Where do my specimens go?

TESTING FOR HIV-1 DRUG RESISTANCE: ViroSeq

When plasma for drug resistance testing arrives from your site to the Pittsburgh-based MTN Virology CORE lab, we test it using the ViroSeq™ HIV-1 Genotyping System by Abbott Molecular Systems. This assay is FDA-approved and the results can be used for patient management.

The assay is complex and requires at least 3 separate, contained laboratory rooms (an RNA only “Clean” room, a DNA amplification “Dirty” room, and a sequencing room) and several specialized pieces of laboratory equipment, the most expensive being the ABI-3100 Genetic Analyzer (Figure 1). The procedure takes about 3 days, not including data analysis. The samples can be batched and are run on 96-well plates, which make centralizing resistance testing more efficient when the sample number from each site is relatively low.

Despite the time and cost involved in genotyping, the results obtained are essential for determining whether participants who become infected in the trials have drug resistant HIV-1. This procedure enables us to analyze every base in key regions of the reverse transcriptase and protease genes of HIV-1, and identify whether any changes have occurred. By comparing sequences from participant HIV-1 to known reference sequences, we can evaluate whether any changes seen are mutations associated with drug resistance to tenofovir or FTC (emtricitabine), or whether the changes are common polymorphisms.

Figure 1. ABI-3100 Genetic Analyzer

The ABI PRISM® 3100 Genetic Analyzer is a multi-color fluorescence-based DNA analysis system with 16 capillaries operating in parallel. Photo from Applied Biosystems product brochure.
ViroSeq™ HIV-1 Genotyping Protocol

The following is a general description of how we obtain a DNA sequence from the plasma sample you send us:

The plasma samples are thawed and prepared for testing. First, the plasma sample undergoes high speed ultracentrifugation to pellet virus. RNA is then extracted from the virus, reverse transcribed to DNA, and amplified (RT-PCR).

A spin column system followed by separation through an agarose gel is used to purify then quantify PCR product. The sequencing reaction is prepared by adding each DNA product to 7 different sequencing primers in separate wells of a 96-well plate (Figure 2). This means we can run 12 samples per plate (10 clinical samples, 1 negative control and 1 positive control).

Next, the pre-sequencing products are purified using the Centri-Sep Strip method, which is also a spin-column based purification procedure. The purified products are spun in a Speed-Vaccuum until completely dry, then re-suspended in formamide.

Finally, the ABI-3100 Genetic analyzer is cleaned and primed, and the plate is prepared and inserted into the machine for sequencing. The sequencer can run two columns of samples in approximately 2.5 hours, so to run a 96-well plate may take ~15 hours of continuous machine time.

Figure 2. Primers Used in ViroSeq

The gene region covered by ViroSeq. Figure from ViroSeq product brochure.
Data Analysis

ViroSeq software is used to analyze the data from the ABI-3100 Genetic Analyzer. The data is presented as a graph called an "electropherogram" and also as a list of each base (A, T, C and G) in order of 5’ to 3’ from the gene region sequenced (Figure 3). The data must be re-analyzed manually (by eye) to make sure each peak is distinct and has been correctly identified. With experience, you learn to differentiate between a correct peak and background “noise.”

Figure 3. ViroSeq Sequence Analysis Software

Example sequence analysis screen from the ViroSeq™ software.

Patient Report

Finally, when the data has been double checked, we use the ViroSeq software to generate a patient report. The report will let us know what resistance mutations, if any, are present in the sample, and what drugs the patient may be resistant to as a result of having these mutations (Figure 4).

Figure 4. ViroSeq Sample Antiviral Drug Resistant Report