Next Generation Sequencing-based HIV-1 Drug Resistance Monitoring System


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Introduction
The most common cause for therapeutic failure in people infected with Human Immunodeficiency Virus (HIV) is the resistance of HIV to antiretroviral drugs. Objective of this study was to compare two sequencing-based HIV-1 drug resistance monitoring systems: an CLIP-based system (TruGene HIV-1 Genotyping Kit) and a novel Next Generation Sequencing (NGS)-based test (Sentosa SQ HIV-1 Genotyping Assay).

Materials and Methods
We used a newly developed automated NGS-based integrated workflow, comprised of 1) a customized version of the epMotion 5075 (Eppendorf) robotic liquid handling system for nucleic acid extraction and NGS library preparation (Sentosa SX101); 2) Ion Torrent instruments for template preparation and deep sequencing; 3) kits for RNA extraction, HIV NGS library preparation, template preparation and deep sequencing, and 4) data analysis and reporting software. Reporting includes 86 Drug Resistance Mutations (DRMs) across the Reverse Transcriptase (RT), Protease (PR) and Integrase genes. 111 prospective EDTA plasma clinical samples from patients infected with HIV-1 were tested for this study.

Results
All 111 HIV-1 positive samples were tested on both systems. 97.3% (108/111) samples were subtyped as CRF01_AE. In total, 647 DRMs were detected (435 in the RT gene, 199 in the PR gene and 13 in the Integrase gene). The Sentosa SQ HIV Genotyping Assay detected 100% (199/199) of all DRMs in the PR gene and more than 98% DRMs (427/435) in the RT gene. The Integrase gene was not included into the comparison study because it is not covered by the TruGene test. In total, 130 DRMs were detected by the Sentosa SQ HIV Genotyping Assay, that were not found by TruGene and 8 DRMs were missed by the Sentosa HIV Genotyping Assay (but detected by TruGene). Mutation detection rate for the HIV PR gene was 100% (95%CI: 98.11-100%) for the Sentosa SQ HIV Genotyping Assay and 90.45% (95%CI: 85.57–93.80%) for the TruGene system. In the RT gene 98.16% (95%CI: 96.41-99.07%) of DRMs were recorded by the Sentosa SQ HIV Genotyping Assay and 74.48% (95%CI: 70.18-78.35%) by TruGene. Overall DRM detection rates aggregated were 98.74% (95%CI: 97.53-99.36%) for the Sentosa SQ HIV Genotyping Assay and 79.5% (95%CI: 79.02-79.62%) for the TruGene HIV-1 Genotyping Kit. All HIV strains were carrying 1 or multiple DRMs in 61, 16 and 9 AA positions of the RT, PR and Integrase genes respectively. The most prevalent DRMs in the RT gene were: M184V was present in 48.7% (54/111) of the samples, K103N in 29.7% (33/111), Y181C in 27.9% (31/111), G190A and D67N (both 18.9% (21/111)). In the PR gene: M36I 91.9% (102/111), K20R 21.6% (24/111) and L10I 20.7% (23/111).

Conclusions
Timely detection and reporting of DRMs is critical for drug regimen and can minimize the development of resistance to antiviral drugs. In this perspective the NGS-based workflow appears as a promising new tool for detecting clinically relevant variants in HIV-1. Given its high sensitivity (up to 5% mutation frequency) compared to Sanger sequencing-based systems and the comparatively short turnaround time of 2.5 days the workflow provides comprehensive, clinically relevant information for optimal selection of HIV treatment regimens.