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

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INTRODUCTION

The most common cause for therapeutic failure in individuals infected with the Human Immunodeficiency Virus (HIV) is the development of resistance to antiretroviral drugs. The objective of this study was to compare two sequencing-based HIV-1 drug resistance monitoring systems: a 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 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 [1]; 3) kits for RNA extraction, HIV NGS library preparation, template preparation and deep sequencing, and 4) data analysis and reporting software (**Fig. 1**).

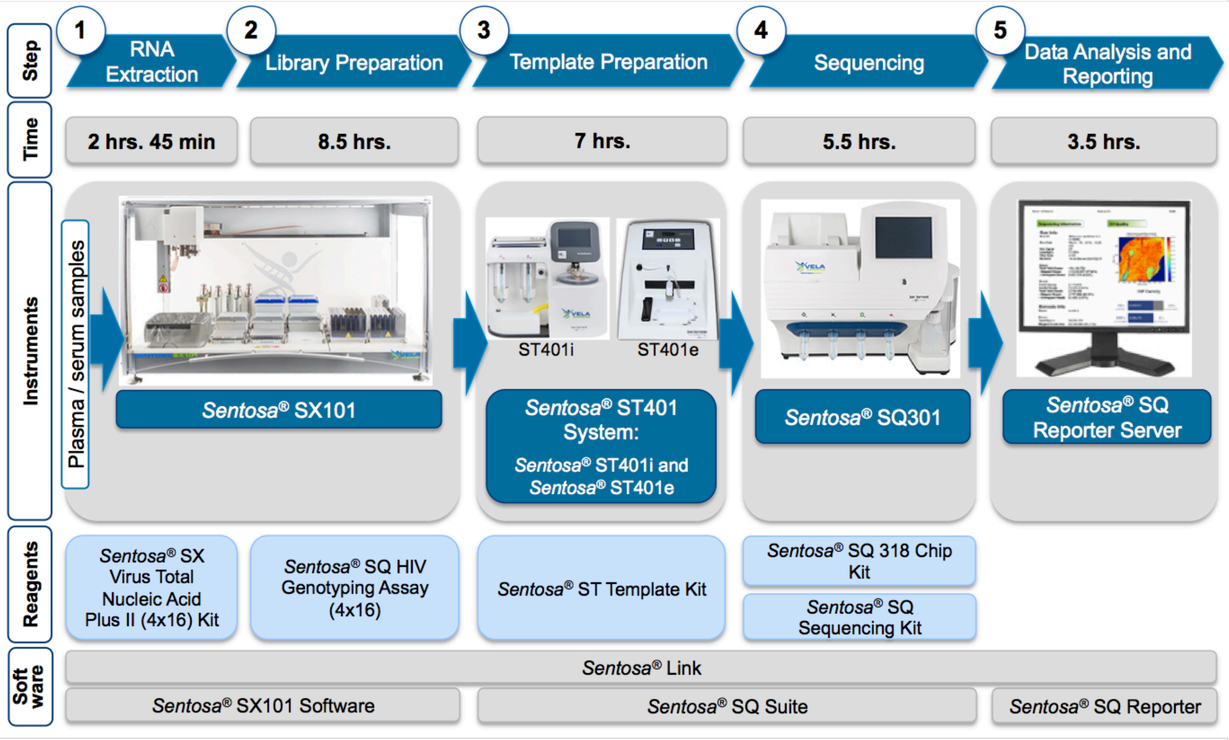

Figure 1. *Sentosa*® SQ HIV Genotyping Assay Workflow.

Reporting includes 86 Drug Resistance Mutations (DRMs) across the Reverse Transcriptase (RT), Protease (PR) and Integrase genes (**Fig. 2**). 111 prospective EDTA plasma samples from patents infected with HIV-1 were tested in this study.

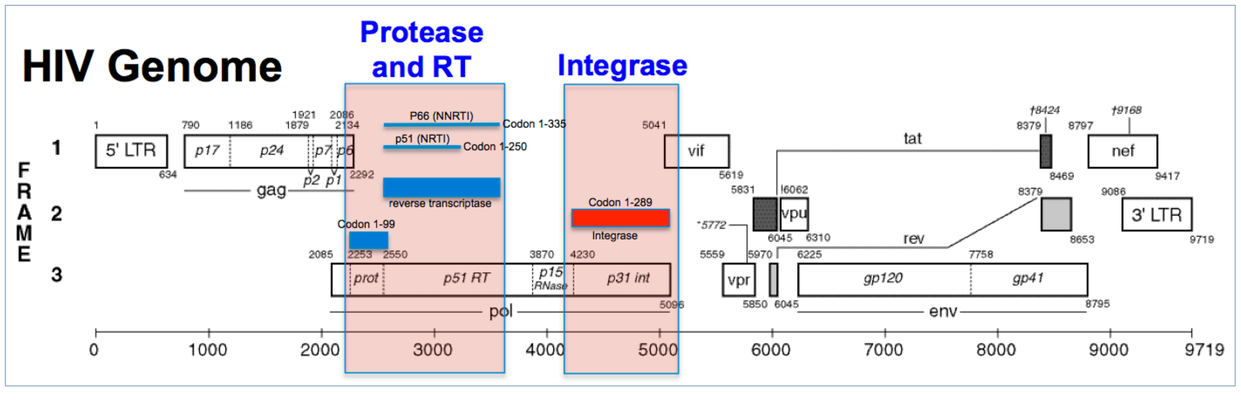

Figure 2. Regions targeted by the *Sentosa*® SQ HIV Genotyping Assay.

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 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
Protease	*Sentosa*® SQ HIV Genotyping Assay	199	199	100.00%	98.11 – 100.00%
	TruGene HIV-1 Genotyping Kit	199	180	90.45%	85.57 – 93.80%
Reverse Transcriptase	*Sentosa*® SQ HIV Genotyping Assay	435	427	98.16%	96.41 – 99.07%
	TruGene HIV-1 Genotyping Kit	435	324	74.48%	70.18 – 78.35%
Overall	***Sentosa*® SQ HIV Genotyping Assay**	**634**	**626**	**98.74%**	**97.53 – 99.36%**
	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
Reverse Transcriptase	M184V	48,7% (54/111)	3TC, FTC (NRTI), ddI
	K103N	29.7% (33/111)	NVP and EFV (NNRTI)
	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), ddI
Protease	M36I	91.9% (102/111)	Increases the replication fitness of viruses with PI-resistance mutations
	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

CONCLUSION

Timely detection and reporting of DRMs is critical for drug regiment and can minimize the development of resistance to antiviral drugs. In this regard the newly developed 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.

REFERENCES

1) *Loman N. et al.* Nat. Biotechnol. 2012 May 30(5):434-9.