Next-Generation Sequencing for HCV Genotyping and Optional Identification of RAVs

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BACKGROUND

Despite the advent of highly efficient DAAs for the treatment of HCV infection accurate genotyping remains an essential part of pre-treatment laboratory assessment. Line probe assays can give incorrect genotype results with some genotypes, which can be overcome by deep sequencing. In addition, RAV determination can provide substantial value for guiding therapy decisions in some patient subgroups. The newly developed method presented here combines both analytical tools.

OBJECTIVES

Objective of this study was to compare a line probe based test (VERSANT HCV Genotype 2.0 LiPA) on the AutoBlot 3000H platform [1] and a newly developed Next Generation Sequencing (NGS)-based integrated workflow on the *Sentosa*® SQ system, which provides genotyping and concomitantly determines RAVs.

MATERIALS & METHODS

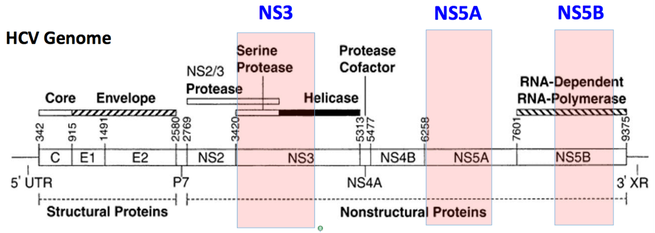
The *Sentosa*® SQ NGS workflow, comprised of 1) a customized version of the epMotion 5075 (Eppendorf) robotic liquid handling system for nucleic acid extraction and NGS library preparation (*Sentosa*® SX101); 2) Ion Torrent instruments for template preparation and deep sequencing [2]; 3) kits for nucleic acid extraction, HCV NGS library preparation (*Sentosa*® SQ HCV Genotyping Assay), template preparation and deep sequencing; 4) assay specific application, and 5) data analysis and reporting software. The system determines RAVs in combination with genotypes and respective subtypes.

Clinical samples: This study included a cohort of 346 prospective and retrospective EDTA-plasma (n=134) and serum (n=212) samples from patients with chronic HCV. 16 samples were excluded from the analysis due to poor quality or low viral load.

RESULTS

In contrast to the widely used 5'UTR region, the limitations of which are well recognized, we have employed target sequences from the HCV NS3, NS5A and NS5B regions (**Fig. 1**).

Figure 1. Regions targeted by *Sentosa*® SQ HCV Genotyping Assay



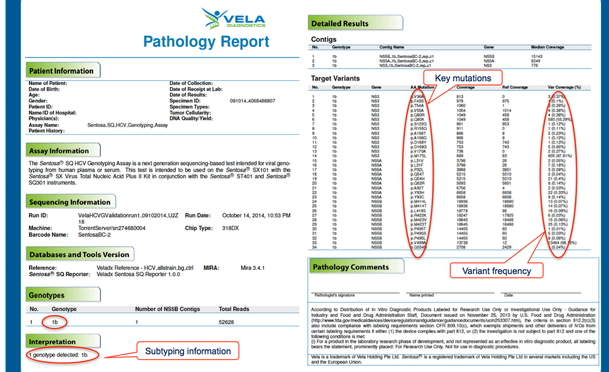
Genotype (GT) distribution in the population tested was as follows: 35 GT1a, 44 GT1b, 3 GT1c, 18 GT2, 125 GT3, 12 GT4, 8 GT5, and 85 GT6 and 1 mixed infected sample (GTs 3 and 6). In 47/346 (13.58%) of the samples GT was undetermined by VERSANT. In 19/299 (6.35%) of the samples, discordant results between the two methods were obtained. All discordant samples and samples with indeterminate GTs were subjected to Sanger sequencing. Confirmation testing by Sanger sequencing indicated that the ability to correctly determine HCV genotypes was 93.65% (95%CI: 90.29 – 95.89%) for VERSANT and 99.39% (95%CI: 97.82 – 99.83%) for the *Sentosa*® SQ HCV Genotyping Assay (**Table 1**). Among the 19 discordant samples, 10 GT6 were erroneously classified as GT1b by line probing, 6 GT3 as GT4, 2 GT3 as GT6, and 1 GT1c as GT1a. GT distribution among the 47 samples with undetermined GT by VERSANT was as follows: 5 GT1a, 1 GT2, 19 GT3, 1 GT4, 20 GT6 and 1 mixed infected (GTs 2 and 3).

Clinical sensitivity aggregated was 86.42% (95%CI: 82.40 – 89.63%) for VERSANT and 100% (95%CI: 98.90 – 100.00%) for the *Sentosa*® SQ HCV Genotyping Assay.

Table 1. HCV genotyping accuracy of *Sentosa*® SQ HCV Genotyping Assay.

Genotype	Number of samples tested	Number of samples correctly identified	Clinical genotyping correctness		
			Percent correctly identified	95% confidence interval	
1a	35	35	100%	90.11%	100.00%
1b	44	42	95.45%	84.87%	98.74%
1 (non 1a and 1b)	3	3	100%	43.85%	100.00%
2	18	18	100%	82.41%	100.00%
3	125	125	100%	97.02%	100.00%
4	12	12	100%	75.75%	100.00%
5	8	8	100%	67.56%	100.00%
6	85	85	100%	95.68%	100.00%
Overall	**330**	**328**	**99.39%**	**97.82%**	**99.83%**

Figure 2. An example of a Pathology Report generated by *Sentosa*® SQ Reporter. RAVs are routinely reported for GT1.



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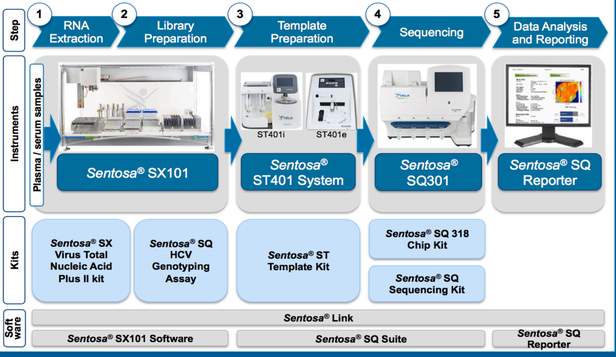
SUMMARY

The newly developed automated workflow based on Ion Torrent deep sequencing technology (**Fig. 3**) accurately determines HCV genotypes and simultaneously reports RAVs.

Figure 3. *Sentosa*® SQ HCV Genotyping Assay Workflow.



CONCLUSIONS

In conclusion, considering the crucial role of correct genotyping in HCV treatment management, automated HCV NGS appears as a highly reliable tool for differentiating HCV GTs, which can help to prevent diagnostic errors potentially leading to suboptimal treatment. Not least, the library generated DNA contigs are fully user accessible for further sequence analysis thereafter, e.g., enabling assessment of additional mutations specific to the case under investigation (**Fig. 2**). This added feature may prove useful as an additional tool for therapy guidance in some difficult to treat HCV patient groups.

REFERENCES

1) Comanor L et al. J Clin Virol. 2003 Sep;28(1):14-26.
2) Loman N. et al. Nat. Biotechnol. 2012 May;30(5):434-9.