Performance of the newly designed Sentosa SQ HCV Genotyping v2.0 assay in HCV genotyping and resistance testing within the PEPSI study

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BACKGROUND

The Sentosa® SQ HCV Genotyping Assay v2.0 from Vela diagnostics) is a next generation sequencing-based test intended for qualitative identification of Hepatitis C Virus (HCV) genotypes 1 to 6 and the detection of resistance-associated substitutions (RAS) in HCV genotypes 1a/1b and 3. We used the research use only assay for genotyping and resistance detection in the NS3, NS5A and NS5B genes of HCV for samples within the PEPSI study(1). The further enhancement of version 2 is the extended length of sequence for NS5B starting at aminoacid (aa) 1 (instead of aa 339 in version 1) up to aa 565, and the inclusion of genotype 3 in resistance analysis.

METHODS

220 samples (so far) were analyzed with the HCV version 2 assay. The assay is validated by Vela for viral loads above 1000 IU/ml. Beside the included analysis in the Vela system (quality-controls, reads, coverage, genotype, RAS and variant frequency Figs 1 and 2) we reinterpreted the alignment files of the IonTorrent using the torrent suite (5.6) to map the sequences (s. Fig.3). We generated then consensus sequences with a minimum of 100 / 20 / 4 reads for each base with individual minority cut-offs at 30 / 20 / 15 / 10 / 5 and 2%. Sequence-interpretation was performed with the geno2pheno [hcv] 0.92 online tool (http://hcv.geno2pheno.org), see Fig 4. Sequence quality, predicted genotypes, success rates per genotype and resistance mutations for NS3, NS5A and NS5B were analyzed.

RESULTS

Sequences for all except one sample could be generated. In four of four samples below 1000 IU/mL sequences were achieved (two genotype 1a with 170 and 450 IU/mL ok for all three genes; one genotype 1a and one genotype 3a, 210 and 460 IU/mL, only with NS5A sequences). There were drop-outs for single genes (NS3 23/220, NS5A 7/220 and NS5B 3/220) mainly in non-1/non-3 genotypes (s. Fig 5). Genotype-distribution is shown in Fig 6. No RAS were detected in genotypes 2, 4 and 6. 26/40 1b samples showed resistance, two samples to all three classes. 41/80 1a samples had resistance mutations, NS3 Q80K (30x), NS5A (6x 28VT, three 93H) and NS5B (one 553V or 316Y and two 556R). 12/63 3a samples had NS5A mutations (six 30K, two 30V, four 93H), one 80K NS3 and none NS5B mutations. Overall 132 RAS were detected (63/45/24 for NS3 / NS5A / NS5B) including 24 mutations in minorities below 10% of the population.

RESULTS

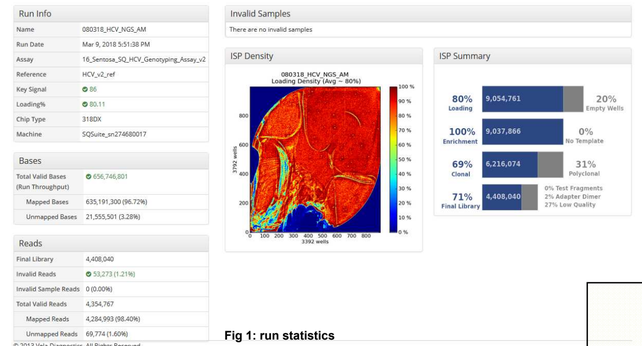

Fig 1: run statistics

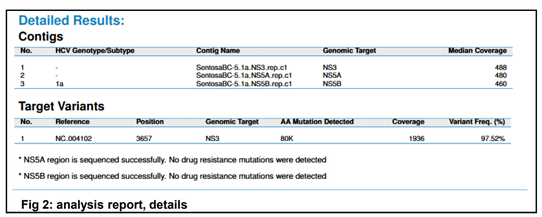

Fig 2: analysis report, details

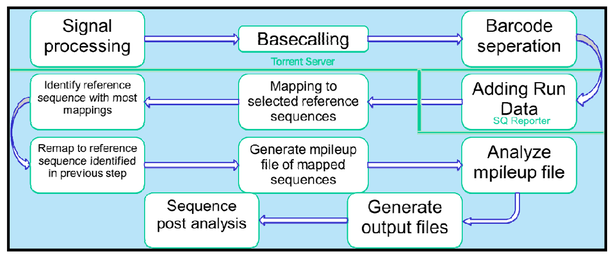

Fig 3: MIB NGS pipeline for Vela

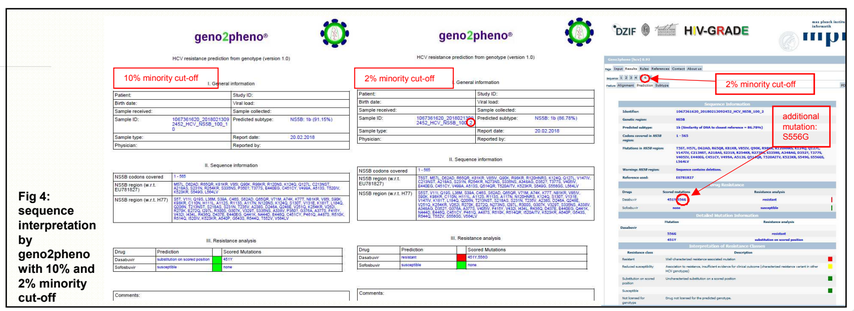

Fig 4: sequence interpretation by geno2pheno with 10% and 2% minority cut-off

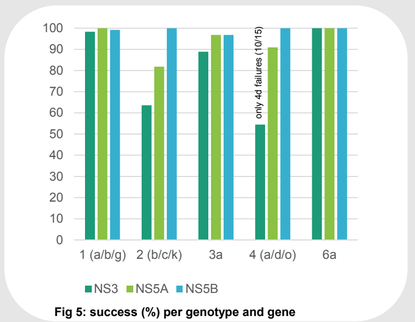

Fig 5: success (%) per genotype and gene

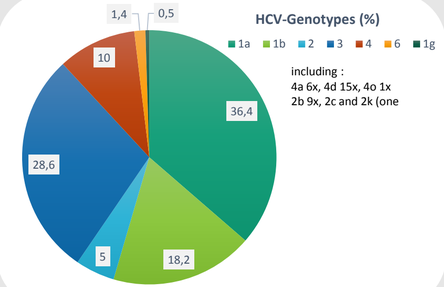

Fig 6: detected HCV genotypes, percentages

CONCLUSIONS

The Sentosa® SQ HCV Genotyping Assay v2.0 performed excellent, even in samples with low viral load. Designed to genotype all HCV strains and RAS in genotype 1a/1b and 3 we got success rates per gene of 97,5% (552 sequenced genes / 14 failures). In non-1a/b or 3 genotypes the success rate was 82,4% (108/19). With regard to the high genetic variability of HCV and the diversity of its genotypes and subtypes this is an exceptionally good success rate, which is reached otherwise only by stepwise approach using multiple in-house protocols. While for many sequencing protocols to determine the occurrence of RASs the HCV genotype has to be known on advance, this assay allows to gather this information in one step. Launch of HCV version 2 assay is planned for august 2018.

(1) Kalaghatgi P, Sikorski AM, Knops E, et al. Geno2pheno[HCV] – A Web-based Interpretation System to Support Hepatitis C Treatment Decisions in the Era of Direct-Acting Antiviral Agents. Menéndez-Arias L, ed. PLoS ONE. 2016;11(5):e0155869. doi:10.1371/journal.pone.0155869.

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