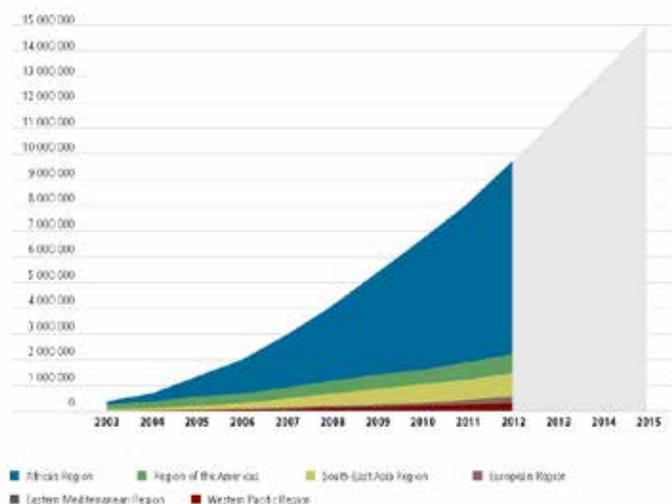


# SURVEILLANCE OF HIV DRUG RESISTANCE

## INTRODUCTION

Human Immunodeficiency Virus type 1 (HIV-1) is the etiologic agent responsible for the Acquired Immunodeficiency Syndrome (AIDS). The virus belongs to the lentivirus family of retroviruses and produces chronic infection damaging the host's immune system. The clinical course of HIV-1 infection is remarkable due to a high degree of strain variability, both genotypically and phenotypically. This heterogeneity is a result of the relatively high frequency of viral replication errors as a result of the retroviral reverse transcriptase (RT) lacking a proofreading function. When the virus replicates, it can produce genetic variations that may be compatible with continued viral growth and survival as well as resistance to antiretroviral drugs (ARVs).

As HIV infection has already reached global epidemic proportions, efforts supported by domestic and international funding have propelled the use of ARVs, totaling 9.7 million recipients within low- and middle-income countries at the close of 2012.<sup>1</sup> (Fig. 1) However, while antiretroviral therapy (ART) is reducing mortality rates from AIDS-related causes, the widespread and long-term usage of ARVs raises concerns with regards to viral mutation and the emergence of ARV resistance among evolving strains of HIV.



**Figure 1.** Actual and projected numbers of people receiving antiretroviral therapy in low- and middle-income countries, and by WHO Region, 2003-2015

## White Paper

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Treatment of HIV-1 infections with potent ARVs can result in a profound and durable suppression of HIV-1 replication resulting in plasma HIV-1 RNA concentrations below levels of detection. However, while some mutations introduced into the HIV-1 genome during replication will compromise infectivity, others display compatibility with virus infectivity in combination with ARV resistance. As such, the long-term success of ART in developing countries should be complemented by HIV drug resistance (HIVDR) monitoring programs to ensure favorable treatment outcomes, and to minimize treatment failure and transmission of HIVDR.<sup>2</sup>

## TREATMENT FAILURE

Antiretroviral therapy has increased in developing countries as a result of various sources of multinational support, including the U.S. President's Emergency Plan for AIDS Relief (PEPFAR) and the Global Fund to Fight AIDS, Tuberculosis and Malaria.<sup>2</sup> However, long-term usage of ARV drugs have led to HIV strains displaying single- or multi-drug resistance within ARV-treated and ARV-treatment-naïve populations, resulting in treatment failure.

Treatment failure has been defined broadly as a failure to achieve or maintain viral suppression via:

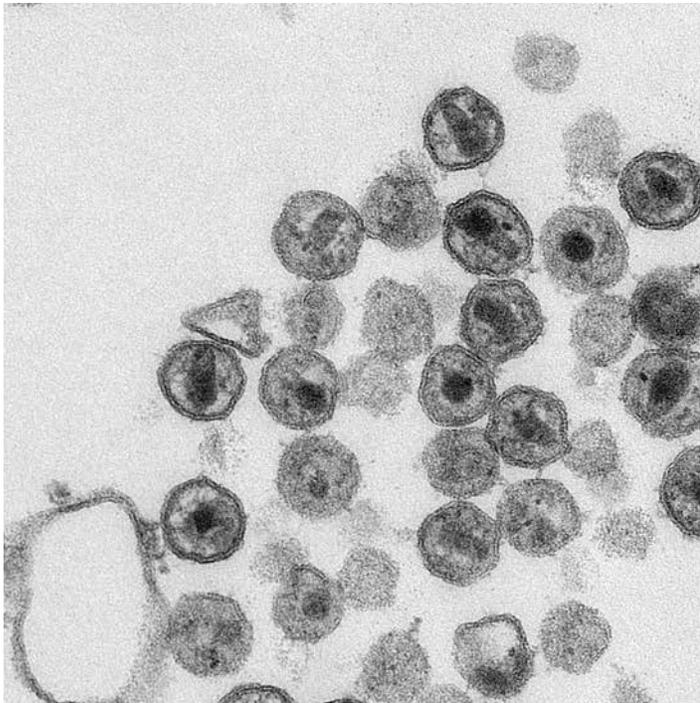
- Detectable HIV RNA in plasma
- A decrease in CD4+ cell count
- Progression of disease, including clinical signs and symptoms related to immunodeficiency

Frequently, treatment failures are a result of several factors such as poor adherence to treatment regimens, drug potency, emergence of virus strains resistant to drugs, and pharmacokinetic issues related to a specific drug.

The emergence of ARV-resistant HIV strains has resulted in a global initiative to monitor for changes in resistance to one or more drugs in each of six classes, including: Nucleoside reverse transcriptase inhibitors (NRTI), non-nucleoside reverse transcriptase inhibitors (NNRTI), protease inhibitors (PI), fusion inhibitors, entry inhibitors,

and integrase strand transfer inhibitors (INI). Likewise, genotyping and mutation analysis have shown that resistance to a given drug can generate cross-resistance to other drugs of the same class. However, treatment failure does not always result in resistance to all drugs in a regimen.

Since HIV-1 replication occurs rapidly, large numbers of viral variants are generated, including those that have diminished sensitivity to ARVs. Mutations that result in resistance to ARVs can be present in HIV-1 infected individuals before anti-retroviral therapy (ART) is initiated due to the transmission from an individual having had prior therapy or spontaneous mutations. Once drug therapy is initiated, the pre-existing population of drug resistant viruses will rapidly predominate as a result of selective pressure. Some mutations selected by ARVs directly affect viral enzymes and cause resistance by decreased drug binding, where others have indirect effects. Treatment with different ARVs may select for variants that harbor similar mutations. In addition, treatments can select for the outgrowth of HIV-1 variants that are resistant to drugs to which the patient has not yet been exposed, known as cross-resistance.



HIV virions. Photo courtesy: CDC

Having knowledge of the viral resistance genotype is a key factor in identifying new treatment strategies. Retrospective and prospective intervention-based studies have shown the usefulness of resistance testing. These studies have indicated that the presence of drug resistance is an independent risk factor for treatment failure. Resistance testing is recommended for persons with HIV infections when they enter into care, regardless of when therapy is begun, with repeat testing being performed at the initiation of ARV therapy. HIV drug resistance testing should also be performed when changing ARV regimens as a result of virologic failure.

## GENOTYPING MIXED HIV-1 POPULATIONS

Surveillance of HIVDR by molecular genotyping is an essential element in ensuring ongoing ART efficacy in resource-limited countries.<sup>3</sup> However, a major difficulty with evaluating HIVDR in resource-limited countries has been the costly expense of commercial genotyping assays, which detect HIV-1 group M subtype B viruses, representing the predominant strains typically isolated in resource-rich countries.<sup>3</sup>

In response to the need for a cost-effective HIV-1 genotyping assay for surveillance of virus strains frequently isolated within resource-limited countries, the CDC developed an optimized HIV-1 Pan Group M Drug Resistance Genotyping Assay. Three primers for one step reverse transcriptase (RT) and PCR and two for nested PCR were designed to detect both the RT and protease regions from the *pol* gene, while 4 additional primers were designed specifically for sequencing. When validated and compared with commercial HIV-1 group M subtype B genotyping assays, the CDC assay out-performed commercial assays in use with dried blood spots (ideal for resource-limited areas), and detected more mixture bases, with minimal background noise observed in sequence chromatographs.<sup>3</sup>



The Division of Global HIV/AIDS (DGHA) within the Center for Global Health is responsible for strengthening surveillance systems, building high quality laboratories, and programs to prevent and control human immunodeficiency virus (HIV) infection and acquired immunodeficiency syndrome (AIDS). Program staff work in collaboration with governmental and nongovernmental partners at different country levels, applying well-integrated multidisciplinary programs of research, surveillance, technical assistance, and evaluation. Photo credit: James Gathany

To facilitate rapid production and effective distribution of the assay, the CDC partnered with ATCC for scale-up and distribution of a ready-to-use kit to WHO-designated and CDC-supported PEPFAR Genotyping Laboratories, taking advantage of ATCC's long-standing history and expertise in global distribution of biological reagents & research materials. The licensed assay, labeled the ATCC® HIV-1 Drug Resistance Genotyping Kit, is indicated for use in detecting HIV-1 genomic mutations in the protease and part of the RT regions of the HIV-1 *pol* gene that confer resistance to specific types of ARVs. Further, the kit may be used to sequence and genotype the protease and RT regions of the *pol* gene, which represent the primary targets of ARVs and the principle locations of ARV-resistance mutations. Indications for use of the HIV-1 Drug Resistance Genotyping Kit encompass the following populations:

- HIV-1 infected individuals at drug therapy failure (non-suppressive viral load) before therapy switch
- HIV-1 infected individuals upon clinical presentation at diagnosis or before initial drug therapy

The ATCC HIV-1 Drug Resistance Genotyping Kit is comprised of two modules, providing reagents for RT-PCR, nested PCR in the first module, and sequencing in the 2<sup>nd</sup> module. The entire protease and two-thirds of the RT gene are amplified to generate a 1.1kb amplicon that is used as a sequencing template for six overlapping and bi-directional primers that generate a consensus sequence of 1.04 kb. Commercially available software is used to trim the ends of the individual primer sequence files and assembles them into a consensus sequence. Once the sequence is edited and verified, the resulting final consensus sequence is submitted to the Stanford HIV database (<http://hivdb.stanford.edu>) where the drug resistance profile is generated.

## HIV DRUG RESISTANCE DATABASE

The HIV drug resistance database (HIVDB) is an open access, public database curated by Stanford University, providing a wide range of tools to upload and analyze HIV mutations associated with ARV resistance. The HIVDB offers three primary types of content, including:

- Modules to query the database, allowing investigators to evaluate genotype-treatment correlations, genotype-phenotype correlations, or genotype-clinical outcome correlations
- Interactive programs; allowing for analysis of user-submitted mutations or complete sequences
- Educational resources, including drug resistance summaries and the surveillance drug resistance mutation list

The HIVdb program represents one of the interactive tools provided by the HIVDB, which allows investigators to upload protease and RT sequences, returning inferred levels of resistance to 19 commonly used protease and RT inhibitors.<sup>4,5</sup> The easy-to-use graphical interface allows investigators to enter multiple sequences in FASTA format by inputting data into a text box or uploading a text file. Output from the HIVdb includes:

- Summary data, which provides subtyping of the submitted sequence based on codon location, insertions/deletions, and comparison to and distance from the database reference sequences
- Sequence quality assessment, identifying areas of poor quality represented by stop codons, frame shifts, ambiguous nucleotides, or unusual residues
- Protease inhibitor (PI) resistance interpretation, classifying major, minor, and other mutations, and providing estimates for the level of PI resistance associated with the submitted sequence

Other interactive tools provided by the HIVDB include the HIValg program, combining the power of three algorithms (HIVdb, ANRS, and Rega) to interpret genotypic resistance; the HIVseq program, which reports the prevalence of the mutations in a submitted sequence within the HIVDB with regard to subtype and treatment history; and, MARVEL, the Mutation ARV Evidence Listing, linking specific mutations to specific drugs.

## CONCLUSION

The World Health Organization (WHO) reports that there are over 25 million people eligible for ART in low- and middle-income countries.<sup>1</sup> Currently, only about 39% of these individuals are receiving treatment, but the WHO goal is to reach a total of 15 million by 2015. As WHO achieves its goals for treatment, the corresponding need for surveillance of HIV drug-resistance should grow at an equivalent rate.

Surveillance of strains via rapid PCR-based genotyping, in particular, strains more closely associated with resource-limited countries, is made possible with technology developed by CDC and supported by ATCC. Genotyping provides scientists and clinicians with a genetic map. This genetic map aids in monitoring the development and transmission of drug resistance mutations among an ARV-treated population or an ARV-treatment-naïve population recently diagnosed with HIV-infection.

The ATCC HIV-1 Drug Resistance Genotyping Kit allows for rapid detection and sequencing of HIV strain variants in the field, specifically WHO-designated and CDC-supported PEPFAR Genotyping Laboratories, and provides additional information to the scientific community through the Stanford HIV Drug Resistance Database to further research related to HIV ARV drug resistance. These tools endeavor to support researchers around the globe, to positively impact the success of HIV treatment within resource-limited populations, and to assist in the global initiative to understand and stop the spread of HIV and ARV-resistant strains.



The Stanford HIVDB strives to connect researchers around the globe with data on HIV drug resistance genotyping data.

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