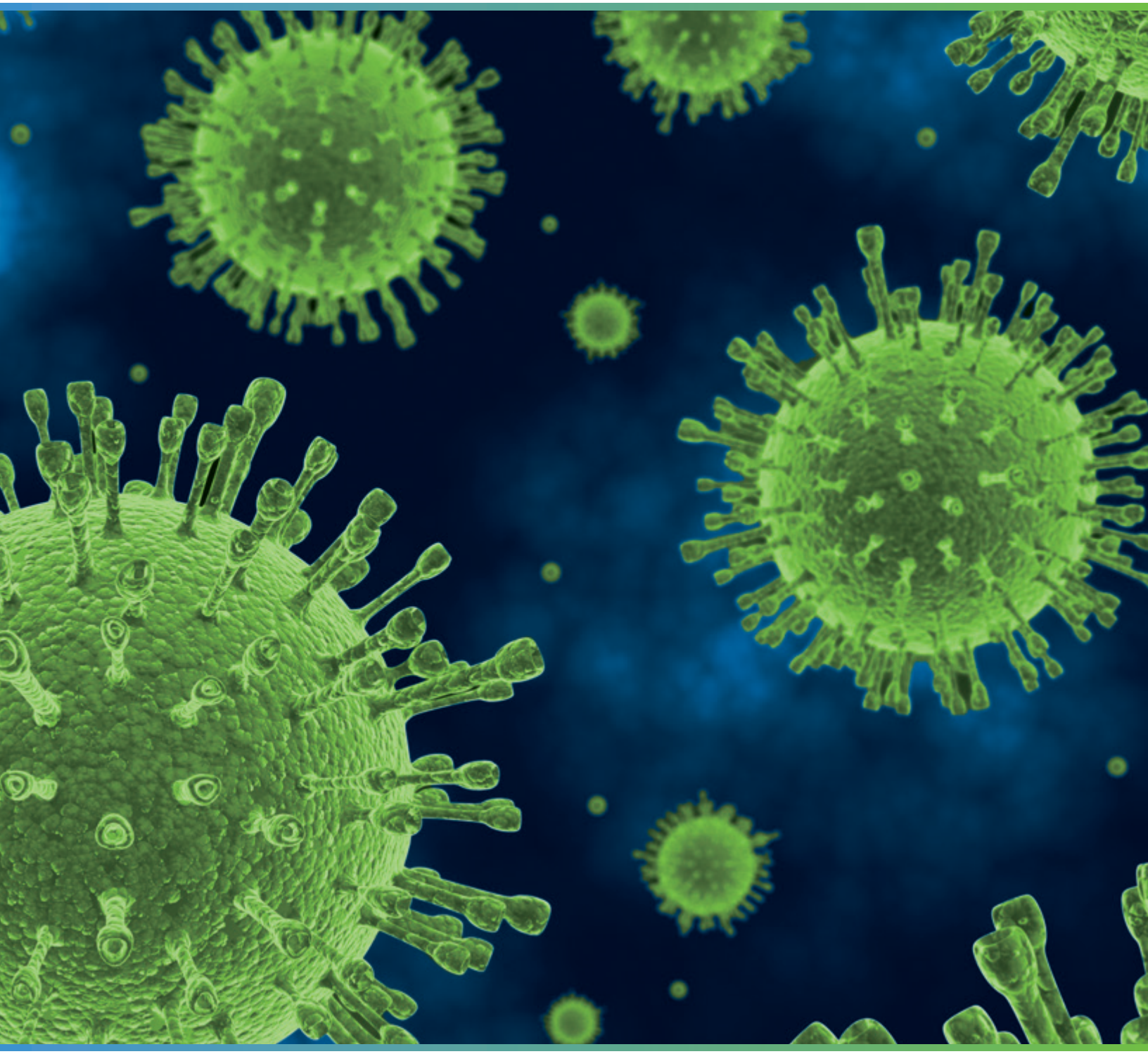
VELA DIAGNOSTICS



Simultaneous genotyping and RAS-calling with the *Sentosa*® SQ HCV Genotyping Assay

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- **Delivers genotyping & RAS identification accuracy in a single, efficient workflow**
- **Reliable, automated solution** – full workflow automation combined with built-in controls give you confidence in your results
- **Speeds time to next steps** – simultaneously analyze genotyping and RAS-calling with minimal hands-on-time using our automated solution
- **Expert assay design** – multiplexed sequencing design targets key regions indicated in AASLD guidelines including genotypes 1 through 6 as well as 70 subtypes enabling discovery of potential new relationships between subtypes and novel therapeutics

Overview

Prior to 2011, therapies for treatment of the Hepatitis C Virus (HCV) focused on interferon-based therapy. In 2011 it was found that the genotype of the virus is clinically important, both in response of interferon-based therapy and to other potential therapies. Current AASLD guidelines recommend specific combination therapies for each genotype as well as subtypes 1a & 1b[1] . The introduction of these targeted therapies has reduced adverse effects and increased cure rates from 45% to 93% in genotype 1, for example[2]. Further research to develop additional genotype-specific targeted therapies will continue to improve outcomes for this disease. This research makes accurate determination of HCV genotype critical in the development of novel therapies for HCV.

Accurate HCV Genotyping and RAS analysis

The *Sentosa*® SQ HCV Genotyping Assay uses next-generation sequencing technology to deliver accuracy in the detection of Hepatitis C Virus genotypes 1-6, and their associated subtypes as well as resistance-associated substitutions (RASs). Compared with traditional methods for HCV genotyping such as line-probe assays (LiPA), this next-generation sequencing-based assay eliminates genotyping errors (Table 1) while simultaneously delivering additional information about RASs found in the sample. As shown in an external study comparing HCV genotyping results using NGS, Sanger Sequencing, and LiPA, the *Sentosa* SQ HCV Genotyping assay delivered 100% accurate genotyping assignment while the LiPA approach yielded 11% inaccurate genotyping assignments, potentially leading to incorrect future action (Table 1).[3]

Speeds Time to Next Steps

Reliable and reproducible, this assay is designed for routine use with the *Sentosa* SQ workflow. By integrating genotyping and RAS identification into a single automated workflow, you eliminate running multiple assays and are able to move to the next steps more quickly. This fully automated solution requires less than 2.5 hours of hands-on-time, delivering results from sample to answer in just 2 days. Both Laboratory Information System (LIS) connectivity and system and extraction controls are integrated into the workflow, giving you confidence in the results while removing many user interactions with the system to further streamline the workflow (Figure 1).

This ready-to-use platform can be running your lab in less than two weeks. Vela Diagnostics offers a reagent rental model as an alternative to capital equipment purchase. Please contact your sales representative for more information.

Sample number	Viral Load (IU/mL)	Genotype by VERSANT HCV Genotype 2.0 Assay (LiPA)	Genotype by *Sentosa* SQ HCV Genotyping	Genotype by Sanger Sequencing
1	364,100	1b	6	6
2	397,650	1b	6	6
3	3,130,050	1b	6	6
4	1,978,350	1b	6	6
5	1,997,600	1b	6	6
6	73,150	1b	6	6
7	96,800	1b	6	6
8	821,150	1b	6	6
9	44,000	4	3	3
10	2,253,900	4	3	3
11	78,650	4	3	3
12	111,100	4	3	3
13	5,529,700	4	3	3
14	155,650	4	3	3
15	550	6	3	3
16	1,042,800	6	3	3

Table 1: Genotyping accuracy with NGS compared with LiPA analysis. 150 samples were analyzed using both next-generation sequencing (Sentosa® SQ HCV Genotyping Assay) and line probe assay analysis (VERSANT® HCV Genotype 2.0 Assay). These samples were randomly selected archived serum or plasma samples from 143 Asian and 7 African patients with chronic HCV infection, viral loads ranging from $5.50x10^2$ to $1.04x10^8$ IU/mL (median $6.10x10^6$). The genotype (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 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 LiPA and 100% (95%CI: 97.5-100) for Sentosa NGS. Correct GT subtype calls were found to be 89% for LiPA and 100% for NGS. Among the 16 discordant samples, 8 GT6 were wrongly classified as GT1b with LiPA, 6 GT3 as GT4, and another 2 GT3 as GT6.[4]

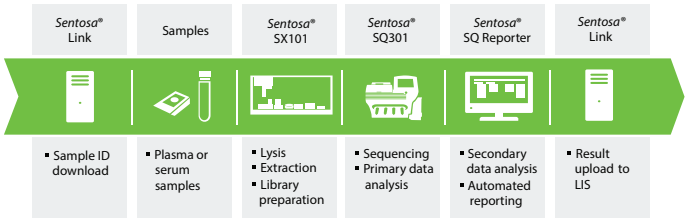

Figure 1: Single, automated NGS workflow for HCV Genotyping & RAS analysis. Workflow diagram details the steps involved from sample through analysis using the *Sentosa*® SQ HCV Genotyping Assay. This workflow requires less than 2.5 hours of hands on time and delivers results in 2 days.

Expert sequence design

The *Sentosa*® SQ HCV Genotyping Assay employs a multiplexed sequencing design targeting 3 therapeutically important regions of the Hepatitis C Virus genome: NS3, NS5A, and NS5B . This sequencing strategy thus maximizes sequencing reads on the most informative regions of the HCV genome. By targeting the NS5B region of the HCV genome instead of the 5'UTR, typically targeted by traditional HCV genotyping assays, even recombinant strains can be identified. This approach enables correct genotyping of recombinant viruses while simultaneously identifying clinically relevant RASs(Fig. 2). Additionally, this approach overcomes many of the uninterpretable results seen with LiPA (6.7% uninterpretable results with LiPA) by providing direct sequencing results.[7]

Furthermore, this assay design enables you to identify subtypes beyond 1a and 1b, delivering subtypes for 70 of the subtypes for Hepatitis C with accuracy. This advances your research, allowing you to discover potential new relationships between subtypes and therapeutics under investigation. Additionally, DNA contigs are readily available for further sequence analysis enabling assessment of additional mutations specific to the specimen under investigation. This may prove useful for further bioinformatics analyses in conjunction with monitoring resistance to novel drug treatments in your research.

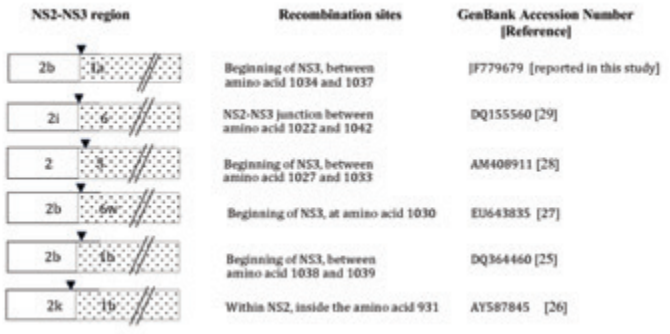

Figure 2: Schematic shows recombinant HCV strains identified to date. This depicts recombinant sites for the identified species, demonstrating that in these cases the recombination occurs prior to NS3, leading to accurate genotyping with the *Sentosa* SQ HCV Genotyping Assay while still covering all 3 drug target regions for RAS testing.[6]

For more information, visit VelaDX.com/HCV

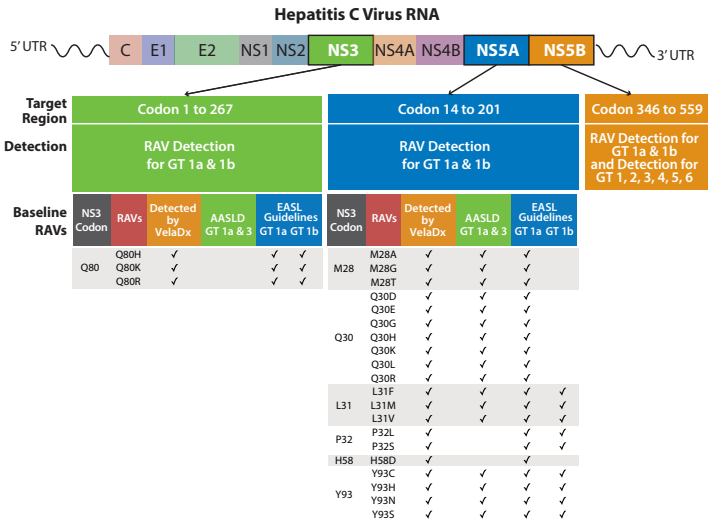

Figure 3: Illustration of Hepatitis C Virus RNA genes and regions targeted by the *Sentosa* SQ HCV Genotyping Assay design. Major RASs are identified and reported through the *Sentosa* SQ Reporter Software in accordance with the guidelines as well as targeted gene regions corresponding to the genotyping information.

Ordering Information

Product Name	Pack Size	Item Number
Sentosa® SQ HCV Genotyping Assay	4x16 tests	690019
Sentosa® ST Template Kit	8 runs	690007
Sentosa® SQ Sequencing Kit	8 runs	690005
Sentosa® SQ 318 Chip Kit	8 runs	300301
Sentosa® SX Virus Total Nucleic Acid Plus II Kit	4x16 tests	300352
Sentosa® SX101	1	400089
Sentosa® NGS Starter Kit	1	400105
Sentosa® SQ301 (120V) with SQ301 Minicentrifuge	1	690026
Sentosa® ST401	1	690027
Sentosa® Link	1	400045
Sentosa® SQ Reporter (software, server and perpetual license)	1	690014

Sentosa® SQ HCV Genotyping Assay Specifications

Analytical sensitivity	>1,000 HCV IU/mL for genotypes 1a, 1b, 2, 3 & 4 >2,000 HCV IU/mL for genotypes 5 & 6
Analytical specificity	No cross-reactivity with HAV, HBV, HIV, CMV, EBV, BKV, Dengue virus or genomic DNA
Reproducibility	99.2% (95% confidence interval: 97.20%-99.79%)
Controls	1 system control, 1 extraction control
Amplicons targeted	NS5B, NS3, & NS5A;
Automated result calling	GT 1 through 6 with RAS reporting for GT1a, 1b & 3. Sequence information for NS5B, NS3 & NS5A accessible in BAM files.
Coverage/target	>200x for genotyping, >500x for RAS calling
Sample types supported	Plasma & serum
Sample input required	530 uL
Sample throughput	15 samples/run, 80 samples/week
Time to results	2 days
Hands on time	Less than 2.5 hours

References

1. AASLD/IDSA HCV Guidance, Panel (September 2015). "Hepatitis C guidance: AASLD-IDSA recommendations for testing, managing, and treating adults infected with hepatitis C virus.". Hepatology (Baltimore, Md.). 62 (3): 932–54. doi:10.1002/hep.27950. PMID 26111063.
2. Liang, TJ; Ghany, MG (May 16, 2013). "Current and future therapies for hepatitis C virus infection.". The New England Journal of Medicine. 368 (20): 1907–17. doi:10.1056/NEJMra1213651. PMID 23675659.
4. Data from "Next Generation Sequencing (NGS) for HCV genotyping and optional identification of resistance-associated variants". Presented at AASLD Annual Meeting 2015 (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, Na=onal University of Singapore, Singapore;**Vela Research Pte Ltd., Singapore)
5. Simmonds P, Bukh J, et al. (2005). "Consensus proposals for a unified system of nomenclature of hepatitis C virus genotypes". Hepatology. 42 (4): 962–73. doi:10.1002/hep.20819. PMID 16149085
6. Reference: Bhattacharya et al. Virology Journal 2011, 8:458
7. Reference: Siemens VERSANT HCV Genotype Assay 2.0 Package Insert. 26017. Rev 5. 2012-08.

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