Highly Accurate HCV Genotyping by Targeted Next Generation Sequencing

*Kok Siong Poon, *^Evelyn S. Koay, *Cui Wen Chua, *Mui Joo Khoo, **Zhang Rui, **Elian Rakhmanaliev, **Wen Huang and **Gerd Michel

*Molecular Diagnosis Centre, Department of Laboratory Medicine, National University Hospital, Singapore;
^Department of Pathology, National University of Singapore, Singapore; **Vela Research Pte Ltd., Singapore.

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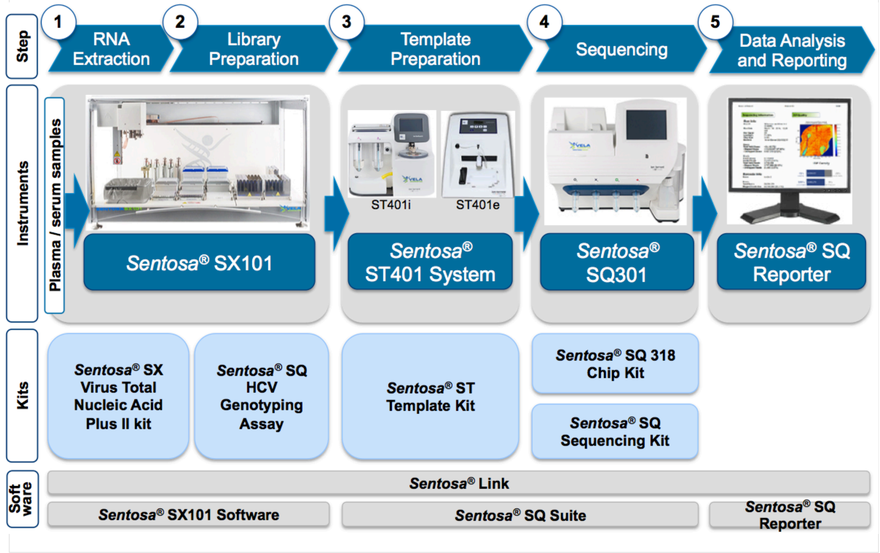
INTRODUCTION

Determination of HCV genotype (GT) provides essential information for decision making on treatment regimens in HCV infection [1]. DNA hybridization employing a line probe test format (VERSANT HCV Genotype 2.0 LiPA), first introduced about 20 years ago, still represents the most widely used laboratory method [2]. The recent fast advancement of next generation sequencing (NGS) technologies allowing for unprecedented speed and accuracy in analyzing viral genomes are opening new ways to further improve diagnostic genotyping of HCV [3].

MATERIAL & METHODS

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



We have used NGS in combination with workflow automation, from sample preparation to result reporting (**Fig. 1**), to demonstrate high accuracy of GT assessment in clinical samples. This newly developed platform based on a customized version of the epMotion 5075 robotic system (Eppendorf, Germany) consists of a continuous robotic process starting with plasma or serum extraction and RT-PCR followed by automated library preparation, Ion Torrent deep sequencing [4] and direct online data analysis to determine HCV GTs. In contrast to the commonly 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. 2**).

RESULTS

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

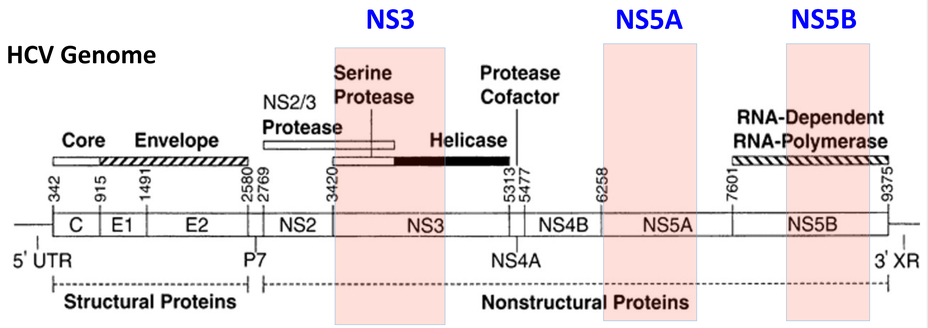
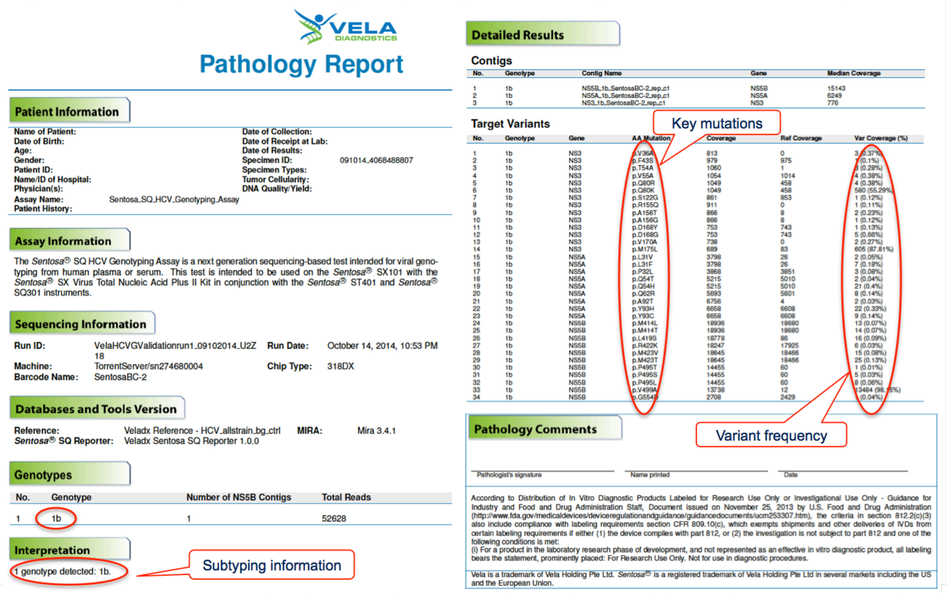


Figure 3. Example of Pathology Report generated by the *Sentosa*® SQ Reporter.



Results were compared to line probe assay data obtained from the same samples. Randomly selected archived serum or plasma samples from 143 Asian and 7 African patients with chronic HCV infection, viral loads ranging from 5.50×10^2 to 1.04×10^8 IU/mL (median 6.10×10^6), were included in the study. GT distribution was as follows: 11 GT1a, 14 GT1b, 12 GT2, 58 GT3, 9 GT4, 7 GT5, and 39 GT6. In 16/150 (11%) of the samples, discordant results between the two methods were obtained. Confirmation testing by Sanger sequencing indicated that the ability to discriminate at the major GT level was 89.3% (95%CI: 83.4 – 93.3) for VERSANT and 100% (95%CI: 97.5-100) for NGS. Correct GT subtype calls were found to be 89% for VERSANT and 100% for NGS. Among the 16 discordant samples, 8 GT6 were wrongly classified as GT1b by line probing, 6 GT3 as GT4, and another 2 GT3 as GT6 (**Table 1**).

Table 1. Resolution of discrepant results: confirmation by Sanger Sequencing.

No.	Viral Load (IU/mL)	Genotype by VERSANT HCV Genotype 2.0 LiPA	Genotype by *Sentosa* SQ HCV Genotyping Assay	Genotype by Sanger Sequencing*
1	364100	1b	6	6
2	397650	1b	6	6
3	3130050	1b	6	6
4	1978350	1b	6	6
5	1997600	1b	6	6
6	73150	1b	6	6
7	96800	1b	6	6
8	821150	1b	6	6
9	44000	4	3	3
10	2253900	4	3	3
11	78650	4	3	3
12	111100	4	3	3
13	5529700	4	3	3
14	155650	4	3	3
15	550	6	3	3
16	1042800	6	3	3

*NS5B has been used as target region for Sanger Sequencing. Results were obtained on serum samples.

CONCLUSIONS

In conclusion, considering the crucial role of correct genotyping in HCV treatment management, workflow automated HCV NGS appears as a highly reliable tool for differentiating HCV genotypes, 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. 3). This additional feature may prove useful for monitoring resistance to novel drug treatments.

REFERENCES

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Contact information

Corresponding author: Dr. Gerd Michel gerd.michel@veladx.com
Web: http://www.veladx.com