

World Health Organization/HIVResNet Drug Resistance Laboratory Strategy

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With rapidly increasing access to antiretroviral drugs globally, HIV drug resistance (HIVDR) has become a significant public health issue. This requires a coordinated and collaborative response from country level to international level to assess the extent of HIVDR and the establishment of efficient and evidence-based strategies to minimize its appearance and onward transmission.

In parallel with the rollout of universal access to HIV treatment, countries are developing protocols based on the recommendations of the World Health Organization (WHO) to measure, at a population level, both transmitted HIVDR and HIVDR emerging during treatment. The WHO in collaboration with international experts (HIVResNet Laboratory Working Group), has developed a laboratory strategy, which has the overall goal of delivering quality-assured HIV genotypic results on specimens derived from the HIVDR surveys. The results will be used to help control the emergence and spread of drug resistance and to guide

decision makers on antiretroviral therapy policy at national, regional and global level.

The HIVDR Laboratory Strategy developed by the WHO includes several key aspects: the formation of a global network of national, regional and specialized laboratories accredited to perform HIVDR testing using a common set of WHO standard and performance indicators; recommendations of acceptable methods for collection, handling, shipment and storage of specimens in field conditions; and the provision of laboratory technical support, capacity building and quality assurance for network laboratories. The WHO/HIVResNet HIVDR Laboratory Network has been developed along the lines of other successful laboratory networks coordinated by the WHO. As of August 2007, assessment for accreditation has been conducted in 30 laboratories, covering the WHO's African, South-East Asia, Western Pacific, and the Caribbean Regions.

Introduction

The World Health Organization (WHO) promotes a public health approach [1] to antiretroviral therapy (ART) scale-up. As a result, the WHO, with the technical input of HIVResNet, has developed a Global HIV Drug Resistance (HIVDR) Prevention and Assessment Strategy [2]. HIVResNet is a group of more than 50 international HIVDR experts, affiliated with universities, laboratories, international and

non-profit organizations. The overall goal of the global strategy is to support country planning of ART programme practices, minimize the emergence of HIVDR, and to restrict the extent to which resistance jeopardizes the effectiveness of the standard first- and second-line recommended ART regimens available in many resource-limited settings. As part of this HIVDR Global Strategy, the WHO and the

Laboratory Working Group of HIVResNet have developed the HIVDR Laboratory Strategy.

WHO/HIVResNet HIVDR Laboratory Strategy

The virology laboratory plays a fundamental role in the HIVDR surveys. Identification of HIVDR can be determined phenotypically, with cell culture-based assays, and genotypically, by DNA sequence analysis of the reverse transcriptase (RT) and protease-coding regions. Genotyping identifies mutations associated with reduced susceptibility to one or more antiretroviral drugs or even to entire classes of antiretroviral drugs. This genetic analysis can also provide information on HIV-1 subtype, although it does not provide sufficient data to correctly characterize some recombinant forms.

Genotypic drug resistance testing is widely used for clinical and surveillance purposes in developed countries and is the technique recommended by the WHO for the scaling up of HIVDR population-based surveys. However, the lack of common standards for quality assurance has remained an impediment to the generation of comparable and reliable results by the different laboratories. In addition, the shortage of basic laboratory infrastructure, the cost and the complexity associated with genotypic testing has limited the development of genotyping laboratories in resource-limited settings.

The overall goal of the HIVDR laboratory strategy is to support WHO-recommended HIVDR surveys through the timely provision of quality-assured genotyping results, by: establishing a WHO/HIVResNet Global Laboratory Network, whereby each country adopting the WHO strategy for national HIVDR surveys has access to quality-assured genotyping laboratory services accredited by the WHO; and developing guidance for the accurate collection, handling, shipment, storage and testing of HIVDR specimens

WHO/HIVResNet Global Laboratory Network

The WHO/HIVResNet Global Laboratory Network includes laboratories at national, regional and global level that have achieved WHO accreditation for performing HIVDR testing.

There are many restrictions to the establishment of the network in low- and middle-income countries, particularly in terms of available resources, adequate infrastructure and access to appropriate training. These factors have been accounted for in the structuring of the WHO/HIVResNet Laboratory Strategy to ensure its effective implementation in these settings.

Other international HIVDR laboratory networks already exist in the HIVDR arena. However, existing networks have been established to support specific

research projects, to provide clinical results or both. The WHO/HIVResNet Laboratory Network is the first to be established with the public health goal of supporting the WHO-recommended HIVDR surveys [2].

The network's operations are based on the successful models provided by other laboratory networks coordinated by the WHO, such as the Global Polio Laboratory Network [3,4], the Measles and Rubella Laboratory Network [5,6], the Global Human Papillomavirus (HPV) Laboratory Network [7], the Influenza Laboratory Network [8] and the Tuberculosis Laboratory Network [9]. The influenza programme supported by the Influenza Laboratory Network was established between 1948 and 1952, with a major focus on annual recommendations for influenza vaccine composition and related activities, and a global alert mechanism for the emergence of influenza viruses with pandemic potential [7]. The Tuberculosis Laboratory Network was formally established as a quality assurance network for drug susceptibility testing in 1994 [8]. Development of the Polio Laboratory Network began in 1986 in the Americas [3] and by 2001 encompassed 147 laboratories globally.

The HIVResNet Laboratory Network is built on the same pyramidal structure common to other WHO laboratory networks, allowing for different categories of membership according to existing capacities and responsibilities. Individual laboratories, based on their capacity and expertise, are expected to perform specific duties in support of the national, regional and global requirements of the network. The global network is organized on three levels: national drug resistance laboratories (NDRLs), regional drug resistance laboratories (RDRLs) and global specialized drug resistance laboratories (SDRLs)

All WHO HIVDR national surveys require that testing occurs in a WHO-accredited laboratory. The WHO accreditation process is initiated only for those laboratories that are designated by national authorities to test specimens collected for WHO-recommended HIVDR surveys. In general, only one laboratory per country is accredited by the WHO. However, the number of accredited laboratories may vary depending on the size of the country, population, HIV prevalence, volumes of patients on ART, existing capabilities in HIV genotyping and available resources in each country. Countries with limited laboratory capacity and focusing on scaling up diagnostic and clinical laboratory tests, such as HIV testing and CD4 cell count, may decide to send their HIVDR survey specimens to an accredited laboratory at the regional level. Populous countries where HIVDR testing capacity already exists may request the accreditation of more than one national laboratory, as the sheer volume of HIVDR survey specimens may be beyond the capacity of a single facility.

Where the transport of specimens from different areas of the country to a single laboratory is difficult, the accreditation of a second national laboratory may also be required.

Tasks and responsibilities of network laboratories

The main task for WHO-accredited laboratories is to deliver quality-assured genotypic results, relevant to national ART programmes, and supporting a public health approach to ART.

National Drug Resistance Laboratories

To be assessed and eventually accredited by the WHO, national laboratories have to be designated by national public health authorities in order to ensure country network effectiveness. The task of NDRLs is to genotype specimens collected during HIVDR surveys and provide, in a timely manner, accurate HIV sequence data to a nationally established HIVDR database. If capacities to perform HIVDR testing in a laboratory do not exist within a country, each country can choose to ship the HIVDR survey specimens to a WHO-accredited regional or specialized laboratory. National AIDS programmes are expected to support the HIVDR surveys, including the laboratory testing, with their own funds or those obtained from donors.

Regional Drug Resistance Laboratories

Each WHO region (African, Western Pacific, South-East Asia, Eastern Mediterranean, and Latin America and the Caribbean) will have one or more regional laboratory, depending on the volume of specimens to be tested in that region. Based on the number of countries implementing WHO HIVDR surveys within a given region, the presence of additional regional laboratories may be warranted. Regional laboratories are 'centres of excellence' in each region and can undertake international responsibilities. They serve as HIVDR-testing reference centres for the purpose of supporting HIVDR surveys for countries within their region that do not

have an accredited NDRL. In addition, RDRLs' responsibilities include: facilitating the training, education and capacity building of laboratory personnel from NDRLs within the region; assisting the WHO with candidate NDRLs assessments within the specified region and provision of technical assistance when necessary; and contributing to the organization of the WHO/HIVResNet Laboratory Network regional meetings, which offer training, opportunities for discussions pertaining to programme development and updates on new methodologies.

Specialized Drug Resistance Laboratories

SDRLs are identified by the WHO based on several criteria such as: internationally recognized experience with HIVDR; experience as public health, clinical or research HIVDR laboratory; recognized expertise on selected key research topics of public health relevance; and the laboratory's capacity, resources, commitment and motivation.

Although SDRLs may be called upon to provide HIVDR testing to countries not served by NDRLs/RDRLs, the primary function is to set standards and provide support and technical assistance to RDRLs/NDRLs. The SDRLs are expected to be competent in both commercial and non-commercial genotyping technologies, and to be responsible for specific functions within the network (see Box 1).

Although the SDRLs, RDRLs and the host governments bear much of the financial responsibility for their work in conjunction with the HIVResNet Laboratory Network, the WHO will consider funding or co-funding several aspects as needed: the initial laboratory assessment visit including the travel and per diem cost of a laboratory expert identified within the network to audit the candidate NDRLs, RDRLs and SDRLs; following the initial laboratory assessment, visits for the provision of technical assistance; shipment and analysis of proficiency panels (PP) to candidate laboratories and accredited NDRLs, RDRLs and SDRLs for the quality

Box 1. The specific functions of SDRLs

- To provide training and technical assistance to those laboratories in developing countries performing HIVDR testing for the WHO-recommended HIVDR surveys.
 - To develop training modules and materials.
 - To set technical standards for laboratory testing within the network.
 - To assist the WHO in coordinating the quality assurance programme for the network.
 - To critically evaluate the capacity of laboratories seeking WHO accreditation.
 - To engage in research to answer operational questions that are relevant for the implementation of the WHO HIVDR strategy. The high cost of the genotyping testing as well as the logistics of specimens collection, shipping and storage in remote areas represents a barrier for the scale up of WHO-recommended HIVDR surveys in many resource-limited settings, highlighting the need for cheaper high-throughput assays (for example, allele-specific real-time PCR assays for key mutations) and the validation of HIVDR-testing protocols using dried fluid spots.
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These tasks may not be equally distributed between the global specialized HIV drug resistance (HIVDR) laboratories (SDRLs), with some laboratories being the sole provider of certain functions, according to availability, commitment and expertise. WHO, World Health Organization.

assurance programme, at least in the initial phase of the global network development; and operational research that is relevant to the goals of the WHO strategy.

In addition, WHO can endorse grant applications of NDRLs, RDRLs and SDRLs with regard to obtaining financial support from other sources to conduct operational research of public health importance in the area of HIV drug resistance.

One of the goals of the WHO/HIVResNet Laboratory Network is to support the transfer of knowledge and expertise from WHO-accredited laboratories to those who have not yet achieved accreditation. For this purpose, WHO facilitates partnering between accredited and non-accredited laboratories, with the aim of providing training and technical assistance.

Standards for the collection, handling, shipment, storage and testing of specimens

Specimen types for HIVDR genotype testing

At present, the specimen type most commonly collected for clinical HIVDR genotyping is plasma; some population-based surveillance systems also use plasma, whereas others use serum [10]. However, for HIVDR surveys under the WHO's strategy, which recommends a population-based approach for HIVDR testing rather than for individual patient monitoring, the use of residual specimens collected for diagnostic or clinical purposes is encouraged where feasible [1].

The use of dried blood spots (DBS) is advantageous in several settings when the volume of residual specimens is limited; when plasma or serum cannot be quickly separated and frozen, and maintaining frozen specimens at a uniform temperature is problematic; or where logistic difficulties limit the likelihood of successful shipment of frozen specimens to the genotyping laboratory. Other alternatives include dried plasma spot (DPS) or dried serum spot (DSS) specimens. Whereas HIV genotyping based on plasma or serum elucidates the HIVDR profile found in the majority of circulating viral population, results generated from whole blood specimens (for example, DBS specimens) also reflect the relative contribution of HIV DNA found in latent cellular reservoirs [11]. Theoretically, this may lead to discordance in results generated from DBS and plasma, but recent studies have shown sequences from plasma and DBS to be highly comparable [12,13].

Plasma

Plasma has been the conventional specimen type used for HIVDR testing in research and clinical applications. Citrate- or EDTA-anticoagulated tubes are used to collect specimens for CD4 cell enumeration and viral load prior to and during ART, and can therefore conveniently be

used for HIVDR surveys. Studies have indicated that the success of viral amplification from plasma is dependent on several factors including viral RNA load, time from blood collection to plasma separation, condition of the plasma after separation, time from plasma separation to freezing, the temperature at which plasma specimens are frozen, and the duration of time before extraction, amplification and genotyping take place [14–17]. Because freeze-thaw cycles can be detrimental to amplification, frozen plasma should be shipped on dry ice and not be thawed prior to genotyping [18]. Studies on DPS are more limited, but it is reasonable to assume that timely blood fractionation and appropriate storage are equally important for the preparation of these specimens [19]. Plasma should be selected as the HIVDR genotyping specimen type only in settings where appropriate processing and shipping are possible.

Serum

Serum is the specimen of choice for HIV antibody testing or HIV serosurveillance, making it relatively easy to organize the collection of serum specimens for surveillance of transmitted HIVDR in diagnostic settings. Studies have shown that viral loads in sera are lower than in plasma by as much as 10-fold, possibly due to the trapping of HIV during clot formation [20–22] (unpublished data, Roche Diagnostic Systems, Inc.). As with plasma, RNAses continue to degrade HIV viruses until serum is frozen. It is important that separation and freezing of serum specimens be managed within the recommended time frame [15].

Dried fluid spots (blood, serum or plasma)

Although plasma and serum are considered the optimal specimens for genotyping, they are not practical for many resource-limited settings. This is because of the limited personnel and laboratory facilities, the common necessity of performing basic clinical or diagnostic tests before aliquoting, and the lack of cold chain and facilities for optimal storage and transport.

DBS have been reported to be suitable for HIV serology and molecular diagnosis [23], viral RNA quantification [24] and CD4 cell enumeration [25]. More recently, DBS have been shown to be comparable to plasma as a specimen for HIVDR genotyping [12,13]. DSS have also been shown to be a feasible specimen for HIVDR genotyping [26]; however, the requirement for blood collection by venipuncture and the subsequent laboratory processing may limit the use of DSS for surveillance in resource-limited settings. Compared with DSS, whole blood collected from a finger prick onto a filter paper card in the form of a DBS would require no further laboratory manipulation. The increasing use of DBS for diagnostic and clinical purposes in paediatric populations, and the small volume required

(50–100 µl), would also allow their use in HIVDR surveys in the HIV-infected paediatric population.

The collection of blood specimens on absorbent paper has unique advantages for large-scale screening programmes and epidemiological surveys. Once a DBS has dried, the RNAses present in blood that degrade HIV RNA become inactive, allowing the virus to be stable for extended periods. As freezing is not required for short-term storage, DBS specimens can be transported more easily in resource-limited countries, which may lack sufficient resources or infrastructure for maintaining frozen specimens during transport. DBS are already extensively used in many resource-limited settings as the specimen type for the HIV serosurveillance, allowing HIVDR testing to be easily embedded in existing HIV surveillance programmes. DBS can also be made from anticoagulated blood drawn for the purposes of CD4 cell enumeration or other routine clinical tests and therefore are a potential specimen candidate for HIVDR monitoring surveys. In addition, specially formulated absorbent papers for blood collection are economical and commercially available.

Specimen processing

The standardization of the collection, handling, shipment and storage of specimens for HIVDR testing is a critical step for producing accurate laboratory results. The laboratory network provides guidance documents that enable standardization of all components of the HIVDR laboratory testing, including specimen collection, handling, shipment and storage (Box 2). Adherence to these recommendations is important for the integrity of the specimens and the quality of the sequence data, and they are to be incorporated into the country's national strategy for HIVDR testing to support HIVDR surveys.

Quality assurance

Quality assurance is paramount to the HIVDR surveys. Although many laboratories in low- and middle-income countries are experienced in genotyping, the absence of standardized methodologies across regions hampers the reliability and comparability of data. The assurance of consistent performance in widely dispersed HIVDR-testing laboratories is addressed in several ways, including standard accreditation criteria, guidance documents, training, use of validated assays, and enrolment in external PP programmes for HIVDR genotyping.

The purpose of the quality assurance programmes is to ensure all network laboratories are held to the same standards, regardless of level of membership, thus allowing for the ongoing improvement of network performance.

To be considered for accreditation, a laboratory must pass the WHO PP successfully.

The WHO PP is developed taking into account the following aspects: PP specimens should be derived from diluted clinical specimens or cell culture propagation; PP should contain specimens 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 specimens; various subtypes of HIV-1 should be represented in the panel (at least one subtype B, one subtype C and at least one non-B/non-C); specimens should have a minimum viral load of 5,000 copies/ml; the PP specimens should be compatible with all commercial assays (all specimens from the panel should be validated using all commercial kits before distribution); and PP may include specimens containing an equal mixture of two defined virus variants.

The panel is distributed at least once a year under the direction of the WHO. Individual laboratory results will be scored by comparing each laboratory's sequence to the consensus sequence derived from all participating laboratories (NDRLs, RDRLs and SDRLs). A minimum of 10 laboratories should test each panel in order to yield a meaningful consensus sequence. The consensus sequence is prepared by first aligning the sequences submitted by all participants in the programme. At each position in the alignment, the nucleotide, or nucleotide mixture, observed in >80% of the submitted sequences is included in the consensus. To pass the PP, the concordance of resistance-associated codons (for major and minor mutations) in the RT and protease regions, as well as the overall nucleotide sequence concordance should be $\geq 99\%$.

Regular contact between the laboratories within the network is critical for reviewing progress, developing testing strategies, reviewing new methodologies and, in general, strengthening the network. Annual participation in PP testing recognized by WHO/HIVResNet is mandatory for all national, regional and specialized laboratories that have achieved accreditation. In addition, all candidate laboratories applying for WHO accreditation are required to enrol in the WHO drug resistance PP programmes. For the current rounds, the National Institutes of Health, on behalf of the WHO, has agreed to develop a PP based on WHO specifications and distribute it to those laboratories seeking WHO accreditation around the world. In the future, this function may be covered by one of the specialized laboratories. As of January 2008, the PP has been sent to 33 laboratories that have applied to achieve WHO accreditation. To date, Quality Control for Molecular Diagnostics (Glasgow, UK), has performed the analysis of the PPs and provided results to the WHO.

WHO HIVDR laboratory accreditation

The primary aim of the accreditation process is to ensure that laboratories achieve the same level of

performance. Only WHO-accredited genotyping laboratories receive full membership within the HIVResNet Laboratory Network. The accreditation process simultaneously provides a learning opportunity for the laboratories, a mechanism for identifying resource and training needs and a measurement of the laboratories' progress. The accreditation process is divided into three phases (application, assessment

visit and accreditation) and provides for yearly performance reviews. Separate accreditation is awarded for HIVDR genotyping from plasma or DBS

To achieve accreditation, candidate national, regional and specialized drug resistance laboratories are required to meet respective mandatory criteria and to pass the minimum scoring of additional accreditation criteria (Box 3). Before a candidate laboratory

Box 2. WHO/HIVResNet key recommendations on specimen storage and transport

Processing of plasma or serum for HIVDR surveys

- Whole blood specimens should be left at room temperature (15–30°C) from the time of collection until centrifugation and separation. Specimens collected for HIVDR testing should be fractionated and serum/plasma refrigerated preferably within 24–48 h (and strictly not beyond 72 h). Aliquots should be frozen within 96 h after collection.
- For situations where HIVDR testing is to be performed on specimens collected for HIV viral load determination, blood should be fractionated and frozen within a maximum of 24–48 h from the time of collection.
- At least 1 ml (500 µl is the minimum volume required for extraction) of plasma or serum should be collected and stored at ≤-20°C.
- Repeated freezing and thawing should be avoided.
- Frozen specimens should always be shipped on dry ice.
- Shipments should be prepared in a manner determined to be appropriate for infectious materials.
- Tubes should be labelled using indelible markers and freezer-proof labels.

Storage* and shipment of DBS for HIVDR surveys

Short-term storage (<30 days) of DBS at the collection sites.

- Make sure the DBS are completely dry before packing.
- To avoid contamination, handle the filter paper by the edges.
- Individually store dry DBS in a gas-impermeable sealed zip-lock plastic bag containing 1–2 desiccant packs (to remove residual moisture) and humidity indicator card.
- Store DBS at room temperature (15–30°C) for a maximum period of 2 weeks, avoiding direct exposure to light.
- Nucleic acid materials in DBS are extremely sensitive to degradation in the presence of humidity/moisture. Check humidity indicator card daily and change the desiccant packs if indicator colour changes from blue to pink.
- Transport DBS at room temperature from the collection site to the processing laboratory for genotyping testing as soon as possible (preferably within 7–14 days).
- In settings where it is not feasible, transport DBS at room temperature to a laboratory with a constant electricity supply and store them at ≤-20°C in a freezer in which the humidity has been evaluated and confirmed as suitable for long-term storage of DBS.

Long-term storage (>30 days) of DBS at the central laboratory.

- Long-term storage of DBS should take place in a central facility. DBS should be stored at ≤-20°C in the original unopened zip-lock bags with desiccant.

Shipment of DBS

- DBS should be shipped as non-infectious material.
- To prepare the DBS for transport, change the desiccants before transport if the bags have remained at the site longer than 7 days before transport, even if the indicator remains blue.
- DBS that have been stored at room temperature should be shipped at ambient temperature.
- DBS that have been frozen should be transported on dry ice or should be allowed to thoroughly equilibrate to room temperature for a minimum of 30 min prior to opening the bag. After equilibrating, the outer bag should be opened and the desiccant replaced with fresh desiccant. The equilibrated DBS should be placed in an envelope and shipped at room temperature.

Amplification and sequencing

- A high level of standardization should be established by laboratories performing genotyping, including quality check of staff training and the use of peer-reviewed standard operating procedures.
- For laboratories that are initiating genotyping plasma and serum, kit-based assays are preferred over home-brew assays.
- Home-brew assays should only be implemented after adequate validation, including evaluation of the assay's performance with various HIV-1 subtypes.
- HIVDR testing using DBS should be performed only in an experienced laboratory.
- Sequencing of both strands is essential.
- The minimal regions for which sequence information must be collected are protease codons 10–99 and reverse transcriptase codons 41–220.

*More systematic studies are needed to better understand the stability of genetic material in DBS (dried blood samples) stored at different temperatures and time periods. WHO (World Health Organization) recommendations will be updated regularly to reflect any new available data. HIVDR, HIV drug resistance.

Box 3. Criteria evaluated for candidate laboratories seeking WHO accreditation**Mandatory criteria**

National HIVDR Laboratories

- The laboratory must be designated by the Ministry of Health as a candidate laboratory to provide HIVDR testing for the national strategy.
- National strategy for the WHO HIVDR surveillance and/or monitoring developed by the Ministry of Health.
- Minimum infrastructure for HIVDR genotyping in place.
- More than 1 year experience in genotyping HIV or RNA viruses and >100 specimens tested.
- Successful participation in WHO proficiency panel programme.

Regional HIVDR Laboratories

- Letter of agreement from the Ministry of Health.
- More than 1 year experience in HIVDR genotyping.
- Minimum infrastructure for HIVDR genotyping.
- Successful participation in WHO proficiency panel programme.

Specialized HIVDR Laboratories

- WHO proficiency panel testing passed successfully.
- Internationally recognized experience and leadership in HIVDR genotyping.

Accreditation criteria

National HIVDR Laboratories

- Complete laboratory infrastructure and equipment for HIVDR genotyping.
- Adequate expertise of laboratory personnel with a minimum of one dedicated laboratory professional in HIVDR genotyping.
- Laboratory experience in genotyping:
 - Number of years of experience in HIV genotyping or in sequence-based genotyping of RNA viruses other than HIV.
 - Number of HIV specimens sequenced.
- Laboratory management structure and financial sustainability.
- Standard operating procedures documented and properly implemented.
- Successful participation in a quality assurance programme other than WHO in the past year.

Regional HIVDR Laboratories

- Regional recognized experience and leadership in HIV laboratory science.
- Complete laboratory infrastructure and equipment for HIVDR genotyping.
- Laboratory experience in HIVDR genotyping:
 - Number of years of experience in HIVDR genotyping.
 - Number of HIV specimens genotyped.
 - Personnel expertise in genotyping, including techniques other than commercially available kits.
- Experience in provision of training and establishment of collaborations in laboratory sciences in the past 3 years.
- Administrative and financial sustainability of the laboratory; willingness to seek funds for carrying out regional HIVDR reference service activities.
- Expertise of laboratory personnel with a minimum of two laboratory technicians trained in HIVDR genotyping.
- All standard operating procedures documented and properly implemented.
- Ability to provide reference virology services to other laboratories.
- Successful participation in quality assurance programmes other than WHO in the past 2 years.

Specialized HIVDR Laboratories

- Laboratory set up for HIVDR genotyping.
- Laboratory conducts original research in HIVDR.
- Laboratory experience in HIV genotyping:
 - Number of years of experience in HIVDR genotyping
 - Number of HIV specimens genotyped.
 - Number of year of experience in performing non-commercial HIVDR assays.
- Experience in the provision of training and the establishment of collaborations in HIVDR in the past 3 years.
- Administrative and financial sustainability of the laboratory and capacity to support WHO HIVResNet activities through the laboratories core budget and/or to secure external funds when required.
- Expertise of laboratory personnel with a minimum of two laboratory technicians trained in HIVDR genotyping.
- Standard operating procedures clearly documented and properly implemented.
- Ability to provide reference virology services to other laboratories.
- Successful participation in quality assurance programmes other than the WHO programme in the last two years.
- Willingness to share information and work cooperatively with the WHO and with other HIVDR Network laboratories.

HIVDR, HIV drug resistance; WHO, World Health Organization

undergoes an assessment visit, it must complete an application which includes a checklist and an assessment questionnaire. The application has to be returned to the WHO along with any additional requested documents. The checklist determines that the laboratory has met minimal requirements to be considered for assessment. All the significant aspects of the checklist will be evaluated during the assessment process. The checklist is also intended to guide the laboratories in preparation for the assessment phase. If the application is satisfactory, an on-site assessment site visit is undertaken to audit laboratory performance and capacity. Assessment visit for accreditation includes an evaluation of the experience and capacity in HIV sequencing, as well as performance indicators such as staff competency, facilities, equipment, management practices, safety and financial sustainability. Laboratories that fail to meet the criteria will be encouraged to respond to recommendations in order to meet accreditation standards.

If the assessment visit is successful, the laboratory is required to pass the next available WHO-recognized PP. Successful sequencing of specimens provided through the global genotyping PP scheme is a key element for receiving and maintaining accreditation.

The WHO/HIVResNet HIVDR Laboratory Network is still in its expansion phase, developing as countries focus activities and resources on accelerated ART scale up and on national plans for HIVDR control. The accreditation process began in November 2005 with the goal of global coverage by 2008–2009. As of August 2007, assessment visits for accreditation have been conducted in 30 laboratories, covering WHO African, South-East Asia, Western Pacific, and the Caribbean Regions. The findings of the assessment visits and the results of the PP were presented in a blinded fashion to an independent committee of international experts in the field of HIV drug resistance and HIV laboratory science.

Thus far, accreditation for 2007 has been granted to four specialized laboratories, two regional laboratories and one national laboratory (Box 4). Although several candidate laboratories demonstrated good standards, they were encouraged to address a number of recommendations in order to receive accreditation.

Conclusion

The need for standardized HIVDR survey methodology requires a collaborative and standard approach for laboratory testing of HIVDR worldwide, including within resource-limited settings. The establishment by the WHO of the global WHO/HIVResNet Laboratory Network is proceeding rapidly. Its development has made use of the successful example of other networks coordinated by the WHO, such as the polio, measles, influenza and tuberculosis networks. The WHO

Box 4. World Health Organization-accredited laboratories as of 2007

Specialized laboratories

- University Medical Center Utrecht, Department of Virology, Utrecht, The Netherlands
- UMR145, AIDS and Associated Diseases, IRD and UM1, Montpellier, France
- Molecular Biology Laboratory, Infectious Diseases Department, Hospital Carlos III, Madrid, Spain
- National Laboratory for HIV Genetics, Public Health Agency of Canada, Ottawa, Canada

Regional Laboratories

- Service de Virologie Immunologie, Centre Hospitalier Universitaire de Fort de France, Martinique
- Clinical Research Laboratory, Burnet Institute, Melbourne, Australia

National Laboratories

- National AIDS Research Institute, Pune, India

HIVDR laboratory accreditation process began in November 2005, with the goal of global coverage by 2008–2009. As of July 2007, with adequate international support, this network will be able to provide reliable data for HIVDR control activities at a national, regional and global level.

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Disclosure statement

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