Executive Summary

- The number of accredited labs worldwide remained at 24 in 17 countries
- 3 to 6 new labs are poised to be accredited in 2011
- External QA programs for plasma-based genotyping are in place and running well
- Over 2000 specimens genotyped and over 80 people trained by the network in 2010
- A bi-regional training workshop for HIVDR genotyping was held in Vietnam in May, 2010
- Operational research studies and assay validation/standardization using DBS specimens for HIVDR genotyping was a major area of activity in 2010
Laboratory Network

At the end of 2009, there were 24 laboratories, located in 17 countries, accredited by WHO for HIVDR genotyping using plasma specimens. The geographical distribution of these laboratories is summarized in Table 1 and represented in Figure 1 below.

Table 1. Distribution of Accredited Laboratories in 2010

<table>
<thead>
<tr>
<th>Region</th>
<th>National</th>
<th>Regional</th>
<th>Specialized</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>AF</td>
<td>3</td>
<td>3</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>SEA</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>WP</td>
<td>3</td>
<td>2</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>EU</td>
<td>0</td>
<td>0</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>AM</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Global</td>
<td>10</td>
<td>7</td>
<td>7</td>
<td>24</td>
</tr>
</tbody>
</table>

Figure 1: Location of WHO HIVDR Laboratories

This map is an approximation of actual country borders
## Table 2. List of Accredited Laboratories

<table>
<thead>
<tr>
<th>Laboratory Name/Institution</th>
<th>Type</th>
<th>WHO Region</th>
<th>Country</th>
<th>City</th>
</tr>
</thead>
<tbody>
<tr>
<td>IMPM-IRD/CREMER</td>
<td>National</td>
<td>AF</td>
<td>Cameroon</td>
<td>Yaounde</td>
</tr>
<tr>
<td>KEMRI/CDC HIV Research Laboratory</td>
<td>National</td>
<td>AF</td>
<td>Kenya</td>
<td>Kisumu</td>
</tr>
<tr>
<td>Bacteriology-Virology UTH A Le Dantec</td>
<td>National</td>
<td>AF</td>
<td>Senegal</td>
<td>Dakar</td>
</tr>
<tr>
<td>Department of Clinical Research Tuberculosis Research Centre (ICMR)</td>
<td>National</td>
<td>SEA</td>
<td>India</td>
<td>Chennai</td>
</tr>
<tr>
<td>National AIDS Research Institute, Indian Council of Medical Research</td>
<td>National</td>
<td>SEA</td>
<td>India</td>
<td>Pune</td>
</tr>
<tr>
<td>National Institute of Health, Department of Medical Sciences</td>
<td>National</td>
<td>SEA</td>
<td>Thailand</td>
<td>Nonthaburi</td>
</tr>
<tr>
<td>Dept. of Microbiology, Siriraj Hospital</td>
<td>National</td>
<td>SEA</td>
<td>Thailand</td>
<td>Bangkok</td>
</tr>
<tr>
<td>Division of Research on Virology and Immunology (DRVI), NCAIDS, Chinese Center for Disease Control and Prevention</td>
<td>National</td>
<td>WP</td>
<td>China</td>
<td>Beijing</td>
</tr>
<tr>
<td>Shanghai Municipal Center for Disease Control and Prevention</td>
<td>National</td>
<td>WP</td>
<td>China</td>
<td>Shanghai</td>
</tr>
<tr>
<td>Key Laboratory of Immunology of AIDS, Ministry of Health</td>
<td>National</td>
<td>WP</td>
<td>China</td>
<td>Shenyang</td>
</tr>
<tr>
<td>AIDS Virus Research Unit, National Institute for Communicable Diseases</td>
<td>Regional</td>
<td>AF</td>
<td>South Africa</td>
<td>Johannesburg</td>
</tr>
<tr>
<td>MRC/UVRI Basic Sciences Laboratory</td>
<td>Regional</td>
<td>AF</td>
<td>Uganda</td>
<td>Entebbe</td>
</tr>
<tr>
<td>Service de Virologie Immunologie Centre Hospitalier et Universitaire de Fort-de-France</td>
<td>Regional</td>
<td>AM</td>
<td>Martinique</td>
<td>Fort de France</td>
</tr>
<tr>
<td>Clinical Research Laboratory, Burnet Institute for Medical Research and Public Health</td>
<td>Regional</td>
<td>WP</td>
<td>Australia</td>
<td>Melbourne</td>
</tr>
<tr>
<td>CLS Genotyping Laboratory, Johannesburg General Hospital</td>
<td>Regional affiliated</td>
<td>AF</td>
<td>South Africa</td>
<td>Johannesburg</td>
</tr>
<tr>
<td>AIDS Research Program-Immunology Reference Laboratory</td>
<td>Regional affiliated</td>
<td>AM</td>
<td>Puerto Rico</td>
<td>Ponce</td>
</tr>
<tr>
<td>NSW State Reference Laboratory for HIV and Molecular Diagnostic Medicine</td>
<td>Regional affiliated</td>
<td>WP</td>
<td>Australia</td>
<td>Sydney</td>
</tr>
<tr>
<td>National Laboratory for HIV Genetics, PHAC</td>
<td>Specialized</td>
<td>AM</td>
<td>Canada</td>
<td>Ottawa</td>
</tr>
<tr>
<td>International Laboratory Branch, GAP, NCHSTP, CDC</td>
<td>Specialized</td>
<td>AM</td>
<td>United States</td>
<td>Atlanta</td>
</tr>
<tr>
<td>Laboratoire de Virologie, CHU</td>
<td>Specialized</td>
<td>EU</td>
<td>France</td>
<td>Bordeaux</td>
</tr>
<tr>
<td>UMR145, AIDS and Associated diseases, IRD and UM1</td>
<td>Specialized</td>
<td>EU</td>
<td>France</td>
<td>Montpellier</td>
</tr>
<tr>
<td>Department Of Virology, University Medical Center Utrecht</td>
<td>Specialized</td>
<td>EU</td>
<td>Netherlands</td>
<td>Utrecht</td>
</tr>
<tr>
<td>Infectious Diseases Department, Hospital Carlos III</td>
<td>Specialized</td>
<td>EU</td>
<td>Spain</td>
<td>Madrid</td>
</tr>
<tr>
<td>Health Protection Agency</td>
<td>Specialized</td>
<td>EU</td>
<td>United Kingdom</td>
<td>London</td>
</tr>
</tbody>
</table>
During 2010, no new labs were accredited. However several labs that applied for accreditation during 2009 completed their pre-qualification proficiency testing (including one in Ethiopia, one in Cote d’Ivoire, and one in the Russian Federation) and 3 labs in Brazil received site visits and are working towards accreditation. Thus it is likely that between 3 and 6 labs will be accredited in 2011.

Currently, the following WHO staff are partly responsible for helping to maintain the WHO HIVDR Lab Network:

- Regional Office for Africa, West (Ouagadougou, Burkina Faso): 1 (0.5 FTE)
- Regional Office for Africa, East and South (Harare, Zimbabwe): 1 (0.5 FTE)
- Regional Office for Africa, Central (Libreville, Gabon): 1 (0.5 FTE)
- Regional Office for Southeast Asia (New Delhi, India): 0
- Regional Office for Western Pacific (Manila, Philippines): 1 (0.5 FTE)
- Regional Office for Eastern Mediterranean (Nasr City, Cairo): 0
- Regional Office for Europe (Copenhagen, Denmark): 0
- Regional Office for Americas (Brasilia, Brazil): 1 (0.5 FTE)
- Headquarters (Geneva): 2 (0.2 FTE)

In addition, WHO uses several consultants and advisors, including the principal investigators in the accredited labs themselves, to address specific issues as they arise (for example, Quality Control for Molecular Diagnostics, Data First Consulting, Tencza Designs).

**External Quality Assurance Program**

In 2010, as in previous years since 2007, a panel of 5 blinded plasma specimens were prepared and distributed to the lab network by the Virology Quality Assurance (VQA) program in Chicago, IL (USA). The 2010 panel was sent to 28 labs including all accredited labs and new labs seeking accreditation; since some labs tested the panel with more than one type of assay, a total of 37 data sets were submitted. Of these, 28 submissions passed after one attempt; of the submissions that failed, 1 passed on repeat testing, 2 failed again, and 6 are pending. A more detailed description of the results from this panel as well as from those tested in previous years is included in a manuscript that has been submitted for publication in a special supplement to *Clinical Infectious Diseases* in 2011.

**Training and Workshops**

A training workshop on HIVDR Genotyping was held in Ho Chi Minh City, Vietnam, May 25 to 28, 2010. The workshop was attended by laboratory technicians and supervisors from 11 countries in the Western Pacific and Southeast Asian regions. Representatives from the 2 accredited Regional DR Laboratories in Australia (Sydney and Melbourne) participated and
helped facilitate the workshop. A list of topics covered is below. Additional details of the workshop topics and materials can be found on the WHO website.

*Module 1*: Introduction to HIV Drug Resistance
*Module 2*: WHO Laboratory Network
*Module 3*: Principles of PCR and HIVDR Sequencing
*Module 4*: Molecular Laboratory Set Up and Workflow
*Module 5*: Sequencing Procedures
*Module 6*: Use of Sequencing Output
*Module 7*: Criteria for In-house Assay Validation
*Module 8*: Dried Blood Spots for HIVDR Genotyping
*Module 9*: A Systems Approach to Laboratory Quality
*Module 10*: Standard Operating Procedures (SOPs)
*Module 11*: Quality Control and Quality Assurance
*Module 12*: Data Management
*Module 13*: Equipment and Supplies
*Module 14*: Stock Management
*Module 15*: Specimen Management
*Module 16*: Biosafety and Waste Management
*Module 17*: Lab Staff Experience and Training

In March 2010, on request from Dr. Michael Jordan of the WHO HIV Drug Resistance Team, Dr. Mary Kearney (HIV Drug Resistance Program, National Cancer Institute, NIH) supported capacity building by conducting a two week training course on HIV genotyping and sequence analysis at the Pasteur Institute in Ho Chi Minh City, Vietnam. Each day included a 1-2 hour presentation covering a wide range of topics from molecular laboratory setup to phylogenetic analysis, and 4 hours of practical education on HIV genotyping. Dr. Kearney made recommendations to improve the lab’s ability to genotype samples with low viral loads by altering methods for viral extraction from plasma, and to genotype samples with high viral loads by adding a dilution step prior to the nested PCR to increase their amplification efficiency. Recommendations to increase throughput included amplifying multiple gene fragments in a single amplicon to reduce the number of PCR reactions and designing primers with degenerate bases to reduce primer/target mismatches. In addition to technical advice, Dr. Kearney also emphasized quality control by teaching rigorous methods to scrutinize sequence data using phylogenetic analyses to ensure that there was no sample mix up or cross-contamination. The technical education provided to the Pasteur Institute in Ho Chi Minh City, Vietnam will result in higher capacity for HIV genotyping at this site and will contribute towards the laboratory achieving WHO accreditation.

Finally, the lab network hosted over 80 trainees from other institutions in 2010. The trainees received training in practical and theoretical aspects of HIVDR genotyping.
Testing Activity (Genotype Assays for HIVDR Surveys)
One of the core functions of the laboratory network is to provide genotype testing of specimens from WHO surveillance and monitoring surveys. In 2010, a total of >2000 specimens were tested, as part of threshold surveillance surveys and monitoring surveys conducted in several countries (including Angola, Ghana, Kenya, Malawi, Nigeria, South Africa, Zimbabwe, China, Vietnam, Thailand).

Operational Research
Operational research on issues relevant to the resource-limited settings where WHO surveys are being performed is an important responsibility of the laboratory Network, particularly the Specialized and Regional labs. Studies being performed in various locations are listed below.

HIVDR genotyping assay development
- An investigation into HIV drug resistance and subtype distribution in the Eastern and Western Highlands of Papua New Guinea (Burnet).
- Optimization of a broadly-sensitive genotyping assay for HIV-1 drug resistance surveillance (CDC).
- Development of in-house genotyping assay methods to replace commercial kits (UMC Utrecht).
- The application of a multiplexed, parallel, high throughput, real-time PCR-based pathogen detection system for HIV drug resistance testing; validation and characterization of DBS technology as applied to HIV drug resistance testing; RNA stability in dried plasma spots to determine optimal storage conditions (PHAC).

HIVDR Genotyping using Dried Blood Spots
Two important studies, one initiated in 2009 and completed in 2010, and one in final planning stages in 2010, are expected to provide important information regarding the use of dried blood spots (DBS) for genotyping.

1. Methods for viral load testing and genotyping from DBS (Hospital Carlos III, Madrid, Spain). Several commercial and in-house RNA extraction methods were compared using viral load measurements. Genotyping was also performed on a subset of the specimens. Results were presented at 2 international conferences in 2009, and a manuscript describing the study and its conclusions is in final stages of preparation, for submission in 2010.

2. Field study of DBS stability and shipping conditions (UVRI, Entebbe, Uganda, and CDC-GAP, Atlanta, GA). Plasma and DBS from 100 patients on antiretroviral therapy will be collected and stored at ambient temperature in Entebbe, Uganda for 2 or 4 weeks, then shipped to the genotyping lab at CDC in Atlanta either on dry ice or at ambient temperature. Amplification rates and sequences will be compared between groups. In addition the study will compare DBS specimens made from EDTA-anticoagulated blood with DBS made directly from a finger prick. Specimen collection is expected to begin in January, 2011.
Additional DBS-related operational research activity includes:

- Evaluation of Dried Blood Spots as Alternative Sample Type in Viral Load and HIV-1 Drug Resistance Genotyping in Patients Receiving Antiretroviral Therapy (CDC)
- Viral load determinations (RealTime Abbott HIV-1 m2000rt) and genotypic resistance testings (in house PCR) in DBS during storage at different temperatures (Hospital Carlos III, Madrid, Spain)
- Viral load quantification (NucliSENS EasyQ HIV-1 assay, version 1.2) and genotypic resistance testing (ViroSeq HIV-1 Genotypic System) in DBS from patients on treatment in Tanzania (Hospital Carlos III, Madrid, Spain).
- Analyzing the feasibility of using DBS for HIVDR genotyping (DRVI, Beijing, China)

Dried Blood Spot-based Genotype Assay Validation

Given the diversity of genotyping methods across countries and labs, it is essential to establish uniform standards for DBS testing as more and more countries are using DBS for their HIVDR surveys. WHO and the HIVResNet are taking a multi-faceted approach to address this need, including:

1. Production and regular updating of WHO guidelines for DBS testing by the WHO HIVResNet DBS Working Group: A laboratory guidance document describing recommended procedures and issues related to the use of DBS for genotyping was finalized in March 2010 and made available on the WHO website. This document provides a detailed protocol as a starting place for labs with limited DBS experience, and a discussion of important considerations related to preparation, storage and shipping of DBS. It will be updated as new developments arise, including possible procedural improvements based on validation studies (see below).

2. Validation of DBS-based assay procedures: A large panel of clinical specimens prepared as plasma and DBS was prepared in 2009 by 2 of the Specialized DR laboratories (Madrid, Spain, and Utrecht, Netherlands) and distributed to 10 labs in the network with DBS experience. Analysis of results, completed in early 2010, demonstrated considerable variability in assay sensitivity and reproducibility between labs. Key variables that are likely to impact performance and that differ between the procedures that performed well were compared to others were identified. Further methodological comparisons are planned for early 2011 to confirm these observations and inform potential protocol improvements.

3. Implementation of EQA panels consisting of DBS specimens: EQA programs are a key component that helps to ensure the quality of laboratory results. The use of EQA and proficiency testing helps to monitor and control lab-to-lab variability. Therefore, DBS-based EQA panels are needed, and will be based on the successful plasma-based program already in place and supported by the VQA (see above). Well-characterized specimens will be used to prepare a large number of DBS cards under optimal conditions. Initially, specimens will be shipped on dry ice or at ambient temperature in parallel, in order to assess whether shipping conditions impact genotyping assay performance. Otherwise, the DBS EQA program will resemble that described above for plasma. The first results of this study are expected in early 2011.
4. **Validation guidelines and accreditation criteria:** An important step towards standardization of existing DBS-based genotyping assays is assay validation performed according to a consistent methodology. While the 10 network labs with the most experience working with DBS have achieved this through the validation panel testing (see point #2 above), other labs that are interested in becoming accredited for DBS-based testing have not. Therefore a more generic description of minimum requirements for assay validation is being developed, which along with definition of other criteria for accreditation will allow for uniform evaluation of candidate labs and assay performance.

**Plans for 2011 and Beyond**

In the coming year, there will be continued focus on DBS-based genotyping in the following areas:

- Optimization of a standardized procedure
- Definition of optimal storage and shipping conditions under field conditions
- Accreditation of several laboratories for DBS
- External QA (Proficiency panels) on DBS

In addition, operational research into lower-cost genotyping assays will be encouraged.

The lab network is expected to grow in 2011 (between 3 and 6 new labs, located in Brazil, Côte d’Ivoire, Ethiopia, and the Russian Federation), and the annual EQA testing using plasma specimens will continue as before.

As the grant from the Bill and Melinda Gates Foundation enters its 5th year of funding, the sustainability of the lab network will take on greater importance. Network labs, especially Specialized and Regional labs, will continue to provide technical assistance and training for capacity building in countries with labs working towards accreditation. WHO offices in countries with testing needs are being encouraged to include requests for laboratory support in their applications to alternative funding sources such as the Global Fund for HIV, TB and Malaria, the President’s Emergency Plan for AIDS Relief (PEPFAR) and the country’s own national AIDS budget.

**Publications and Presentations in 2010**

**WHO guidance and technical documents**

- [DBS protocol](#)
- DBS review article (AIDS Reviews)
- [Lab guidance document](#)
Peer-reviewed publications


Nguyen Bui Duc, Bui Thu Hien, Nick Waggar, Tran Hong Tram, Le Truong Giang, Chunfu Yang, Mitchell I. Wolfe, Nguyen Tran Hien, Nguyen Anh Tuan. HIV drug resistance threshold survey using matched plasma and DBS specimens from voluntary counseling and testing sites in Ho Chi Minh City, Vietnam, 2007-2008 (Submitted to CID)


Conference Presentations


Paul Weidle, J Kiarie, P Intalapaporn, J Stringer, P Muiruri, I Zulu, M McConnell, C Yang, O Bolu, J Johnson, and the NNRTI Response Team. NVP Resistance Mutations among Women Exposed toa sdNVP Intropartum more than 1 Year Prior to Starting NNRTI-based ART are not Associated with Virologic failure. CROI 2010, Feb 16-19, San Francisco.

M McConnell, T Jariyasethppong, N Chantharajwong, C Utenpitak, Z Zhou, J-F, Li, J McNicholl, O Bolu, P Weidle, and T Anekthananon. Low level viremia in Thai women 24 weeks after treatment initiation with NNRTI-based antiretroviral therapy (ART) was not associated with prior single-dose nevirapine exposure or viral resistance mutations. CROI 2010, Feb 16-19, San Francisco.


Conradie F, C Wallis, W Stevens, M Fox, C Van Der Horst, R Wood, M Dehlinger, J McIntyre, I Sanne, and CIPRA-SA Study Team. Virological Failure Rates among Women Exposed to sdNVP Compared to Women not Exposed to sdNVP: Results from CIPRA-SA. CROI 2010, Feb 16-19, San Francisco.

Wallis CL, D Struck; D Perez Bercoff; M Bronze; G Denisov; JC Schmit; TF Rink de Wit; W Stevens on behalf of the ART-A consortium. Evaluation of an automated sequence analysis software programme in high-throughput laboratories. 8th European HIV Drug Resistance Workshop, Italy, 2010.


Acknowledgements
WHO gratefully acknowledges the contributions of the laboratory network to the preparation of this report. The report was prepared by Data First Consulting, Inc., Menlo Park CA USA in consultation with Dr. Michael Jordan and Silvia Bertagnolio at WHO, Geneva, Switzerland.