Ion Torrent Next Generation Sequencing (NGS) for Accurate Genotyping and Detection of Resistance Associated Variants in HCV and HIV

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INTRODUCTION

Detection of resistance-associated mutations is well established in HIV-1 antiretroviral therapy (as Drug Resistance Mutations or DRMs) and is increasingly used in HCV patients selected for treatment (as RAVs) with direct acting antiviral agents (DAAs). Both for DAA treatment and conventional interferon-based therapy accurate determination of HCV genotypes (GTs) is essential. Sanger sequencing has recognized limitations in sensitivity and turn around time. NGS provides excellent accuracy, speed and sensitivity enabling detection of rare mutants, HCV subtypes as well as mixed infections.

MATERIAL & METHODS

We have used NGS in combination with workflow automation on a newly developed *Sentosa*® SQ NGS platform consisting of a continuous robotic process starting with sample extraction and RT-PCR followed by automated library preparation, Ion Torrent deep sequencing and direct online data analysis to determine HCV genotypes and RAVs as well as DRMs in HIV-1 (**Fig. 1**). We employed target sequences from the HCV NS3, NS5A and NS5B regions. For HIV the Reverse Transcriptase, Protease and Integrase genes were selected for NGS.

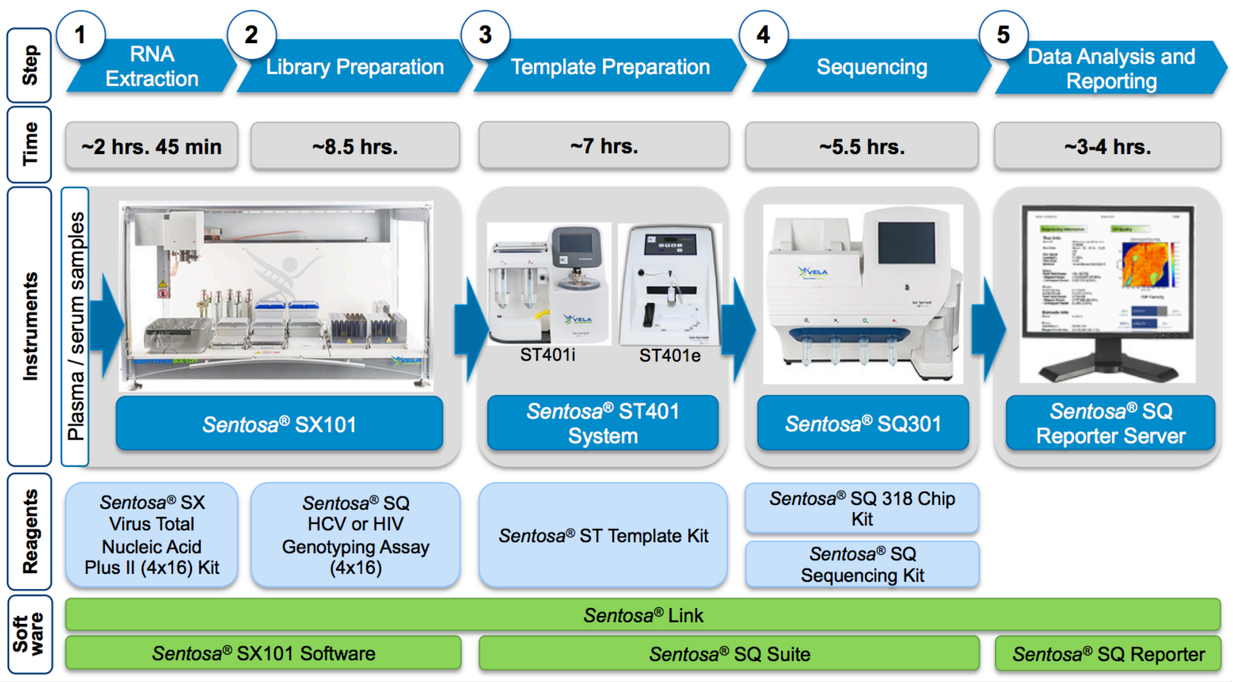

Figure 1. Vela's NGS workflow for the HCV and HIV Genotyping assays.

The data reports include 136 known RAVs in the NS3, NS5A and NS5B genes on HCV GTs 1a and 1b (**Fig. 2**) and 86 DRMs across the Reverse Transcriptase, Protease and Integrase genes on HIV (**Fig. 3**). However, the report does not make direct treatment recommendations, which are left to the investigator.

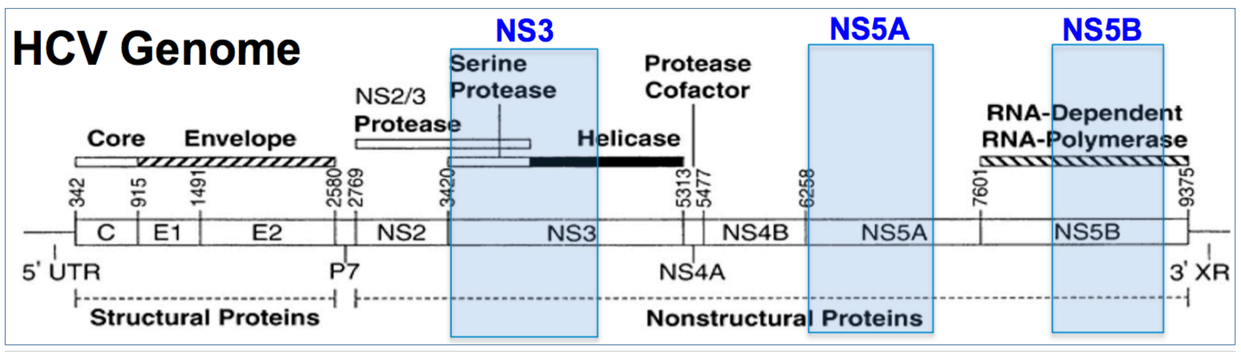

Figure 2. Regions targeted by the *Sentosa*® SQ HCV Genotyping Assay.

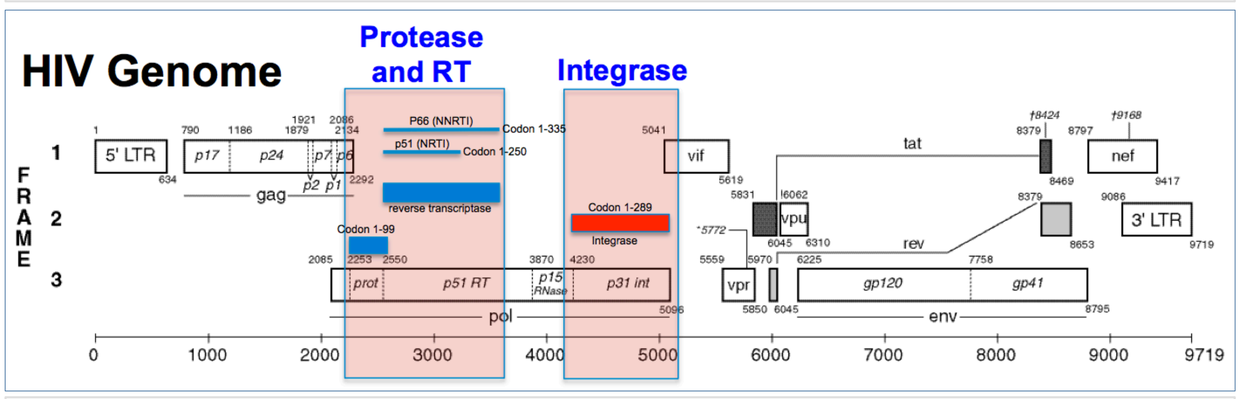

Figure 3. Regions targeted by the *Sentosa*® SQ HIV Genotyping Assay.

RESULTS

346 HCV positive samples were tested on a line probe-based VERSANT HCV Genotype 2.0 LiPA and *Sentosa*® SQ HCV NGS platforms. For 47/346 (13.6%) samples GT results by VERSANT were "indeterminate". In 19/299 (6.4%) of the samples, discordant results between the two methods were obtained. Sanger sequencing confirmed that all 19 discordant samples were incorrectly classified by the VERSANT (**Table 1**).

Table 1. Comparison of the VERSANT and *Sentosa* HCV Genotyping Assays.

Parameter	VERSANT HCV Genotype 2.0 LiPA	*Sentosa*® HCV Genotyping Assay
Clinical Sensitivity	86.4% (95%CI: 82.4-89.6%)	100% (95%CI: 98.9-100%)
Genotyping Correctness	93.7% (95%CI: 90.3-95.9%)	100% (95%CI: 98.7-100%)

56 GT1a and 54 GT1b samples were used for further analysis of RAVs distribution among the GT1 population. 52.7% (58/110) of HCV strains were carrying 1 or more RAVs in 23 positions across all targets. An unequal distribution of 4 mutations across the GT1 subtypes was observed (**Fig. 4**).

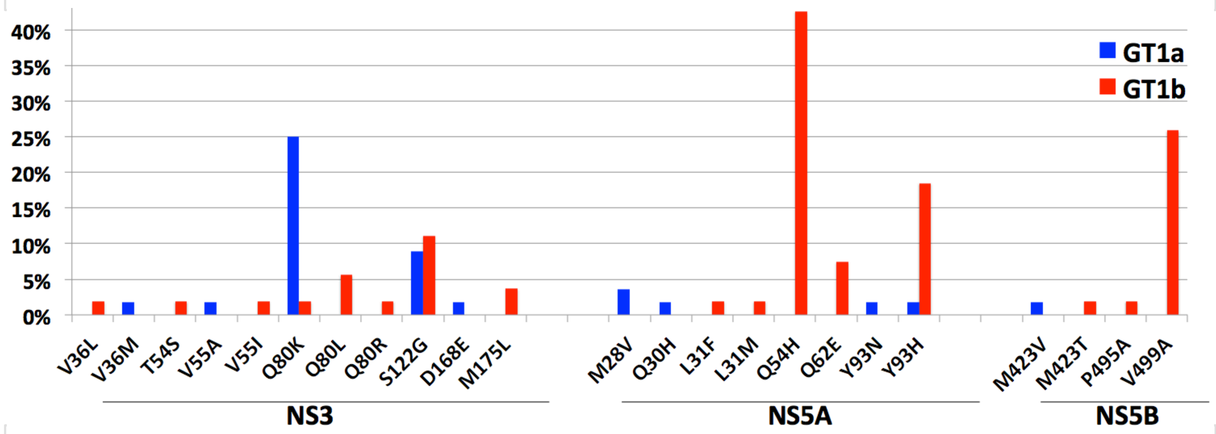

Figure 4. RAVs distribution significantly differs for 1a and 1b subtypes in the tested population. 20 out of 23 RAV were found either in GT1a or GT1b population. Only S122G in the NS3 gene was present in relatively equal proportions for both subtypes.

In an HIV-1 pilot study (n=111) *Sentosa*® SQ NGS platform was compared with a CLIP-based TruGene system. 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 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. 130 DRMs were detected by the *Sentosa*® SQ HIV assay, that were not found by the TruGene and 8 DRMs were detected by the TruGene but not by the *Sentosa*® HIV assay. DRM detection rates for both assays are presented in **Table 2**.

Table 2. DRM detection rates for the TruGene and *Sentosa*® SQ HIV assays.

HIV Gene	Test	Number of DRMs	DRMs Detected	Detection rate	95% Confidence Interval
Protease	*Sentosa*® SQ HIV	199	199	100.00%	98.11 – 100.00%
	TruGene HIV-1	199	180	90.45%	85.57 – 93.80%
Reverse Transcriptase	*Sentosa*® SQ HIV	435	427	98.16%	96.41 – 99.07%
	TruGene HIV-1	435	324	74.48%	70.18 – 78.35%
Overall	***Sentosa*® SQ HIV**	**634**	**626**	**98.74%**	**97.53 – 99.36%**
	TruGene HIV-1	**634**	**504**	**79.50%**	**79.02 – 79.62%**

CONCLUSIONS

Given the crucial role of accurate sequencing analysis in HCV and HIV treatment management, automated NGS workflow appears as a highly reliable tool for differentiating HCV genotyping and RAVs, which can help to prevent diagnostic errors potentially leading to suboptimal treatment.

Considering the pivotal role of DRMs in HIV patients under HAART the *Sentosa*® SQ HIV Genotyping NGS workflow appears as a valuable new tool for detecting clinically relevant HIV variants. Given its high sensitivity compared to Sanger based systems and the comparatively short turnaround time of two days the workflow offers relevant improvements in HIV DRM detection.

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