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

**BACKGROUND**

Despite the advent of highly efficient DAAAs 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® 5X101); 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, and 4) Ion software. The system determines RAVs in combination with genotypes and respective subtypes.

**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 N53, NSA5 and NSB5 regions (Fig. 1).

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**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 (13.5%) 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. 2) All patient samples were subjected to Sanger sequencing.

**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**