for transmission of resistance, and of the mutations most likely to be transmitted.

Together with a large amount of programmatic information, the prevalence of

resistance among persons in their first year of treatment could support programme evaluation. However, in isolation a high level of resistance may or may not be an indication that prescribing procedures, drug supply, and support for adherence needs attention on a programmatic level. Resistance has been seen in all countries where ARV drug treatment is widespread, and some degree of resistance is inevitable.

The prevalence of resistance could be used as a dependent variable in a follow-up assessment treatment failure, but this would require additional follow-up information beyond the scope of routine surveillance.

### **Advantages**

Patients in HIV treatment are accessible, and clinician interest in the results makes specimens easy to obtain.

### **Limitations**

A representative sample from the treated population is difficult to obtain. Individuals whose treatment has led to suppression of viral load cannot be included in a surveillance system for resistance. Capture of information only from those individuals whose clinicians send specimens for resistance testing leads to an overestimate of resistance, since treatment failure is the prime reason for testing.

Strains tested from treated individuals or who are not adherent to their regimens, or from previously treated individuals who are currently not prescribed ARV drugs, is unlikely to demonstrate mutations that may be archived. The inclusion of such persons would lead to an underestimate of resistance among the treated population. A sampling scheme to exclude such individuals would require substantial resources beyond the means of a routine surveillance system.

The proportion of individuals with resistant virus partly depends on the length of time a treatment programme has been in effect and the number of people it reaches. Even with a highly effective programme, persons for whom more than one regimen has failed will accumulate as time passes, with a resulting increase in the prevalence of resistant HIV. It would be difficult to ascertain the proportion of resistance due to

increasing treatment access and increasing time on treatment for many individuals, and the proportion due to programmatic problems.

Detailed information on drug regimens, adherence, and a variety of important clinical and treatment factors for individuals at more than one point in time is necessary both for programme assessment and for an analysis of the contribution of resistance to treatment failure.

### **Recommendations for inclusion in surveillance systems**

Because of the difficulty of obtaining a representative cross-sectional sample in most countries and the necessary data on each individual sampled, inclusion of the treated population in routine HIV drug resistance surveillance is not recommended. However, the inclusion of HIV drug resistance testing in treatment programme monitoring may be appropriate.

### **HIV drug resistance monitoring in persons receiving treatment for HIV**

In a country where treatment access is being expanded, HIV drug resistance monitoring may play a role in a cohort-based evaluation of a new treatment programme at specific sites. If programme monitoring includes a blood draw for evaluation after a specified treatment duration for all, or a representative sample of all, patients beginning treatment at a certain time, HIV drug resistance monitoring could be included as part of this programme evaluation.

The list of persons to be included would be made when treatment began, and blood samples drawn routinely after a specific treatment duration (generally one year) would be utilized. The denominator would be all, or a sample of all, individuals beginning treatment in a particular quarter. The analysis would include the proportion of persons who died, changed regimens or who were taken off drug treatment, and those who were lost to follow-up before the end of the specified time period. Those who changed regimens or were taken off treatment would have drug resistance testing performed before the change was made. Other proportions to be analyzed

## DRAFT GUIDELINES FOR SURVEILLANCE OF HIV DRUG RESISTANCE

would concern those still on the original regimen at the end of the time period, and would include the proportion with viral suppression, the proportion with a detectable viral load and susceptible HIV, and the proportion with a detectable viral load and drug-resistant HIV.

The prevalence estimates would apply only to the site or sites in which the monitoring took place, but could, in combination with other information on drug supply, clinical indicators, and support for adherence, contribute to overall programme evaluation.

At a site with a high proportion of treatment failures, information about the development of specific mutations associated with specific nonB subtypes might also be obtained in the course of this monitoring, but a successful site might not provide sufficient resistant strains for such an evaluation in the early years of the programme.

### **Monitoring of programme issues associated with HIV drug resistance**

The HDRST may wish to work with HIV treatment programme monitoring staff to collect information that will help inform analyses of drug resistance data in both treated and untreated persons with HIV. Programme indicators for analysis of whether programmes are functioning to minimize drug resistance include annual monitoring of a clinical site or area to calculate:

- Proportion of persons starting HIV treatment whose treatment was begun because they met criteria in standard clinical guidelines
- Proportion of persons starting their first regimen who were prescribed appropriate (standard) first regimens
- Proportion of persons switched to a second or later regimen according to standard clinical guidelines
- Proportion of persons starting their second (or later) regimen who were prescribed appropriate (standard) second-line regimens
- Proportion of months in drug supplies for all drugs for standard regimens where adequate (to be defined operationally)
  - At each site

- At the central warehouse

- Proportion of persons starting the first standard regimen still taking the same regimen at the end of the year

Additionally, any measures of adherence collected systematically for all persons in treatment can be analyzed to provide aggregate estimates of programme success in supporting adherence (which minimizes HIV drug resistance).

### **Special studies**

Special studies are conducted on a one-time basis, or at regular widely-spaced intervals. The studies described below characterize certain aspects of drug resistance in treated patients, but could not be used for prevalence estimates. HIV drug resistance phenotyping, which provides additional information, may also be considered for special studies.

In selected sites where a blood draw precedes a change in regimen after clinical failure, such a blood draw could be used for HIV resistance genotyping to indicate the association between resistance and failure at that site. This study would also help to evaluate the particular mutations most often found in resistance-related failure associated with specific subtypes and specific regimens. Such a study would only be possible in sites where it was certain that all participants were currently taking their regimen at the time of the blood draw.

In countries where treatment has been widely available for at least three years, and where clinical resistance testing is available in-country, it may be useful to perform a cross-sectional survey of laboratory specimens sent for HIV drug resistance testing during a specified time period. Because clinicians generally send specimens for testing when they suspect treatment failure, but vary in the frequency with which they do so, the sample would not be representative. It may be useful to examine the prevalence of mutations arising with certain regimens in various subtypes, and to note any unexpectedly high levels of resistance to specific drugs. Collection of additional information about viral suppression in patients whose strains were

## DRAFT GUIDELINES FOR SURVEILLANCE OF HIV DRUG RESISTANCE

not sent for resistance testing would make such a study more complete.

A one-time blood draw for resistance testing of all patients in treatment over a certain period of time would not generate a useful estimate, and would include inappropriate and wasteful genotyping. The strains of persons who were not currently taking their regimens, or who were not currently prescribed treatment, would not demonstrate mutations even if they had archived resistant strains.

When sufficient resources have been made available to reduce to a minimum the programmatic factors known to be associated with treatment failure (including insufficient drug supplies, inappropriate regimens, and insufficient support for adherence), it may be appropriate to initiate special clinical studies on factors associated with treatment failure, including drug resistance. Such studies could include information that cannot be captured by routine surveillance, including follow-up information.

## 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 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.

## **Planning and Implementing Resistance Threshold Surveys**

Before resources are used for sentinel surveillance of resistance, there should be a reasonable indication that transmitted ARV resistance is sufficiently widespread. The resistance threshold assessment surveys will indicate whether HIV drug resistance among persons newly diagnosed with HIV at selected sites may have reached the 5% "threshold", and allow methodology for routine surveillance to be evaluated.

Purposes include:

1. evaluating whether the threshold value of  $\geq 5\%$  prevalence of resistance in newly diagnosed, untreated persons with HIV, may have been reached in a sentinel site(s) where transmitted drug resistance, if present in the country, is likely to be found.
2. evaluating methods of performing resistance surveillance on a wider scale, including:
  - a. the methodology to obtain specimens from all newly diagnosed persons, or a representative sample, in one or more selected sites
  - b. specimen collection and transport procedures
  - c. procedures for data collection or data capture
  - d. laboratory procedures
  - e. methods to ensure confidentiality
  - f. data transfer procedures
  - g. analysis and reporting methods
  - h. the effect of the proposed system on the programmatic functions of the site(s)

The HDRST should decide whether one or more target groups and one or more sites will be included. Generally the first resistance threshold assessment will be confined to one target group and one site type, but may include more than one site. A detailed description of target groups and target site selection is included in the next chapter.

## **Assessment of potential sites**

The needs and capacity assessment performed by the HDRST will have provided information on the number of persons diagnosed yearly at each site and their characteristics. It will also have provided information on the type of specimens collected and the methods for processing, storage, and transport. Information will have been gathered on whether HIV surveillance or special studies operate routinely at the site. This assessment information will allow the team to choose potentially optimal sentinel sites for threshold surveys.

Meetings should be requested with potential site managers and interest gauged. In addition to providing an appropriate target population and adequate data, sites must be selected in which the threshold survey can be integrated relatively easily into the routine work. Training, resource, and staffing needs should be thoroughly discussed in advance. Staff whose work will be affected by the project, especially of whom extra labour will be required, should be included in discussions.

### **Threshold survey site coordinators**

A person responsible for coordinating the work of the site with the collection of data and specimens should be chosen from the staff of each participating site. This position will be required even if data collection and specimen collection will be performed by persons who are not on the staff. The responsibilities of this person include liaising with the data coordinator and the laboratory coordinator of the HDRST. Duties may also include adapting the protocol to site-specific requirements, ensuring that the protocol meets site-specific ethics committee requirements, operations, overseeing data and specimen collection, and coordinating the return of the results to participant's providers.

### **HIV drug resistance threshold survey sampling design**

Resistance threshold surveys utilize a sampling method called lot quality acceptance sampling. This method does not yield an actual prevalence estimate, but is designed to assess whether a minimum

## DRAFT GUIDELINES FOR SURVEILLANCE OF HIV DRUG RESISTANCE

number with the characteristic of interest is found among a sample from a "lot", or group. The group must share common characteristics. The minimum number, or threshold, is chosen in advance based on the proportion of the characteristic in the entire group which would be considered unacceptable, and the group size. If fewer than the minimum number among the sample have the characteristic, the probability is low that an unacceptably high prevalence of the characteristic would be found in the group as a whole.

For the purposes of the HIV drug resistance threshold survey, the "lot" consists of the individuals newly diagnosed with HIV in one or more similar sites and the characteristic of interest is HIV drug resistance.

It is recommended that the threshold value for an "unacceptable" threshold level of HIV drug resistance, which would trigger planning for routine surveillance, be set at  $\geq 5\%$ . The finding of one resistant strain in the threshold resistance survey indicates that the threshold may have been reached. To avoid inappropriate triggering of sentinel surveillance, it is suggested that additional investigations take place to ensure any participant with a resistant strain did not receive ARV drugs prior to the blood draw. The survey should also be repeated in the following year. If a strain with one or more major mutations associated with HIV drug resistance is found in two separate threshold surveys in two successive years, the HDRST should consider instituting sentinel surveillance of HIV drug resistance in one or more areas where antiretroviral drugs are widely used.

HIV drug resistance threshold survey sampling allows a smaller number of persons to be sampled than would be needed to estimate prevalence. If out of the number of persons sampled, no more than the specified number are found with drug resistance HIV, it is unlikely that the proportion of HIV in the group is 5% or more.

### **Sampling method**

It is critical that the persons sampled be representative of persons newly diagnosed

with HIV at the site during the time period selected. Haphazard samples based on convenience cannot be utilized, since persons not included may be different from those who are included. If sequential sampling is used, lists should be kept of the persons tested and the dates and times their specimens were drawn. Once test results are known, the sample should be selected sequentially from persons newly diagnosed with HIV on that list, in the order in which they were diagnosed. Selecting persons in the order in which they return for their results is inappropriate. In many studies, it has been shown that persons who do not return for their results, or who delay in returning, differ from those who return promptly. A strategy of simple or stratified random sampling with replacement may also be based on the list. Because of the small numbers included in the threshold survey, sampling must be methodical.

If it is decided that a certain subgroup who seek HIV testing is more likely than other subgroups to be infected first with resistant strains of HIV, the sampling strategy may be designed to sample that subgroup only.

### **Number to be sampled**

For the purposes of threshold HIV drug resistance surveys, the specified number of persons who should be found with drug resistant strains of HIV is set at zero.

The minimum number for a resistance threshold survey, if sampling is performed so that the samples are characteristic of a target group diagnosed at one or more sites, is 52 if the threshold is set at  $\geq 5\%$ . Finding zero (no) persons with resistant virus among 52 recently infected persons would indicate a 95% probability that the prevalence of resistance in the group was less than 5%.

However, planning should take into account the possibilities of insufficient and non-amplifiable samples. The sample number should be increased by at least 10-20% to ensure an adequate number of specimens. A sample of 70, in the first year of the survey, would allow for additional difficulties that may arise initially.

Planning should also take into account that in many sites, the majority of persons newly



## DRAFT GUIDELINES FOR SURVEILLANCE OF HIV DRUG RESISTANCE

diagnosed with HIV will not be recently infected. Either the sample number should be increased to allow for this possibility, or sampling should be restricted to those likely to be recently infected.

If possible, the HDRST should examine characteristics of HIV positive individuals diagnosed in the previous year at the site or sites to estimate the likely proportion who could have been recently infected. Newly diagnosed persons under the age of 21, or women experiencing their first pregnancy, have a greater likelihood than others of recent infection. If measurements of recent infection using the less sensitive EIA are available, these may also be used. After examining the proportion and absolute numbers of those who may have been recently infected, planners may decide to sample only from that subgroup. Restricting the sample only to persons under the age of 21 or only to women with their first pregnancy, or to specimens with laboratory indications of recent infection, is desirable where feasible. Restricting the sample to newly diagnosed persons without AIDS-defining conditions, although it does not guarantee a sample with recent infection, will at least exclude persons whose infection is likely to be of long duration.

If exclusions are impractical, and an estimate of the proportion recently infected who were diagnosed in the previous year is available, planners may consider increasing the overall sample size proportionately, to insure that 70 recently infected persons are likely to be included even if they cannot be identified as recently infected. For instance, if 70% of individuals in the previous year may have been recently infected, an appropriately selected sample of 100 may be adequate.

### **Resistance threshold assessment site selection**

Because the resistance threshold survey is a partly feasibility study of methodology, it should generally be limited to one to five sites. The site should include one or more of the target groups described in Target Group and Site Selection section, depending on the state of the epidemic in the country. If more than one site is used, the persons

diagnosed at these sites should share similar demographic and HIV exposure risk characteristics. An alternative is to conduct separate resistance threshold surveys for different groups. Sample size calculations should be performed separately for each survey.

Survey sites need not be representative of the population newly diagnosed with HIV. Survey site(s) should be selected to maximize the chance of detecting transmitted drug resistance if it exists; that is, persons diagnosed at the pilot site should be among the most likely to have had HIV transmitted from a partner with access to ARV drugs.

### **Resistance threshold assessment site characteristics**

Survey sites should be chosen from HIV diagnostic testing facilities. Voluntary counselling and testing centres, or sites where the HIV status of pregnant women is assessed routinely in countries with a generalized epidemic, are the most likely to include a relatively high proportion of recently infected individuals. Restricting the survey to persons under 21, if feasible, increases the chances of including recently infected persons. Survey site characteristics should generally include:

1. Location in an urban area
2. High volume of newly-diagnosed HIV-seropositive persons
3. Location in an area in which programmes providing access to HIV drug treatment have been in existence longer than elsewhere in the country, or in which a greater number or higher proportion of persons with HIV are in treatment than elsewhere
4. Availability of specimens drawn at or within one month of HIV diagnosis for resistance testing, *or* easy incorporation into routine site functioning of an additional blood draw within one month of HIV diagnosis
5. Availability of the minimum dataset (and preferably at least some items of the expanded dataset) items in information already captured during the routine functioning of the centre, or ability to

## DRAFT GUIDELINES FOR SURVEILLANCE OF HIV DRUG RESISTANCE

incorporate collection of data items into the routine work of the site

6. Ability to link data collected with specimens collected for resistance testing
7. Ability to follow procedures to ensure confidentiality
8. Reason to expect that transmitting partners of some persons diagnosed in the centre will have had access to treatment with ARV drugs before transmission took place
9. Availability of information on, or reasonable method of ascertaining, whether persons included in the study will not have been previously diagnosed with HIV (> 3 months previously)
10. Ability to incorporate transfer of specimens into routine functioning of the centre
11. Ability to incorporate transfer of data into routine functioning of the centre

*Additional desirable sentinel site characteristics include:*

1. Reason to believe that  $\geq 20\%$  of newly diagnosed individuals at the site could be recently infected. For example:
  - a. Age < 21
  - b. If pregnant, first pregnancy
2. Availability of information on, or ability to collect information on, dates of previous negative HIV tests (if any)
3. Ability to make available specimens, or to incorporate additional specimen collection, for laboratory testing for recent HIV infection, if such a test may be part of the routine surveillance system

The above characteristics will be relevant for the resistance threshold assessment regardless of the state of the HIV-epidemic in a country. In a country with a concentrated epidemic, the threshold assessment should be conducted in a geographic area with a relatively high HIV prevalence.

The above characteristics will also be relevant for resistance threshold assessment

regardless of the state of treatment access in a country. Threshold assessment should take place first in one or more areas where public treatment programmes have been operating for the longest period of time, and/or where the greatest proportion of persons diagnosed with HIV are in treatment.

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, or two different geographic areas where the risk may be different, separate full samples should be chosen from each.

### **Implementation**

Sampling, development and use of instruments, data management, specimen handling, transport, and processing, laboratory processing, and analysis and dissemination of results are described in detail in the respective sections of this document.

### **Successive Resistance Threshold Surveys**

It is recommended that the resistance threshold survey be performed initially in two successive years, so that lessons learned in the evaluation in the first year can be applied, and the methodology altered accordingly, in the second year. After the second year the methodology for expanding surveys to other areas, and for use in routine surveillance, should have been thoroughly evaluated.

If there is no evidence that the threshold has been reached - that is, if there are no persons with HIV strains carrying major mutations associated with resistance found in either of the first two years -- surveys should then be performed every one to three years.

The team should decide in advance if resistance threshold surveillance will be expanded to additional areas once the threshold has been reached in the initial sites. The team may decide to focus first on

## DRAFT GUIDELINES FOR SURVEILLANCE OF HIV DRUG RESISTANCE

initiating sentinel surveillance in the area of the initial sites, and to expand threshold surveillance to other areas only after one or more years of sentinel surveillance confirms a level of resistance  $\geq 5\%$  in the initial area.

### **Interpretation of Results**

The method of HIV drug resistance testing described in these guidelines is genotyping. A recommended list of major mutations associated with HIV drug resistance for the purposes of analysis will be updated regularly on the WHO HIV website. For the purposes of assessing whether the trigger threshold value may have been reached, the analysis should be confined to major mutations associated with HIV drug resistance directly; it should not include polymorphisms indirectly associated with resistance.

### **Use of Results**

The detection of transmitted HIV drug resistance in a threshold resistance survey - that is, one person with drug resistant HIV - means that the resistance prevalence among the newly diagnosed in the country may be  $\geq 5\%$ , and that sentinel surveillance can be considered. Because of the implications for resources and for public

health, an investigation should be performed to insure that the person(s) concerned were not exposed to ARV drugs before the blood draw. If the finding is confirmed, the team should decide whether the sample in the site should be expanded to include a large enough number to perform sentinel surveillance for a prevalence estimate in the particular centre for that year. Sentinel surveillance in that site and possibly others should be considered for the following year.

The public health implications of identifying one or more resistant strains of HIV in a threshold resistance survey should not be overstated. No change in HIV treatment programmes should be recommended, and it should be emphasized that such a finding does not represent a failure on the part of treatment programmes. Some transmitted HIV resistance is inevitable if the expansion of HIV treatment is successful in including a large number of patients. HIV drug resistance threshold surveys are specifically designed to detect resistance in sites where transmitted HIV drug resistance is likely to be seen first. Sentinel surveillance is necessary to provide estimates of the extent of transmission of HIV drug resistance.

## 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 there was no exposure 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, even if resistance surveillance activities are to be carried out by the HDRST.

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.

### **Initiating Sentinel Surveillance**

Sentinel surveillance should begin following the detection of one or more resistant strains of HIV for two successive years in threshold surveys. Planning and site selection for sentinel surveillance should take place before the threshold surveys begin, so that initiation of surveillance will not be delayed. Threshold survey sites should be scrutinized to evaluate whether they meet the criteria for participation in representative surveillance.

If a threshold survey site meets the criteria, the HDRST may wish to plan to implement HIV drug resistance surveillance there as soon as at least one HIV strain with major mutations is found in that site for the second year in a row (if it is confirmed that there was no previous exposure to ARV drugs in either year). If protocols have been written, evaluated during threshold surveys and changed accordingly, and approved by an ethics committee, the threshold survey may simply be continued to reach the required sample size calculated for sentinel surveillance. The larger sample will allow an estimate of prevalence of drug resistance in that site.

An alternative is to perform additional surveys in other sites in the same year, to insure that the threshold has been reached in an additional site before initiating surveillance.

Generally, sentinel surveillance will begin in the year following the detection of at least one drug resistant HIV strain for two successive years in at least one survey site.

#### **Numbers of sites and areas to be included**

*A detailed description of target groups and target site selection is included in the next chapter.*

The HDRST must decide whether sentinel surveillance should be performed in one or more areas in a country, and the numbers of sites and target groups to be included. Issues to be taken into account include the resources available, the competing needs for

which the resources, including staff, could be used, the particular public health uses of surveillance information, and the likelihood that the "resistance picture" will differ among areas and target groups. Sites and groups should be selected to represent the population of newly diagnosed individuals in the country or area, using data from the previous three years if possible. If surveillance is restricted to a few sites or areas, care must be taken that prevalence estimates are not generalized as if they represent a general picture of resistance in the country.

#### **Assessment of potential sites**

The needs and capacity assessment performed by the HDRST as the first step to HIV drug resistance surveillance planning will have provided information on the number of persons diagnosed yearly at each site and their characteristics. It will also have provided information on the type of specimens collected and the methods by which they are processed, stored, and transferred. Information will have been gathered on whether HIV sentinel surveillance or special studies operate routinely at the site.

This assessment information will allow the team to choose potentially optimal sentinel sites for resistance surveillance. Additional information on sites that have participated in the resistance threshold surveys should also be considered. Optimal sites are those that can contribute to a sample that will represent the persons newly diagnosed with HIV in the country or the area, and that can meet operational criteria.

Meetings should be requested with potential site managers and interest gauged well before the year in which sentinel surveillance is to begin. In addition to providing an appropriate target population and adequate data, sites must be selected in which the operation of sentinel surveillance can be integrated relatively easily into the routine work. Training, resource, and staffing needs should be thoroughly discussed in advance. Staff whose work will be affected by the project, especially those

who would be burdened with extra labour, should be included in discussions.

### **Sentinel resistance surveillance site coordinators**

A person responsible for coordinating the work of the site with the collection of data and specimens should be chosen from the staff of each participating site. This position will be required even if data collection and specimen collection for resistance surveillance is performed by persons who are not on the staff. The responsibilities of this person include liaising with the data coordinator and the laboratory coordinator of the HDRST. Duties may also include adapting the protocol to site-specific requirements, ensuring that the protocol meets site-specific ethics committee requirements, operations, overseeing data and specimen collection, and coordinating the return of the results to participant's providers.

## **Sampling and participation**

### **Sample size**

*A detailed description of sample size calculation can be found in Appendix I. The tables in that appendix should be used to select sample numbers. Numbers should be increased by at least 10% to allow for problems with samples and amplification.*

The surveillance sample size should be sufficient to detect a prevalence of resistance of  $\geq 5\%$  if the "true" prevalence is between 4%-6%, with 95% confidence (and 80% power). The actual number will depend on the number of new diagnoses expected at the site or sites; the previous year's new diagnoses should be used to provide this number.

The sample size should also be able to provide a baseline for a comparison with a future sample of the same number performed at the same site. It should allow a 95% chance that if the study shows an increase in resistance prevalence at the site from 5% to 10% in two different years, this is a "true" increase, and an 80% chance that if this difference is seen, the samples are large enough that the confidence intervals around the two estimates will show the difference to be significant.

Ideally, especially if HIV drug treatment has not been widely accessible in the probable HIV positive population in care for  $> 3$  years, the calculation should also take into account the estimated proportion of recently infected persons likely to be included in the sample. If the proportion of recently infected persons in the sample is likely to be less than 70%, the sample size should be increased proportionately, as described in the Resistance Threshold Survey Chapter.

### **Sampling strategy**

The most common sampling strategy will be sequential sampling; that is, testing of specimens from all eligible individuals who are newly diagnosed with HIV starting at a certain time in the order that they present for testing, and ending when the sample has reached the designated size. If consent is required, characteristics of those who refuse should be included in the dataset and analyzed separately. For each refusal, the next person in the sequence should be selected for the sample.

If laboratory specimens for HIV drug resistance genotyping are not taken from the diagnostic blood draw, care must be taken to maintain a list of the sequence of presentation for HIV diagnostic testing of eligible persons. The sequential sampling must be performed using original sequence, not the sequence in which persons return to receive their results or to receive counselling.

If a separate blood draw for the purposes of HIV drug resistance genotyping is to be drawn when HIV positive persons return for results or counselling, a method should be developed for attempting to find individuals who do not return within the specified time period.

### **Eligibility criteria**

Eligibility criteria must include:

- The person must be newly diagnosed with HIV. Generally, "newly diagnosed" is defined as no known previous positive HIV test, or no previous diagnosis earlier than the past three months. (The national protocol developed by the HDRST should state which of these definitions is used.)

## DRAFT GUIDELINES FOR SURVEILLANCE OF HIV DRUG RESISTANCE

- The person must not have had any previous exposure to ARV drugs, or there must be a reasonable presumption that such exposure is extremely rare in persons newly diagnosed with HIV.
- If consent is used, the person must be able to consent unless waivers have been granted for certain groups unable to consent.
- Additional restrictions, including age or residency, may also be included.

## TARGET GROUP AND SITE SELECTION

**Target group/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 need not 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 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,



## DRAFT GUIDELINES FOR SURVEILLANCE OF HIV DRUG RESISTANCE

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.

## **Target Group Criteria**

Resistance threshold surveys and routine surveillance of resistance have a number of criteria in common for the selection of target groups to represent drug-naïve persons newly diagnosed with HIV and the subset of recently infected persons. The selection should be made among groups already targeted for HIV testing. Target groups and the target sites through which they are reached should ideally have the following characteristics:

### **Inclusion of a reasonable proportion of recently infected persons**

The target group selected should have a reasonable chance of including a reasonably high proportion of recently infected persons among the newly diagnosed. This may be achieved by selecting a group with a high proportion of individuals in adolescence or early adulthood, to maximize the possibility of testing relatively recently infected individuals. Indirect indication of recent initiation of sexual activity, such as first pregnancy, may also be used in target group selection. Groups in which most individuals receive HIV-testing for reasons other than clinical illnesses associated with AIDS are also more likely to include relatively recently infected persons.

### **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 numbers of samples to justify the use of surveillance resources.

### **Availability of required data**

Data must be available on participating groups or collected specimens representing these groups to enable programmes to:

- exclude specimens from persons with previous HIV diagnoses
- ensure that only one specimen is sent for resistance testing from each eligible individual
- exclude specimens from persons with previous exposure to ARV drugs

## **Routine data collection**

If possible, some or all of the required information for resistance surveillance should be collected as part of the routine functioning of the site and accessible without medical records abstraction or interview.

## **Additional criteria for resistance surveillance only**

### **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.

### **Integration with HIV surveillance**

If HIV surveillance is routinely performed at a target site, specimen collection for HIV drug resistance surveillance should be as far as possible integrated with the HIV surveillance operations. If HIV surveillance is performed only for a certain duration of time each year, the operations for HIV drug resistance surveillance may have to be extended for a longer period of time in order to collect a sufficiently large number of HIV-positive individuals whose specimens can be sent for resistance genotyping.

### **Comparability from year to year**

For threshold survey to assess whether resistance surveillance should begin, estimates are not generated and compared from year to year. However, when routine surveillance begins, sites should be selected to attempt to achieve collectively a representative sample of all groups making up the population of newly diagnosed with HIV. Some sites, such as those where routine HIV testing of pregnant women is performed, are likely to be selected. Other sites may differ from year to year, particularly if outreach strategies change. Information should be collected, if possible, on relevant characteristics (including age, gender, HIV exposure risk, area of origin, and other relevant characteristics) so that the analysis of trends can include factors which may be associated with apparent rises or falls in drug resistance prevalence. Inclusion of different proportions of groups

with different risks in different years can lead to false estimates of changes in rates of transmission of drug resistance.

<b>Potential target groups/site categories</b>
--

Potential target groups may include:

**Individuals tested at voluntary counselling and testing centres (VCT)**

Persons tested for HIV at VCT are relatively likely to be representative of new HIV diagnoses unless selected sites provide services to specific risk groups.

VCT sites are relatively likely to include a sufficient number of recently infected persons if outreach to persons at risk who do not yet have clinical illness is performed and is successful.

VCT sites are likely to have a relatively high volume of new HIV diagnoses.

Much of the required information may be available as part of routine functioning.

Programmes to improve outreach and bring new subgroups in for testing may reduce comparability from year to year, but information may be collected to adjust for such changes in the analysis.

**Pregnant women**

*Pregnant women should be targeted only where HIV testing is performed routinely as part of PMTCT or other antenatal programmes.*

*To maximize chances of including recently infected persons, target women 21 years or under, and/or first pregnancies, if numbers are sufficient.*

Pregnant women tested at sites where routine HIV testing is provided are relatively likely to be representative of new HIV diagnoses.

Because first pregnancy occurs at a young age for a high proportion of women in many countries, and because HIV testing is less often associated with clinical illness than in other groups, this group may include a high proportion of recent infections.

Even in a country with a generalized HIV epidemic, several sites may be needed in order to achieve a sufficiently large number of pregnant women newly diagnosed with

HIV. In a country with low HIV prevalence or a concentrated epidemic, numbers of HIV diagnoses among pregnant women will be relatively low and targeting this group may have limited utility.

Much required information may be routinely recorded but may reside in written records requiring extra resources for transcription, unless the target sites are part of an HIV surveillance programme.

Duplication of specimens within the year is unlikely. However, potential inclusion of some of the same women in successive annual samples may be a potential problem unless it is possible to ensure exclusion of pregnant women who tested positive for HIV during a previous pregnancy. Particular care must be taken to include only new HIV diagnoses if this group is targeted.

Comparability from year to year is often good in programmes operating in a specific area with an established methodology.

**Blood donors**

*Blood donors should be targeted only where blood donation is voluntary and unpaid, and where large numbers of HIV positive specimens are found among donors.*

Persons who know they are at risk may avoid blood donation, or may be discouraged from participation, so blood donors may be less representative than pregnant women or persons who seek HIV testing at VCT.

Because of exclusion of ill individuals from blood donation, relatively high numbers of recent infections may be included.

In countries where the Nucleic Acid Testing (NAT) assay is used to screen blood units, a more precise selection of recently-infected individuals can be performed.

In some countries, information recorded at each donation may allow the dates of previous negative HIV tests to be accessed for newly HIV-positive individuals, which allows identification of HIV seroconversions. In many countries, retrieval of previous testing information linked to particular individuals is not possible.

Numbers of HIV-positive specimens among this group may be high in a generalized epidemic.

In many countries, insufficient information is routinely recorded to identify previous donation and HIV testing history. In such countries, exclusion of previously diagnosed persons, and assurance that only one specimen per person is sent for resistance testing, may not be possible.

Comparability from year to year is possible, but information may not be available to evaluate changes in the risks of the newly diagnosed group of blood donors.

### **Persons being screened for potential military recruitment**

*Potential military recruits should be targeted only in countries with universal conscription or where a high proportion of the male population, or a representative proportion, is recruited to the military. All specimens from potential recruits, both from those who go into the military and those who do not, are included.*

Except in countries with universal conscription, potential military recruits may not be representative of the young male population, and may fail to provide a reasonable representative subgroup with new HIV diagnoses. Collection of specimens for HIV drug resistance testing should be performed at the time of screening for potential military recruitment. If screening is performed only on persons already in the military, or accepted into the military, the sample will be less representative.

A high proportion of potential recruits diagnosed with HIV may be recently infected, because of the relatively young age of potential recruits and because HIV testing is less often associated with clinical illness in a routinely screened population than in some other groups.

In a country with a generalized epidemic, numbers of HIV diagnoses among this group may be high. In a country with low HIV prevalence or a concentrated epidemic, numbers among this group will be relatively low and targeting of this group may have limited utility.

Required information may not be routinely recorded or accessible, but duplication is unlikely if persons are only evaluated once for potential military entry.

Comparability from year to year may be good unless recruitment strategies change.

### **Persons newly diagnosed with sexually transmitted infections (STI)**

*Persons diagnosed with STI should be targeted only if HIV testing is routinely performed when STI are diagnosed.*

Persons with new STI may represent persons with particular risks for HIV, and may not represent the newly diagnosed population as a whole.

A high proportion of persons diagnosed may be recently infected if facilities and outreach are available to individuals at risk at a relatively young age.

In a country with low HIV prevalence or a concentrated epidemic, numbers of HIV diagnoses among this group may be higher than among pregnant women, military recruits, or blood donors.

Required information may not be routinely recorded or accessible unless an STI surveillance system is also in place. Specimen duplication may be highly likely and information on previous diagnoses may not be available.

Comparability from year to year may be variable, but information may be available to control for differing risks in the analysis.

### **Persons newly diagnosed with illnesses associated with HIV, such as tuberculosis**

*Persons newly diagnosed with HIV-associated illness should be targeted only if HIV testing is routinely performed when the illness is diagnosed.*

Individuals with HIV-related illnesses are unlikely to be representative of all new diagnoses of HIV.

A low proportion of persons diagnosed may be recently infected, because of the length of time often seen between HIV infection and development of a clinical illness. However, in countries with a high prevalence of TB infection, a reasonable proportion of recent infections may still be seen in a TB clinic.

In a country with low HIV prevalence or a concentrated epidemic, numbers of HIV diagnoses among this group may be higher

than among pregnant women, military recruits, or blood donors.

Minimal required information may be recorded, but may not be accessible unless a TB surveillance system is in place. Specimen duplication is unlikely, but information on previous diagnoses may not be available.

Comparability from year to year may be good if the programme is established and functions comparably from year to year. Information may be recorded routinely which will help to assess comparability in the analysis.

#### **Individuals tested at other sites**

Individuals tested at other sites, such as occupational clinics, may be targeted if certain conditions are met. Such groups are suitable if HIV testing is routinely performed and there is a high volume of HIV diagnoses, and if they represent a definable subgroup (e.g., miners, truckers, sex workers) within the newly diagnosed population. Occupational clinics may be also appropriate for resistance threshold surveys if they offer HIV testing to a subgroup whose partners may have had access to treatment as part of their employment.

Persons diagnosed at special clinics are unlikely to be representative of all new diagnoses of HIV.

A high proportion of persons diagnosed may be recently infected if routine testing is required and a high proportion of the targeted subgroup is young.

If routine testing has been instituted because of a known high prevalence among an occupational group, numbers of new HIV diagnoses in this group may be high.

Required information may be recorded in medical records, but may not be easily captured for surveillance purposes without additional resources for medical record abstraction.

If serial testing is performed on a regular basis, information about previous negative HIV tests as well as previous positive tests may be available.

Comparability from year to year may be good if the programme is established and functions comparably from year to year.

#### **Persons whose HIV is diagnosed at central HIV diagnostic laboratories**

Central HIV diagnostic laboratories, either national or regional, may include HIV specimens from many or all of the target groups discussed above. Such a laboratory may be used as a central collection site. Provided specimens can be separated and frozen within 72 hours of the blood draw, and provided necessary information can be made available from the sites where specimens are collected. Necessary information will allow the data manager to distinguish new diagnoses from repeat tests or confirmatory tests, to allow a representative selection from representative groups, and to provide the minimal, and preferably the expanded, data requirements.

#### **Target group selection**

Generally, **persons diagnosed at VCT clinics** and **pregnant women** diagnosed where routine HIV testing is available are likely to be the most representative groups and to have a reasonable proportion of recent infections. A high number of specimens should be available at VCT clinics, and at sites serving pregnant women if the epidemic is generalized. In low-prevalence countries or in concentrated epidemics, numbers among pregnant women are likely to be insufficient. Required information is likely to be recorded and accessible at VCT clinics and possibly at sites where pregnant women are tested.

#### **Target groups in countries with low-level or concentrated epidemics**

In countries with low-level or concentrated epidemics, the initial target group should be individuals tested at VCT, which will often be linked to preventive or treatment services for groups at higher risk for HIV such as injecting drug users. In such countries, pregnant women, blood donors, and persons being screened for military recruitment are likely to have a low prevalence of HIV.

Persons with STI or with illnesses often associated with HIV, such as TB, who receive HIV testing routinely when diagnosed with these illnesses, are a second possibility. Persons in occupational or

## DRAFT GUIDELINES FOR SURVEILLANCE OF HIV DRUG RESISTANCE

specialist clinics with a known high prevalence of HIV may be a third possibility. Both these possibilities are less desirable for routine surveillance in terms of representativeness, but would be appropriate for resistance threshold surveys if some of their partners may be relatively likely to have access to HIV treatment.

### **Target groups in countries with generalized HIV epidemics**

In countries with generalized epidemics individuals receiving HIV testing at VCT remain an important target. Pregnant women, especially if they are routinely being HIV-tested in connection with PMCTC programmes, are a second important target group and may be more representative. Not only do they provide reasonable

representation of new HIV diagnoses, but the prevalence and characteristics of resistance among newly diagnosed pregnant women specifically is relevant to consideration of regimens for prevention of vertical transmission.

The subgroup of pregnant women < 21 years of age, or presenting with their first pregnancy, may yield a higher proportion of recently infected individuals than in other groups, if sufficient numbers are available. Blood donors and persons being screened for potential military recruitment are other potential targets, if site selection criteria can be met. The subset of individuals < 21 years of age in these groups may also yield a higher proportion of recently infected individuals.

## 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.

**Demographic and basic clinical data items to be collected for HIV drug resistance surveillance**

**Minimum dataset**

The minimum dataset to be collected includes:

- Unique subject identifier
- Unique site identifier
- Date of blood draw for HIV drug resistance testing specimen
- Age group
- Gender
- Date of HIV diagnosis
- Previous HIV diagnosis > 3 months before specimen collection for resistance surveillance (yes/No/Not known)
- Previous ARV drug treatment (Yes/No/Not known)

**Expanded dataset**

The expanded dataset to be collected for sentinel surveillance of resistance if at all possible includes the following list. \*Starred items constitute the expanded dataset for HIV drug resistance threshold surveys; both starred and unstarred items constitute the expanded dataset for sentinel surveillance:

- ARV treatment history (Yes/No)\*
- Date and result of any previous HIV test (with positive or negative result)\*
- First pregnancy/≥second pregnancy (for pregnant women)\*
- Date of birth\*
- [Age on day specimen is drawn\* (if date of birth is not available)]
- Area of residence
- Lab/clinical evidence of recent infection
- Lab/clinical evidence of stage of HIV infection,
- CD4 count closest to diagnosis (if available)
- Viral load closest to diagnosis (if available)
- Additional risk or subgroup factors relevant to the country or area.

**Capturing data from HIV surveillance or serosurvey systems**

If routine HIV surveillance or an HIV serosurvey system is already set up at a selected site, the information collected can also be utilized for the resistance surveillance system. With the exception of information on previous ARV treatment, all needed data items, including information to determine whether this is a new diagnosis, may be collected as part of HIV surveillance. It may be possible to add the ARV treatment history item and additional necessary items to the HIV surveillance form. A copy of this form, rather than a separate resistance surveillance form, should be used for resistance surveillance, or, if possible, data can be downloaded directly from a local database.

Special HIV studies may also collect the basic information needed for resistance surveillance, including information on previous drug experience. Data collected may be captured for resistance surveillance as above.

If clinical information which is not available until after diagnosis, such as CD4 count or viral load, is not routinely collected in the HIV surveillance system and is desired for the surveillance dataset, additional medical records abstraction may be needed.

**Advantages**

Capturing data from an existing HIV surveillance system has several advantages. Staff are trained to capture and record data items correctly, and monitoring and evaluation systems to ensure data quality may already be set up. The need for additional training and monitoring resources will be minimized.

HIV surveillance is being strengthened and expanded in many countries. Several of the data items needed for HIV drug resistance surveillance are also advantageous to a general HIV surveillance system. The National AIDS Committee may agree to expand the system to collect these items routinely.



### **Limitations**

The format in which data are collected may not be the optimal format for HIV drug resistance surveillance. However, early planning in the country to ensure that the formats are compatible can minimize potential problems.

If HIV prevalence is estimated by serosurveys, the surveys may not last for a long enough period of time each year to include a sufficient number of HIV positive individuals. Special arrangements will have to be made to extend the period of data collection to include a sufficient number of persons newly diagnosed with HIV for the purposes of HIV drug resistance surveillance.

### **Special data collection for resistance surveillance**

A specially designed instrument for resistance surveillance can be used in sites where data collection is not already occurring for HIV serosurveillance or special studies. Some items may be available on the laboratory request form for the HIV diagnostic test, and it may be possible to add additional items to this form. Generally a supplemental process will be needed for some items. Efforts should be made to design instruments compatible with data needs for general HIV surveillance and programme monitoring.

Even if data collection is already occurring for other purposes, the HDRST may decide that data collection on a specially designed instrument is preferable.

If a special blood draw is performed, participating individuals may be interviewed at the time of consent or when blood is drawn. In care and treatment centres that are also diagnostic centres, medical records abstraction can be used to collect some items.

### **Advantages**

Use of methods formulated especially for drug resistance surveillance to collect demographic and other data may be seen as an advantage in some areas.

More complete information may be obtained in an interview than from some routine

surveillance systems. If the HIV drug resistance testing laboratory is collecting data as well as performing tests, it is convenient to collect information at the same time as a special blood draw and send it with the specimen.

At HIV care and treatment centres or other clinically oriented centres such as STI or TB clinics, most data items may be available from medical records and can be abstracted.

### **Limitations**

Dedicated data entry using a special instrument requires additional training and staff time, and requires the setting up of a data quality monitoring system. Use of a special instrument for the resistance survey or surveillance system may require duplicate data entry if the data items are already being entered on another form for another purpose.

Interviews require extra time and training. Scheduling and follow-up can add labour if many individuals do not keep their appointments.

If an interview after HIV diagnosis is the main method of capturing data, the data will not be available for persons lost to follow-up.

Medical records abstraction requires special training and extra labour. Variation in recording of information in medical records may lead to incomplete or incorrect information being abstracted.

### **Developing the data collection system**

The optimum data collection system will differ among countries, areas, and sites. To develop data collection methods, planners should first evaluate routine information collection, and note procedures during which additional data could be collected, in participating centres.

An attempt should be made to develop a uniform data collection method compatible with the least burden on site staff, a minimum need for resources, and a reasonable means for continual monitoring of data quality. If possible, resistance threshold surveys should be conducted using such a minimal system, so that the

## DRAFT GUIDELINES FOR SURVEILLANCE OF HIV DRUG RESISTANCE

same system can be used year after year in routine surveillance. It should never be assumed that site staff, who may already be overburdened, can fill in extra forms during the course of their ordinary duties.

Further discussion of data management issues can be found in the next chapter.

## 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 global database.

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).

### **Data Management in HIV Drug Resistance Surveillance**

Data management includes plans, procedures, and infrastructures for the collection, flow, integration, and analysis of surveillance data. A data management system should be implemented by the HDRST for sites and laboratories, with data flowing to a central data centre. The system should minimize the labour and resources required for data entry and data capture, ensure collection of appropriate, complete, and accurate data, organize the data in a form suitable for analysis, produce reports for dissemination, and ensure confidentiality, the protection of privacy, and data security.

Data for the resistance threshold survey and for resistance surveillance will consist of:

- data for the determination of eligibility
- basic demographic data on individual participants
- specimen tracking data
- clinical laboratory results (if available), including previous HIV testing information, CD4 count, viral load
- HIV resistance genotyping data (typically the sequence from the relevant portions of the HIV genome)
- other laboratory data, such as results of the less sensitive EIA/STARHS algorithm (if available)

Indicator data for the monitoring and evaluation of the operation of all HIV drug resistance surveillance operations will also be collected and analysed.

Piloting data management methods during the resistance threshold surveys will facilitate data management during routine surveillance.

### **The National HIV drug resistance surveillance data centre**

#### **Overview of the data centre**

A data centre must be identified to coordinate the data management system. The centre will receive and collate data from HIV testing sites, clinical sites, and laboratories must be identified. The data centre will generally be housed in the same location as the general HIV surveillance system, but could also be

located at an HIV treatment programme site or a reference laboratory.

Analyses will be performed at the data centre. Reports will be generated for participating sites and laboratories, and for public health professionals, governmental departments, and the general public. Data for the monitoring and evaluation systems will be collected, analyzed, and reported at the data centre. A national data coordinator, who will be a member of the HDRST and will generally be based at the data centre, should be identified.

The data centre will be responsible for a number of functions, overseen by the national data coordinator.

#### **The National HIV Drug Resistance Data Coordinator**

The national data coordinator should if possible have experience in HIV surveillance, and should be familiar with the HIV diagnostic system in the country or area. Expertise in database development, data transfer, and the production of analyses and reports is important. The data coordinator functions may be performed by a team or by one person. Partnerships with local experts, universities, or international agencies, including WHO HIVResNet, can provide needed support. The coordinator or team should work closely with the HDRST statistician(s).

The data coordinator, or coordinating team, should:

1. Design an overall plan for the flow of data, and coordinate data management operations among sites, laboratories, and the data centre itself.
2. Prepare paper forms and electronic templates for use by sites and laboratories.
3. Prepare a specimen tracking data entry system for use at sites and/or processing laboratories.
4. Help sites to identify methods of adding additional variables to existing forms or databases if necessary, and capturing data from existing systems.
5. Develop user-friendly methods of transmitting data from sites and laboratories to the data centre, incorporating error-checking to allow immediate correction of problems.

6. Prepare manuals incorporating standard operating procedures to be used in participating sites and laboratories.
7. Provide training in data entry, quality assurance, and the system for data flow to site staff, and especially the site data managers.
8. Develop a system for linking HIV resistance genotyping data with other participant data.
9. Convert into electronic form any data that arrives on paper.
10. Develop manual and electronic methods to check completeness and accuracy, and ensure that the system linking different types of data is operating successfully.
11. Query sites and laboratories to resolve problems, keeping logs of queries so that there can be follow-up if queries are not answered promptly.
12. Respond by telephone or e-mail to questions from sites and laboratories.
13. Ensure timely analysis, reporting, and feedback on performance to participating sites and laboratories.
14. Integrate the data into a coherent database ready for use in analysis, policy-making, and dissemination.
15. Work with statisticians to produce regular analyses of the data.
16. Regularly disseminate results, including reports for use by public health officials and medical professionals.
17. Create appropriate access and security systems, including encryption and password protection measures.
18. Provide any data management training required for site, laboratory, and/or other surveillance programme staff.
19. Regularly review and evaluate data handling at sites and laboratories, the overall flow of surveillance data, and data centre operations, to detect and correct problems.

### **Data Security**

Security systems should be developed to provide user-friendly access to the data only for authorized staff. Unauthorized access should be prevented. For authorized staff, these systems should allow varying levels of access, allowing individuals access to specified databases, specified tables within those databases,

and specified variables within the tables. In addition to these specifications, three levels of data access should be specified:

1. the ability to view aggregate data only
2. the ability to view individual data
3. the ability to edit individual data.

Additional authorized users, including site and laboratory staff, should be allowed to request reports (or generate them on-line if a web-based system is used).

### **Eligibility determination**

In countries where HIV/AIDS surveillance utilizes names or unique identifiers generated by a repeatable algorithm from personal information, the national data centre should work with HIV surveillance staff to match new potential participant information to the HIV/AIDS reporting dataset, to identify evidence of previous diagnosis or treatment.

Information on previous testing history or previous treatment may also be collected routinely at participating sites, where the local system may be searched. The HDRST may also decide to request information on eligibility to be collected on HIV laboratory testing request forms or to interview forms connected with the project.

Available information on eligibility should be collected systematically and a system developed to ensure as far as possible that specimens from previously diagnosed or treated persons are not sent for HIV drug resistance surveillance. Methods used in countries where resistance surveillance is taking place include placing a coloured dot specimens drawn at HIV testing sites from persons believed to be eligible, or use of a code to note eligibility on the laboratory request form.

HIV diagnostic laboratories may have additional databases with some identifying information that can be queried to ensure that only newly diagnosed persons with HIV are included in HIV drug resistance surveillance.

Methods of insuring that not more than one specimen from a single participant is sent for HIV drug resistance surveillance should be developed for all participating sites and HIV diagnostic laboratories.

The data centre should work with sites and laboratories to standardize methods for matching potential participant

information to previously gathered information in any existing database to determine eligibility, and to standardize recording and transferring of interview information on eligibility to ensure as far as possible that ineligible specimens are not sent for testing.

The clinical and demographic database should include an eligibility field, to ensure that persons whose specimens receive HIV drug resistance genotyping, but who are subsequently found to be ineligible, are not included in the analysis.

### **The participant Identifier**

The data centre should develop a method to create a unique HIV drug resistance surveillance participant identifier (RPID) for each participant. The RPIDs should be used on forms and in databases to prevent anyone other than HIV drug resistance and HIV surveillance staff to identify participants.

The RPID may be assigned at the site, at the processing laboratory, or at the resistance laboratory. A system of labels distributed by the centre, to be placed on forms and specimens, may facilitate this process.

In areas where HIV diagnostic specimens are used for HIV drug resistance genotyping, the RPID may be assigned by the HIV diagnostic laboratory after HIV infection is confirmed, or the identifier assigned by the HIV surveillance system may be used as the RPID. In areas where a special blood draw is performed after HIV infection is performed, the RPID may be assigned at the site, or the identifier used by the HIV surveillance system may be used.

The RPID plus a site identifier will be included both in the demographic/clinical database, the specimen tracking database, and the HIV drug resistance genotyping database to link data for each participant.

### **Clinical reports**

If results are to be returned to the participant's physician, the data coordinator must also work with the laboratory to ensure that a "clinician-friendly" report of individual results will be generated either at the data centre or the HIV drug resistance genotyping laboratory. The data coordinator will work

with the sites to arrange the system of providing the individual reports.

### **Data management at HIV diagnostic and clinical sites**

An HIV drug resistance data manager should be identified for each HIV diagnostic or clinical site where participants are selected and specimens obtained. The data manager may be part of the site staff, or may work directly for the HDRST. He or she may manage data at more than one site. Data managers will receive training, suggested techniques, and consultation as needed from the national data coordinator, and will work with the data centre to develop appropriate data management strategies for their sites.

The data manager will ensure that all data items available at the site are properly recorded or downloaded from existing databases. Participant information may already be present in paper or electronic records held by the site as part of its normal operations, or it may be recorded on a new form or newly entered into a computer at the site.

### **Determining eligibility at the site**

The national data coordinator should work with data managers at participating sites and HIV diagnostic laboratories to create methods to determine, confirm and record eligibility for all prospective participants as far as possible, following criteria set by the HDRST. Site-specific methods of determining and recording other eligibility requirements developed within the country (e.g., age over 18 or lack of specific clinical signs) should also be developed.

In many HIV surveillance sites, site operations may preclude questioning persons presenting for HIV testing. At these sites, the default will be "unknown" for eligibility variables, but a method should be developed for recording information if it happens to be known and for retrieving information in other ways. Methods of determining eligibility could include searching site records and databases for previous diagnostic or clinical information.

If HIV diagnostic specimens are used for HIV drug resistance testing, a method of

identifying eligible specimens before sending them for diagnostic testing should be agreed with the national data coordinator. Methods include color-coding, marking of tubes, or marking HIV diagnostic laboratory request forms. At sites where nearly all specimens are considered potentially eligible because of lack of information, the system should mark specimens known to be ineligible.

### **Consent**

If a special blood draw is utilized or consent is required for other reasons, the national data coordinator should work with the site data manager to develop a method of recording and storing the consent records. Consent forms should be checked against laboratory specimens before shipment to ensure that specimens from non-consenting persons are not tested. Specimens from non-consenting persons should be considered ineligible, and identified using methods described in the Eligibility section.

### **HIV drug resistance participant identifiers (RPID)**

Identifiers assigned at sites or laboratories for other purposes, including return of participant HIV results, HIV test specimen tracking, or HIV surveillance may be used as temporary identifiers which will later be linked to the RPID. Alternatively, the RPID may be assigned at the site, if a special blood draw for resistance testing is performed after HIV status is known. However, if HIV diagnostic specimens are aliquoted for resistance testing after a positive result is known, the site will generally be informed by the HIV diagnostic laboratory of the RPID for each participant.

### **Specimen labels and specimen tracking data**

If RPID are assigned at the site, the data manager should ensure that RPID are included on specimen labels and/or relevant laboratory forms.

Specimen tracking data to be recorded at the site will generally include the date and time of blood draw, and the RPID if assigned by the site. The identification number or specimen number used in the routine operations of the site should also be recorded for later linkage.

If HIV drug resistance results are to be returned to participants' providers, a system should be developed to link results to each participant without breach of confidentiality.

### **Demographic, clinical, and interview data collected at the site**

In most areas, demographic and clinical data should be captured as far as possible from the HIV surveillance system and other routinely operating systems at the site, rather than recorded especially for the HIV drug resistance surveillance system. The data centre will coordinate linkage of specimen tracking data and HIV drug resistance data to basic demographic and clinical data.

If interview information is collected for the purposes of HIV drug resistance testing either at the HIV diagnostic testing blood draw, or at a subsequent blood draw, this information must be recorded at the site and transferred to the data centre.

Either the identification number assigned in the routine functioning of the site, or the RPID, or both, will be recorded with these data for later linkage.

### **Data transfer from the site and the processing laboratory to the data centre**

Participant and specimen data must be transmitted to the data centre on a regular, agreed-upon schedule. Transmission will be by a method and in a format negotiated with the centre. Transfer can be performed by postal mail, fax, or electronically, depending upon circumstances. Transmission frequency can be set to accommodate each site's needs. Formats for sending the data, such as paper forms or electronic templates, will be determined by HDRST and the data centre in collaboration with each site.

### **Retention of records at participating sites**

Paper or electronic copies of all data sent to the data centre, and of shipping manifests with specimen details, should be retained for at least 12 months after transmission. This will allow the data centre and the laboratory to discover problems and initiate queries. When these records are no longer needed, they should be destroyed in a way that protects participant confidentiality.

**Participant registry**

Participants may be identified at the site, or subsequently after diagnostic specimens are tested at the HIV diagnostic laboratory. Using a method approved by the ethics committee, a confidential list of identifiers representing the persons whose specimens were sent for HIV drug resistance testing, and the actual identity of the corresponding participant, should be securely maintained at the site through an agreed time period (at least 12 months).

Participant information should not leave the site and or be included in any shared database. It should be available only to specified staff, and should be protected against loss or unauthorized access. The purpose of the list is to deal with subsequent problems or queries, to make sure that a participant's data and specimens are labelled and linked by with the same identifiers, and to enable the return of results to participant's providers.

**Site data quality assurance**

The site data manager, working with the national data coordinator, should develop data quality assurance methods. These may include duplicate data entry for a certain percentage of records, and the checking of data in the database against other data systems. Data quality assurance systems may already be in place for recording of data in VCT or sites participating in HIV surveillance. These systems may be expanded to include any new data items collected for resistance surveillance.

**Specimen manifests**

If HIV diagnostic specimen aliquots are to be used for HIV drug resistance testing, the usual system of specimen shipment from the site to the HIV testing laboratory should be used. If specimens are being sent directly from the site to the HIV drug resistance testing laboratory, they should be labelled with the RPID, and a specimen manifest should accompany them and be copied to the national data centre (as described in the next section)

**Data management in the HIV diagnostic or processing laboratory**

If specimens are sent through one or more HIV diagnostic or processing laboratories to the HIV drug resistance testing laboratory, the data management system must include these laboratories.

If HIV seropositive diagnostic specimens are aliquoted for HIV drug resistance surveillance, the HIV diagnostic laboratory will generally require a data manager. Occasionally a processing laboratory will be used to process specimens specially drawn at participating sites for HIV drug resistance testing before the specimens are sent on. The information below must also be recorded at the processing laboratory.

**Specimen tracking**

Checking specimens received from sites for appropriate labelling and shipping manifest listings, must be performed. Additional specimen tracking information must be recorded, generally including the date and time of the first positive EIA, and possibly the time the specimen was centrifuged and separated. Because the majority of diagnostic specimens will not be HIV seropositive, "batch" methods of recording time and date of receipt and other times for batches of specimens should be developed with the national data manager. Data should be recorded for individual specimens in the resistance data system only after the specimen is known to be HIV seropositive and eligible for drug resistance testing.

The date and time specimens are aliquoted and frozen, and the date and time of shipment to the HIV drug resistance genotyping laboratory are also important for evaluation purposes if problems with amplification are identified in the resistance laboratory. These data may be recorded in a database, or on the shipping manifest to be sent with the aliquoted specimens to the HIV drug resistance testing laboratory.

**Eligibility determination in the diagnostic laboratory**

HIV diagnostic testing laboratories may have databases with information on previous test results, or in some cases on related clinical test results such as CD4



counts. Methods should be developed with the national data coordinator to query these databases for information on potentially eligible specimens. Specimens identified as belonging to persons previously diagnosed as HIV-infected should not be aliquoted or sent for HIV drug resistance testing.

### **The RPID**

In countries where HIV diagnostic specimens are used for drug resistance testing, the RPID will generally be assigned after a specimen is confirmed as HIV seropositive. Numbers and labels will generally be provided by the national data centre, and will be placed on the aliquot tube sent to the HIV drug resistance testing laboratory, the initial HIV testing laboratory request form and the shipping manifest. The RPID will also be recorded in the laboratory in the specimen tracking database.

A method must be worked out to record in a database and/or on paper forms both the site-specific HIV testing ID or the laboratory accession number assigned to the specimen for routine HIV testing and the RPID assigned after the positive test result. The specimen shipping manifest, is often used for this purpose.

If the RPID is assigned at the diagnostic or processing laboratory, the RPID should be included in information sent to the site regarding the HIV test result for the individual.

### **Specimen manifests**

Specimen manifests should accompany specimens sent to the HIV drug resistance laboratory. If the information is recorded electronically, a paper copy should still accompany the specimens. The manifest may be simply a list of RPIDs and dates, or may be used to record additional information required by the HIV drug resistance laboratory or the national data centre. Three copies are generally made, with one copy accompanying the specimen, one copy retained by the laboratory, and one copy sent to the national data center as soon as the specimens are transferred.

## **Data management at the HIV Drug Resistance Testing Laboratory**

HIV drug resistance testing laboratories should also identify a data manager to liaise with the national data centre.

The resistance laboratory data manager will ensure that specimens received from sites and processing laboratories are checked against the shipping manifest, and are labeled properly with site identifiers and RPIDs. The shipping manifest should be checked for any additional required information such as collection dates. If there are discrepancies or label information is incomplete, the laboratory should query the diagnostic laboratory or the site for missing information. Queries should be logged and followed up if no initial response is received. The national data coordinator will provide guidance in setting up query and logging systems and support in resolving problems.

The resistance laboratory data manager should ensure that resistance testing results are properly recorded, electronically or on paper and transferred to sites and to the central data centre as agreed. If results are to be returned to the participant's physician, the HIV drug resistance testing laboratory may generate a "clinician-friendly" report for each participant if this is not done at the data centre. The format will be designed by the national data manager, the HIV drug resistance testing laboratory, and participating sites.

An internal system of data quality checks should be created to minimize missing and faulty data, whether resulting from the assay or from the process of recording results.

### **HIV drug resistance data transmission**

Results should be transmitted to the national data centre on a regular, agreed-upon schedule. Transmission will typically be electronic, by a method (e.g., web, e-mail, direct export) and in a format negotiated with the centre. Tools for sending the data, such as electronic templates or software for laboratories to use locally, will be provided by the national data centre.

Paper or electronic copies of all results generated at the laboratories and sent to the data centre, and of shipping manifests, should be retained for at least 12 months. When these data copies are no longer needed, they should be destroyed in a way that protects participant confidentiality.

#### **Development and implementation of the data management system**

Data management operations may be performed in a variety of ways, depending upon the needs, capacities, and preferences of a particular country. National Data Centre staff, working under the national data coordinator, will coordinate all the functions listed, but may require assistance. Assistance may be available from universities or experts within the country, or from international organizations or partner countries with experience in HIV drug resistance surveillance.

WHO HIVResNet may also be able to provide assistance in planning and implementing the data management system. It is planned that support which will be available through WHO HIVResNet will include:

- Resources and tools available from the WHO HIVResNet website.

- Web-based systems to be developed by HIV ResNet, for receiving, checking and correcting genotypic data and producing interpretations and reports.
- Technical assistance in the form of expert consultation and advice.
- Direct provision of some data management functions.
- Help in regular evaluation of national data centre operations, should be evaluated on a regular basis.

#### **Global sharing of HIV drug resistance data**

The WHO Global Surveillance Programme recommends that the HDRST consider sharing selected HIV drug resistance data items in a global WHO HIVResNet database. Collation of HIV drug resistance data internationally will allow comparisons over time among countries and worldwide mapping of trends and variations in HIV drug resistance prevalence. Information on plans for this global network will be available through the WHO HIV website. Standard methods are being developed to ensure that data from different countries can be collected and analyzed comparably. Each country's right to maintain control over its own data and specify the extent to which these data can be used in shared analyses will be respected.

## 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 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 prevalence, prevalence of key "indicator" mutations, and estimates of trends.

### **Electronic transfer of sequence information from the HIV resistance genotyping laboratory**

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. The sequence can be transferred either as a single file containing a concatenation of protease and the relevant portion of the RT region of the genome, or as two separate files, one for protease and one for RT. Although the start and stop positions (sequence range) are not an intrinsic part of the sequence, they should accompany the nucleotide sequence.

### **Deriving data from the nucleotide sequence**

Data for use directly in analyses and clinical reports will generally be derived from the nucleotide sequence of the national data centre. All analyses of cumulative results must begin with original sequence data rather than a set of summary mutation lists. This will allow analyses to be redone using new criteria to take advantage of new drug resistance knowledge and the standardization of approaches to tracking resistance.

#### **Sequence quality checking**

Before programmes are run, each sequence should be evaluated for inclusion in the analysis based on its quality. A more complete explanation of this section is found in the Appendix II and the HIV drug resistance laboratory section.

Sequences containing frame shifts should be excluded unless the sequencing reaction containing the frame shift is re-run.

Sequences containing more than two highly ambiguous nucleotide or at least one highly ambiguous nucleotide and one stop codon should be excluded unless the poor quality sequence can be trimmed without affecting the overall integrity of the remaining part of the sequence.

Sequences containing more than 3% nucleotide mixtures or known to have a low signal should be excluded from

analysis unless the sequencing reaction is redone.

The following types of data can be derived from the nucleotide sequence text using programmes which will be available from WHO HIVResNet or other programmes available over the web. The names, versions and last date of modification of programmes used should be noted.

#### **Amino acid differences from a standard reference sequence**

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.

#### **Drug resistance mutations**

Additional programmes, which will be regularly updated on the WHO HIV website, can be applied to this file. A list of drug resistance mutations found in each strain can be derived from the complete list of amino acid differences by a programme selecting only mutations at those positions considered in the current scientific literature to be associated with drug resistance. Further classification of drug resistance mutations can produce lists of mutations associated with drug resistance to individual drugs and drug classes for use in reports of HIV drug resistance prevalences.

During a resistance threshold survey, a list of drug resistance mutations in the strain should be generated immediately to determine whether the resistance threshold may have been met or whether additional information is needed. However, such lists should not be maintained because they will become outdated. New mutation lists should be generated for each strain whenever an analysis is performed. In resistance threshold surveys among newly diagnosed persons, a finding of more than one major mutation associated with resistance should trigger an investigation to ensure that previous treatment has not taken place. The finding of a large number of mutations in different drug classes is very likely to indicate previous treatment rather than transmitted resistance.

### Drug resistance interpretations

Drug resistance interpretation algorithms should be agreed between the national data centre, the resistance genotyping laboratory, and the HDRST. Several interpretation algorithms are available over the web; countries participating formally in WHO HIVResNet are encouraged to use the HIVResNet algorithm once it becomes available. If results are to be returned, clinical reports may be produced using one of these interpretations. Suggested report formats will be available from WHO HIVResNet.

### Sequence subtype

The subtype should be reported as one of the 9 pure subtypes (A, B, C, D, F, G, H, J, K), one of the two common circulating recombinant forms (CRF01\_AE and CRF02\_AG), one of the many less common circulating recombinant forms, or an uncharacterized recombinant form to be indicated by concatenation the subtypes present within a sequence as determined by the methods described below.

The HIV subtype can be determined by one of the following approaches: (i) comparing the sequence to a list of reference nucleotide sequences and assigning to it the subtype of the closest matching sequence, (ii) creating a phylogenetic tree using an unknown sequence and a list of reference nucleotide sequences, (iii) using a programme such as RIP (Los Alamos Sequence Database) or the NCBI subtyping tool that uses a sliding window to detect local matches to reference sequences enabling the detection of recombinant sequences containing a combination of subtypes, or (iv) using a bootscanning programme (e.g. Simplot) that uses a sliding window to perform phylogenetic analyses to identify recombinant sequences.

### HIV drug resistance analyses

For resistance threshold surveys, the strains tested should be evaluated for the presence of one strain with major mutations associated with resistance to drugs used routinely in the country. If such a strain is found two years in a row, and no evidence is found that the persons

involved received previous treatment, sentinel surveillance is to be considered.

When sentinel surveillance is taking place, prevalences should be calculated which include confidence intervals (See Appendix I). A summary prevalence of mutations associated with resistance in the strains tested during the year, and separate prevalences to individual drug classes, should be calculated. Care must be taken not to imply that a prevalence calculated on a small or unrepresentative sample actually represents the prevalence of drug resistance in the country.

### Recommended Reporting of HIV Drug Resistance Surveillance Results

Reports should include:

- Overall annual prevalence estimates of HIV drug resistance (with confidence intervals)
- Annual prevalence estimates of resistance to specific drug classes
- Annual prevalence estimates of resistance and of specific mutations associated with specific HIV-1 subtypes
- Annual prevalence estimates of resistance to specific drugs in use in recommended first and subsequent regimens in the country or area
- Prevalence estimates of key "indicator" mutations
- Estimates of trends in the above prevalence estimates, if numbers are sufficient and the samples are representative and comparable from year to year
- If numbers are sufficient:
  - Area-specific estimates of the above bullet points
  - Subgroup-specific estimates of the above bullet points

### Global analyses of HIV drug resistance

WHO HIV ResNet will establish a global database to which countries performing HIV drug resistance surveillance are encouraged to contribute. Data analysis and dissemination of reports on a global level are planned. The global system will include web-based tools for analysis and communication with other countries and with the WHO HIVResNet global data centre.

## 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.

### **Collecting specimens at HIV diagnosis**

HIV diagnostic specimens – specimens that are collected for routine HIV testing – can be a convenient source for HIV resistance surveillance. Since blood is being drawn for routine HIV testing purposes, some ethics review boards have ruled that HIV resistance genotyping can be performed on the same specimen without the necessity of an additional consent process. The use of HIV diagnostic specimens ensures that specimens from all individuals who test positive for HIV infection, including those who may not return to receive their HIV results, will be included in HIV drug resistance surveillance activities. Alternatively, at sites where routine HIV diagnosis involves point-of-care or rapid HIV testing strategies, it may be more feasible to collect dried blood spots which could be forwarded for HIV resistance genotyping from those individuals who are identified as infected.

#### **Potential specimen types**

In most settings the use of HIV diagnostic sera is preferred for HIV drug resistance surveillance. 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. If a clinical specimen is drawn at the time of diagnosis using rapid HIV testing methods, other specimen types may be obtained.

### **Advantages and limitations of specimen collection at diagnosis**

#### **Advantages**

Collection of dried blood spots from the blood draw for HIV diagnosis, or aliquoting HIV diagnostic sera, ensures the earliest possible specimen for resistance testing. This may maximize the chances of finding transmitted mutations, and of identifying recent infection by laboratory methods.

The use of HIV diagnostic specimens and/or specimens collected at the time of HIV

diagnosis, ensures the earliest possible specimen for resistance testing. As the length of time transmitted resistance mutations remain detectable is still largely unresolved, the collection of the earliest possible specimen may maximize the chances of finding transmitted mutations. It also maximizes the chances of identifying recent infection by laboratory methods in the same specimen.

Collecting resistance surveillance specimens at diagnosis ensures that specimens will be available from all eligible individuals, including those who do not return promptly for their HIV results or for further treatment.

As discussed in the Ethical Considerations chapter, each country and site involved in HIV drug resistance surveillance programme must ensure that procedures for the appropriate protection of human rights are in place. In some countries, it may be determined that the use of HIV diagnostic specimens for resistance testing does not require an informed consent process. This is an advantage in terms of labour and record-keeping.

Collection of specimens at the time of the diagnostic test minimizes labour for the blood draw itself, since a special blood draw is not needed. Fewer laboratory supplies are used. Use of diagnostic specimens ensures that sequential sampling (following the sequence in which persons present themselves for HIV testing and are diagnosed) can be performed, increasing the likelihood of a representative sample.

#### **Limitations of specimen collection at diagnosis**

Because of time limitations required for specimen stability, the use of diagnostic specimens for HIV resistance genotyping will generally require the initial processing and storage of all diagnostic specimens, including those which subsequently test HIV-negative. Processing, aliquoting and storing specimens that will not be genotyped will require extra labour and materials. Subsequent discarding of the specimens shown to be HIV negative after the diagnostic test may appear wasteful. In rapid testing sites where a confirmatory blood draw is used immediately to confirm

an initial test on oral fluid, labour and materials needed are minimized.

Most HIV diagnostic algorithms rely on collected sera, meaning that blood plasma, widely regarded as the specimen of choice for resistance genotyping, will not be available.

HIV diagnostic algorithms may be modified in some sites to ensure the quality of the specimen for resistance testing and minimize potential sources of molecular contamination.

### **Collecting specimens for resistance genotyping after HIV diagnosis**

After an HIV diagnosis has been made, a special blood draw can be performed for the purpose of resistance testing at the time the person returns to receive the result, or an appointment can be made for the blood draw to occur shortly afterwards.

If all HIV-positive individuals who return for their result are scheduled at a fixed time after diagnosis to have blood drawn for clinical purposes, an aliquot or DBS from that blood draw can be utilized for the purpose of resistance testing. In practice, because all individuals will not return for appointments as scheduled, or will not have blood drawn for clinical purposes, a special blood draw may still be needed for some individuals.

#### **Potential specimen types**

Plasma is the usual material for HIV resistance genotyping and is preferred if a special blood collection allows planners to choose the specimen type. Dried plasma spots, which can be stored and transported at room temperature, should be considered when quick separation is possible but quick freezing of specimens, or transport of specimens on dry ice, cannot be performed. Dried blood spots and sera may also be considered if neither the collection of plasma or dried plasma spots is possible.

#### **Advantages of a blood draw after HIV diagnosis**

The main advantage of drawing blood for resistance testing after HIV diagnosis is that specimens are only collected from

individuals who have tested positive for HIV. Far fewer specimens will be collected and stored.

A special blood draw by staff dedicated to resistance surveillance may ensure that specimens are handled and transported optimally. A greater choice is available to planners of the type of specimens to be used. Blood plasma, which is considered optimal for resistance genotyping, can be obtained. A greater volume may also likely to be obtained using a dedicated blood draw.

#### **Limitations of a blood draw after HIV diagnosis**

Individuals who refuse the extra blood draw will not be represented in the survey, which may bias the results. If some individuals do not return as scheduled for their HIV diagnostic result, they may also be lost to the survey. Extra resources may be needed to track individuals who do not return. Those who are lost to follow-up will not be represented in the survey, which may bias the results.

Sequential selection of persons who test positive for HIV is more difficult utilizing an additional blood draw after HIV diagnosis. Selection of individuals in the order that they return for counselling and a subsequent blood draw may bias the sample, since those who return promptly may differ from those who do not. Keeping a record of the order in which the initial diagnostic tests were drawn, and using this list for the sequential sampling, should be performed, but makes sampling more complex.

A special blood draw after HIV diagnosis may mean a substantially longer time between infection and resistance testing for some individuals. It is possible that some mutations which would have been seen in a diagnostic specimen will not be found in a later specimen, and that recent infection will no longer be detectable by laboratory methods if the specimen is drawn months after diagnosis.

Extra staffing resources will be needed for a special blood draw. These include not only phlebotomists, but staff to attempt to locate and reschedule persons who do not return



for their HIV result or for a subsequent blood draw appointment.

Ethics review committees generally require a consent process for a special blood draw. This may add an extra burden for staff in countries where consent would not be needed to use a diagnostic specimen.

### **Specimen types for HIV Drug Resistance Surveillance**

#### **Sera**

As sera are almost universally used in the diagnosis of HIV infection, diagnostic sera represent the most convenient specimens for large population based resistance surveillance programmes. If blood collected for HIV diagnosis can be processed into sera and expeditiously tested (within 72 hour), 1-2 ml of sera from those individuals who test positive for HIV infection can be aliquoted and stored for future resistance genotyping. If specimens can be processed and transported within 48 hours of the blood draw, they may be transported at 4C.

This method for obtaining specimens is facilitated when the HIV diagnostic laboratory is in the same location as the HIV resistance genotyping laboratory, or when courier systems exist for rapid transport. The aliquots for HIV drug resistance genotyping should be frozen at -70C (or at -20C if the lower temperature is not feasible) and transported on dry ice to the genotyping laboratory. Under IATA regulations, infectious clinical samples should be shipped as "hazardous goods". Long term storage of specimens should be at -20C (although -70C is preferable).

#### **Advantages of sera**

Centrifugation and separation of sera are performed routinely at HIV diagnostic laboratories for HIV diagnostic purposes. Infection controls procedures will already be in place for these procedures.

Aliquoting diagnostic sera for the purposes of HIV drug resistance genotyping, does not require additional training.

The use of HIV diagnostic sera ensures that specimens from all individuals who test

positive for HIV infection, including those who may not return to receive their HIV results, will be included in HIV drug resistance surveillance activities.

Since sera is being drawn for routine HIV testing purposes, some ethics review boards have ruled that HIV resistance genotyping can be performed on the same specimen without the necessity of an additional consent process.

Sera have been used successfully for HIV resistance genotyping in routine surveillance systems in the US and Canada.

#### **Limitations of sera**

In some areas, volume drawn for HIV testing may be insufficient to provide enough sera for HIV resistance genotyping.

If diagnostic sera are used, the time frame for separation, aliquoting, freezing, and transport of specimens may be burdensome to staff with other duties. Shipping on dry ice is an added expense.

Virus may not be amplifiable for resistance testing from poorly handled specimens. HIV diagnostic laboratory procedures may need to be modified in order to ensure the quality of the sera available for resistance testing and to minimise potential sources of molecular contamination.

#### **Plasma**

In most countries HIV drug resistance genotyping is presently performed mainly on blood plasma. Blood plasma is the preferred specimen type in most HIV drug resistance genotyping laboratories because of extensive experience with this specimen type, and for reasons listed under the advantages below. Despite this preference, the fact that plasma is not routinely collected for HIV diagnostics may limit its utility for large population based surveillance programme. For surveillance purposes, its use will generally be limited to those situations where drug resistance surveillance specimens are collected post diagnosis with a special blood draw.

Nucleic acid extraction and subsequent amplification procedures generally work best with EDTA or Citrated plasma. Heparinized plasma should NOT be used.

## DRAFT GUIDELINES FOR SURVEILLANCE OF HIV DRUG RESISTANCE

Plasma should preferably be separated from whole blood within approximately 72 hours after collection and stored at  $-70^{\circ}\text{C}$  (or at  $-20^{\circ}\text{C}$  if the lower temperature is not possible) for optimal stability. Plasma specimens should remain frozen during transport (i.e. shipment on dry ice). If specimens can be processed and transported within 48 hours of the blood draw, they may be transported at  $4^{\circ}\text{C}$ .

Centrifugation, pipetting, and aliquoting must be performed following standard laboratory biosafety precautions at a local laboratory equipped to manipulate infectious clinical samples, with adequate sample storage- and inventory facilities.

Plasma samples are to be considered infectious. Under IATA regulations, infectious clinical samples should be shipped as "hazardous goods". These facts have a major impact on the logistics and cost of sample shipment to other laboratories.

### **Advantages of Plasma**

The clinical relevance of HIV genotyping has only been proven with plasma derived HIV resistance profiles. Genotypic information obtained from plasma-derived HIV reflects the actively replicating virus population that forms the majority virus population.

If viral load determinations are being performed as part of routine clinical evaluation after diagnosis, procedures will already be in place for prompt processing of blood plasma, and it may be possible to use left over viral load specimens for HIV drug resistance genotyping.

### **Limitations**

The need for quick processing, special handling, and storage at  $-20^{\circ}\text{C}/-70^{\circ}\text{C}$  impose an extra cost in terms of handling, storage, and transport to the HIV resistance genotyping laboratory.

As plasma is not routinely collected for diagnostic purposes its use will be limited to post diagnostic resistance sampling.

### **Dried Plasma Spots**

An alternative to the using fresh frozen blood plasma for HIV drug resistance surveillance is the use of Dried Plasma Spots (DPS) consisting of fixed amounts of patient

plasma spotted onto filter paper, and subsequently dried at room temperature. DPS have demonstrated to be applicable for viral load determination using various assays and extraction procedures. The viral load on the DPS remained stable at room temperature and has been shown to be comparable to VL determinations on fresh/frozen plasma.

As with the preparation of frozen plasma, DPS should be prepared by immediately spotting onto absorbent specimen collection paper, and plasma separated from whole blood within 6 to 72 hours of collection. Alternatively DPS could be created in bulk from frozen plasma stored at  $-20^{\circ}\text{C}$  (preferably at  $-70^{\circ}\text{C}$ ) in order to maintain the stability of the viral genome. Thus the blood samples must be centrifuged, pipetted, and spotted onto filter paper at a local laboratory equipped to manipulate infectious clinical samples.

Once dried, DPS are no longer considered infectious and can be shipped at room temperature as non-infectious material.

### **Advantages**

The advantages of DPS are similar to those of plasma, with the added advantages that DPS are considered non-infectious and do not require refrigeration for transportation and storage. Therefore DPS are considerably easier to handle than other specimen types.

### **Limitations**

Although DPS technology has proven to be reliable for the determination of plasma viral load, the need for larger PCR products in resistance genotyping may be problematic, particularly for low viral load specimens. The stability of viral genetic material in DPS specimens needs to be systematically evaluated specifically as it relates to HIV drug resistance genotyping.

DPS cannot be made until blood has been separated and plasma is available for spotting. The need for relatively quick processing may constitute an extra burden on laboratory staff. The necessity to perform spotting in addition to plasma preparation may make it more difficult to reliably

generate specimens of the quality needed for resistance genotyping.

As with whole blood plasma, DPS will generally only be available for surveillance if a special blood draw, with the attendant limitations listed under Blood Plasma, is performed for surveillance purposes.

### **Dried blood spots**

Dried blood spots (DBS) refer to specimens collected by directly applying several drops of blood, freshly drawn by either a lancet finger or heel stick from patients, into absorbent specimen collection (filter) paper. The blood is allowed to uniformly soak through the paper (avoiding caking and/or clotting) and is subsequently air dried at room temperature for several hours before shipping and storage.

If blood is routinely collected for HIV diagnostic testing at a centre selected for HIV resistance surveillance, a DBS for the purpose of resistance genotyping may be made during the collection of the HIV diagnostic specimens. The DBS can be stored on-site or transported to the HIV resistance testing laboratory at room temperature. When specimens test HIV-positive, the corresponding DBS will be used for resistance genotyping if eligibility criteria are met; other DBS will be discarded.

DBS may be the only practical specimen in those situations where routine HIV diagnostics includes point-of-care or rapid HIV testing strategies. In these situations, a DBS could be collected for all patients and forwarded for resistance testing on those individuals where HIV infection is confirmed.

Alternatively, if tubes of blood are sent directly to the HIV diagnostic laboratory to be processed for HIV testing, a DBS may also be made at that laboratory by directly spotting left over blood from the diagnostic tube. Nucleic acid extraction and subsequent amplification procedures for HIV resistance genotyping work best with EDTA or citrated blood. Heparinized blood should *not* be used. Blood should be spotted within 72 hours of the blood draw.

Blood spots should be collected on appropriate filter paper labelled so as to link with the minimum dataset for each

individual. The amount of blood used should be sufficient to soak through the paper completely. The filter paper must then be dried completely at room temperature before storage. Each DBS should be stored and transported in a separate envelope avoiding extremes of humidity and temperature. Specimen collection, shipment and storage techniques have been published by the National Committee for Clinical Laboratory Standards.

### **Advantages of DBS**

If DBS are taken from a confirmatory blood draw collected in rapid testing sites after the first reactive test, the DBS have similar advantages to diagnostic sera, in that all newly diagnosed persons can be included in drug resistance surveillance. In addition, a collection from the confirmatory blood draw means that fewer specimens need to be processed and handled than if diagnostic sera are used.

DBS are easier to handle and transport than other types of specimens used for resistance testing. They can be stored at room temperature, so that freezing is not required, nor advisable.

DBS are no longer infectious and can be shipped at room temperature as non-infectious material. This has a major impact on the cost and logistics of sample collection, storage and shipment of clinical samples for resistance determination.

DBS may be the only practical specimen in situations where routine HIV diagnostics includes point-of-care or rapid HIV testing strategies.

### **Limitations of DBS**

Preparation of DBS, though simple, requires extra training and quality assurance procedures. If sufficient blood is not collected to soak through the filter paper, or if the paper is not properly air-dried, virus may not be amplifiable for resistance testing. At present the use of DBS for HIV drug resistance genotyping has not yet been evaluated systematically, though successes have been reported.

The nucleic acid extraction procedures used for viral load determination, which have been widely successful with DBS, are the

## DRAFT GUIDELINES FOR SURVEILLANCE OF HIV DRUG RESISTANCE

same as those used for genotyping. However, given that viral load assays use only short PCR fragments, the efficiency of using larger fragments from DBS must be systematically evaluated. It is expected that DBS can be used for genotyping, but an operational study may be needed in specific sites.

If DBS are not taken from an existing specimen but are collected from a fingerstick for the purposes of HIV drug resistance surveillance, consent may be needed and some persons may not be included.

## 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.

### **HIV Drug Resistance Testing Procedures**

HIV drug resistance can be determined using either phenotypic or genotypic approaches. Phenotypic drug susceptibility determination resembles traditional antimicrobial drug susceptibility testing but the laboratory assays are complicated, laborious, and expensive. Recombinant phenotypic tests can be obtained using highly specialised commercial service laboratories (e.g. Virco, Virologic and Viralliance). Resistance determination by nucleotide sequence analysis (genotyping) of portions of HIV genome known to be associated with HIV drug resistance is currently most commonly used for clinical care by laboratories worldwide. Genotyping provides a genetic profile of the complete protease region and much of reverse transcriptase (RT). In addition to using this profile for HIV drug resistance determinations, the genetic profile can also be used to determine the viral subtype.

Genotyping identifies the majority virus populations in the viral quasispecies. The sensitivity of detecting a mutation is approximately 30%. This means that if a viral variant is present in less than ~30% of the virus population in a sample, it is unlikely to be detected. More sensitive technologies, for instance using real-time PCR technology or hybridization, have been developed recently. However, most of these methods are only applicable to a single position in the sequence and do not result in a complete genotypic profile.

Genotyping results in the identification of mutations associated with reduced susceptibility to one or more individual antiviral drugs or even to antiviral drug classes. It has been demonstrated that access to genotyping results can be useful in the clinical management for the selection of the appropriate salvage therapies. Since the number of mutations known to be associated with antiviral drug resistance is already more than 150 and various interactions between mutations have been identified, the interpretation of a genotypic

resistance profile for clinical purposes can be very complex. Therefore, several different genotyping interpretation algorithms have been developed for clinical application. None of these have been specifically developed for epidemiological (surveillance) purposes. In a very recent initiative, researchers from various groups involved in drug resistance surveillance have started to develop an epidemiological genotyping interpretation algorithm, which will later be available for use in HIV drug resistance surveillance. An interim minimum list of mutations associated with drug resistance which are not generally seen among untreated persons, and can be used for HIV drug resistance surveillance analyses, is found in the HIV Drug Resistance Data Analysis section.

Specimen collection, genotyping procedures and developing a sample repository are separate functions which need not both be performed at the same laboratory.

For this programme, one of three genotypic testing procedures is recommended. Laboratories performing genotyping should be aware that successfully implementing these procedures is not a trivial process and requires not only training but continuing experience. Laboratories in which a substantial number of specimens will not be genotyped yearly should not participate in HIV drug resistance surveillance even if other criteria are met.

### **Specimen Collection**

#### **The HIV drug resistance specimen collection laboratory services manual**

The HIV Resistance specimen collection procedures manual (in development) includes:

- General guidelines for laboratory setup.
- Minimal requirements for laboratory equipment, computing supplies, staffing, and training.
- Minimum personnel requirements.
- Protocols for sample collection, storage and shipment from the proposed biological materials.

## DRAFT GUIDELINES FOR SURVEILLANCE OF HIV DRUG RESISTANCE

- Protocols for appropriate documentation.

### Genotyping Procedures

#### The HIV drug resistance genotyping laboratory services manual

The HIV Resistance Genotyping laboratory procedures manual (in development) includes:

- General guidelines for PCR laboratory setup.
- Minimal requirements for laboratory equipment, computing supplies, staffing, and training
- General instructions for PCR use
- Minimum personnel requirements
- Protocols for nucleic acid extraction from the proposed biological materials (including DPS and DBS)
- Protocols for appropriate documentation
- Protocols for nucleic acid sequencing, including sequence quality control criteria. For example, these include
  - Contamination control
  - Accurate performance with negative/positive controls
  - tracking of data quality
  - the final sequence should have no stop codons, <5% ambiguities, etc.

### HIV Drug Resistance Surveillance Specimen repository

It is strongly suggested to set up a specimen repository in-country to support possible retrospective analyses. An international specimen repository under WHO HIVResNet may also be set up to allow additional analyses of the unique global cohorts collected during surveillance of HIV drug resistance.

The HIV drug resistance specimen repository manual will include:

- Minimal requirements for laboratory equipment, computing supplies, staffing, and training.
- Minimum personnel requirements.

- Protocols for sample receipt, storage and shipment of the proposed biological materials.
- Details of a database for sample retrieval.
- Protocols for the protection of privacy and ethics.
- Protocols for use of the stored samples.
- Appropriate documentation.

### Available Methodologies

#### Commercial sequencing kits

##### HIV Viroseq Kit (Abbott Diagnostics), developed by Applera:

*HIV-1 drug resistance genotyping kit for the viral RT and protease genes.* The kits consist of protocols and reagents for sample extraction, amplification and sequencing of the protease gene and the relevant part of RT (aa 1-320).

##### Requirements

A PCR grade laboratory setup is required.

(Expensive) sequence detection hardware (Applera) is needed. Capillary electrophoresis equipment is recommended, as it is easier and less complicated to use, and generally better suited for diagnostic use. However, training is still needed.

Gel based sequencers equipment are an alternative, but require extensive training and experience and are less suitable for diagnostic use.

Sequence analysis software is provided by the company. Initial studies demonstrate good results for various subtypes but this is still under investigation.

##### HIV-1 TrueGene kit (Bayer Diagnostics):

*HIV-1 drug resistance genotyping kit for the viral RT and protease.* The kits consists of protocols and reagents for sample extraction, amplification and sequencing of the protease (aa 4-99) gene and the part of RT (aa 40-250).

##### Requirements

A PCR grade laboratory setup is required.

Sequence detection hardware (Bayer) is needed.

Moderate experience and training is needed, but the system is suited for diagnostic use.

Sequence analysis software is provided by the company. Performance on viral subtypes is still under investigation.

**‘Home Brew’ sequencing methods:**

Several laboratories specialising in HIV-1 drug resistance have developed “in-house”, non-kit-based approaches or so called “home-brew” methods. These methods usually require Applera reagents and (expensive) sequencing hardware (Applera) is needed.

Requirements

A PCR grade laboratory setup is required.

Extensive inter-laboratory variation is found in all aspects of the sequencing procedure.

Performance on various subtypes may vary among laboratories.

Less standardised (within- and between laboratories) reagents and procedures are available than with commercial kits.

A considerable advantage is that reagent price per sample is significantly less than for commercial kits. “Home-brew” methods are more flexible than kit-based methods, in that changes (such as alternative primers) can be more easily implemented as needed. Sequence analysis software is not provided.

**Choice of resistance testing methodology**

For surveillance purposes the use of one of the three sequencing based genotyping assays listed above is preferred.

Genotyping is a complex technology. If laboratory capacity is not presently available in-country and planners wish to develop such capacity, an international partner laboratory can help in protocol development including setup of a PCR grade laboratory environment. In addition, laboratory technicians should be trained to perform genotyping and extensive quality control and quality assurance protocols. The international partner laboratory can either provide HIV drug resistance genotyping, or help to develop laboratory capacity in-country, or both.

To allow the collection of comparable genotyping information, all laboratories participating in WHO HIVResNet should have detailed laboratory protocols for procedures.

Sequence data analysis, sequence interpretation and data collection should be standardized and simple.

**Internal and External Quality Control**

Quality control is an essential part of genotyping given the number of laboratory steps involved in the procedure and the risk for sample contamination or mix up. Quality control should include both internal and external quality controls for procedures and reagents.

**Internal Quality Control**

Internal quality control (QC) measures include: the use of positive and negative control reagents during sample extraction, amplification and sequencing. Quality control of each step in the process is highly recommended. In addition, phylogenetic analysis of all patient sequences is recommended to check for contamination control. A list of free phylogenetic analysis software available on the internet, and a brief guide to their use to check for contamination, will be available on the WHO HIV website.

**External Quality Control**

External quality control programmes (proficiency testing) are also an essential step to monitor constant quality of the laboratory performance and to monitor inter-laboratory variation. External QC programmes should monitor each step of the assay procedure. The specimens should resemble clinical specimens used for HIV resistance surveillance as closely as possible, including the sample type. Participating HIV resistance genotyping laboratories should participate in the external quality control programme. Test specimen will be indicated through HIVResNet at least every twelve month.

As an addition external quality control measure, electropherograms will also be distributed regularly to evaluate each



## DRAFT GUIDELINES FOR SURVEILLANCE OF HIV DRUG RESISTANCE

participating laboratory's analysis of sequence results.

### Training

Training of laboratory personnel is essential for the success of HIV drug resistance surveillance. All aspects of the laboratory procedures should be included in the training programme. Special attention should be given to:

- Communication with local processing laboratories
- Documentation of procedures used
- Biosafety procedures
- Sample storage, retrieval, shipment
- Nucleic acid extraction
- Nucleic acid amplification
- Sequence analysis
- Sequencing reactions
- Use of the sequencing hardware
- Use of the sequencing software
- Electropherogram analysis
- Sequence Data analysis and management
- Daily Quality Control of the assays and procedures
- Data backup

### Transport regulations

Under IATA regulations, infectious clinical specimens should be shipped as "hazardous goods". These facts have a major impact on the logistics and cost of specimen shipment to other laboratories. The IATA rules are updated yearly in January. To date, in the current 2003 IATA rules ([www.IATA.org/dangerousgoods](http://www.IATA.org/dangerousgoods)), HIV testing specimens are defined as diagnostic rather than clinical specimens; less stringent procedures may apply to diagnostic specimens. However, the UN recommendations follow the ICAO technical instructions "Transport of dangerous goods" and have legal power. These are published biannually.

Import - Export regulations for laboratory specimens change frequently in many countries and differ between individual countries.

Guidelines for safe handling and transport can be found in the WHO "Guidelines for the safe transport of infectious substances and diagnostic specimens" available throughout the website.

### HIV Drug Resistance Genotyping Data

Genotypic resistance tests produce the following types of data:

#### DNA sequence trace data

These data are contained within individual samples files containing 300-500 nucleotides in several standard formats. These data can also be viewed as a chromatogram.

#### Edited assemblage

This is obtained by assembling several overlapping DNA sequence trace files using software provided by the sequencing manufacturer. The edited assemblage also contains information on which positions have mixtures either because a trace data file, containing evidence of two nucleotides or overlapping trace files, each contained a different nucleotide. The assignment of a mixture is done partly by manufacturer's software and partly by manual review.

#### Nucleotide sequence text file

A text file should be created by exporting the nucleotide sequence from the edited assemblage in text format. The nucleotide sequence text contains a string of letters to represent unambiguous (A, C, G, T) nucleotides or mixtures (R, Y, M, K, S, W, B, D, H, V, N). This file should be sent to the national data center together with associated data (RPID, site ID, date of arrival of specimen in laboratory, condition of specimen, volume of the specimen).

For specimens from which virus could not be amplified, the tracking data should be recorded and sent to the local data center despite the fact that the nucleotide sequence is not available.

Individual nucleotide sequences can be stored in a variety of formats. The "fasta" format contains a single-line header beginning with ">" followed by a single line that contains the nucleotides. The header can contain the identifying data and possibly additional data such as the sequencing

range, list of mutations, number of problem positions (e.g. positions with stop codons, frame shifts, highly ambiguous nucleotides, or highly atypical mutations). Another option is a standard spreadsheet programme or one or more tab-delimited text files.

Several files derived from the nucleotide sequence file will be created through programmes run at the national data centre. These include a list of amino acids differing from the consensus sequence HBX2, lists of mutations associated with resistance, drug resistance interpretations, and the subtype.

### **Storage of data in the HIV drug resistance testing laboratory**

The DNA sequence trace data, the edited sequence assemblage, and the nucleotide sequence text should all be stored by the sequencing laboratory for at least one year after the sequence is sent to the data centre. Although nearly all subsequent analyses will be based on the nucleotide sequence text, the DNA sequence trace data and the edited assemblage will be needed for quality control purposes.

The nucleotide sequence text should be stored either as a single file containing a concatenation of protease and RT or as two separate files, one for protease and one for RT. Although the start and stop positions (sequence range) are not an intrinsic part of the sequence, they should accompany the nucleotide sequence.

### **Quality assurance**

An assessment of sequence quality can be made using the nucleotide sequence text by noting frame shifts, stop codons, the number of ambiguous nucleotides, and the presence of atypical mutations. Stop codons and frame shifts are unequivocal indications of sequencing error and some experts would choose to exclude sequences containing them. Nucleotide ambiguities are common, occurring at about 1% of positions in sequences from treated persons. However, a significantly greater number of ambiguities or the presence of many highly ambiguous nucleotides (e.g. B, D, H, V, N) might suggest that the sequence is of poor quality and contains inaccuracies. A run of several consecutive atypical mutations (e.g.

mutations that are seen in <0.1% of sequences) would also suggest that a sequence contains inaccuracies. This type of assessment can be made using a variety of programmes including the HIVseq and HIVdb programmes on the Stanford HIV Reverse Transcriptase and Protease Sequence Database site.

Analyses for assessment of sequence quality may be performed at the HIV drug resistance testing laboratory or the national data centre, or both.

Transmitted resistant variants will often be present as part of a mixture of wildtype and mutant forms. Significant differences between laboratories or over time within the same laboratory, could reflect artifactual differences in measured rates of drug resistance. WHO HIVResNet or the national data centre may require access to the raw data if significant findings are based on the frequencies of nucleotide mixtures, in order to recreate sequence text files using standard software.

## APPENDIX I

### Sample Size Calculations for sentinel surveillance

**Estimation in each area or site.** In this case the surveillance sample size should be sufficient to detect a prevalence of resistance of  $\geq 5\%$  if the "true" prevalence is between 4%-6%, with 95% confidence. The actual number will depend on the number of new diagnoses at the site or sites; the previous year's new diagnoses should be used to provide this number. Use this number to find the "n" in the second column, and find the appropriate row. For representative sampling of all new diagnoses in the country, large numbers must be used.

*This assumes simple random samples. For cluster samples, c will be larger by a proportion depending on cluster homogeneity. However, stratification may reduce sampling errors.*

Given p and n			Result	Given p and c			With rounding
p	Number of new HIV diagnoses (n)	c		p	c	Number required in sample	
0,025	150	0,024985		0,025	0,025	150	150
0,06	100	0,046547		0,06	0,05	87	90
0,5	100	0,098		0,07	0,05	100	104
0,5	400	0,049		0,08	0,05	113	118
				0,09	0,05	126	131
				0,1	0,05	138	144
				0,5	0,05	384	400

Required Sample Sizes				
		Precision Desired: + or -		
		2,5%	5%	10%
Proportion to be estimated	1%	63	16	4
	2,5%	156	39	10
	5%	304	76	19
	10%	576	144	36
	25%	1.200	300	75
	50%	1.600	400	100

At least 10% should be added to these numbers to perform actual surveillance of HIV drug resistance, to allow for problems with transport, virus amplification, and inclusion of persons who are later found to be ineligible.

**Estimation between consecutive surveys.** The sample size should also be able to provide a baseline for a comparison with a future sample of the same number performed at the same site. It should allow a 95% chance that if the study shows an increase in resistance prevalence at

the site from 0% to 5% in two different years, this is a “true” increase, and an 80% chance that if this difference is seen, the samples are large enough that the confidence intervals around the two estimates will show the difference to be significant. Then these numbers are depicted in the Figure 1, where there is a correlation between the sample size, and the power. Three situations were calculated based on actual rate of resistance and the future rate to be estimate in the next survey.

This computation assumes that the difference in proportions is -0,04 (specifically, 0,01 versus 0,05)

### **Selection of the prevalence required to be detected**

This prevalence “effect” used in calculations should be the smallest prevalence that would be important to detect, in the sense that any smaller effect would not be of clinical or substantive significance. The prevalence to be detected is also selected to represent a “reasonable effect”, in the sense that an effect of this magnitude could be anticipated in this field of research.

### **Precision for estimating the effect size**

A second goal of this study is to estimate the difference between the two populations. Based on these same parameters and assumptions, the study will enable us to report the difference in proportions with a precision (95,0% confidence level) of approximately plus/minus 0,03 points.

Specifically, an observed difference of -0,04 would be reported with a 95,0% confidence interval of -0,07 to -0,01.

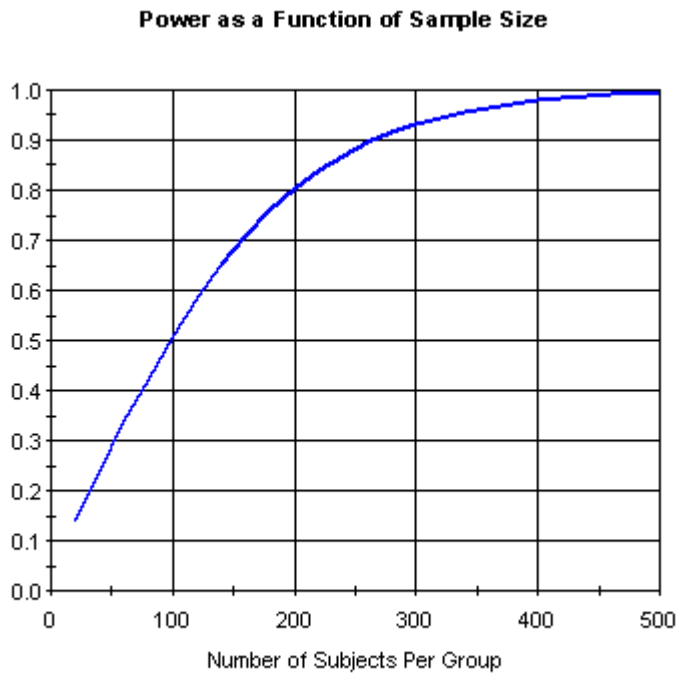
The precision estimated here is the approximate expected precision. Precision will vary as a function of the observed proportions (as well as sample size), and in any single study will be narrower or wider than this estimate.

### *Notes*

Power computation: Normal approximation (unweighted mean p)

Precision computation: Log method

Using three different situations we have modelled the estimation of power in function of sample size when the initial resistance rate was 0%, 5%, and 10 % and were changed to 5%, 10%, and 15%, respectively.



**Figure 1-** graphic showing the relation between sample size and power of the analysis to estimate a jump from 0% to 5% in the resistance prevalence in a population, in two distinct studies done with identical sampling methodology.

## APPENDIX II

### THE HIV DRUG RESISTANCE MUTATION LIST FOR ANALYSIS OF SURVEILLANCE DATA

1. For surveillance among newly diagnosed persons, lists of relevant drug resistance mutations should be developed locally in consultation with HIVResNet or recognized experts. Mutations associated with resistance to drugs not in use in the country generally should not be included in the basic list of mutations for calculation of prevalence of resistance or for determining that the "resistance threshold" has been met. However, these mutations should be included in a more general description of results.  
Appropriate lists of HIV drug resistance mutations to be used in epidemiologic analyses are currently being developed by international groups, but have not yet been published for general use. A standard updated list will be maintained on the WHO HIV website. In the interim, the list below may be used.
2. This interim list is based on the premise that drug resistance surveillance should focus on well-characterized drug resistance mutations that are non-polymorphic in untreated persons. For practical purposes, non-polymorphic sites should be sites that are mutant in <0.5% of isolates from untreated persons.
  - a. Twenty-three positions in protease are commonly listed as being associated with drug resistance. These include positions 10, 20, 24, 30, 32, 33, 36, 46, 47, 48, 50, 53, 54, 60, 63, 71, 73, 77, 82, 84, 88, 90, and 93. However nine of these positions – 10, 20, 33, 36, 60, 63, 71, 77, and 93 – are highly polymorphic in untreated persons with rates of mutation ranging from >1% to >50% depending upon subtype. In addition, the V82I substitution occurs commonly in subtype G and as an uncommon polymorphism in several other subtypes (e.g. B and C).
  - b. Thirty-three positions in RT are commonly listed as being associated with drug resistance. These include 18 nucleoside RT inhibitor mutations at positions 41, 44, 62, 65, 67, 69, 70, 74, 75, 77, 115, 116, 118, 151, 184, 210, 215, and 219 and 15 non-nucleoside RT inhibitor mutations at positions 98, 100, 101, 103, 106, 108, 179, 181, 188, 190, 225, 227, 230, 236, and 238. At the NRTI drug-resistance positions, E44D, T69S/A, and V118I occur in  $\geq 1\%$  of sequences from untreated persons. At the NNRTI drug-resistance positions A98S, K101R, K103R, V106I, V179I, and K238R among others occur commonly in untreated persons, whereas A98G, K101Q, V108I, V179D/E occur in  $\geq 0.5\%$  of untreated persons. F227L and P236L are extremely rare mutations that also occur in untreated persons.
3. Surveillance should focus on mutations within the first 240 amino acids of RT because the remainder of the RT (amino acids 241-560) is sequenced less frequently and because there are few mutations beyond this range. Y318F is an uncommon delavirdine associated mutation. G333E/D are common polymorphisms that occur with a slightly increased frequency during antiretroviral treatment.
4. HIV-1 protease and RT are highly variable and additional treatment-associated mutations have been recently reported.
  - a. In an analysis of 2,244 subtype B protease isolates from 1,919 persons, mutations at an additional 22 positions were found to be associated with treatment (T Wu et al, J Virol, 2003). Thirteen of these 22 newly described treatment-associated positions (positions 11, 22, 23, 45, 58, 66, 74, 75, 76, 79, 83, 85, 85) were highly conserved in untreated persons whereas the remainder were polymorphic in untreated persons. Several of these mutations have also

## DRAFT GUIDELINES FOR SURVEILLANCE OF HIV DRUG RESISTANCE

- been described in other recent publications containing analyses of large databases. The phenotypic and clinical impact of these mutations is not yet known because they rarely occur in the absence of other known drug-resistance mutations and have not been studied *in vitro*.
- b. In an analysis of 1,210 subtype B RT isolates from 1,124 persons, mutations at an additional 9 positions were significantly associated with NRTI treatment: K20R, T39A, K43E/Q/N, E203D/K, H208Y, D218E, H221Y, D223E/Q, L228H/R (M Gonzales et al, AIDS, 2003). The first three mutations are polymorphic in untreated persons occurring in 4%, 4%, and 1% of untreated persons. The remaining six occur only in treated persons and are particularly common in persons receiving multiple courses of NRTI therapy, perhaps explaining their delayed recognition. These newly identified mutations nearly always occurred in combination with other previously characterized NRTI-resistance mutations, suggesting that they act primarily as accessories to increase NRTI resistance or to compensate for the decreased replication associated with other NRTI-resistance mutations. The precise phenotypic effect of these mutations alone and in combination with other mutations has not yet been published.
  - c. Treatment-associated mutations at positions that are conserved in untreated persons should be noted but should not yet be used to characterize the frequency of drug resistance in a population. The rates of mutation in treated and untreated persons according to viral subtype can be found through queries at the Stanford HIV RT and Protease Sequence Database (<http://hivdb.stanford.edu> Mutation profiles: Protease, RT, and Position Summary pages) and are being compiled onto a single summary page.
5. Analyses should note whether a mutation is present as part of a mixture. Mutations that are present as part of a mixture with wildtype should be considered differently from mutations present in the absence of wildtype because mutations present as part of a mixture are more likely to represent sequence artefact. This is particularly true for mutations present as a minority variant. Unfortunately, for most analyses it will be difficult to know which mutations were present as minor as opposed to major variants. Secondary analyses using the original DNA sequence trace data would be needed if it were deemed essential to make this distinction.
  6. Analyses should note the presence of more than one drug-resistance mutation. The presence of more than one drug-resistance mutation in an untreated isolate is highly suggestive of transmitted drug resistance (or unreported previous treatment). In contrast, a single drug-resistance mutation could represent a sequencing artefact (if present as part of a mixture) or a rare polymorphism (<0.5%).