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 (Vela diagnostics) is a NGS-based test for qualitative identification of Hepatitis C Virus (HCV) genotypes 1 to 6 and the detection of resistance-associated substitutions (RAS) in HCV subtypes 1a/1b and 3. We used the research use only assay for genotyping and resistance detection in the NS3, NS5A and NS5B genes 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

75 samples (so far) were analysed. Beside the included analysis in the Vela system we interpreted the raw data using the torrent suite (5.6) to map the sequences with a minimum of 100/20/4 reads for each base with 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). Predicted genotype and resistance mutation for NS3, NS5A and NS5B were analysed.

RESULTS

Sequences for all samples could be generated with declining quality below 1000 IU/mL (three samples; one 1a with 450 IU/mL ok for all three genes; one 1a and one 3a, 250 and 460 IU/mL, only with NS5A sequence). There were drop-outs for single genes (NS1 12/75, NS5A 4/75 and NS5B 2/75) mainly in non-1/3 genotypes. Genotype-distribution was 1a (25), 3a (23), 4d (9), 1b (8), 2b (4), 4a (3) and each of 1g, 2c and 6a. No RAS were detected in genotypes 2b/c, 4a/d and 6a. Seven of eight 1b samples showed resistance, one sample to three classes. 14/25 1a samples had resistance mutations, NS3 Q80K (11x), NS5A (3x 28VT, one 58S) and NS5B (one 553V and 556R). 6/23 3a samples had NS5A mutations (4x 30K, one 30V, two 93H), none had NS3/NS5B mutations. Overall 32 RAS were detected (15/12/5 for NS3/NS5A/NS5B), additionally 7 RAS in minorities between 10% and 2% of population.

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 96.4% (168 sequences/6 failures). In non-1a/b or 3 genotypes the success rate was 78.9% (57/12). 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 2018.


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