Is there a need for HCV resistance testing in routine diagnostics and patient treatment? - Routine HCV genotyping and resistance testing and performance of the Sentosa SQ HCV Genotyping v2.0 assay

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BACKGROUND

The need of HCV resistance testing in routine diagnostics is still a subject of debate. We evaluated the recently updated Sentosa® SQ HCV Genotyping Assay v2.0 from Vela diagnostics, a next generation sequencing (NGS)-based assay for identification of HCV genotypes 1 to 6 and 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

274 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.

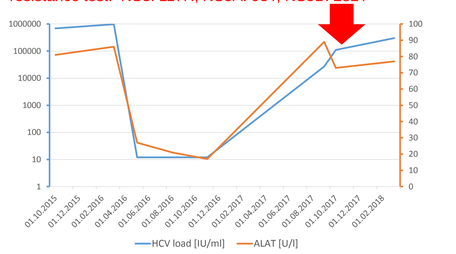
RESULTS

Sequences for 274/276 samples could be generated, only missing two samples with low viral loads (<1000 IU/mL) and a low number of drop outs for single genes (NS3 25/274, NS5A 10/274 and NS5B 3/274) mainly in non-1, non-3 genotypes (s. Fig 5). Genotype distribution was 1a (101), 3a (80), 1b (51), 4d (16), 2b (10), 4a (8), 6a (3), 2a (2) and one of 1g, 2c, 2k, 4o and 4r each as shown in Fig 6. Overall NS3, NS5A and NS5B genes showed RAS in 21,9%, 16,8% and 9,9%, respectively. 33 of 51 1b genotype samples showed resistance, two samples were resistant to three classes. 50/100 genotype 1a samples had resistance mutations, mainly NS3 Q80K (35x) but also NS5A (12x 28VT and 7x 93HN) and NS5B (3x556G; 2x553V, 316Y and 556R). Twelve out of 80 samples with genotype 3a had NS5A mutations (6x 30K, 2x 30V and 6x 93H), except one 80K the genotype 3a samples had no NS3 or NS5B mutations. Overall 167 RAS were detected. (71, 60, 36 for NS3, NS5A and NS5B) including 35 RAS in minorities with a cut-off below 10% and greater than 2% of population.

Patient example 1

83 years old, male, HIV negative
Fibro-Scan 11/2015: 7.7
HCV VL at treatment initiation: 980.000 IU/mL, genotype 1b
therapy: Harvoni (Ledipasvir/Sofosbuvir) 8w, 21.03.16 – 15.05.16

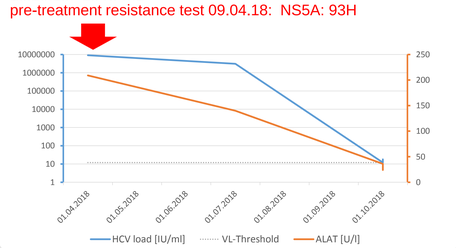
relaps: 11.09.2017 VL: 27.000 IU/mL
09.10.17: 690.000 IU/mL, genotyping: 1b
resistance test: NS3: 117H, NS5A: 93Y, NS5B: 282T



Patient example 2

34 years old, male HIV negative
IVDU, HCV diagnosis 2008, IL28B: C/T
HCV VL at treatment initiation: 3 100 000 IU/mL, genotype 3a
therapy: Epclusa(Velpatasvir/Sofosbuvir) + Ribavirin for 12 weeks (treatment start 10.07.18)

EOT: 02.10.2018 VL: <12 IU/mL (detected)
16.10.18: 18 IU/mL
pre-treatment resistance test 09.04.18: NS5A: 93H



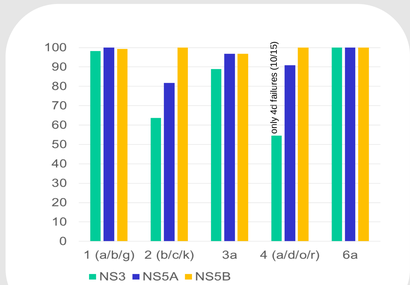


Fig 5: success (%) per genotype and gene

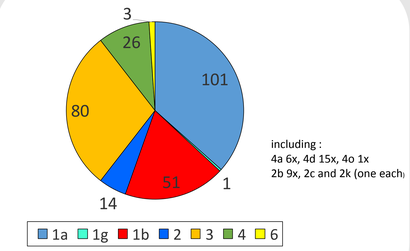


Fig 6: detected HCV genotypes, percentages

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 96,9% (21 failures / 696 sequenced genes). In non-1a/b or 3 genotypes the success rate was 81,3% (24/129). 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 in advance, this assay allows to gather this information in one step. Launch of HCV version 2 assay as Research Use Only (RUO) kit was in August 2018. CE/IVD approval is expected Q1/2019. A further improved assay is in development and the required documents are compiled for FDA approval. FDA approval is planned for 2019. A relative high proportion of investigated sequences showed RAS at a minority cut-off of 10%. Lowering this cut-off didn't significantly increase the number of detected RAS. Those RAS led to restrictions for single drugs or whole drug-classes. The relatively high rates of RAS clearly indicate the necessity of resistance analyses before starting an HCV-therapy and in case of treatment failure to avoid therapy failures and unnecessary given drugs avoiding side-effects and costs.

RESULTS

Fig 1: run statistics

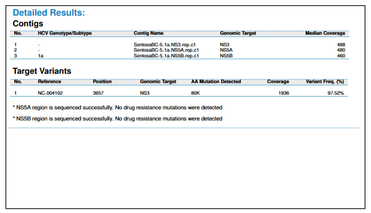


Fig 2: analysis report, details

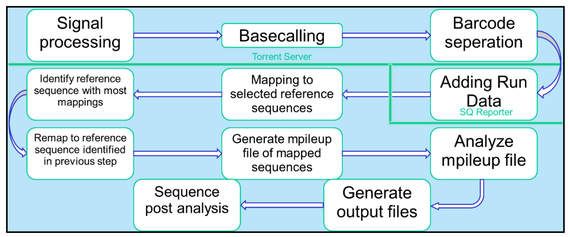


Fig 3: MIB NGS pipeline for Vela

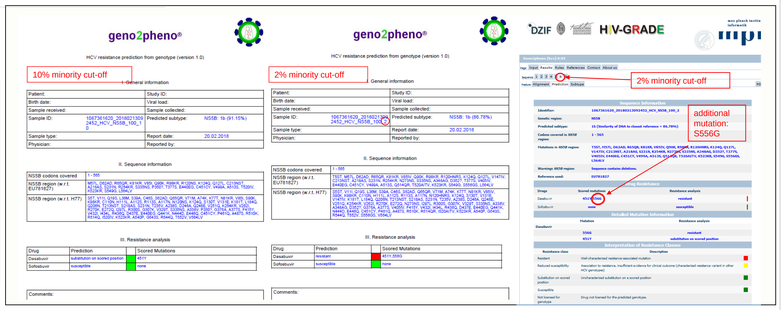


Fig 4: sequence interpretation by geno2pheno with 10% and 2% minority cut-off

(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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1559

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