

## **RESISTANCE TESTING**

### ***TEST METHODS***

There are two types of tests: phenotypic and genotypic assays.

#### ***Phenotypic Assays***

*In vitro*, resistance is based on the ability of the virus to grow in the presence of an inhibitor compared with a more susceptible control virus (wild-type).

These assays measure the ability of HIV to replicate at different concentrations of a tested ARV agent. The method involves isolation, amplification, and insertion of the reverse transcriptase (RT) and protease genes from the patient into a backbone laboratory clone by cloning or by recombination. Replication is then monitored at various drug concentrations and compared with a reference. Results are reported as the IC<sub>50</sub> (50% inhibitory concentration) for the test strain relative to that of a reference or a wild-type strain.

#### ***Genotypic Assays***

The genotype is defined as the nucleotide sequence from which a protein's amino acid sequence can be deduced. This sequence can be compared to a reference wild-type genotype. Any change from the wild-type is usually reported as a change in amino acid at a specific residue (codon) of the protein.

Genotypic assays identify mutations into the RT and protease gene. The methodology is: 1) amplification of the RT and/or protease gene by RT PCR; 2) DNA sequencing of amplicons generated for the dominant species; and 3) reporting of mutations for each gene.

The results of these assays can be obtained in one to two weeks and are reproducible. Limitations are viral load >500 - 1,000 copies/mL and identification of mutations present in >10% - 20% of plasma virions.

Due to the continuous rising of the number of mutations associated with resistance, only assays allowing the complete determination of nucleotides sequences of the RT *and* protease gene should be used.

Commercial kits exist, but many laboratories utilise their own sequencing methods.

#### ***Interpretation of Genotypic Assays***

Resistance to ARV drugs is due to complex combinations of mutations in the HIV genes coding for the RT or protease. The interpretation of the mutations observed and of their combinations needs the expertise of the analyst and algorithms regarding each ARV. As the interpretation algorithms are quickly evolving with time, the following links should be consulted:

Table from IAS-USA updated at <<http://www.iasusa.org>>; see also <<http://hivdb.stanford.edu/pages/seqAnalysis.html>>.

Table from ANRS updated at <<http://www.hivfrenchresistance.org>>.