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Diagnostic Tools for Immunological and Virological Monitoring

Next Generation Sequencing for Detection of Drug Resistance Mutations in HIV-1

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Introduction: Resistance of Human Immunodeficiency Virus (HIV) to antiretroviral drugs is the most common cause for therapeutic failure in people infected with HIV. We compared two sequencing-based HIV-1 drug resistance monitoring (DRM) systems. Subtypes and resistance-associated mutations were determined using an established 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 an automated NGS-based integrated workflow (Sentosa SQ System), comprised of a robotic liquid handling system, kits for RNA extraction and library preparation, Ion Torrent based deep sequencing system, bioinformatics analysis tools and reporting software. Reporting includes 86 known Nucleoside RT Inhibitor (NRTI), Non-Nucleoside RT Inhibitor (NNRTI), Protease Inhibitor (PI) and Integrase Inhibitor (INI) resistance mutations across the Reverse Transcriptase (RT), Protease (PR) and Integrase genes; sequences obtained can be blasted against those contained in established DRM databases.

Results: This pilot study included 111 HIV-1 samples from 8 consecutive instrument runs. All samples were tested by both methods. The majority of the samples were subtyped as CRF01_AE (n=108). In total, 634 drug resistance mutations were detected (199 mutations in the PR gene and 435 mutations in the RT gene). The Sentosa SQ HIV Genotyping Assay detected 100% (199/199) of all mutations in the PR gene and more than 98% mutations (427/435) in the RT gene. In total, 130 mutations were detected by the Sentosa SQ HIV Genotyping Assay, that were not found by TruGene and 8 mutations 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 HIV-1 system. In the RT gene 98.16% (95% CI: 96.41–99.07%) of mutants were recorded by the Sentosa SQ HIV Genotyping Assay and 74.48% (95% CI: 70.18–78.35%) by TruGene. Overall mutation 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 resistance-associated mutations in 61 AA positions of the RT gene and 16 AA positions of the PR gene. The most prevalent mutations in the RT gene were: M184V was present in 48.7% (54/111) of the samples, K103N in 29.7% (33/111), Y181C was found in 27.9% (31/111), G190A and D67N (both 18.9% (21/111)). In the PR gene: M36I 91.9% (102/111), K20R 21.8% (24/111) and L10I 20.7% (23/111).

Conclusions: Considering the pivotal role of DRM in HIV patients under HAART the newly developed Sentosa SQ HIV Genotyping NGS workflow appears as a promising new tool for detecting clinically relevant HIV variants. Given its high sensitivity (up to 5% mutation frequency) compared to Sanger based systems and the comparatively short turnaround time of 2.5 days the workflow offers relevant improvements in HIV DRM.

No conflict of interest