Sequencing Technology Comparison for Detection of HIV-1 **Mutations in the Protease and Reverse Transcriptase Regions**

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INTRODUCTION

Resistance of HIV to antiretroviral drugs is the most common cause for therapeutic failure in people infected with Human Immunodeficiency Virus (HIV) [1]. 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).

MATERIAL & METHODS

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



Figure 1. Vela's NGS workflow for the HIV Genotyping Assay.



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All 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 that 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 rates for both assays are presented in **Table 1**.

Table 1. Mutation detection rates for the TruGene HIV-1 Genotyping and Sentosa[®] SQ HIV Genotyping Assays.

HIV Gene	Test	Number of Mutations	Mutations Detected	Detection rate	95% Confidence Interval
Protoco	Sentosa [®] SQ HIV Genotyping Assay	199	199	100.00%	98.11 – 100.00%
FIULEASE	TruGene HIV-1 Genotyping Kit	199	180	90.45%	85.57 – 93.80%
Reverse	Sentosa [®] SQ HIV Genotyping Assay	435	427	98.16%	96.41 – 99.07%
Transcriptase	TruGene HIV-1 Genotyping Kit	435	324	74.48%	70.18 – 78.35%
Ovorall	Sentosa [®] SQ HIV Genotyping Assay	634	626	98.74%	97.53 – 99.36%
Overall	TruGene HIV-1 Genotyping Kit	634	504	79.50%	79.02 – 79.62%

All HIV strains were carrying one 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 and PR genes are listed in **Table 2**.

Table 2. The most prevalent mutations in the tested population.

Gene	Mutation	Percentage	Resistance to / Effect
	M184V	48,7% (54/111)	3TC, FTC (NRTI), ddl
Dovoroo	K103N	29.7% (33/111)	NVP and EFV (NNRTI)
Transcrintase	Y181C	27,9% (31/111)	NVP, ETR, RPV, EFV (NNRTI)
	G190A	18.9% (21/111)	NVP, EFV (NNRTI)
	D67N	18.9% (21/111)	AZT, d4T (NRTI), ddl
	M36I	91.9% (102/111)	Increases the replication fitness of viruses with PI-resistance mutations
Protease	K20R	21.6% (24/111)	Increases the replication fitness of viruses with PI-resistance mutations
	L10I	20.7% (23/111)	Either reduce PI susceptibility or increase the replication of viruses containing PI-resistance mutations

RESULTS

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uple of a QC Report generated by Sentosa[®] SQ Reporter.

CONCLUSIONS

porting of DRMs is critical for optimal selection of HAART prevent or minimize the development of resistance to antiviral itivity (up to 5% mutation frequency) and the comparatively ime of 2.5 days make this NGS-based workflow a promising ing relevant mutations in HIV-1.

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