

# 661 Sensitivity of the ViroSeq™ HIV-1 Genotyping System for detection of the K103N resistance mutation in HIV-1 subtypes A, C and D

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## ABSTRACT

**Background:** Detection of HIV-1 drug resistance mutations is complicated by the wide variation in plasma viral load, the need to isolate and reverse transcribe HIV-1 RNA prior to analysis, and the natural genetic diversity of HIV-1 viruses. The ViroSeq™ HIV-1 Genotyping System (ViroSeq) and other population sequencing-based assays detect mutations present in the major viral population in a test sample. These genotyping methods also detect some mutations that are present at lower levels.

**Methods:** We compared detection of the K103N resistance mutation in subtype A, C and D HIV-1 using ViroSeq and a sensitive and quantitative point mutation assay, LigAmp, which measures the percentage of K103N-containing variants in the viral population (% K103N).

**Results:** Both assays were used to analyze 305 samples collected from Ugandan and Malawian women 6-8 weeks after administration of single dose nevirapine (NVP): 146 with subtype A, 64 with subtype C, and 95 with subtype D. ViroSeq detected K103N in 100% of samples with >20% K103N, 77.8% of samples with 10-20% K103N, 71.4% of samples with 5-10% K103N, and 16.9% of samples with 1-5% K103N. The sensitivity of ViroSeq for detection of K103N was similar for subtypes A, C and D. Detection of K103N at levels between 1-20% in 107 (35.1%) of the 305 samples was unlikely to represent false positives in the LigAmp assay, since K103N was detected in only 1 of 238 available samples (0.4%) collected from these women prior to NVP administration.

**Conclusion:** The ViroSeq system reliably detects the K103N mutation at levels above 20%, and frequently detects the mutation at lower levels. Further studies are needed to compare the sensitivity of different assays for detection of HIV-1 drug resistance mutations, and to determine the clinical relevance of HIV-1 minority variants.

## INTRODUCTION

HIV-1 variants with the K103N mutation are often selected in women who receive a single dose nevirapine for prevention of HIV-1 mother-to-child transmission. We evaluated the sensitivity of ViroSeq for detection of K103N in 305 African women 6-8 weeks after single dose nevirapine. This included 241 Ugandan women in the HIVNET012 trial (146 subtype A and 95 subtype D) and 64 Malawian women in the NVAZ trial (all subtype C). The level of K103N was quantified using a sensitive point mutation assay, LigAmp.

## METHODS

**Analysis of K103N with the LigAmp assay**  
 The LigAmp assay involves mutation-specific ligation of two adjacent oligonucleotides hybridized to a DNA template. Ligated oligonucleotides are quantified in a second step using a real-time PCR-based detection method (Figure 1). A standard curve is analyzed in each experiment and the % K103N is plotted against the cycle threshold. The standard curve is used to determine the % K103N in each sample. In this study, the LigAmp assay was performed using PCR products remaining after genotyping from the ViroSeq system.

### HIV genotyping with the Viroseq system

HIV-1 genotyping was performed with the ViroSeq system according to the manufacturer's instructions. Identification of K103N as present or absent during sequence editing was performed using guidelines provided with the ViroSeq system, recognizing that mutation identification at low levels may be influenced by a variety of factors.

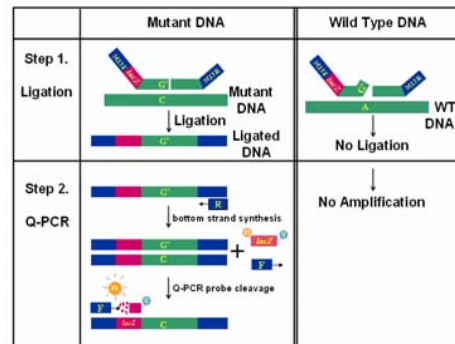


Figure 1. Overview of the LigAmp assay

## RESULTS

### Representative Results

Figure 2 shows electropherograms from nine plasma samples analyzed with the Viroseq system. Codon 103 in HIV-1 reverse transcriptase is shown. The samples were determined to be K103N positive or negative by Viroseq. The % K103N in each sample was measured using the LigAmp assay. In three samples, K103N was not detected by ViroSeq, but was detected by LigAmp (range 1.3-5.0 % K103N).

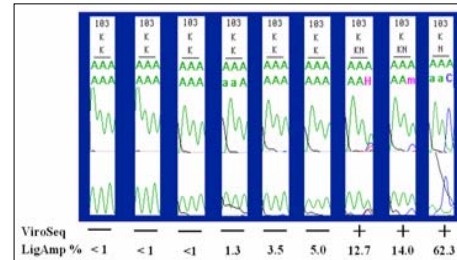


Figure 2. K103N detection using ViroSeq and LigAmp

### Detection of K103N in Women after Single-Dose Nevirapine

305 plasma samples collected from women after single-dose nevirapine were analyzed with the LigAmp assay to quantify the % K103N-containing variants in each sample. Those results were compared to results obtained with the ViroSeq system in previous studies. ViroSeq detected K103N in 79 (25.9%) of the 305 samples and LigAmp detected K103N at >1% in 140 (45.9%) of the 305 samples. ViroSeq detected K103N in all of the samples with >20% K103N, in 77.8% of samples with 10-20% K103N, in 71.4% of samples with 5-10% K103N, and in 16.9% of samples with 1-5% K103N. Similar results were obtained for samples with subtypes A, C, and D (Figure 3).

K103N was detected at levels between 1% and 20% in 107 (35.1%) of the 305 samples. These results were unlikely to represent false positives in the LigAmp assay, since K103N was detected at a level > 1% in only 1 (0.4%) of 238 available samples from these women collected prior to NVP administration. Peaks suggesting the presence of the K103N mutation were visible in ViroSeq electropherograms for all of the samples with 5-20% K103N and many of the samples with 1-5% K103N.

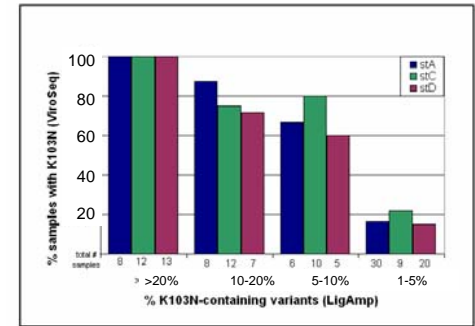


Figure 3. Sensitivity of K103N detection by ViroSeq

## CONCLUSIONS

Previous studies have documented high sensitivity and specificity of the ViroSeq system for detection of HIV-1 drug resistance mutations, and excellent performance of ViroSeq for analysis of diverse HIV-1 strains. This report demonstrates that ViroSeq consistently detects the K103N mutation in plasma samples with subtypes A, C and D at levels over 20% of the viral population, and often detects the K103N mutation at lower levels. Sensitive point mutation assays, such as LigAmp, have recently been used to detect and quantify HIV-1 minority variants. However, the clinical significance of HIV-1 minority variants (e.g. those below the level of detection of current FDA-cleared HIV genotyping assays) is not known. Further studies are needed to determine the sensitivities of different assays for detection of HIV-1 drug resistance mutations, and to determine the clinical relevance of HIV-1 minority variants.

### LigAmp References:

Nat Methods (2004) 1:141-7.  
 J Infect Dis (2005) 192:24-9.