

WHO HIVDR Laboratory Network Annual Report for 2010

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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

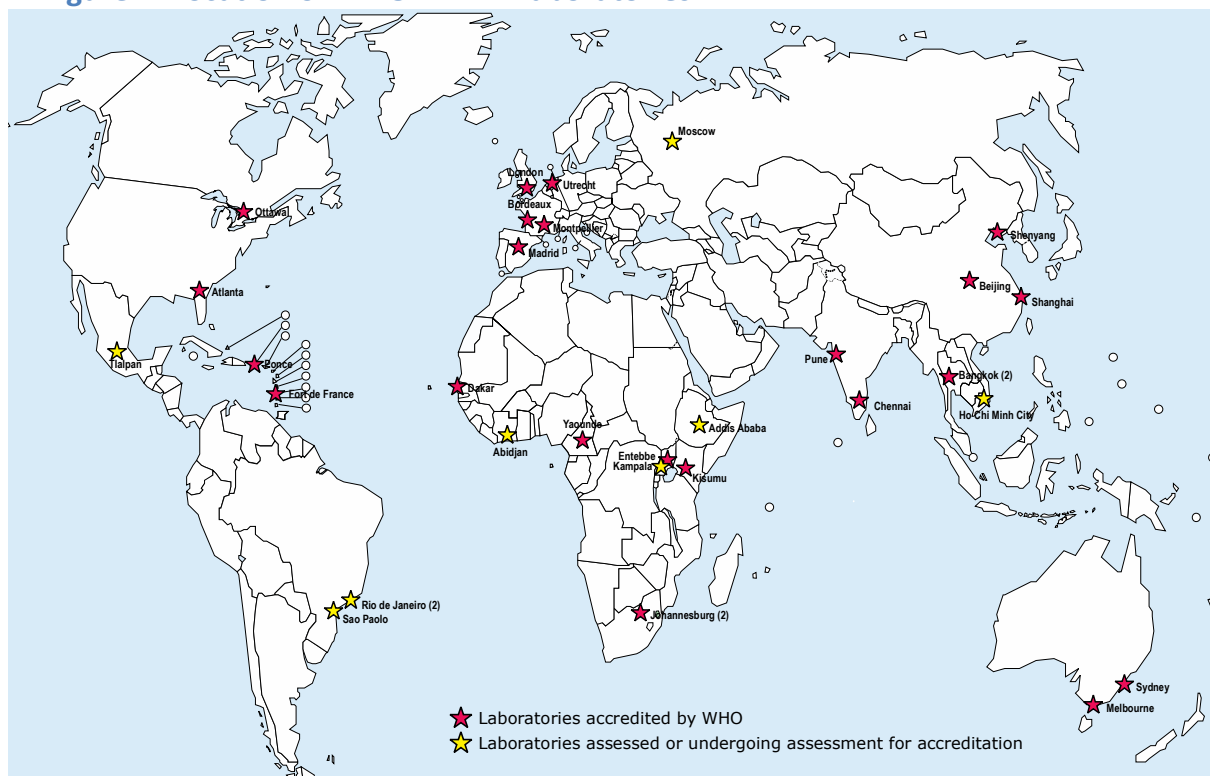
Laboratory Network

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

Region	National	Regional	Specialized	Total
AF	3	3	0	6
SEA	4	0	0	4
WP	3	2	0	5
EU	0	0	5	5
AM	0	2	2	4
Global	10	7	7	24

Figure 1: Location of WHO HIVDR Laboratories



This map is an approximation of actual country borders

Table 2. List of Accredited Laboratories

Laboratory Name/Institution	Type	WHO Region	Country	City
IMPM-IRD/CREMER	National	AF	Cameroon	Yaounde
KEMRI/CDC HIV Research Laboratory	National	AF	Kenya	Kisumu
Bacteriology-Virology UTH A Le Dantec	National	AF	Senegal	Dakar
Department of Clinical Research Tuberculosis Research Centre (ICMR)	National	SEA	India	Chennai
National AIDS Research Institute, Indian Council of Medical Research	National	SEA	India	Pune
National Institute of Health, Department of Medical Sciences	National	SEA	Thailand	Nonthaburi
Dept. of Microbiology, Siriraj Hospital	National	SEA	Thailand	Bangkok
Division of Research on Virology and Immunology (DRVI), NCAIDS, Chinese Center for Disease Control and Prevention	National	WP	China	Beijing
Shanghai Municipal Center for Disease Control and Prevention	National	WP	China	Shanghai
Key Laboratory of Immunology of AIDS, Ministry of Health	National	WP	China	Shenyang
AIDS Virus Research Unit, National Institute for Communicable Diseases	Regional	AF	South Africa	Johannesburg
MRC/UVRI Basic Sciences Laboratory	Regional	AF	Uganda	Entebbe
Service de Virologie Immunologie Centre Hospitalier et Universitaire de Fort-de-France	Regional	AM	Martinique	Fort de France
Clinical Research Laboratory, Burnet Institute for Medical Research and Public Health	Regional	WP	Australia	Melbourne
CLS Genotyping Laboratory, Johannesburg General Hospital	Regional affiliated	AF	South Africa	Johannesburg
AIDS Research Program-Immunology Reference Laboratory	Regional affiliated	AM	Puerto Rico	Ponce
NSW State Reference Laboratory for HIV and Molecular Diagnostic Medicine	Regional affiliated	WP	Australia	Sydney
National Laboratory for HIV Genetics, PHAC	Specialized	AM	Canada	Ottawa
International Laboratory Branch, GAP, NCHSTP, CDC	Specialized	AM	United States	Atlanta
Laboratoire de Virologie, CHU	Specialized	EU	France	Bordeaux
UMR145, AIDS and Associated diseases, IRD and UM1	Specialized	EU	France	Montpellier
Department Of Virology, University Medical Center Utrecht	Specialized	EU	Netherlands	Utrecht
Infectious Diseases Department, Hospital Carlos III	Specialized	EU	Spain	Madrid
Health Protection Agency	Specialized	EU	United Kingdom	London

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, Cote 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

Silvia Bertagnolio, Neil T. Parkin, Michael Jordan, James Brooks, J. Gerardo García-Lerma. Dried blood spots for HIV-1 Drug Resistance and Viral Load Testing: A Review of Current Knowledge and WHO Efforts for Global HIV Drug Resistance Surveillance. *AIDS Rev.* 2010;12:3-16.;

Chunfu Yang, Neil Parkin, Karidia Diallo and Silvia Bertagnolio. HIV-1 Drug Resistance in Resource-Limited Settings: Public Health Approach for Population-Based Resistance Surveillance and Implication for Health System Strengthening. *Clinical Microbiology Reviews* (in preparation).

Arredondo M, Garrido C, Parkin N, Zahonero N, Bertagnolio S, Soriano V and de Mendoza C. Comparison of HIV-1 RNA measurements in plasma and dried blood spots (DBS) using the Abbott real Time Viral Load assay, and automatic viral load methodology. (In preparation, to be submitted to *J Clin Microbiol*)

Johannessen A, Garrido C, Zahonero N, Naman E and de Mendoza C. HIV-1 drug resistance testing from dried blood spots collected in rural Tanzania using the ViroSeq HIV-1 Genotyping System. *J Antimicrob Chemother* 2010. (epub ahead of print) doi:10.1093

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EK Rottinghaus, R Ugbenga, K Diallo, O Bassey, A Azeez, J DeVos, I Wurie, J Aberle-Grasse, J Nkengasong, N Knight, C Yang. Dried Blood Spots are a Suitable Alternative Sample Type for Viral Load and HIV-1 Drug Resistance Genotyping in Patients Receiving Antiretroviral Therapy (in preparation).

Zhiyong Zhou, Nick Wager, Joshua R DeVos, Erin Rottinghaus, Karidia Diallo, John Nkengasong, Chunfu Yang. Optimization of a broadly-sensitive genotyping assay for HIV-1 drug resistance surveillance using plasma and DBS from ARV naïve and experienced patients in PEPFAR supported countries (in preparation).

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Conference Presentations

Miguel Arredondo, Angélica Corral, Natalia Zahonero, Carolina Garrido, Maria de la O López, Vincent Soriano, and Carmen de Mendoza. Evaluation of Viral Load and Drug Resistance on Dried Blood Spots Following Automatic Nucleic Acid Isolation under Different Storage Conditions. Poster THPE0459. *AIDS 2010* (Vienna).

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