Evaluation of a sequence-based method for hepatitis C genotyping (Deepchek® SingleRound PCR and NS5B/5'UTR sequencing) and comparison with VERSANT HCV Genotype 2.0 assay (InnoLiPA)

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Introduction

Adequate hepatitis C virus (HCV) genotyping and subtyping is important for choosing the optimal antiviral treatment regime and the duration of treatment. The VERSANT HCV genotype 2.0 assay (InnoLiPA, Siemens) is frequently used for genotyping of HCV. However, sequencing of part of HCV genome can provide more detailed information on HCV genotype (gt), subtype and genetic variation. We compared two genotyping methods: the Deepchek® for sequencing of the NS5B and 5'UTR region (ABL, Luxembourg) and the InnoLiPA.

Methods

162 plasma samples from patients with different HCV genotypes, previously tested with InnoLiPA, were selected and genotyped with Deepchek®.
Both assays were performed according to manufacturer's instructions.
Deepchek-kits for HCV genotyping by sequencing for this study were derived from ABL, Luxembourg.
InnoLiPA-kits were derived from Siemens.
For the Deepchek assay, HCV RNA was isolated on Magnapure 96 with MP96 DNA and viral NA Large volume kit. Amplifications and sequencing amplification were performed with Deepchek SingelRound PCR and NS5B/5'UTR sequencing kit. Before sequencing, amplification products were checked on e-gel. In case of positive NS5B product, this part of the viral genome was sequenced. In case NS5B was not amplified, 5'UTR was sequenced. Sequencing was performed with the Sanger method on ABI3730 cycle sequencer. Sequences were analysed with the Deepchek software and also uploaded and blasted in the Los Alamos database (https://hcv.lanl.gov/content/index, NS5B and 5'UTR) and Geno2Pheno (http://www.geno2pheno.org/, NS5B). Genotyping results of the different methods were compared.

Results

Table 1. Samples

genotype InnoLIPA	total numer samples	number NS5B typing	number 5'UTR typing
1a	n=42	n=38	n=4
1b	n=27	n=23	n=4
1	n=4	n=3	n=1
2	n=22	n=19	n=3
3a	n=32	n=27	n=5
3b	n=2		n=2
3c	n=2	n=2	
3	n=3	n=3	
4	n=22	n=15	n=7
5	n=2	n=2	
6	n=1	n=1	
untypable	n=3	n=1	n=2

Of the 162 serum samples, 134 (83%) were typed on NS5B and 28 on 5'UTR (when typing on NS5B did not succeed). There was no specific genotype-relationship whether typing by sequencing on NS5B did or did not succeed (table 1).

With Deepchek, in all samples a test result for genotyping was obtained whereas 3 samples were 'untypable' with InnoLIPA. These 3 samples were genotype 3 (1x 3b, 2x 3h) by sequencing.

Sequences obtained from NS5B were 380-400 nt and from 5'UTR were 210-230 nt. In 3 samples (gt 1, 2 and 4) amplification of 5'UTR generated a sequence of only 78 nt, However the HCV genotype agreed between both methods.

Results

The overall agreement on the HCV genotype level between the InnoLiPA and the Deepchek sequencing method was 95,7% (figure 1).

Figure 1. Agreement InnoLIPA and Deepchek per genotype

Discordant results

Seven samples showed discordant genotyping results:

- Two samples were 2k/1b recombinant samples (gt 2 on 5'UTR, gt 1b on NS5B). Genotyping on 5'UTR only does not reveal this recombinant strain.
- One sample was gt 1b with InnoLiPA and 3a by sequencing
- One sample was gt 1a with InnoLiPA and gt 6 by sequencing
- Two samples were gt 4 by InnoLiPA but gt 3k with sequencing
- One sample with gt 5a by InnoLiPA was typed as 3h by sequencing.

Discrepancy analysis by sequencing of the core-region: genotyping of core agreed best with seguencing NS5B (5/5) or 5'UTR (5/5), whereas InnoLiPA agreed only in 1/5 (table 2). Both recombinant strains were typed on core as 1b/2k. The 3 untypable samples with InnoLiPA were all gt 3 by sequencing (2x 3h, 1x 3b).

Subtyping within genotype 1

From the 73 samples with gt 1, 41 were subtyped as gt 1a and 26 were subtyped as 1b with both methods (table 3). Four samples were gt 1 (no subtype) with InnoLiPA and these were all typed as gt 1a with sequencing. Two other gt 1 samples (InnoLiPA) were discordant at the genotype level (gt 6 and 3a by sequencing).

Table 2. Discordant test results

	InnoLIPA	Deepchek software		Geno2Pheno	Los Alamos blast			discrepancy analysis
nr	5UTR	NS5B	5UTR	NS5B	NS5B	5UTR	remark	genotype
W102	1b	3a (100%)	3a (100%)	3a (93%)	3e/ 3a (99%)	3a (93%)		3a (97%)
W086	1a	failed	6v (34%)	failed	failed	1a (98%)		1a (99%)
W026	2	1b (100%)	2a (63%)	1b (93%)	1b/2k (97%)	2 a/e/c / 5 (99%)	recombinant	1b/2k (98%)
W013	2	1b (100%)	nd	1b (95%)	1b (98%)	nd	recombinant?	1b/2k (98%)
W003	4h	3k (100%)	3k (100%)	3k (93%)	3k (94%)	3k (99%)		3k (97%)
W123	4	3k (96%)	3k (77%)	3k (93%)	3k (73%)	3 (96%)		3k (95%)
W008	5a	3h (100%)	3h (100%)	3h (91%)	3h (92%)	3h (99%)	LiPA bands did not agree	3h (96%)

nd = not done

Table 3. Subtyping of gt1

genotype InnoLIPA	genotype Deepchek	number of samples
1	1a	n=4
1a	1a	n=41
1a	6v	n=1
1b	1b	n=26
1b	3a	n=1

Conclusion

- **In all 162 samples a genotyping test result was obtained with HCV Deepchek ® for genotyping.**
- **HCV genotyping by sequencing with Deepchek® agreed in >95% with genotyping with InnoLiPA.**
- **Discrepancy analysis on core correlated well with genotyping by sequencing on NS5B or 5'UTR.**