M Shakirzyanova et al., J AIDS Clin Res 2017, 8:10 (Suppl)
DOI: 10.4172/2155-6113-C1-024

Validation of viroseq HIV-1 integrase genotyping assay at NYC public health laboratory

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Statement of the Problem: Integrase strand transfer inhibitors together with protease and reverse transcriptase inhibitors are the drugs recommended by NIH for pre- and post-HIV-1 exposure prophylaxis. As part of the Ending the AIDS Epidemic (ETE) initiative in New York State, the NYC Public Health Laboratory has validated the ViroSeq HIV-1 Integrase Genotyping Assay for detection of the drug resistance mutations in the HIV-1 integrase gene. This assay allows us to provide the clinicians with complete drug resistance information. Meanwhile, the sequence data allows performing the phylogenetic analysis of virus from newly infected patients and following up of incident cases that may be out of care.

Methods: RNA was isolated manually from plasma samples (provided by NYC VA Hospital and Sonic Reference Laboratory Austin, TX), or recombinant viruses' supernatants (Harvard Medical School). The viral DNA was amplified by RT-PCR, quantified on agarose gel, purified and used for cycle sequencing PCR. The sequencing was performed on ABI3500 genetic analyzer.

Results: The limit of detection results were generated with four randomly chosen supernatants diluted at several levels with negative human plasma. The LOD was 500 gene copies per mL (cp/ml) for two of the supernatants (Y143C; V151I and L74M, V151I, N155H), 1000 gene cp/ml for one (G140S; Q148H; V151I) and 2500 gene cp/ml for another one (V151I, N155H and G163R). The reproducibility was performed with virus harboring E92Q/N155H/V151I mutations. All sequencing results showed the same mutations at different dilution levels, which confirmed the high reproducibility of the assay. Blinded sample validation was performed on 40 samples. All of drug resistance mutations revealed in our study was matched with the keys provided by the supplier of the samples.

Conclusion: ViroSeq HIV-1 Integrase Genotyping Assay detects integrase resistant mutations with the reasonable analytical sensitivity and the high reproducibility and 100% accuracy.

Biography

M Shakirzyanova is a licensed Medical Laboratory Scientist with a PhD in Microbiology and a research background in Virology, Molecular Biology and Microbiology. During her Post-doctoral Fellowship, she studied HIV transmission and monitored SAIDS in monkey (Indian Rhesus Macaque) model. She conducted analysis of the co-receptor switch in R5-tropic SHIV-infected macaques. She has expertise in protocol development, reporting and analysis of experimental data. Her current research is monitoring of HIV-1 drug resistance in HIV-1 positive patients on anti-retroviral therapy.

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