WHO/HIVResNet HIV Drug Resistance

Laboratory Strategy

July, 2010
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# Abbreviations

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<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>ART</td>
<td>Antiretroviral therapy</td>
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<tr>
<td>ARV</td>
<td>Antiretroviral drugs</td>
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<tr>
<td>DBS</td>
<td>Dried blood spot</td>
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<tr>
<td>DFS</td>
<td>Dried fluid spot</td>
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<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
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<tr>
<td>DPS</td>
<td>Dried plasma spot</td>
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<tr>
<td>DSS</td>
<td>Dried serum spot</td>
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<td>EDTA</td>
<td>Ethylenediamine tetra-acetic acid</td>
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<tr>
<td>EWI</td>
<td>Early warning indicator</td>
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<tr>
<td>HIV</td>
<td>Human immunodeficiency virus</td>
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<td>HIVDR</td>
<td>HIV Drug resistance</td>
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<td>HIVResNet</td>
<td>HIV Drug Resistance Network</td>
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<td>MOH</td>
<td>Ministry of Health</td>
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<td>NDRL</td>
<td>National HIV Drug resistance Laboratory</td>
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<tr>
<td>PCR</td>
<td>Polymerase chain reaction</td>
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<td>PP</td>
<td>Proficiency panel</td>
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<tr>
<td>RDRL</td>
<td>Regional HIV Drug resistance Laboratory</td>
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<tr>
<td>RNA</td>
<td>Ribonucleic acid</td>
</tr>
<tr>
<td>RT</td>
<td>Reverse transcriptase</td>
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<tr>
<td>SDRL</td>
<td>Specialized HIV Drug resistance Laboratory</td>
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<tr>
<td>SOP</td>
<td>Standard operating procedures</td>
</tr>
<tr>
<td>WB</td>
<td>Whole blood</td>
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<td>WHO</td>
<td>World Health Organization</td>
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1. WHO/HIVResNet Global HIV Drug Resistance Strategy

The WHO/HIVResNet Global HIV Drug Resistance (HIVDR) Prevention, Surveillance and Monitoring strategy was developed by the World Health Organization (WHO) beginning in 2000. In coordination with the plans for universal access to antiretroviral therapy (ART), the strategy consists of a coordinated plan for HIVDR prevention and assessment on a national, regional, and global level, to support ART and HIV prevention planning on a population basis.

The emergence of HIV strains that are resistant to antiretroviral drugs (ARVs) is not a new problem, but it has recently received increased attention. Concerns about the observed increase in the transmission of drug resistant HIV strains (DR-HIV) have been raised as a consequence of the joint international effort to provide appropriate treatment to millions of persons living with HIV/AIDS in resource-limited countries [1]. If ARVs are not effectively delivered, HIVDR could become widespread, leading to an increase in therapeutic failures, transmission of resistant virus, and a decrease in therapeutic options, treatment program effectiveness and survival.

In general, the lack of reliable data and standardized methodologies for sample collection, specimen manipulation and analysis make interpretation of results difficult. Subsequently, the application of this information towards public health action is particularly challenging. Currently available drug resistance data are insufficient in quantity and quality to allow for an accurate assessment of the extent of HIVDR emergence during treatment, and of DR-HIV transmission, on a national and global level. The cost of HIVDR testing poses an additional constraint. In resource-limited countries, HIVDR testing is not generally available, or is too costly to be used for routine monitoring of patients receiving ARVs. In resource-limited countries, WHO recommends that HIVDR testing be performed for public health assessments, and that capacity not be expanded to allow individual clinical monitoring until more basic diagnostic and clinical tests (e.g. viral load and CD4 count) are made widely available. For HIVDR surveillance and monitoring, countries with no capacity for HIVDR testing may choose to utilize a WHO-accredited regional laboratory.
WHO is approaching universal access by developing a national strategy for HIVDR prevention and assessment in countries where ART is widely available or where ART is being rapidly scaled up.

**WHO public health principles for minimizing emergence of HIV drug resistance**
- Appropriate access to ART, prescribing practices and usage
- Fostering adherence
- Supporting prevention of HIV transmission, and
- Appropriate action based on surveillance and monitoring results.

**Program elements to implement these principles**
- Use of standard highly active ART regimens
- Quality assurance for ARVs
- Adequate and continuous drug supplies
- Standardized individual treatment records
- Support for and monitoring of adherence
- Removal of barriers to continuous access to care
- Prevention programs to reduce HIV transmission from ARV-treated patients
- Surveillance for transmission of DR-HIV in recently infected individuals
- Routine population-based monitoring of key measures that may be affected programmatically to minimize the emergence of HIVDR.

The essential package of the WHO/HIVResNet Global HIVDR Program includes six elements, which are described below.

### 1.1. NATIONAL HIVDR WORKING GROUP

A national HIVDR working group should be established within the Ministry of Health (MOH) to integrate the essential HIVDR strategy into the country’s ART and HIV prevention plans. The national work plan should include:
- adaptation of country-specific protocols for HIVDR assessment;
- development of a budget and a funding plan;
- data analysis and interpretation;
- collection, reporting, and dissemination of annual reports;
• evidence-based recommendations for public health action to restrict development of HIVDR;
• partnerships between national and international agencies for all aspects of the work.

1.2. HIVDR EARLY WARNING INDICATORS

As part of the recommended strategy to assess the HIVDR situation in a country, WHO recommends collecting HIVDR Early Warning Indicators (EWI) with the objective of assessing the extent to which ART programs have been optimized for the prevention of HIVDR. These measures, along with other more specific studies for monitoring the emergence of DR-HIV at sentinel sites and for surveillance for transmission of drug resistant strains, will produce country-specific data that will help national decision makers to make appropriate program adjustments and improve strategies for short and long term ART regimens. The HIVDR EWI are based on measurements of ART program factors associated with HIVDR prevention. The EWI are assessed at a clinic level, and summarized on a national level by recording the percentage of clinics meeting the target. For instance, the first indicator, monitoring of standard ART prescribing practices, is reported in one of two ways:

• Percentage of ART clinics in the country where 100% of patients starting ART are initially prescribed a standard potent regimen annually, or
• Percentage of ART clinics in the country in which 100% of patients are taking a standard potent ART regimen at a specific point in time.

The HIVDR early warning indicators

• standard ART prescribing practices
• percentage of patients lost to follow up
• patient retention on first-line therapy after 12 months
• drug pick up
• patient appointment keeping
• pill count/adherence
• of adequate and continuous drug supplies.

1.3. SURVEILLANCE OF TRANSMITTED HIVDR

The WHO HIVDR strategy evaluates transmitted DR-HIV using a minimum resource strategy (HIVDR threshold survey) focusing on geographical areas within each country where HIVDR is
most likely to emerge first. The method categorizes the overall prevalence of transmitted DR-HIV as well as proportions with resistance to relevant drugs and drug classes in untreated, recently infected populations in specific geographical settings. Separate prevalence categorizations are made for each setting, and, if relevant, for subgroups within each setting.

The HIVDR threshold survey

- Results are used to classify the prevalence of transmitted HIVDR to individual drugs and drug classes as < 5%, 5-15%, or > 15%. HIVDR prevalence classifications will trigger specific recommendations for action by the HIVDR working group.
- To classify HIVDR as above a minimum of 47 consecutive specimens is required. To ensure that 47 specimens will be amplifiable each survey should collect at least 60 specimens from eligible consecutively diagnosed individuals. It is recommended that more extensive (expensive) surveillance strategies be considered only if transmitted HIVDR appears to be rising substantially, if resources are available, and if the action taken would be different if more precise estimates could be obtained.
- Specimens and information already being collected for routine purposes are used for the surveys, if possible. Generally, consecutive HIV-seropositive diagnostic specimens from HIV serosurveillance performed in antenatal clinics, voluntary counseling and testing centers, or other HIV diagnostic settings are used, along with information collected routinely at these sites.

1.4. MONITORING HIVDR EMERGING DURING TREATMENT

The HIVDR monitoring survey is a minimum resource, sentinel clinic-based strategy for monitoring the emergence of HIVDR during the first year of treatment in persons starting first-line ART, and for determining the factors associated with lack of viral suppression and HIVDR emergence.

Specific objectives of the HIVDR monitoring survey

- At each ART site, estimate the proportion of the population achieving HIVDR prevention, as measured by viral load suppression, 12 months after starting first-line ART.
- Identify specific resistance-associated mutations and mutation patterns in populations not achieving prevention of HIVDR on first-line ART.
• Identify ART program factors potentially associated with success or failure of HIVDR prevention.
• Report and disseminate results and recommendations.
• Support optimal ART program functioning at sentinel sites.
• Apply lessons learned to other ART program sites.
• Suggest studies or evaluations to provide additional information on program factors associated with HIVDR emergence, or methods for optimizing program functioning.
• Support planning and decision-making to optimize ART effectiveness.

1.5. HIVDR DATABASE

The WHO HIVDR Database application, supplied by the WHO, is designed to store epidemiological information and genotypic data from HIVDR surveillance and monitoring surveys. The WHO HIVDR Database will produce country-specific annual HIVDR reports, and selected data will contribute to the WHO HIVResNet regional and global database.

1.6. WHO/HIVResNet LABORATORY STRATEGY

Specimens collected during WHO recommended surveillance and monitoring surveys must be tested for HIVDR exclusively in genotyping laboratories accredited by the WHO/HIVResNet. The genotyping laboratories accredited by the WHO constitute the HIVResNet Laboratory Network. Countries may use an accredited WHO regional laboratory for genotyping specimens from the HIVDR surveys or, if capacity already exists within the country, an accredited national laboratory.
2. The WHO HIVResNet Laboratory Strategy

2.1. BACKGROUND

HIVDR can be determined *phenotypically*, for example in cell culture-based assays, and *genotypically*, by DNA sequence analysis of the reverse transcriptase (RT) and protease (PR) coding regions. Genotypic drug resistance testing is widely used for clinical and surveillance purposes by laboratories in developed countries and is the technique recommended by the WHO for HIVDR surveillance and monitoring. Genotyping is performed either by using commercial HIVDR genotyping kits, that include reagents, controls and software to generate results, or by using in-house developed or “home-brew” assays. For home-brew assays, laboratories select their own primers for amplification and sequencing, and use generic reagents and software for further sequence analysis. A large variety of home-brew sequencing assays are used in different laboratories and several methods have been published.

Participation in external quality assurance programs is a key component of efforts to ensure the quality of genotyping results. Surveys have been performed in experienced genotyping laboratories showing that the quality of data may vary [2-7]. Factors that contribute to the quality of the results include the type of assay/kit used, the level of experience of the technician performing the analysis, and the viral subtype present in the clinical sample. Results from sequential rounds of proficiency testing have demonstrated that over time, the quality of genotyping results tends to improve [8].

Although in resource-limited settings many laboratories are experienced in genotyping, the lack of standardization limits the production of comparable and reliable results. Existing networks have made attempts to standardize practices and procedures, but there is still a need to develop a common approach for quality assurance. In resource-limited settings, the lack of infrastructure and the cost associated with genotype testing limit the development of genotyping laboratories. Nevertheless, a number of laboratories have been established in resource-limited settings and have active collaborations with several centers of excellence in Europe or North America.

2.2. PRINCIPLES OF THE WHO/HIVResNet LABORATORY STRATEGY

The WHO/HIVResNet Laboratory Strategy functions to support national, regional, and global HIVDR surveillance and monitoring by the timely provision of accurate genotyping results in a
standardized format, which meets the WHO specifications. The aim of the WHO HIVDR laboratory strategy is to ensure:

- accurate collection, handling, shipment and storage of specimens collected in countries implementing HIVDR surveillance and monitoring surveys;
- availability of quality-assured HIV genotyping laboratory services producing comparable and reliable results at the national, regional and global level.

2.2.1. Elements of the WHO/HIVResNet Laboratory Strategy:

1. **National strategy for HIVDR surveillance and monitoring laboratory support.** The national HIVDR working group should designate a WHO-accredited laboratory to perform testing for the HIVDR surveys. This laboratory can be located within the country or at the regional or global level. In countries where a national laboratory is developing capacity and proceeding towards accreditation, an interim solution is to utilize an accredited regional laboratory.

2. **WHO/HIVResNet Laboratory Network.** The HIVResNet Laboratory Network is responsible for ensuring the delivery of quality-assured HIV genotyping data at the national, regional and global level. This is achieved through standardization of laboratory procedures in all laboratories accredited by the WHO for HIVDR surveillance and monitoring surveys.

   Only WHO accredited genotyping laboratories receive full membership of the HIVResNet Laboratory Network. The Network includes different categories of membership, with different tasks and responsibilities:

   - National HIVDR Laboratories (NDRL; usually one per country)
   - Regional HIVDR Laboratories (RDRL; usually one per WHO region)
   - Specialized HIVDR Laboratories (SDRL).

   In addition, full membership can also be achieved by laboratories providing training on behalf of WHO.

   The Network is coordinated by the WHO, in consultation with the HIVResNet Advisory Group (AG), which is composed of representatives of the specialized and regional laboratories.

3. **Standards for specimen collection, handling, shipment, and storage.** Standardization of collection, handling, shipment and storage of specimens for HIVDR testing is a
critical step for production of accurate, comparable results. Accurate genotypic testing depends on appropriate methods of specimen collection and handling, suitable transportation from the collection site to the central laboratory and storage under appropriate conditions.

One of the responsibilities of the national HIVDR working group is to ensure that the national plan for specimen collection, handling, shipment and storage is developed according to WHO HIVResNet guidelines, before HIVDR surveillance and monitoring surveys begin. The preparation and implementation of the plan require close cooperation between virologists, epidemiologists and clinicians at the national level and between the accredited genotyping laboratories and local laboratory staff. The Laboratory Network provides guidance documents on specimens collection/handling/shipment/storage and laboratory procedures, to support standardization of all components of the Laboratory Strategy.

4. **Laboratory technical support for capacity building.** One of the goals of the WHO/HIVResNet Laboratory Network is to maximize transfer of knowledge and expertise from WHO accredited laboratories to those that have not achieved WHO accreditation. For this purpose, the WHO facilitates the link between the accredited (twinning laboratory) and non-accredited (trainee) laboratories. The accredited laboratory will provide training and technical assistance to the non-accredited laboratory. The accredited laboratory will also offer HIVDR testing of HIVDR surveillance and monitoring specimens while the non-accredited laboratory is participating in the training scheme.

2.3. **STRUCTURE AND FUNCTION OF THE WHO/HIVResNet LABORATORY NETWORK**

One of the main functions of WHO-accredited laboratories is to genotype HIV specimens according to WHO requirements either from serum, plasma or dried fluid spots (DFS). The global WHO/HIVResNet Laboratory Network of genotyping laboratories is being developed by WHO to deliver accurate and comparable genotypic results for HIVDR surveillance and monitoring. The results will be stored in national, regional and global databases and analyzed to
facilitate targeting of resources to minimize the development and spread of drug resistance, and
to guide decisions of policy makers on ART at the national, regional and global level.

The WHO/HIVResNet Laboratory Network aims to ensure high-quality data by:

- developing and updating laboratory guidance to describe the complete process of
  specimen collection, handling, shipment and storage, and HIVDR testing (Annex 1-2);
- developing and coordinating an integrated and harmonized quality assurance scheme
  that operates in all accredited laboratories within the Network (Annex 3);
- assisting in capacity building and in training of laboratories that are seeking to improve
  their infrastructure and ability to achieve WHO accreditation;
- engaging in research to develop simple and affordable methods for HIVDR testing;
- assisting WHO in the assessment of laboratories seeking WHO accreditation.

The Network is coordinated by the WHO, to provide countries and regions with technical
assistance. The network is a key WHO implementing partner for the global Strategy for
HIVDR prevention, surveillance and monitoring. The Network has been largely supported by
the members' host governments, nongovernmental organization, foundations, and other
contributions.

A laboratory is accredited with full membership in the Network when it has demonstrated
that it meets the criteria listed in this document. A laboratory that is awarded full membership
must demonstrate, at regular intervals, that it continues to meet the criteria. The main
responsibility of the full members of the WHO/HIVResNet Laboratory Network is to provide
high-quality sequence data to the Network in a timely manner. Assessment for full membership
takes place through review of procedures and documentation, genotyping results produced by
the laboratory, laboratory assessment visits conducted by the WHO Advisory Group, and
successful participation in a quality assurance system recognized by the WHO. The categories
of full membership are:

- specialized, regional and national laboratories
- twinning laboratories

A laboratory may participate in one or more of these categories.

The WHO/HIVResNet Laboratory Network consists of three levels of institutions that perform
HIVDR genotyping, which are national, regional and specialized. In addition to these three
categories, network full membership can also be achieved by accredited laboratories acting as a twinning laboratory in the WHO/HIVResNet Laboratory Network. The overall structure of the Laboratory Network is shown in Figure 1:

![Figure 1. Structure of the HIVResNet Laboratory Network.](image-url)

### 2.3.1. National HIVDR Laboratories

**Requirements**

A NDRL is a national institution designated by the national MOH and *accredited* by the WHO for the purpose of supporting the country's HIVDR surveillance and monitoring surveys. Upon such recognition by the WHO, a NDRL becomes a member of the WHO/HIVResNet Laboratory Network. Although a NDRL is preferably a public health laboratory, with an active role in HIV surveillance, other types of laboratories may also be designated by the MOH as candidate laboratories for HIVDR genotyping.

**Tasks and Responsibilities**
1. The NDRL conducts genotyping of specimens collected during HIVDR surveillance and monitoring surveys and provides accurate HIV sequences to the National HIVDR Database in a timely manner.

2. Performs viral load testing for all HIVDR monitoring surveys; until such time as a WHO quality assurance system is in place for HIV viral load testing and by default as monitoring surveys use remnant specimens it is unlikely that sufficient remnant specimens will be available to be divided for HIVDR testing at the accredited genotyping laboratory and at a second viral load testing facility.

3. The NDRL participates in a WHO-recognized quality assurance program for genotyping and the lab is able to support the cost of the testing and shipment of the annual proficiency panel.

4. The NDRL alerts the WHO HIVResNet Laboratory Network about any HIV isolate that cannot be sequenced and forwards the isolate to a regional or specialized laboratory.

5. Send HIV genotyping results, in a FASTA file, to the national HIVDR working group, to the WHO regional and headquarters virologist for ongoing quality assurance.

6. To support the county in performing HIVDR analysis using the WHO list of transmitted drug resistance for surveys of transmitted DR-HIV and the Stanford HIVDR algorithm for HIVDR monitoring surveys and surveys to assess HIVDR in infants less than 18 months of age.

### 2.3.2. Regional HIVDR Laboratories

**Requirements**

A RDRL is an institution designated by the national MOH and accredited by the WHO for the purpose of supporting the region's HIVDR surveillance and monitoring surveys. Upon such recognition by the WHO, the RDRL becomes a member of the WHO/HIVResNet Laboratory Network. Ideally, there should be at least one accredited RDRL in each WHO region. The presence of more than one laboratory for each region may be warranted. The RDRL should preferably be located in the same region as that of the surveyed countries. Experience as public health laboratories, although not compulsory, is an asset.

**Tasks and Responsibilities**

1. The RDRL functions as a genotyping facility for countries within the region that do not have an accredited NDRL. It should provide support and back-up to at least two NDRLs
in countries that are implementing WHO-recommended HIVDR surveillance and monitoring surveys.

2. Performs viral load testing for relevant HIVDR monitoring surveys; until such time as a WHO quality assurance system is in place for HIV viral load testing and by default as monitoring surveys use remnant specimens it is unlikely that sufficient remnant specimens will be available to be divided for HIVDR testing at the accredited genotyping laboratory and at a second viral load testing facility.

3. The RDRL may serve as the NDRL in its own country.

4. The RDRL, in coordination with a designated Specialized HIVDR Laboratory, facilitates the training, education and capacity-building of laboratory personnel from NDRLs within the region. The RDRL hosts laboratory technicians from candidate laboratories and trains them to become competent in HIV genotyping.

5. Representatives from the RDRL are available to visit the NDRLs for technical assistance when necessary.

6. Representatives from the RDRL are available to participate in assessment of candidate laboratories within the specified region, including on-site inspection visits.

7. Representatives from the RDRL participate in HIVResNet Laboratory Network regional meetings. These meetings will be organized at least once a year and offer an excellent opportunity for discussion of program development and problem solving.

8. A RDRL provides good quality sequence results in a timely manner to the Network.

9. Send HIV genotyping results, in FASTA file format, to the national HIVDR working group, to the WHO regional and headquarters virologist for ongoing quality assurance.

10. To support the country in performing HIVDR analysis using the WHO list of transmitted HIVDR for threshold surveys of transmitted DR-HIV and the Stanford HIVDR algorithm for HIVDR monitoring surveys and surveys to assess HIVDR in infants less than 18 months of age.

2.3.3. Specialized HIVDR Laboratories

Requirements
A small number of laboratories are identified by the WHO based on:

- the excellence of their performance;
- their recognized expertise on selected key topics relevant to the development of the HIVDR Laboratory Network;
- their capacity, resources, commitment and motivation.
Experience as public health laboratories, although not compulsory, is an asset. As with the national and regional laboratories, candidate laboratories are also assessed for accreditation as a SDRL by the WHO.

Tasks and responsibilities

The SDRL must be willing to:

1. be represented in the WHO HIVResNet Laboratory Network Advisory Group and contribute actively to the development of the WHO HIVResNet Laboratory Network;
2. provide support, technical assistance and back-up to National or Regional HIVDR Laboratories, where needed;
3. host laboratory technicians from candidate laboratories and train them to become competent in HIV genotyping, when not possible at a RDRL;
4. provide high quality sequence and HIV viral load results for HIVDR monitoring surveys in a timely manner to the Network;
5. serve as a RDRL to countries within a region that do not have an accredited RDRL;
6. serve as a NDRL to specified countries where there is no national accredited genotyping laboratory, and:
   a. the regional laboratory is not able to assist; and/or
   b. a special relationship between the SDRL and the specified country is already in place.

In addition, SDRLs should actively participate in one or more of the four core activities listed below. These may not be equally distributed between the laboratories, with some laboratories being the sole provider of certain functions, according to availability, commitment and expertise. Nevertheless, each SDRL must be willing to take responsibility for at least one of the four core activities.

SDRL Core Activities

1. Quality assurance system
   - Coordinate the participation of accredited laboratories in a WHO-recognized quality assurance program for genotyping, including proficiency panels (PP).
   - Coordinate the performance evaluation of laboratories participating in any WHO-recognized proficiency testing program.
   - Assist in the development and supply of WHO-recognized PP.
- Harmonize WHO-recognized quality assurance systems and identify methods for attaining comparative results.
- Coordinate the development and distribution of standardized reagents and validation panels for all laboratories in the Network, as needed.

2. Capacity building/training
- Coordinate the development of training materials and educational programs for laboratories within the HIVResNet Laboratory Network.
- Organize technical workshops at the regional level, as necessary.

3. Operational research
- Participate in collaborative studies to develop and validate methodologies aimed at improving the feasibility of genotype testing under field conditions.
- Participate in research aimed at improving the sensitivity, specificity, applicability, turn-around-time and reporting of HIVDR testing in surveillance and monitoring surveys of adult and pediatric populations.

4. Dried fluid spot activities

Function as a genotyping reference laboratory for dried fluid spot (DFS) specimens by:
- coordinating the development of a validated protocol for DFS specimens and sharing the protocol among the laboratories in the Network, in order to reach consensus;
- performing HIVDR testing for countries without an accredited laboratory for DFS testing;
- coordinating the development of a DFS-based PP (e.g. dried plasma or blood spots) which is more affordable and easier to ship than standard frozen plasma.

2.3.4. WHO HIVResNet Laboratory Advisory Group

The Advisory Group includes a select number of experts that represent the SDRLs and RDRLs. The coordination of the HIVResNet Laboratory Network is carried out by the WHO in consultation with the Advisory Group. Each of the WHO Regions has a Regional Laboratory Coordinator, who reports to the laboratory coordinator of the HIVResNet Laboratory Network at the WHO Headquarters in Geneva, Switzerland. The HIVResNet laboratory coordinator shares results with the Advisory Group. Quality assurance of results generated by network laboratories will be assured by the regular feeding of results, requests and queries, and feedback of analysis, comments and technical advice.
**Tasks and Responsibilities**

1. Harmonize and support the HIVResNet Laboratory Network by providing technical advice, assistance with advocacy and resource mobilization.

2. Assist in the assessment and strengthening of laboratories that have the capacity to function as National, Regional or Specialized HIVDR Laboratories.

3. Meet yearly to review the technical performance of the Network and provide assistance in policy formulation.

4. Provide updates to the Laboratory Strategy document, as needed.

**2.3.5. Role of the WHO**

The WHO has four specific areas of responsibility.

1. **Coordination**
   - Facilitate the linkages, communication and flow of data between NDRL, RDRL and SDRL, when necessary.
   - Organize laboratory assessments and accreditations.
   - Organize the annual meeting for RDRLs and SDRLs.
   - Communicate information relevant to the Network, as needed.
   - Assure quality of results by reviewing sequences forwarded by network laboratories and supporting country and regional analyses.

2. **Financial Support**

   Although the SDRL, RDRL and often the host governments bear much of the financial responsibility for assistance to countries in conjunction with this project, the WHO will consider funding the following as needed:
   - costs related to the initial laboratory assessment visit including the cost of travel and per diem of a laboratory expert identified within the Network to visit the candidate NDRL or RDRL. Subsequent visits for the provision of technical assistance may also be supported;
   - shipment of PP to NDRLs and RDRLs in the context of the proficiency testing scheme;
   - small operational research studies related to core Network priorities (e.g. DBS stability)
• letters of endorsement for grant applications may be provided by the WHO to the NDRLs, RDRLs and SDRLs, with regard to obtaining financial support from other sources.

3. **Formal status**

As SDRLs, RDRLs and NDRLs are not formally designated by the WHO as collaborating centers, they have no "official" WHO status. An official relationship, however, may be important for continued governmental funding of these laboratories and international recognition of their work.

4. **Logo**

The SDRL/RDRL network provides work on behalf of the WHO. In order to obtain recognition for its service, it may be possible for a SDRL/RDRL to use a logo specifically designed for the HIVResNet Laboratory Network, which will state the year of the laboratory accreditation.

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3. **Laboratory Accreditation in the WHO/HIVResNet Network**

Accreditation provides documentation that the laboratory has demonstrated the capacity to provide quality assured HIV protease and reverse transcriptase sequences to the National HIVDR database in a timely manner. The accreditation process also provides a learning opportunity, a mechanism for identifying resource and training needs, and a measure of progress.

Evaluation for full membership takes place through laboratory assessment, review of procedures and documentation, review of genotyping validation and proficiency panel testing results and successful participation in a WHO-recognized quality assurance system. When a laboratory becomes accredited, it is awarded membership in the Global WHO/HIVResNet Laboratory Network. A member laboratory must demonstrate that it continues to meet the criteria for an accredited laboratory at regular intervals.
3.1. ACCREDITATION AND SCORING SYSTEM

The accreditation of HIVDR laboratories will be done in the context of the WHO Global and Regional Strategy for Prevention, Surveillance and Monitoring of HIVDR, which is a component of the strategic information for achieving Universal Access to HIV Prevention, Treatment and Care. The need for Regional and Specialized HIVDR laboratories is based on the Global and Regional Strategy.

To apply for WHO accreditation, laboratories must meet the national, regional, or specialized mandatory application criteria (see Table 1), which will be verified during the assessment audit before accreditation can be awarded. To obtain accreditation, each lab must achieve a passing score (e.g. ≥ 85/100 for NDRL) on the accreditation criteria and pass the WHO recognized PP test. Evidence is required that the laboratory actually meets the criteria.

Details of the minimum requirements for application and criteria for accreditation as a NDRL, RDRL, or SDRL can be found in Annex 4.

3.1.1. Criteria for application and accreditation of WHO/HIVResNet Twinning Laboratories

A candidate twinning laboratory must first be accredited by the WHO before it can become a twinning laboratory in the WHO/HIVResNet Laboratory Network and offers training and technical assistance to laboratories that are not yet accredited. In order to be considered for accreditation, a laboratory must meet the application criteria described below.

1. Appropriately equipped laboratory (see Annex 4 for SDRL)
2. Adequate experience in HIVDR genotyping (see Annex 4 for SDRL)
3. Adequate experience in providing training (see Annex 4 for SDRL)
4. Available to provide training and support to one or more candidate HIVDR laboratories at the national or regional level that have not achieved WHO accreditation and who intend to improve their expertise.
## Table 1: Summary table of application and accreditation criteria for NDRL, RDRL and SDRL

(For further explanation of the criteria, refer to Annex 4)

### A. Application criteria

<table>
<thead>
<tr>
<th>National HIVDR Laboratories</th>
<th>Regional HIVDR Laboratories</th>
<th>Specialized HIVDR Laboratories</th>
</tr>
</thead>
<tbody>
<tr>
<td>Designation by the Ministry of Health.</td>
<td>Letter of agreement from the Ministry of Health.</td>
<td>Laboratory conducts original research in HIVDR.</td>
</tr>
<tr>
<td>Laboratory experience:</td>
<td>Laboratory experience in HIV genotyping:</td>
<td>Laboratory experience in HIV genotyping:</td>
</tr>
<tr>
<td>• &gt;1 year in sequence-based genotyping of HIV or other RNA viruses.</td>
<td>• &gt;3 years experience;</td>
<td>• &gt;5 years experience;</td>
</tr>
<tr>
<td>• ≥ 100 specimens genotyped annually</td>
<td>• ≥ 200 specimens genotyped annually;</td>
<td>• ≥ 300 specimens genotyped annually in the last three years;</td>
</tr>
<tr>
<td></td>
<td>• adequate general knowledge of sequencing, including techniques other than commercially available kits.</td>
<td>• &gt;2 years experience in performing non-commercial HIVDR assays.</td>
</tr>
<tr>
<td>National Strategy for the WHO HIVDR surveillance and/or monitoring developed.</td>
<td>Regionally recognized experience and leadership in HIV laboratory science.</td>
<td>Internationally recognized experience and leadership in HIVDR testing.</td>
</tr>
<tr>
<td>Adequate experience in provision of training and establishment of collaborations in laboratory sciences in the last three years</td>
<td></td>
<td>Adequate experience in the provision of training and the establishment of collaborations in HIVDR in the last five years.</td>
</tr>
<tr>
<td>Administrative and financial sustainability of the laboratory; capacity to seek funds for carrying out regional HIVDR reference service activities.</td>
<td></td>
<td>Administrative and financial sustainability of the laboratory and the capacity to support WHO HIVResNet activities through the laboratories core budget and/or to secure external funds when required</td>
</tr>
<tr>
<td>Adequate expertise of laboratory personnel with a minimum of two laboratory technicians trained in HIVDR genotyping.</td>
<td></td>
<td>Adequate expertise of laboratory personnel with a minimum of two laboratory technicians trained in HIVDR genotyping; willingness to participate in lab site visits under the direction of WHO HIVResNet.</td>
</tr>
<tr>
<td>All standard operating procedures must be available and properly implemented.</td>
<td>All standard operating procedures must be available and properly implemented.</td>
<td></td>
</tr>
</tbody>
</table>
### B. Accreditation criteria

<table>
<thead>
<tr>
<th>National HIVDR Laboratories</th>
<th>Regional HIVDR Laboratories</th>
<th>Specialized HIVDR Laboratories</th>
</tr>
</thead>
<tbody>
<tr>
<td>WHO-recognized proficiency panel testing passed successfully.</td>
<td>WHO-recognized proficiency panel testing passed successfully.</td>
<td>WHO-recognized proficiency panel testing passed successfully.</td>
</tr>
<tr>
<td>Designation by the Ministry of Health.</td>
<td>Letter of agreement from the Ministry of Health.</td>
<td>Laboratory conducts original research in HIVDR.</td>
</tr>
<tr>
<td>Complete laboratory infrastructure and equipment for genotyping*.</td>
<td>Complete laboratory infrastructure and equipment for genotyping*.</td>
<td>Complete laboratory infrastructure and equipment for genotyping*.</td>
</tr>
</tbody>
</table>
| Adequate expertise of laboratory personnel with a minimum of one dedicated laboratory professional in HIVDR genotyping*. | Adequate expertise of laboratory personnel with a minimum of one dedicated laboratory professional in HIVDR genotyping*. | Laboratory experience in HIV genotyping:  
  • >5 years experience;  
  • ≥ 300 specimens genotyped annually in the last three years;  
  >2 years experience in performing non-commercial HIVDR assays. |
| The laboratory has a clear and accountable management structure with financial sustainability*. | The laboratory has a clear and accountable management structure with financial sustainability*. | Internationally recognized experience and leadership in HIVDR testing. |
| Laboratory experience in genotyping*:  
  • >2 years of experience in HIV genotyping or in sequence-based genotyping of RNA viruses other than HIV;  
  • ≥100 HIV specimens, HIV or other RNA virus, genotyped by sequencing annually. | Laboratory experience in HIV genotyping:  
  • >3 years experience;  
  • ≥ 200 specimens genotyped annually; adequate general knowledge of sequencing, including techniques other than commercially available kits. | Adequate experience in the provision of training and the establishment of collaborations in HIVDR in the last five years. |
<p>| Laboratory procedures demonstrate the use of standards of operational procedures*. | Regionally recognized experience and leadership in HIV laboratory science. | Administrative and financial sustainability of the laboratory and the capacity to support WHO HIVResNet activities through the laboratories core budget and/or to secure external funds when required. |
| Adequate experience in provision of training and establishment of collaborations in laboratory sciences in the last three years | Adequate expertise of laboratory personnel with a minimum of two laboratory technicians trained in HIVDR genotyping; willingness to participate in lab site visits under the direction of WHO HIVResNet. | |
| Administrative and financial sustainability of the laboratory; capacity to seek funds for carrying out regional HIVDR reference service activities. | All standard operating procedures must be available and properly implemented. | |</p>
<table>
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<tr>
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<td></td>
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<td></td>
</tr>
<tr>
<td></td>
<td>All standard operating procedures must be available and properly implemented.</td>
<td></td>
</tr>
</tbody>
</table>

*These criteria are scored by WHO and the advisory group to categorize laboratories in 1) accredited (≥ 85/100) and 2) not accredited (< 85/100)*
3.2. CATEGORIES OF WHO ACCREDITATION

3.2.1. Candidate laboratory
A candidate laboratory is an institution at the national, regional or global level that has expressed interest in joining the WHO/HIVResNet Laboratory Network and has fulfilled all of the application criteria.

3.2.2. Accredited laboratory
To achieve accreditation, a lab must meet the application criteria, achieve the passing score of ≥85 on the accreditation criteria and pass a WHO recognized PP test. The accreditation is for one year and re-assessment for the continuation of accreditation will occur on an annual basis.

3.2.3. Laboratories failing to achieve accreditation
A laboratory that does not meet the application criteria or, in case of candidate NDRL, a laboratory that meets the application criteria, but receives a score of <85/100 on the accreditation criteria and/or does not pass the PP test is considered not accredited. Arrangements must be made for an accredited laboratory to perform tests on all specimens. A laboratory that does not achieve accreditation may work with the Regional Laboratory Coordinator or another accredited laboratory to:
- identify areas where improvement is needed
- develop and implement a work plan
- monitor laboratory progress
- continue steps to achieve accreditation.

A laboratory with the status of "not accredited" is eligible to re-apply for the accreditation assessment between one and two years after the previous assessment, if the laboratory expects that all criteria can be met. If the laboratory does not re-apply within two calendar years of the previous assessment, it will no longer be eligible to re-apply.

3.2.4. Suspended laboratory
An accredited laboratory that does not pass the yearly proficiency panel as part of its reaccreditation process has the opportunity to repeat the panel. If the laboratory has passed two previous panels, they may continue to test specimens unless an investigation after the panel failure reveals a systematic problem. If despite retesting the laboratory is unable to receive a
passing score on the proficiency panel, the laboratory is suspended. After receiving the status of suspension, a laboratory may re-apply for accreditation only once and at the discretion of the WHO.

### 3.3. ACCREDITATION PROCEDURE

The procedure to award accreditation is divided into four different phases.

#### 3.3.1. Application phase

An application process has been developed in order to maximize the efficiency of the assessment. Prior to performing an assessment, a set of application criteria is used to determine if all crucial elements that must be evaluated during the assessment are in place.

An **Application checklist (Annex 4)** for candidate NDRL/RDRL/SDRL is publicly available through the WHO website. This will allow laboratories to perform a self-assessment to determine their readiness for assessment. This checklist is intended to provide guidance to the laboratory regarding the requirements for proceeding to the laboratory assessment site visit.

The application checklist, including items specified on the checklist, should be sent to the WHO. This can be done either directly to the HIV Global HIVDR Program (WHO headquarters) or through the WHO Country/Regional Office. All application criteria should be fulfilled, and requested documentation provided, in order for the laboratory to be considered for further assessment. If the initial application is deficient in any required documentation, the laboratory can apply again when the missing elements have been provided. The WHO headquarters will notify the applicant as to whether all criteria have been met and if so, will begin the assessment procedure. The application review process usually takes between one to two months.

#### 3.3.2. Assessment phase

If the application is considered satisfactory, the candidate laboratory will:

- Obtain a pre-assessment questionnaire (**Annex 5**) from the WHO website and complete it in its entirety. This questionnaire is used to collect information about the laboratory including standard operating procedures (SOPs), specific training of personnel and evidence confirming the laboratory’s adherence to expected performance standards.
questionnaire should be returned to the WHO, along with all the requested materials, including SOPs or laboratory protocols as requested in the questionnaire.

- After the questionnaire and requested documentation have been reviewed and found to be satisfactory, the WHO will organize the shipment of a WHO-recognized PP to the candidate laboratory.

- If the candidate laboratory achieves a passing score on the PP, an on-site assessment visit will be organized by the WHO and/or a representative from the HIVResNet Laboratory Network Advisory Group. During this visit the questionnaire will be audited. In special circumstances, the WHO headquarters, in consultation with the Advisory Group, may waive the site visit.

3.3.3. Accreditation phase

The WHO and the HIVResNet Laboratory Network Advisory Group will evaluate the laboratory assessment documents and provide a decision on accreditation. Accreditation is the responsibility of the WHO following consultation with the Advisory Group. The WHO and the Advisory Group will meet yearly and evaluate the performance of candidate laboratories, and make decision whether assessed laboratories meets the criteria for WHO accreditation. Based on this evaluation, a candidate NDRL/RDRL/SDRDL will be granted a status of “accredited”, or “not accredited.” Accredited laboratories become members of the HIVResNet Laboratory Network, and is specific for assay and specimen type (i.e. TruGene, ViroSeq or in-house, and for plasma and/or serum and/or DBS).

3.3.4. Maintain accreditation status and membership in network

The accreditation status of all accredited laboratories will be reviewed annually. This review will be based on the laboratory’s performance during the preceding year, the results of continued proficiency testing (at least once per year) and will be supplemented by a field assessment visit when needed. Accreditation is awarded for the upcoming calendar year by the WHO headquarters in consultation with the Advisory Group, and is specific for assay and specimen type (i.e. TruGene, ViroSeq or in-house, and for plasma and/or serum and/or DBS).

- Accredited laboratories submit all final sequences from HIVDR surveys to WHO headquarters for analysis. All data are reviewed annually for overall quality.
• The results from a PP provided by a WHO-approved quality assurance program must be submitted and evaluated yearly.

• Ideally, all NDRLs should be visited by WHO representatives (from the Regional Offices and/or HQ), the Regional Laboratory Coordinator and/or by WHO/HIVResNet Advisory Group representatives at least once per year for an annual review visit. The review visit can be waived at the discretion of the WHO in consultation with the Advisory Group. It is expected, however, that even a high performing NDRL or RDRL should receive at least one accreditation visit every three years.

3.3.5. Reporting of accreditation results and annual review of accreditation

It is the responsibility of the WHO Regional Office to make an official announcement of the accreditation and annual review results. The WHO Regional Office should inform:

• WHO headquarters Global Laboratory Network coordinator
• Advisory Group
• director of the laboratory
• director of the institute to which the laboratory belongs
• national HIVDR working group
• national authorities through the office of the WHO representative.

A list of currently accredited HIVDR genotyping laboratories can be found on the WHO website: http://www.who.int/hiv/topics/drugresistance/laboratory/en/index.html.
Figure 2: Overview of the WHO accreditation process. Candidate laboratories receive accreditation visits and perform proficiency panels. After review of their application by WHO and its advisory group, a decision is made to accredit or not accredited the laboratory based on criteria outlined in this document. Accredited laboratories must pass a proficiency panel yearly. Failure to pass the proficiency panel during the reaccreditation phase will result in loss of accreditation and designation as a suspended laboratory. Once a laboratory is suspended, it can only reapply for accreditation after consultation with the advisory group.

4. Guidelines for the Return of Results to National HIVDR Working Groups and to WHO

After completion of HIVDR testing, the accredited laboratory returns results to the national HIVDR working group, as well as the WHO region and WHO headquarters. Genotyping results are returned in FASTA format to permit uploading into the WHO HIVDR Database. Sequences should be returned in a single aligned FASTA file; sequences should be trimmed by the laboratory so that data include only the protease and RT region and of equal length. Gaps and insertions should be removed. In addition to the FASTA file laboratories may choose to return an interpretation using the Stanford algorithm for HIVDR monitoring surveys and the WHO
mutations list for surveys of transmitted drug resistant HIV (Stanford CPR tool). Additionally, laboratories return the completed HIVDR specimen manifest forms to national HIVDR working groups for countries completing WHO HIVDR monitoring surveys.

4.1. DATA OWNERSHIP

National, regional, and specialized laboratories are reminded that results of national HIVDR surveillance and monitoring activities belong to the country from which the specimens were obtained and sequence results may never be published under any circumstances without the express consent of the country. Additionally, specimens may not be used for research projects for which they were not initially intended and any remnant specimens must be destroyed after the results have been finalized and quality assured by the national programme, the WHO region and WHO headquarters or per CAP guidelines, where applicable.

4.2. DATA FLOW FROM ACCREDITED LABORATORIES

The WHO HIVDR quality-assurance process incorporates quality-control functions for sequences and complements the WHO HIVResNet laboratory network quality-control efforts. The data flow permits communication between accredited laboratories, countries and the WHO region and headquarters to solve potential problems and to build capacity at country level. VL and HIVDR testing results are first sent from the genotyping laboratory to the HIVDR national working group, and the WHO regional and headquarters virologist. There are queries and cleaning of data, if necessary, and discussions between the regional and headquarters virologist, the accredited laboratory and country counterparts to arrive at the final agreed upon quality-assured dataset. Once the final quality assured dataset has been finalized it is sent to the country WG by the accredited drug resistance testing laboratory. Only the final quality assured dataset is used in country analyses. Figure 3 provides an overview of the data flow process.

Figure 3. Data flow for viral load and HIVDR genotyping from WHO accredited national, regional or specialized laboratories to national HIVDR working groups.
Viral Load and HIVDR Data Flow and Processing

Genotyping Laboratory

HIVDR National Working Group

Regional & Headquarters Virology

Queries and cleaning data
Laboratory data
Results and analyses
Identification of mutations associated with HIV drug resistance (HIVDR) is performed by nucleotide sequence analysis (genotyping) of relevant portions of the HIV genome, typically the complete protease region and most of the reverse transcriptase (RT). In addition to using this profile for HIVDR determinations, the genetic profile can also provide information on HIV-1 subtype, although complete characterization of some recombinant forms may not be possible.

Genotyping by population sequencing identifies the predominant virus populations in the viral quasispecies. An individual variant sequence must be present at levels above approximately 20%, depending on the method used, to be detected. This means that if a particular variant in the virus population of a specimen is present at a level below the threshold of detection for the assay method, it will not be detected reliably. More sensitive technologies, for instance real-time allele-specific polymerase chain reaction (PCR) or hybridization, have been developed recently. However, most of these methods are only applicable to detection of mutations at a limited number of positions in the sequence and do not result in a complete genotypic profile.

Genotyping identifies mutations associated with reduced susceptibility to one or more antiviral drugs. It has been demonstrated that access to genotyping results can be useful in the clinical management of HIV infection by providing information that can be used to guide the selection of appropriate subsequent therapies. Since the number of mutations known to be associated with HIVDR 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. The algorithms have been developed mainly using information on HIV-1 subtype B strains, although it is recognized that the natural polymorphisms in non-subtype B strains can influence the final results of drug resistance interpretation algorithms. The WHO HIVResNet has recently published a list of mutations suitable for surveillance of transmitted DR-HIV (Bennett DE et al, PLOS One, 2009). The Stanford HIVDR algorithm is used for monitoring surveys and for surveys to assess HIVDR in infants less than 18 months.

**Specimen types for HIVDR genotype testing and specimen choice**

Presently, the specimen type most commonly collected for HIVDR genotyping is plasma. For HIVDR surveillance surveys appropriate specimen types include plasma, serum or dried blood spots, dried serum spots, or dried plasma spots. For HIVDR monitoring surveys, remnant
plasma from a routine blood draw is the most common specimen type and may be used at both baseline and endpoint. Dried blood spots (DBS) may be considered for HIVDR monitoring surveys as a baseline specimen type but are not suitable endpoint specimens because on average population on ART will have lower viral loads and there exists significant concern that patients with viral loads between 1,000 and 5,000 copies/ml will be misclassified as undetectable when in reality their viral load is >1,000 copies/ml. For additional information including recommended procedures for collection, storage, shipping, and processing of DBS (as baseline monitoring specimens or for HIVDR surveillance) for HIVDR genotyping, refer to the WHO manual for HIV drug resistance testing using dried blood spot specimens: 

Dried plasma spot (DPS) and dried serum spot (DSS) specimens are not appropriate specimen types for HIVDR monitoring surveys.

**Selection of specimen types for surveillance of transmitted HIVDR and monitoring of HIVDR emerging during treatment**

The WHO HIVDR surveillance and monitoring methods are based on the genotyping of remnant specimens collected routinely for another purpose:

- for **surveillance of transmitted HIVDR** remnant HIV diagnostic specimens or specimens collected for HIV serosurveillance are most commonly used.

- for **monitoring of HIVDR emerging during ART**, clinical specimens routinely collected for CD4 counts or full blood counts are often used. In HIVDR monitoring surveys remnant specimens approximately 150 patients initiating first line ART are genotyped. Initiation of ART is referred to as baseline. At baseline, only genotyping is performed for the survey. After 12 months on ART remnant specimens from routine clinical blood draws are subjected to viral load testing. Specimens with viral loads >1,000 copies/ml are subjected to genotyping. At the endpoint on ART at 12 months or at time of switch to second line therapy, remnants from the same specimen are used for both viral load testing and genotyping.

- Selection of specimen type is based on the specimen types routinely collected, the remnant specimen volume available, and the processing and shipping constraints. Clinical and basic HIV surveillance requirements must always take precedence over
provision of specimens for HIVDR surveys. This will mean that in many settings no processing of the remnant specimen for HIVDR genotyping can be done until after routine testing is complete.

**SPECIMEN SELECTION**

**Plasma**

Plasma has been the conventional specimen type used for HIVDR testing in research and clinical applications. Tubes of anti-coagulated blood are used to collect specimens for CD4 counts, which means in many clinical settings it may be possible to separate plasma from remnant CD4 specimens for the purpose of HIVDR monitoring, but separation will generally not be possible until after the CD4 count has been performed.

The presence of anticoagulants in the blood collection tubes prevents the formation of a blood clot, which can absorb a proportion of the virus present in the blood. The prevention of blood clot formation helps to maximize the amount of virus that is available for amplification and genotyping. This is an important consideration for specimens collected from treated individuals in which viral loads may be relatively low. However, RNAses in the blood remain active to degrade HIV RNA until the plasma is separated and frozen or dried onto filter paper as a "spot". Degradation is more rapid at higher temperatures, so separation and freezing or spotting of plasma or whole blood within a reasonable time frame is important.

Research studies have indicated that success of RT-PCR amplification of genetic material from virus in plasma is dependent on several factors including viral RNA load, time from blood collection to plasma separation, condition of the plasma after separation (specifically presence or absence of hemolysis), time from plasma separation to freezing, storage temperature, and the time interval before extraction, amplification, and genotyping. It is important that frozen plasma specimens are shipped on dry ice and not thawed until genotyping is performed, because each freeze-thaw cycle is detrimental to amplification. Evidence regarding amplification from dried plasma spots is more limited, but it is reasonable to assume that quick centrifugation and separation are also important for preparation of these specimens. Plasma should be selected as the HIVDR genotyping specimen type only in settings where appropriate processing and shipping are possible.
Serum

Since serum is the specimen type generally used for HIV diagnostic testing or HIV serosurveillance, it might be easier to organize the collection of serum specimens for HIVDR surveillance and monitoring. Blood from which serum is separated is drawn into a tube without anticoagulants, and the formation of the clot, which absorbs some of the HIV, lowers the amount of virus available for genotyping. Studies have shown that viral load in serum is initially lower than in plasma by as much as tenfold. As with plasma, RNAses may degrade HIV RNA until the serum is separated and frozen, and degradation is more rapid at higher temperatures. It is important that separation and freezing of serum specimens are managed within the recommended time frame.

The success of RT-PCR amplification from sera depends on the same factors described above for plasma.

Dried blood spots (DBS)


Recommendations for specimen selection for HIVDR surveillance and monitoring

- If routine specimens are collected in EDTA- or citrate-anti-coagulated tubes, consider collection of plasma or DBS. The choice depends on volume available and other storage/shipment constraints.
- If whole blood is drawn in the absence of an anticoagulant, consider collection of serum, DBS, only if blood spotting can be performed immediately after the blood draw.

PLASMA AND SERUM SPECIMEN PROCESSING

It is important that specimens are processed and stored as soon as possible to ensure the quality of the specimens for genotyping (see figures 1 and 2). Centrifugation, pipetting, and aliquoting
must be performed following standard laboratory biosafety precautions at a laboratory equipped to manipulate infectious clinical samples, with adequate sample storage and inventory facilities.

Collection
During the time between collection and separation, whole blood specimens should be kept at room temperature (15-30°C). If room temperature is >30°C, an isotherm box should be used to store specimens between 15°C and 30°C.

Centrifugation and separation

HIVDR Surveillance
Whole blood specimens collected for HIVDR testing should be left at room temperature (15-30°C) from the time of collection until centrifugation and separation. After separation, plasma/serum specimens should be maintained constantly at refrigeration temperature (4°C) until aliquots are frozen. Plasma/serum for genotyping must be processed and frozen at -20°C to -80°C within 48 hours after the blood draw. Note: freezing at -80°C is preferable but -20°C is acceptable and not having a -80°C freezer is not an impediment to storing specimens for HIVDR surveillance and monitoring surveys.

All processing, centrifugation, pipetting, and aliquoting, must be performed following standard laboratory bio-safety precautions at a local laboratory equipped to manipulate infectious clinical specimens, with adequate specimen processing and storage facilities. Approximately 1 ml of plasma can be obtained from 2-3 ml of whole blood. Plasma specimens should be considered potentially infectious and transported according to international regulations.

Transport methods depend on a country’s infrastructure. Frequently, field staff is responsible for transporting clinical specimens from an ART site to a central laboratory for CD4 counts or other tests. Additional resources may be required to provide more timely transport for specimens if processing and storage conditions at the ART site are not suitable.

Because plasma/serum for HIVDR testing should be frozen within 48 hours after the blood draw, plasma processing should be completed as quickly as possible.

Plasma specimens should only be frozen at the sentinel monitoring site when maintenance of the frozen state can be guaranteed during the period of transport to a national storage laboratory or to the HIVDR testing laboratory. "Cooler" boxes and ice packs are never sufficient to maintain the frozen state; dry ice or liquid nitrogen must be used for transport within the country once
specimens have been frozen. If local logistics do not permit proper plasma collection, processing and storage, DBS should be strongly considered as the specimen type for the survey, especially at baseline.

After freezing, if appropriate freezer facilities are available for storage centrally, survey plasma/serum specimens may remain frozen for up to three years before being sent to the genotyping laboratory. Transport to the genotyping laboratory must take place on dry ice or liquid nitrogen, and thawing of plasma specimens should be avoided. Therefore, adequate preparation for shipping should include the use of a courier service experienced in the transport of clinical specimens on dry ice and in topping-up dry ice in route and during processing in customs, or transport in liquid nitrogen containers.

All customs import and export permits must be obtained prior to shipping. An acknowledgement/notification system should be set up involving the Survey Coordinator, the transport system, and the receiving genotyping laboratory, to ensure all specimens are delivered promptly and arrive frozen. Notifications may be sent by email or fax using the shipping manifests for this purpose.

**HIVDR Monitoring Surveys**

Whole blood specimens collected for viral load or HIVDR testing should be left at room temperature (15-30°C) from the time of collection until centrifugation and separation. After separation, plasma specimens should be maintained constantly at refrigeration temperature (4°C) until aliquots are frozen. Plasma for viral load and genotyping must be processed and frozen at -20°C to -80°C within 48 hours after the blood draw. Note: freezing at -80°C is preferable but -20°C is acceptable and not having a -80°C freezer is not an impediment to storing specimens for HIVDR monitoring surveys.

All processing, centrifugation, pipetting, and aliquoting, must be performed following standard laboratory bio-safety precautions at a local laboratory equipped to manipulate infectious clinical specimens, with adequate specimen processing and storage facilities. Approximately 1 ml of plasma can be obtained from 2-3 ml of whole blood. Plasma specimens should be considered potentially infectious and transported according to international regulations.
Transport methods depend on a country’s infrastructure. Frequently, field staff is responsible for transporting clinical specimens from an ART site to a central laboratory for CD4 counts or other tests. Additional resources may be required to provide more timely transport for specimens if processing and storage conditions at the ART site are not suitable.

Because plasma should be frozen for viral load and HIVDR testing within 48 hours after the blood draw, plasma processing should be completed as quickly as possible.

Plasma specimens should only be frozen at the sentinel monitoring site when maintenance of the frozen state can be guaranteed during the period of transport to a national storage laboratory or to the HIVDR testing laboratory. "Cooler" boxes and ice packs are never sufficient to maintain the frozen state; dry ice or liquid nitrogen must be used for transport within the country once specimens have been frozen. If local logistics do not permit proper plasma collection, processing and storage, DBS should be strongly considered as the specimen type for the survey, especially at baseline.

NUCLEIC ACID EXTRACTION FROM LIQUID PLASMA OR SERUM

High quality purified nucleic acid is essential for successful genotyping. Most extraction methods have been evaluated and applied using plasma, and are generally designed to isolate either RNA, DNA, or both, from specific types of specimens. In the selection of the extraction method/technology, the type of clinical specimen to be used and the type of nucleic acid to be isolated should be taken into consideration. Standard nucleic acid extraction procedures can be used to isolate HIV RNA from plasma/serum, either through manual or automated procedures. Many commercially available methods exist and examples of well established procedures for extraction are listed below. Several of these methods can also be used to extract RNA from whole blood (WB).

<table>
<thead>
<tr>
<th>Method (kit)</th>
<th>Supplier</th>
<th>Automated/ Manual</th>
<th>Input volume</th>
<th>Plasma/WB</th>
</tr>
</thead>
<tbody>
<tr>
<td>HighPure</td>
<td>Roche Diagnostics</td>
<td>Manual</td>
<td>200μl</td>
<td>Plasma/WB</td>
</tr>
<tr>
<td>QIAamp RNA blood mini kit</td>
<td>QIAgen</td>
<td>Manual</td>
<td>140μl</td>
<td>WB</td>
</tr>
<tr>
<td>QIAamp viral RNA mini kit</td>
<td>QIAgen</td>
<td>Manual</td>
<td>140μl</td>
<td>Plasma</td>
</tr>
</tbody>
</table>
Extraction recommendations

Use an established, commercially available extraction method that has been validated for the type of specimen being used and the type of nucleic acid to be collected. Minimum input volume should be 100µl of specimen. Larger volumes, up to 1ml are preferred in order to increase the amplification sensitivity of the genotypic assay.

AMPLIFICATION AND SEQUENCING

Once purified, the nucleic acid needs to be amplified by PCR and subsequently sequenced. Presently there are two commercial genotyping kits available, ViroSeq HIV-1 (Abbott Molecular) and TruGene HIV-1 (Siemens Healthcare Diagnostics). Many experienced genotyping laboratories have developed their own in-house ("home-brew") amplification and sequencing procedures and reagents. HIVDR testing through genotyping is a complicated procedure that requires a high level of technical experience and a properly designed molecular laboratory. Given the high number of laboratory manipulations that are involved, the procedure is prone to variation and needs a high level of standardization, both in terms of the persons performing the laboratory test as well as in the analysis and interpretation of the genotyping result. Furthermore, extensive genetic differences of HIV-1 exist between the various HIV-1 subtypes. This means the primers used for amplification and sequencing of specimens must be validated for adequate performance on various subtypes.

The performance of both the commercially available kits on various HIV-1 subtypes has been evaluated and published [11-13]. For in house assays, this information may not always be easily accessible. Given the heterogeneous distribution of the various subtypes throughout the world, it is essential that adequate performance of home-brew protocols as well as commercial assays be demonstrated for a range of subtypes. Both commercial genotyping kits come with dedicated

<table>
<thead>
<tr>
<th>Extraction method</th>
<th>Manufacturer</th>
<th>Automation</th>
<th>Volume</th>
<th>Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>NucliSens®</td>
<td>BioMerieux</td>
<td>Automated/Manual</td>
<td>100µl-1000µl</td>
<td>Plasma/WB</td>
</tr>
<tr>
<td>MagnaPure LC</td>
<td>Roche Diagnostics</td>
<td>Automated</td>
<td>100µl-1000µl</td>
<td>Plasma/WB</td>
</tr>
<tr>
<td>Roche Monitor</td>
<td>Roche Diagnostics</td>
<td>Manual</td>
<td>200µl-500µl *</td>
<td>Plasma</td>
</tr>
<tr>
<td>HIV VL kit</td>
<td>Roche Diagnostics</td>
<td>Manual</td>
<td>200µl-500µl *</td>
<td>Plasma</td>
</tr>
<tr>
<td>ViroSeq™ kit</td>
<td>Abbott Diagnostics</td>
<td>Manual</td>
<td>500µl *</td>
<td>Plasma</td>
</tr>
</tbody>
</table>

* Requires ultra-centrifugation of sample prior to extraction.
software to support the analysis and editing of the electropherograms. This software may not be applicable to the analysis of sequence data obtained from in-house assays.

*For laboratories that are considering implementation of HIVDR genotyping, it is advisable to start by using a commercial kit. Before implementing commercial assays, laboratories should also obtain information on the performance of these assays on the local circulating variants. Once the assays are established, home-brew assays should be evaluated by comparison to the commercial kit procedures prior to implementation for routine use.*

Amplification and sequencing recommendations:

- Kit based sequencing procedures are preferred over home brew assays for laboratories that first initiating genotyping.
- "Home-brew" assays should only be implemented after adequate validation, including evaluation with regard to performance with various HIV-1 subtypes.
- A high level of standardization should be established by laboratories performing genotyping, including staff training, peer reviewed SOP, workflow, etc.
- The minimal regions for which sequence information must be collected are:
  - Protease: codons 10 to 99
  - RT: codons 41 to 240

**DATA MANAGEMENT AND TRACEABILITY OF RESULTS**

All specimen handling and manipulation should be traceable for each step of the procedure. This means that the administrative process should be well defined and described. This includes:

- collection and registration of relevant demographic, epidemiological and clinical information at the moment of specimen collection;
- registration of a unique specimen identification code;
- registration of all subsequent specimen codes while processing the specimen in the laboratory;
- registration of the final result in relation to the original specimen identification;
- all laboratory results, including the raw sequencing data (electropherogram files) should be stored at the laboratory, backed-up regularly and traceable at any moment;
- raw laboratory data must be stored for a minimum of five years.
PREFERRED GENOTYPING METHODOLOGIES

For the HIVDR HIVResNet program, one of three genotypic testing procedures is recommended; several “home-brew” protocols are available with different primers sets for amplification and/or sequencing. Laboratories performing genotyping should be aware that the successful implementation of these procedures is not a trivial process.

Commercial sequencing kits

1. ViroSeq™ HIV Genotyping Kit (Abbott Molecular)

The kit consists of protocols and reagents for sample extraction, amplification and sequencing of the entire protease coding region and most of the RT region (amino acids 1-320). Initial studies demonstrate good results for various subtypes but this is still under investigation [15].

Requirements

- PCR grade laboratory design.
- Ultracentrifuge for concentrating virus.
- Sequence detection hardware - capillary electrophoresis equipment is recommended as it is simple to use and generally well suited for diagnostic use. Training is required for the proper use of the equipment.
- Gel based sequencers are an alternative to capillary systems, but require extensive training and experience and are less suitable for diagnostic use.
- Sequence analysis software (provided by Abbott).

2. TruGene™ HIV-1 genotyping kit (Siemens Healthcare Diagnostics)

The kit consists of protocols and reagents for amplification and sequencing of the protease coding region (amino acids 4-99) and part of the RT region (amino acids 40-250).

Requirements

- PCR grade laboratory design
- Sequence detection hardware (Siemens). Moderate experience and training required, though the system is well suited for diagnostic use.
- Sequence analysis software provided by the company. Performance on non-B subtypes remains under investigation.
3. "Home-brew" sequencing methods

Several laboratories specializing in HIV-1 drug resistance have developed “in-house” methods (often referred to as “home-brew” methods), which use reagents that are not marketed in the form of a genotyping kit. These methods usually require purchasing commercial reagents and (expensive) sequencing hardware is needed.

Requirements

- PCR grade laboratory design.
- Extensive inter-laboratory validation of all aspects of the sequencing procedure, including extraction and sequence hardware.
- Performance on various subtypes may vary per laboratory and is dependent on the primers included in the specific "home-brew" protocol.
- Less standardized reagents and procedures than for commercial kits, both within and between laboratories.

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, particularly in that changes (such as alternative primers) can be more easily implemented when required. 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, a WHO accredited laboratory can assist with protocol development. All laboratories accredited for HIVResNet Laboratory Network should have detailed and approved laboratory protocols for procedures to allow the collection of comparable genotyping information.

HIVDR interpretation

Most of what is currently known pertaining to the more than 150 relevant mutations contributing to clinical resistance, has been derived from studies on subtype B HIV infections.
More recently, consideration has been given to adapting HIVDR interpretation algorithms used in clinical management to accommodate non-subtype B variants. The WHO has published a list of HIVDR mutations to be used for HIVDR surveillance (Bennett DE et al. Drug Resistance Mutations for Surveillance of Transmitted HIV-1 Drug-Resistance: 2009 Update, PLoS one 4(3): e4724. 2009). This HIVDR surveillance mutations list is to be used for all surveys assessing transmitted drug resistant HIV. Accredited laboratories are expected to use this mutations list when reporting results for HIVDR surveillance surveys. For HIVDR monitoring surveys mutations are assessed using the Stanford HIVDR algorithm.
ANNEX 2: Laboratory Safety

Guidelines to prevent transmission of HIV in the laboratory

Universal precautions should be observed for ALL blood and body fluid specimens. These precautions are described in the supplement to the Morbidity and Mortality Weekly Report [25] and further expanded [26, 27] and then updated [28]. Every laboratory should have a copy of these guidelines and observe the recommendations. Additional guidance for safe laboratory practices is provided here.

1. Employ appropriate personal protection equipment (PPE) to prevent skin and mucous membrane exposure when contact with blood or other body fluids of any person is anticipated.
   - Wear gloves when performing venipuncture and other vascular access procedures.
   - Use gloves for performing fingerstick tests on children and adults and/or heelstick tests on infants.
   - Change gloves and wash hands after contact with each patient.
   - Place all specimens of blood and body fluids in containers that will prevent leakage during transport. Avoid contaminating the outside of the container and the laboratory form, which accompanies the specimen. *(Note: Whole blood dried on filter paper has not been shown to present a hazard when mailed in paper envelopes. See the specimen collection and storage section for more information).*
   - Wear gloves when processing blood and body fluid specimens. Remove gloves and wash hands with soap and water upon completion of specimen processing.

2. If hands or other skin surfaces become contaminated with blood or other body fluids, wash them immediately and thoroughly with soap and water.

3. Employ a biological safety cabinet for procedures that have a high potential for generating droplets (blending, sonicating, vigorous mixing).
4. Use mechanical pipetting devices to manipulate all liquids in the laboratory. DO NOT PIPETTE BY MOUTH.

5. Take precautions to prevent injuries caused by needles, scalpels, and other sharp instruments.
   - Do not recap needles, bend or break needles by hand, or remove needles from disposable syringes.
   - Discard all sharp instruments in puncture resistant containers located close to the work area.
   - Limit use of needles and syringes to situations in which there is no alternative.

6. Decontaminate laboratory work surfaces at least daily with a freshly prepared chemical germicide such as a 1:10 dilution of household bleach (this dilution has a final concentration of 0.5% sodium hypochlorite). If bleach is to be used, dilutions should be mixed daily as bleach looses its effectiveness within 24 hours. Other commercially available disinfectants can also be used (dilute as indicated by manufacturer).

7. To decontaminate equipment that may come in contact with blood or body fluids:
   - disinfect refrigerators by cleaning thoroughly and then by wiping with 1:10 dilution of household bleach;
   - disinfect centrifuge components by swabbing head, bowl trunion and carriers with 70% ethanol;
   - autoclave or soak specimen racks in a 1:10 dilution of household bleach for five minutes and then rinse thoroughly with water;
   - discard as hazardous waste any disposable components of instrument systems that come in contact with patient specimens. Clean non disposable components with 70% ethanol;
   - allow disinfectant to remain in contact with surfaces for at least five minutes at an ambient temperature for optimal effectiveness against blood or serum;
   - if equipment needs maintenance, clean and decontaminate it in the laboratory before transporting it to the manufacturer for repair.

8. Use special precautions in handling microbiological laboratory waste, pathology waste and blood specimens or blood products.
• Incinerate or autoclave all waste before disposal in a sanitary landfill. Solutions containing bleach may corrode the autoclave, therefore these solutions may be poured down a drain connected to a sanitary sewer.
• After decontaminating, carefully pour down a drain connected to a sanitary sewer bulk blood, suctioned fluids, excretions, and secretions.

9. Wash hands thoroughly after completing laboratory activities. Remove protective clothing before leaving the laboratory.

In Case of a Spill
To decontaminate spills of blood and body fluids:
• wear disposable gloves;
• cover visible blood or body fluids with paper towels and soak with a 1:10 dilution of household bleach. Allow to stand for at least five minutes;
• discard contaminated towels in infectious waste containers;
• wipe down the area with clean towels soaked in a 1:10 dilution of household bleach.
ANNEX 3: WHO HIVResNet External Quality Assurance and Internal Quality Control

To receive accreditation, a laboratory should participate in a WHO-recognized genotyping external quality assurance program that includes proficiency panel testing (PP). The WHO and its partners and/or selected Specialized HIVDR Laboratories (SDRL) will be responsible for developing and distributing PP to accredited laboratories and those applying for accreditation. Initially, however, laboratories should enroll in an existing, nationally or internationally-recognized PP program. Laboratories should successfully pass a minimum of one PP per year. PP programs should take into account the distribution HIV genetic variants and subtypes in different areas of the world, as well as other virological characteristics.

The recommended characteristics for the HIVDR genotyping PP are to:

- PP should include only HIV viruses derived from diluted clinical samples or cell culture propagation;
- PP should contain samples with several mutant codons in both the RT and protease regions, as well as wild-type codons;
- PP should include a minimum of five different samples;
- various subtypes should be represented in the panel. At least one subtype B and one subtype C virus should be included. At least one non-B, non-C virus should be included.
- samples should have a minimum viral load of 5,000 copies/ml;
- the viruses chosen should be compatible with all commercial assays. All samples from the panel should be validated using all commercial kits before distribution;
- PP may include a maximum of one sample containing an equal mixture of two defined virus variants;
- A minimum of ten laboratories should test each panel and return results for analysis in order to yield a meaningful consensus sequence.

Data analysis and scoring
A consensus sequence is prepared by first aligning the sequences submitted by all participants in the program. At each position in the alignment, the nucleotide, or nucleotide mixture, observed in >80% of the submitted sequences is included in the consensus. If no nucleotide, or mixture,
is observed in >80% of the sequences, then that position is not included as part of the consensus sequence during the analysis.

Nucleotide sequence concordance of each laboratory’s results with the consensus sequences, over the region spanning amino acids 10-99 of protease (PR) and 38-240 of reverse transcriptase (RT), is reported as the number of concordant nucleotides out of the total number of unambiguous bases in the consensus (a maximum of 879).

Concordance at the major and minor drug-resistant mutation (DRM) sites is also determined. DRM sites are defined by IAS-USA and are periodically updated (http://www.iasusa.org/resistance_mutations/). DRM site scores are calculated as the number of concordant DRM codons out of the total number of DRM codons not containing an ambiguity in the consensus. As of December 2009, there are 38 DRM sites in PR and 30 in RT. Laboratories will be provided with a summary of overall sequence concordance and DRM site scores for each specimen in the panel, and average scores for each dataset, and sequence alignments for each specimen PR and RT with discrepancies highlighted and scores calculated.

The definition of “concordance” is dependent on the context and involvement of mixtures:

- When mixtures are not present in either the consensus or the test sequence, the same base must be reported to be considered concordant, whether or not the change results in an amino acid mutation or if it is considered “wild-type” (same as the consensus subtype B reference) or “mutant” (any other amino acid).

- If a mixed base is present in either the consensus or the test sequence, it is treated according to the impact on the encoded amino acid(s), as outlined in Table 1. In addition, for comparisons involving mixtures to be counted as concordant the represented bases in the mixture must be compatible with the unmixed base or corresponding mixture (e.g. R vs. A or C vs. Y, but not G vs. Y or R vs. M).
**Scoring matrix for positions involving mixtures (1 = concordant, 0 = discordant)**

<table>
<thead>
<tr>
<th>Test Sequence</th>
<th>Consensus</th>
<th>Wild-type</th>
<th>Mutant</th>
<th>Mixed* (A, wt)</th>
<th>Mixed (A, mut)</th>
<th>Mixed (B, wt+mut)</th>
<th>Mixed (C, &gt;1 mut)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wild-type</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Mutant</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Mixed* (A, wt)</td>
<td></td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Mixed (A, mut)</td>
<td></td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Mixed (B, wt+mut)</td>
<td></td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Mixed (C, &gt;1 mut)</td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

*mixture types:

A: the mixture results in codons that only encode one amino acid; discrepancies at the mixed base position are not counted

Example: consensus = GTR (WT = GTA), test sequence = GTG; or consensus = ACG, test sequence = ACR; count both as concordant. However if consensus = GAR, test sequence = GAM, count as discrepant.

B: the mixture results in the presence of 2 or more amino acids, one of which is the wild-type.

Example: consensus = AYT (WT = ATT), test sequence = ACT: count as concordant; however if the test sequence = ATT, counted as discrepant.

C: the mixture results in 2 or more amino acids, none of which is the wild-type.

Example: consensus = TWC (WT = ACC), test sequence = WCC or TRC: count as concordant

If frameshift mutations are encountered in any test sequence, they will be handled as follows:

- **Deletion** (missing nucleotide): a dash will be put into the test sequence, and the rest of the sequence aligned against the consensus sequence as normal. When calculating the alignment score, each dash would be given a gap penalty of 10 (i.e. the alignment score reduced by 10) and be flagged in a separate column on each report.

- **Insertion** (extra nucleotide): dashes would be put in the sequence to which the participant sequence is being aligned (i.e. the consensus sequence) and a gap-opening penalty of 10 would be given. This process will be done separately and will not be
represented in sequence form on any of the worksheets and only the scores will be shown. This will also be flagged in a separate column on each worksheet.

- In both these cases these insertions and deletions will not affect the DRM site score (unless they occur in a DRM codon), but would affect the alignment scores and the overall decision on the success/failure of the participant.

Internal Quality Control

Internal quality control (IQC) measures include the use of positive and negative control reagents during sample extraction, amplification and sequencing. Although IQC is commonly intended for these procedures, some measures of good laboratory practice (regarding collection, handling, shipment and storage of samples) need to be stressed during the training of technical personnel and have to be verified during the accreditation process and at subsequent site visits.

Control of each step of the genotyping process is essential. Each new patient sequence should be checked for potential contamination. This may be done by calculating the DNA distance matrix using software provided with any of several available phylogenetic analysis programs (for example BioEdit, Phylip, or MEGA.). This analysis will require training for the local team.

Recommendations:

- IQC, consisting of positive and negative control materials should be present throughout the entire laboratory procedure, from the start of specimen extraction to the final sequencing. For example, it is recommended that a limited number of samples be processed in each extraction, amplification and sequencing round (a maximum of twelve samples including a positive and a negative control). This recommendation pertains in particular to laboratories using manual processes and may not be practical for laboratories that use automated instrumentation for these activities.

- Laboratory results for specimens can only be accepted when the results of the internal controls meet the predetermined acceptance criteria.

- The quality of the sequence should be taken into consideration when accepting or rejecting a laboratory result. Characteristics of acceptable sequences are:
  1. Less than 5% of the raw sequence file requires editing
2. Less than 2% of nucleotides consist of mixed nucleotides.

- Contamination control should be performed for each specimen. The sequence from every specimen must be verified through phylogenetic analysis to be sufficiently different from previously amplified virus sequences, including reference viruses (for example positive control viruses) used in the laboratory. The mechanism by which the laboratory makes this determination may vary depending on the genotyping system being employed. For example, some commercial systems include a tool for making this assessment.
ANNEX 4: Checklist for Application to the WHO for HIV Drug Resistance Laboratory Accreditation

National HIVDR Laboratories

A. IN ORDER TO APPLY FOR ACCREDITATION, THE FOLLOWING MANDATORY CRITERIA HAVE TO BE MET:

<table>
<thead>
<tr>
<th>Ministry of Health designation</th>
</tr>
</thead>
<tbody>
<tr>
<td>National plan for HIVDR surveillance and/or Monitoring implementation</td>
</tr>
<tr>
<td>Minimum infrastructure for HIVDR genotyping in place</td>
</tr>
<tr>
<td>At least one year experience in genotyping HIV or RNA viruses AND &gt;100 specimens tested</td>
</tr>
</tbody>
</table>

B. ADDITIONAL ACCREDITATION CRITERIA TO BE EVALUATED DURING THE ASSESSMENT:

| 1. Laboratory facilities and infrastructure |
| 2. Expertise in assigned laboratory personnel |
| 3. Administrative and financial sustainability of the institution |
| 4. Laboratory experience in genotyping |
| 5. Standard Operation Procedures for all steps of the work |
| 6. Successful participation in External HIVDR Proficiency Testing Programs in the past |

The candidate laboratory will achieve WHO accredited if it meets all the mandatory criteria (A) AND achieves a passing score of ≥85/100 of the additional criteria (B) AND successfully passes the WHO HIVDR proficiency panel programme.
EXPLANATION OF THE APPLICATION MANDATORY CRITERIA (A)

1. Designation by Ministry Of Health

Application of candidate national HIVDR laboratories is a responsibility of the Ministry of Health. The laboratory must be nominated by the MOH as the candidate national laboratory for the purpose of genotyping specimens collected during WHO HIVDR surveillance and monitoring surveys. A letter of nomination from the MOH is required.

2. National strategy for HIVDR surveillance and/or monitoring implementation

The country where the laboratory is located must have a national strategy for the implementation of the WHO recommended HIVDR surveillance of transmitted HIVDR and/or monitoring of HIVDR that emerges during treatment.

3. Minimum laboratory infrastructure and equipment for HIVDR genotyping

The laboratory must have minimum infrastructure for HIVDR genotyping as described below.

- Separation of work areas, with workflow plan consistent with molecular diagnostic work (relevant anti-contamination laboratory spaces for PCR)
- Adequate equipment
- Adequate laboratory and office space available and used efficiently
- Electrical power backup
- Reliable and well-documented specimen logistics and storage procedures and capacity
- SOPs in place covering all aspects of laboratory procedures

4. Laboratory experience in genotyping

The laboratory has a minimum of one year experience in sequence-based genotyping of HIV or other RNA-viruses AND >100 specimens tested.
## ACCREDITATION CRITERIA (B)

### 1. Complete laboratory infrastructure and equipment for genotyping

<table>
<thead>
<tr>
<th></th>
<th>No/Yes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Office and laboratory space is clean, well kept and adequate to current work size</td>
<td></td>
</tr>
<tr>
<td>Appropriate equipment in place, including electric power back up</td>
<td></td>
</tr>
<tr>
<td>Space configuration, workflow and contamination control is consistent with good laboratory practices</td>
<td></td>
</tr>
<tr>
<td>Adequate PCR and sequencing capacity, including editing</td>
<td></td>
</tr>
<tr>
<td>Adequate freezer storage capability</td>
<td></td>
</tr>
<tr>
<td>Reliable and well-documented specimen receipt and storage procedures</td>
<td></td>
</tr>
<tr>
<td>Computational capability, including hardware, software and Internet access</td>
<td></td>
</tr>
<tr>
<td>Equipment is functioning and in good condition</td>
<td></td>
</tr>
<tr>
<td>Equipment is maintained regularly, as recommended, and dates recorded</td>
<td></td>
</tr>
<tr>
<td>Temperature monitoring records are kept regularly for incubators, refrigerators, PCR machine and freezers</td>
<td></td>
</tr>
<tr>
<td>Inventories are maintained and adequate time is allowed for replenishing supplies</td>
<td></td>
</tr>
<tr>
<td>Minimum biosafety level of 2, in the areas where the specimens are handled</td>
<td></td>
</tr>
</tbody>
</table>

### 2. Adequate expertise of laboratory personnel

<table>
<thead>
<tr>
<th></th>
<th>Yes/No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laboratory supervisor with graduate degree and specific training in the area of molecular virology</td>
<td></td>
</tr>
<tr>
<td>Minimum of one dedicated laboratory professional (technician level) with a specified qualification and training (in country and/or external training) in HIVDR genotyping</td>
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</tr>
<tr>
<td>Dedicated safety officer</td>
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<tr>
<td>Sufficient number of trained staff to adequately manage the workload</td>
<td></td>
</tr>
<tr>
<td>Laboratory expertise in editing of viral sequences</td>
<td></td>
</tr>
<tr>
<td>Test results critically reviewed by supervisor</td>
<td></td>
</tr>
</tbody>
</table>

### 3. The laboratory

<table>
<thead>
<tr>
<th></th>
<th>Yes/No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clear and accountable laboratory management structure</td>
<td></td>
</tr>
<tr>
<td>Financial sustainability of the HIVDR activities in the laboratory</td>
<td></td>
</tr>
</tbody>
</table>
### 4. Laboratory experience in genotyping

<table>
<thead>
<tr>
<th>Yes/No</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;1 years of experience in HIV genotyping or in sequence-based genotyping of RNA viruses other than HIV</td>
</tr>
<tr>
<td>≥100 specimens, HIV or other RNA virus, genotyped by sequencing annually</td>
</tr>
</tbody>
</table>

### 5. Demonstrate the use of standard operating procedures (SOP) covering all procedures, including:

<table>
<thead>
<tr>
<th>Yes/No</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Specimen receipt, assessment, and storage</td>
</tr>
<tr>
<td>2. Internal quality control</td>
</tr>
<tr>
<td>3. All steps of genotyping tests, including sequencing</td>
</tr>
<tr>
<td>4. Handling and manipulation of infectious human material, including the handling of infectious waste</td>
</tr>
<tr>
<td>5. Workflow</td>
</tr>
<tr>
<td>6. Detection, containment and control of molecular contamination</td>
</tr>
<tr>
<td>7. Data management</td>
</tr>
</tbody>
</table>

### 6. Proficiency panel testing

Successful participation in HIVDR proficiency testing programs from providers other than the WHO in the past year (copies of the reports are requested)  

In addition, the laboratory must pass a WHO recognized proficiency panel before being accredited (see Annex 3).
Documentation to be submitted to the WHO

The laboratory should submit in hard copy and electronic copy the following documentation:

<table>
<thead>
<tr>
<th>Required documents</th>
<th>Y/N</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Letter of support from the MOH, indicating that the laboratory has been identified to test specimens collected during WHO recommended HIVDR surveillance and monitoring surveys</td>
<td></td>
</tr>
<tr>
<td>2 Maintenance records and service contract for major equipments</td>
<td></td>
</tr>
<tr>
<td>3 Map of the genotyping facility</td>
<td></td>
</tr>
<tr>
<td>4 CVs of genotyping laboratory personnel (including supervisor) documenting qualifications and experience in molecular biology</td>
<td></td>
</tr>
<tr>
<td>6 Description of the management structure of the genotyping laboratory personnel</td>
<td></td>
</tr>
<tr>
<td>7 Information on the financial sustainability of HIVDR genotyping activities in the past years</td>
<td></td>
</tr>
<tr>
<td>8 Record/documentation of the sequencing tests performed in the last two years for HIV or other RNA viruses. Both in house methods and commercial kits will be considered</td>
<td></td>
</tr>
<tr>
<td>9 Copies of the reports of proficiency panel testing from providers other than WHO in the past year</td>
<td></td>
</tr>
<tr>
<td>10 Copies of Standard Operating Procedure (SOPs) including: (1) Specimen receipt, assessment, and storage; (2) Internal quality control; (3) All steps of genotyping tests, including sequencing; (4) Handling and manipulation of infectious human material, including the handling of infectious waste; (5) Workflow; (6) Detection, containment and control of molecular contamination; (7) Data management</td>
<td></td>
</tr>
</tbody>
</table>

Together with the application checklist, the laboratory has to complete a pre-assessment questionnaire of lab equipment and infrastructure. Both the electronic copy and the hard copy of the questionnaire and application checklist, along with the documentation specified on the checklist, should be sent to the WHO Global HIVDR Program c/o: Dr Silvia Bertagnolio, Avenue Appia, 20; HIV/HTM; World Health Organization,1211-Geneva, Switzerland. bertagnolios@who.int; +41 22 7913958
If the initial application is deficient in any required documentation, the laboratory can apply again when the missing criteria have been fulfilled. The WHO HQ will then notify the applicant whether all criteria have been met and will begin the assessment procedure.

Upon completion of the checklist and fulfillment of all the application mandatory criteria, an assessment visit will be scheduled. If the assessment visit shows that the majority of the accreditation criteria are met, WHO will coordinate shipment of a WHO recognized proficiency panel (PP).

The WHO and the Advisory Group of the HIVResNet Laboratory Network will evaluate the performances of the assessed laboratory and provide recommendations regarding accreditation.
Regional HIVDR Laboratories

Documentation to be submitted to the WHO

1. Complete laboratory infrastructure and equipment for HIV genotyping
The laboratory should have a well functioning and fully operational laboratory organized for HIVDR genotyping with ALL the following elements in place:

**Space**
- Adequate office and laboratory space
- Appropriate equipment available
- Space configuration, workflow and contamination control is adequate and consistent with good laboratory practices
- Adequate PCR and sequencing capacity including editing programs
- Space is clean and well kept
- Relevant anti-contamination laboratory spaces for PCR
- Adequate freezer storage
- Electrical power back up
- Reliable and well documented specimen logistics and storage procedures
- Computational capability, including hardware, software and internet access

**Equipment**
- Equipment is functioning and in good condition
- Equipment is maintained regularly, as recommended, and dates of maintenance/inspection are recorded
- Records are kept on daily temperature readings of incubators, refrigerators, and freezers

**Others**
- Inventories are maintained and adequate time is allowed for replenishing supplies
- Minimum laboratory biosafety level of 2

*The lab must provide:*
- Copy of critical equipment (sequencer, thermocycler, etc) maintenance records of last 2 years
- Map of the genotyping facility of the laboratory
• Questionnaire for the collection of basic information on HIV sequencing laboratory capacities and equipment (document attached).

2. Regionally recognized experience and leadership in HIV laboratory science
The laboratory must identify a minimum of three public health HIV laboratories within the region as references (please specify the contact persons and contact details for each of the lab; and type of collaboration)

The laboratories will be contacted by the WHO to confirm the suitability of the laboratory to function as a regional laboratory.

3. Laboratory experience in HIV genotyping
The candidate laboratory must prove internationally recognized experience in HIVDR testing, and must provide description of:

- Years of experience of HIV genotyping for clinical use, research or epidemiology
- Number of specimens genotyped annually in the last 3 years
- Years of experience in performing home brew HIVDR assays
- Number of methods in use for sequencing
- Years of experience in sequencing from Dried Blood Spots

4. Adequate experience in provision of training and establishment of collaborations in laboratory sciences in the last 3 years
The candidate laboratory must document and submit description and evidence of:

- List experience in providing training to national/international partners (specify number of persons trained, institution of origin, and date of training) on HIVDR genotyping or HIV science in the last 3 years
- Describe capacity to host visiting scientists upon the request of WHO HIVResNet for the purpose of HIVDR training (regardless of nation of origin)
- List collaborations on HIVDR genotyping or HIV science with laboratories in countries in or outside the region the last 3 years.

5. Adequate personnel expertise in genotyping
The candidate laboratory must prove CVs and material (publication, thesis, training certificate, etc) documenting qualifications, training, and experience of staff and supervisor in the field of
general molecular biology; specify experience in use of techniques other than commercially available kits.

Please describe:

- Number of laboratory technicians trained in HIVDR genotyping, with competency in HIV genotyping assessed and documented for each qualified technician. A minimum of two technicians with documented appropriate training in HIVDR genotyping.
- Number of laboratory staff members competent in editing and aligning viral sequences.
- Are personnel competent in drug resistance mutation interpretation using recognized and accepted interpretation algorithms?
- Are personnel willing to participate in laboratory site visits under the direction of WHO HIVResNet?

Candidate laboratories are required to provide:

- CVs of the laboratory staff and supervisor
- Documentation of the experience and training of the supervisor and staff in the field of general molecular biology (to include information about number of publications in the last 3 years, thesis, training certificate, etc).

6. Administrative and financial sustainability

The laboratory must provide documentation to show:

- Evidence of administrative and financial sustainability of the laboratory (Information on resources/budget that were available to the genotyping laboratory during the last 2 years and source/s of funding must be provided)
- Clear and accountable laboratory management structure (organigram of the management and personnel of the genotyping laboratory must be provided)

7. Ability to provide reference virology services to other laboratories

- Capability to rapidly catalogue, store and distribute HIV blood specimens/isolates referred from the laboratory network
- Accessibility to infectious material arriving from all or most regions (i.e. no severe import restrictions)

8. Laboratory procedures clearly documented and implemented

The candidate laboratory must provide a copy of Standard Operating Procedures, including:
1. Specimen receipt, assessment and storage
2. Internal Quality Control
3. All steps of genotyping tests, including sequencing and workflow
4. Handling and manipulation of infectious human material, including the appropriate handling of infectious waste
5. Disinfection procedures
6. Detection, containment and control of molecular contamination to laboratory equipment
7. Data management

9. Proficiency panel testing
The laboratory must have documentation of successful completion of PP testing from providers other than the WHO in the last two years (copies of the reports are requested). In addition, the laboratory must pass a WHO recognized proficiency panel before being accredited (see Annex 3).

10. Letter of agreement from the Ministry Of Health
A letter of agreement from the MOH is required, indicating that the candidate laboratory has been identified to test specimens collected from other countries during WHO recommended HIVDR surveillance and monitoring surveys and to provide training and capacity building to other laboratories in the region.

The Application Checklist, and items specified on the checklist, should be sent to the WHO. This can be done either directly to the HIV Global HIVDR Programme (WHO HQ) (Dr Silvia Bertagnolio; email: bertagnolios@who.int; mail address: Avenue Appia, 20 - World Health Organization, Geneva) or through the WHO Country/Regional Office.

If the initial application is deficient in any required documentation, the laboratory can apply again when the missing criteria are fulfilled. WHO HQ will then notify the applicant whether all criteria have been met and will begin the assessment procedure. Upon completion of the application form and fulfillment of all application criteria, the laboratory will:

- Obtain a pre-assessment questionnaire from the WHO. The laboratory should complete this questionnaire in its entirety and return it to the WHO.
Following review of the questionnaire and requested documentation, the WHO will coordinate an assessment site-visit to audit the questionnaire and the shipment of a WHO-recognized Proficiency Panel (PP).

The WHO and the Advisory Group of the HIVResNet Laboratory Network will evaluate the assessed laboratory and provide a decision on accreditation.
Specialized HIVDR Laboratories

It is expected that the SDRL will be well established in the international scientific community, with existing national and international responsibilities and experience in reference virology, training, surveillance, and relevant scientific publications. Ideally, a SDRL should already host WHO reference activities outside of HIVDR, and have sufficient medical, scientific and technical resources to respond to demands for training, laboratory testing and advice on short notice.

1. Complete laboratory set up for HIVDR genotyping

The laboratory should have a well-functioning and fully operational laboratory set-up for HIVDR genotyping with ALL the following elements in place:

1. Office and laboratory space is adequate, clean and well kept
2. Appropriate equipment in place, including electric power back up
3. Space configuration, workflow and contamination control is consistent with good laboratory practices
4. Adequate PCR and sequencing capacity, including editing
5. Adequate freezer storage
6. Reliable and well-documented specimen storage procedures
7. Computational capability, including hardware, software and Internet access
8. Equipment is functioning and in good condition
9. Equipment is maintained regularly, as recommended, and dates recorded;
10. Temperature monitoring records are kept regularly for incubators, refrigerators, PCR machine and freezers
11. Inventories are maintained and adequate time is allowed for replenishing supplies
12. Minimum biosafety level of 2, in the areas where the specimens are handled

The lab must provide:
- Copy of critical equipment (sequencer, thermocycler, etc) maintenance records of last 2 years
- Map of the genotyping facility of the laboratory
• Questionnaire for the collection of basic information on HIV sequencing laboratory capacities and equipment (document attached).

2. Internationally recognized experience and leadership in HIVDR genotyping
The candidate laboratory must provide:

• List of 5 public health laboratories, as references (please specify the contact persons and contact details for each of the lab; and type of collaboration)

The laboratories will be contacted by WHO to confirm the suitability of the candidate lab to function as specialized laboratory

3. Laboratory Experience in HIVDR genotyping
The candidate laboratory must prove internationally recognized experience in HIVDR testing, and must provide description of:

• Years of experience of HIV genotyping for clinical use, research or epidemiology
• Number of specimens genotyped annually in the last 3 years
• Years of experience in performing non-commercial, home brew HIVDR assays
• Years of experience in the design and validation of novel HIVDR assays and relevant surveillance technologies.

Experience in providing laboratory support to large scale HIVDR surveillance programs, although not mandatory criteria, is warranted and will be considered positively during the assessment.

4. Experience in the provision of training and the establishment of collaborations in HIVDR genotyping
The candidate laboratory must document and submit description and evidence of:

• experience in providing training to national/international partners (specify number of persons trained, institution of origin, and date of training) on HIVDR genotyping in the last 3 years
• capacity to host visiting scientists upon the request of WHO HIVResNet for the purpose of HIVDR training (regardless of nation of origin)
• collaborations on HIVDR genotyping with laboratories in limited-resource countries in the last 3 years.
• Experience with organizing DR workshops
• Production of training materials/modules for educational programs on HIVDR (attach the material to the application)

5. Adequate expertise of genotyping laboratory personnel
The candidate laboratory must provide CVs and material (publication, thesis, training certificate, etc) documenting qualifications, training, and experience of staff and supervisor in the field of general molecular biology; specify experience in use of techniques other than commercially available kits.
Please describe the following:
• Number of laboratory technicians trained in HIVDR genotyping, with competency in HIV genotyping assessed and documented for each qualified technician.
• Number of laboratory staff members competent in editing and aligning viral sequences.
• Has the laboratory supervisor specific training in the area of molecular virology?
• Are personnel competent in drug resistance mutation interpretation using recognized and accepted interpretation algorithms?
• Describe experience in performing laboratory assessment/evaluation.
• Are personnel willing to participate in laboratory site visits under the direction of WHO HIVResNet?
• Are personnel able to critically review SOPs from other laboratories using different platforms (TruGene, ViroSeq, "home brew")?

6. Administrative and Financial Sustainability of the Institution
The candidate laboratory must provide:
• Information on resources/budget that were available to the genotyping laboratory during the last 2 years and source/s of funding;
• Organigram of the management and personnel of the genotyping laboratory
• Capacity to support WHO HIVResNet activities through the laboratory’s core budget and/or to secure external funds when required

7. Ability to provide reference virology services to other laboratories
The candidate laboratory must prove:
• Description of lab experience as reference virology service to other laboratories;
• Description of mechanism to catalogue, store and distribute HIV blood specimens/isolates referred from the laboratory network;
• Accessibility to infectious material arriving from all or most regions (i.e. no severe import restrictions)

8. Laboratory procedures are clearly documented and in place
The candidate laboratory must provide a copy of Standard Operating Procedures, including:

1. Specimen receipt, assessment and storage
2. Internal Quality Control
3. All steps of genotyping tests, including sequencing
4. Handling and manipulation of infectious human material, including the appropriate handling of infectious waste
5. Disinfection procedures
6. Workflow
7. Detection, containment and control of molecular contamination to laboratory equipment
8. Data management

9. Laboratory conducts original research in HIVDR
The candidate laboratory must prove to conducts original research in laboratory aspects of HIVDR, as demonstrated by:

• Laboratory’s peer-reviewed publications from the last five years in which laboratory staff are included as authors
• List of funding awards for ongoing collaborative HIVDR research in the last 5 years

Laboratories which conduct research and/or coordinate activities on the following topics will receive special consideration, as these topics are pertinent to the aim of the HIVResNet Laboratory Network:

• Design and validation of novel DR assays and relevant surveillance technologies (e.g. point mutation assays; cheap methodologies for genotyping; genotyping using dried fluid spots; etc)
• Development, distribution and evaluation of quality assurance systems
• Production and organization of training materials/modules and educational programs for HIVDR
• Reference laboratory activities for HIVDR surveillance (list of number of specimens tested for the HIVDR surveillance and number of countries assisted)
• Dried fluid spot (DFS) research including DFS method development and testing (please attach description of planned studies, if applicable)

10. Willingness to share information and work cooperatively with WHO and with other HIVDR Network laboratories

• Description of previous collaborative work with WHO in the field of HIV, and specifically on HIVDR
• If applicable, description of any potential problem that may occur by sharing data/information with WHO and other laboratories in the network
• Attach a detailed description on potential role and function of the candidate laboratory within the HIVResNet Lab Network

11. Proficiency panel testing.
The laboratory must have documentation of successful completion of PP testing from providers other than the WHO in the last two years (copies of the reports are requested).
In addition, the laboratory must pass a WHO recognized proficiency panel before being accredited (see Annex 3).
ANNEX 5: QUESTIONNAIRE FOR THE COLLECTION OF BASIC INFORMATION ON HIV SEQUENCING LABORATORY CAPACITIES AND EQUIPMENT

Laboratory Name:..............................................................................................................
Address:..............................................................................................................................
City:.................................................................................................................................
Country:............................................................................................................................
Director of Department or Institution:............................................................................
Director of the Laboratory:..............................................................................................
Contact person for HIVDR assessment visit:.................................................................
Position of contact person:..............................................................................................
Phone:.........................................................Fax:..........................................................
Email:...............................................................................................................................
Date of completion of the questionnaire:...........................................................................

As part of efforts to organize the WHO/HIVResNet Global Laboratory Network, we are trying to evaluate the existing capacities for HIV drug resistance sequencing of your laboratory. Please answer the following questions by checking the appropriate boxes or filling in the appropriate number or text. Please note that a separate questionnaire addresses the collection, handling, storage and transport of specimens and should be completed if appropriate.

A. GENERAL INFORMATION

1 a. Is the laboratory performing HIV-1 drug resistance (DR) sequencing? □ Yes □ No
If Yes, please continue with question 2. If No, please answer Question 1 b.

1 b. Is the laboratory performing sequencing of RNA viruses? □ Yes □ No
If Yes, please specify organisms/viruses being sequenced:____________________________
If no RNA viruses are sequenced, please answer Question 1 c:

1 c. Is the laboratory performing DNA sequencing (non-HIV, non-RNA)? □ Yes □ No
If Yes, please specify organisms/viruses being sequenced:________________________________________

If no DNA sequencing is performed, please answer Question 1 d:

1 d. Is the laboratory performing general molecular diagnostic work? □ Yes □ No
If Yes, please specify:____________________________________________________________________

If no molecular diagnostic work is performed, please do not continue with the questionnaire.

2. For what purpose is HIVDR sequencing performed (Check all that apply)?
   □ Clinical care
   □ Research
   □ Public health/Epidemiological purposes

3. How many years of experience does the laboratory have performing HIVDR sequencing?
   _________ years

4. How many HIV-1 DR sequencing tests did the laboratory perform in each of the past two years?
   Number performed last year:___________________________ year: _______
   Number performed year before last:_____________________ year: _______

5. How many sequencing tests did the laboratory perform on RNA viruses other than HIV-1 in each of the past two years?
   Number performed last year:___________________________ year: _______
   Number performed year before last:_____________________ year: _______
6. Is the laboratory integrated into the Ministry of Health? □ Yes □ No

7. If not, is it a private laboratory? □ Yes □ No

Specify ______________________________
____________________________________________________

B. PERSONNEL

8. Indicate the personnel (scientific and technical staff) available to perform HIVDR sequencing, specify ability to perform in house home brew assays, and include qualifications and training undertaken. Indicate the time dedicated to sequencing per month by each individual. Attach CV of the staff working in the genotyping lab.

<table>
<thead>
<tr>
<th>Name</th>
<th>Qualification/training*</th>
<th>Time dedicated</th>
</tr>
</thead>
<tbody>
<tr>
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</tbody>
</table>

* E.g.: General technician, Technician Molecular Diagnostics, Biomedical Scientist, Physician, else

9. Is a safety officer in place? □ Yes □ No

10. Please provide a management structure of the laboratory/Institution (attach separate file).

11. Please provide indications for the financial sustainability of the laboratory:

____________________________________________________________________________
____________________________________________________________________________
____________________________________________________________________________
____________________________________________________________________________

C. QUALITY SYSTEM MANAGEMENT
12. Does the laboratory have Standard Operating Procedures (SOPs) in place for HIVDR sequencing?  
□ Yes  □ No  

*If Yes, please provide name/number of SOPs for all steps of the HIVDR sequencing procedure.  
Please submit copies of HIVDR SOPs for review along with this questionnaire.*

<table>
<thead>
<tr>
<th>SOP number</th>
<th>SOP name/title</th>
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<tbody>
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</tbody>
</table>

13. Does the laboratory participate in an External Quality Assurance program for HIVDR sequencing?  
□ Yes  □ No  

*If Yes, please specify:  
Name Program: __________________________________________________________  
Name Provider: __________________________________________________________  
Date of participation: __________________________________________________  
Please submit a summary of Proficiency Panel results from the last 2 years along with this questionnaire.*

14. Indicate the frequency of power failure: ____________________________________________  
Is back-up available in case of power failure? □ Yes  □ No

D. BIOSAFETY

15. Does the laboratory have well-documented procedures for the handling and manipulation of infectious human material, including the handling of infectious waste?  
□ Yes  □ No
If Yes, please provide name/number of the SOP or Laboratory Protocol and submit a copy for review:
Name/number: ____________________________________________

16. Are laboratory disinfection procedures in place? □ Yes □ No

17. If Yes, please list the workspace/equipment disinfected, procedures and disinfecting agents and frequency in the following table:

<table>
<thead>
<tr>
<th>Workspace/equipment</th>
<th>Procedure/disinfecting agent</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
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</tbody>
</table>

E. DEDICATED MOLECULAR DIAGNOSTIC WORKSPACE AND WORKFLOW

18. Are there separate laboratories/rooms/work areas for molecular diagnostic activities? □ Yes □ No

If Yes, please answer questions (18a) and (18b):

18a. Is there a dedicated room for specimen extraction and master mix preparation which remains free of contaminating DNA (pre-amplification)? □ Yes □ No

18b. Is there a separate, dedicated room for PCR amplification and handling amplification products/high-copy number DNA (post-amplification)? □ Yes □ No

19. Is a strict "unidirectional workflow" respected by all laboratory personnel? □ Yes □ No

If Yes, please submit copies of SOPs/laboratory procedures for workflow and provide a map of the genotyping facilities.
20. Are procedures in place for cleaning and molecular decontamination of the laboratory?

□ Yes  □ No

*If Yes, please submit copies of SOPs/ laboratory procedures for cleaning and molecular decontamination.*

If a written document is not available, please use the table below to report the type and frequency of cleaning and molecular decontamination procedures/strategy used by the laboratory.

<table>
<thead>
<tr>
<th>Workspace/equipment</th>
<th>Procedure/decontaminating agent</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
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</tbody>
</table>

**F. EQUIPMENT**

21. Indicate the type, year of purchase and the frequency of the maintenance and calibration of the equipment present in the pre-amplification area.

<table>
<thead>
<tr>
<th>Pre-amplification Area Equipment</th>
<th>Type</th>
<th>Year of purchase</th>
<th>Maintenance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bench with sink / tap water</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biohazard flow, class IIb</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dead Air Cabinet</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
(preparation mixes) |  
| Dead Air Cabinet (RNA extraction) |  
| Freezer - 20 ° C |  
| Microcentrifuge 12,500-15,000 g |  
| Vortex |  
| Dedicated set of micropipettes |  
| Ultracentrifuge² 21,000-25,000 g |  

² in case extraction procedure requires pelleting of virus

22. Are the centrifuges anti-aerosol? □ Yes □ No

23. Indicate the type, year of purchase and the frequency of the maintenance and calibration of the equipment present in the post-amplification area.

<table>
<thead>
<tr>
<th>Post-amplification Equipment</th>
<th>Type</th>
<th>Year purchase</th>
<th>Maintenance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bench with sink / tap water</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dead Air Cabinet (nested reaction)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thermal cyclers</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Agarose gel apparatus</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Photo documentation of agarose gel</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
DNA sequencer

Computer

Computer programs
(editing)

Microcentrifuge
450-550 g

Vortex

Dedicated set of
micropipettes

Freezer
-20 °C

Refrigerator
4 °C

<table>
<thead>
<tr>
<th>Equipment</th>
<th>Type</th>
<th>Year purchase</th>
<th>Maintenance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Freezer</td>
<td>-20 °C</td>
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<td></td>
</tr>
<tr>
<td>Freezer</td>
<td>-80 °C</td>
<td></td>
<td></td>
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<tr>
<td>Autoclave</td>
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</tbody>
</table>

24. Indicate the type, year of purchase and the frequency of maintenance and calibration of additional equipment.

25. Describe how the freezer temperature control is organized:
26. Indicate the presence of the following materials for Bio-Safety in the separate workspaces by a “+”.

<table>
<thead>
<tr>
<th>Workspace</th>
<th>Gloves</th>
<th>Paper lab coats</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-amplification/extraction</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-amplification/mix preparation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Post-amplification</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other:_____________________</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

27. If paper lab coats are used, how frequently are they changed?

27a. If paper lab coats are NOT used, are cloth lab coats used: □ Yes □ No

27b If Yes, how frequently are the cloth lab coats cleaned?

28. Supplies:

28a. Are current inventories maintained? □ Yes □ No

28b. System in place for replenishing supplies? □ Yes □ No

29. Computational capability

29a. Is a computer available in the laboratory? □ Yes □ No

29b. Is internet access available? □ Yes □ No

G. SPECIMEN

30. What types of specimens does the laboratory use for HIVDR sequencing (check all that apply)?

□ EDTA plasma

□ Citrate plasma

□ Serum

□ Dried Blood Spot (DBS)
31. If Dried Blood/Plasma/Serum Spots are used for HIVDR sequencing, please indicate the type of membrane and manufacturer:
   □ Membrane 903 filter, Manufacturer:_______________________________________
   □ Membrane FTA filter, Manufacturer:______________________________________
   □ Other membrane, Manufacturer:__________________________________________

32. If Dried Blood/Plasma/Serum Spots are used for HIVDR sequencing, please indicate storage conditions and detailed information on the processing of the specimen, including extraction, amplification and sequencing (quantities used, conditions,..). If an SOP or written Laboratory Protocol is available you may submit a copy and indicate “see enclosed document” in the space below.
__________________________________________________________________________
__________________________________________________________________________
__________________________________________________________________________
__________________________________________________________________________
__________________________________________________________________________

33. If Dried Blood/Plasma/Serum Spots are used for HIVDR sequencing, please indicate the number of specimens tested/annually in the last 2 years and years of experience in genotyping using DBS/DPS/DSS
__________________________________________________________________________
__________________________________________________________________________
__________________________________________________________________________

H. SPECIMEN REGISTRATION
34. Indicate the information present on the stored specimens used for HIVDR sequencing?
   □ Unique specimen identification code
   □ Patient identification code
   □ Identification code for the specimen collection center
   □ Specimen collection date
   □ Specimen collection time
35. Indicate the number of aliquots stored for each patient: ______________
Volume: ______________

36. Indicate the system used for specimen registration (check all that apply)

- paper registry?
- computer registry?

37. Indicate the information collected in the registry:

- Type of specimen
- Unique specimen identification code
- Patient identification code
- Patient date of birth
- Patient age group
- Patient antiretroviral treatment history
- Number of pregnancies (for women)
- Other patient data: ______________________________________________________
- Identification code for the specimen collection facility
- Specimen collection date
- Specimen collection time
- Date specimen was sent to the sequencing lab
- Date specimen was received in the sequencing lab
- Specimen viral load
- Condition of the specimen
- Specimen volume/number of dried fluid spots
- HIV confirmation date
- Specimen storage location
- Other: ______________________________________________________

I. SEQUENCING METHODS
38. Please list HIV-1 RNA extraction method, manufacturer (if applicable), specimen type and starting volume for all specimen types used for HIVDR sequencing.

<table>
<thead>
<tr>
<th>Extraction method</th>
<th>Manufacturer</th>
<th>Specimen type</th>
<th>Starting volume</th>
</tr>
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<tbody>
<tr>
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</tbody>
</table>

39. Method for HIV-1 DR sequencing (list all methods used).

- □ Kit-based assay: ______________________________ Version: __________
  : ______________________________ Version: __________
- □ Home-brew (in-house developed) assay

40. If kit-based HIV genotyping assay: describe deviations from the standard procedure, if any:
________________________________________________________________________
________________________________________________________________________

41. If home-brew (in-house developed) assay: please provide information on primers and method:

  a. RT and PCR primers: □ from a published reference □ designed by laboratory
  b. Sequencing primers: □ from a published reference □ designed by laboratory
  c. RT assay conditions: □ from a published reference □ designed by laboratory
  d. Primary PCR assay conditions: □ from a published reference □ developed by laboratory
  e. Secondary PCR assay conditions: □ from a published reference □ developed by laboratory

*If applicable, lab must provide documentation of references used*

42. Has the method been validated in the laboratory? □ Yes □ No
If Yes, please attach a summary of the method validation including information on the specimen types validated, the number of specimens tested, and the method of evaluation or reference assay used for comparison.

43. Minimal region sequenced on both strands:
   for protease: codons ________________________________
   for reverse transcriptase: codons _______________________

44. Was the minimal analytical detection limit determined for the sequencing method?
   □ Yes  □ No
   If Yes, indicate the minimal analytical detection limit:______________________________

45. Is the viral load of specimens submitted for genotyping known?  □ Yes  □ No

46. What is the minimal viral load for sequencing? _______________ HIV-RNA copies/ml

47. Is the preservation time for reagents controlled?  □ Yes  □ No

48. How are specimens or derivatives kept cooled during sequencing?____________________

49. What is the mean turn-around time for sequencing? ______________________________

50. Is a Positive Run Control included in every run?  □ Yes  □ No
   If Yes, indicate the step(s) in which positive controls are included, the specimen type of the control and the viral load, if known:

   Step:_________ Type of specimen:______________ VL:______________
   Step:_________ Type of specimen:______________ VL:______________

51 If a Positive Run Control is included in every run, please answer to the question below:

One positive control per _________ specimens is used.
What measures are in place in case of a negative result in the positive control?: ___________
52. Is a Negative Run Control included in every run? □ Yes □ No
   One negative control per _________ specimens is used.
   What measures are in place in case of a positive result in the negative control?: ______
   ________________________________________________________________

53. Are filter tips used for reaction set up? □ Yes □ No
   If yes, indicate the steps in which they are used: ________________________________
   ________________________________________________________________

J. SEQUENCE EDITING

54. Indicate the software used for sequence editing: ________________________________

55. Which of the following are taken into account when evaluating the raw sequence data?
   (check all that apply)
   □ Signal intensity; limit___________________________________________________
   □ Signal/Noise Ratio
   □ Reading forward and reverse strand
   □ Amount of editing needed, limit___________________________________________
   □ Other, specify:_________________________________________________________

56. How is sequence editing performed (check all that apply)?
   □ Manual reading
   □ Software-associated editing; specify software_________________________________
   □ Other, specify:_________________________________________________________

57. Are the edited sequence results confirmed by a second/independent person? □ Yes □ No
   If Yes, please provide documentation of this procedure_________________________
58. Does a supervisor critically review results? □ Yes □ No

K. DATA MANAGEMENT

59. Indicate the information registered during the processing/sequencing of the specimen
   □ Dates of different steps in processing of the specimen (extraction, amplification and sequencing)
   □ Detailed information on the processing of the specimen (quantities used, conditions,..)
   □ Results of each step
   □ Other attempts in case of failure
   □ Personnel performing different steps of specimen processing
   □ Storage of interim material (RNA, PCR product,..)
   □ Lot numbers of kits and materials

60. What type of registry are these data records kept in?
   □ paper registry
   □ computer/electronic registry

61. Are the data/results archived? □ Yes □ No
   If Yes, for what length of time are the data/results kept?
   □ Specimen registries time:__________________
   □ Laboratory processing registry time:__________________
   □ Raw sequence data time:__________________
   □ Final sequence result time:__________________ format: __________

62. Are back-up procedures for sequences data in place? □ Yes □ No

Describe the back-up method and the frequency of back-up:

_____________________________________________________________________________
_____________________________________________________________________________
_____________________________________________________________________________

__________________
ADDITIONAL REMARKS, IF ANY:

_____________________________________________________________________________
_____________________________________________________________________________
_____________________________________________________________________________
_____________________________________________________________________________

ASSESSEMENT DONE BY:.................................................................
DATE OF ASSESSMENT VISIT:.........................................................
TIME SPENT ON ASSESSMENT:.......................................................
References


