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Background

- Introducing Next Generation Sequencing (NGS) in HCV patient management leads to a more precise detection of strains related to virological failure.
- Since new direct antiviral agents (DAAs) are increasingly being evaluated and approved for the management of chronic viral hepatitis C, efficient and reliable data management software to analyze and optimize patient management is now required. We developed **DeepChek®-HCV**, a sequencing data analysis system for chronic hepatitis C patients.
- DeepChek®-HCV performs within minutes clinically-relevant analyses (including high resolution subtyping, genotyping and drug resistance assessment) from NGS or Sanger sequencing data targeting key regions of interest of Hepatitis C Virus (Fig. 1).

DeepChek®-HCV handles:



- ✓ 5'UTR (Subtyping)
- ✓ NS3 (Genotyping & Drug resistance)
- ✓ NS5A (Genotyping & Drug resistance)
- ✓ NS5B (Subtyping + Genotyping & Drug resistance)

Fig. 1: Types of regions and analyses supported by DeepChek®-HCV v1.0

Methods

- We used DeepChek®-HCV, a secured and validated software application, in process of CE-IVD marking, to perform within minutes automated downstream analysis, high-resolution subtyping and HCV resistance interpretation of NGS (5'UTR and NS5B samples sequenced by the Roche 454 GS Junior technology) and Sanger sequencing data.
- The results and the user-friendliness (Fig. 2) of the system were compared to a pipeline developed by the Vall d'Hebron Institut of Research (VHIR-HUVH - patent ID EP13382278, CDTI project MINECO; Ref. IDI-20110115) and a third methodology provided via internet by the Oxford University (http://evolve.zoo.ox.ac.uk/evolve/HCV_Subtyping_Tool.html).
- Besides high resolution subtyping (Fig. 3), DeepChek®-HCV was also used to assess drug resistance from NS5A, NS5B and NS3 virtual datasets, manually generated from several resources.
- Those assessments were determined using 3 different algorithms (Geno2Pheno, IAS and an In-vivo/In-Vitro literature review) for the main DAAs including: Boceprevir, Daclatasvir, Faldaprevir, Simeprevir, Sofosbuvir and Telaprevir.

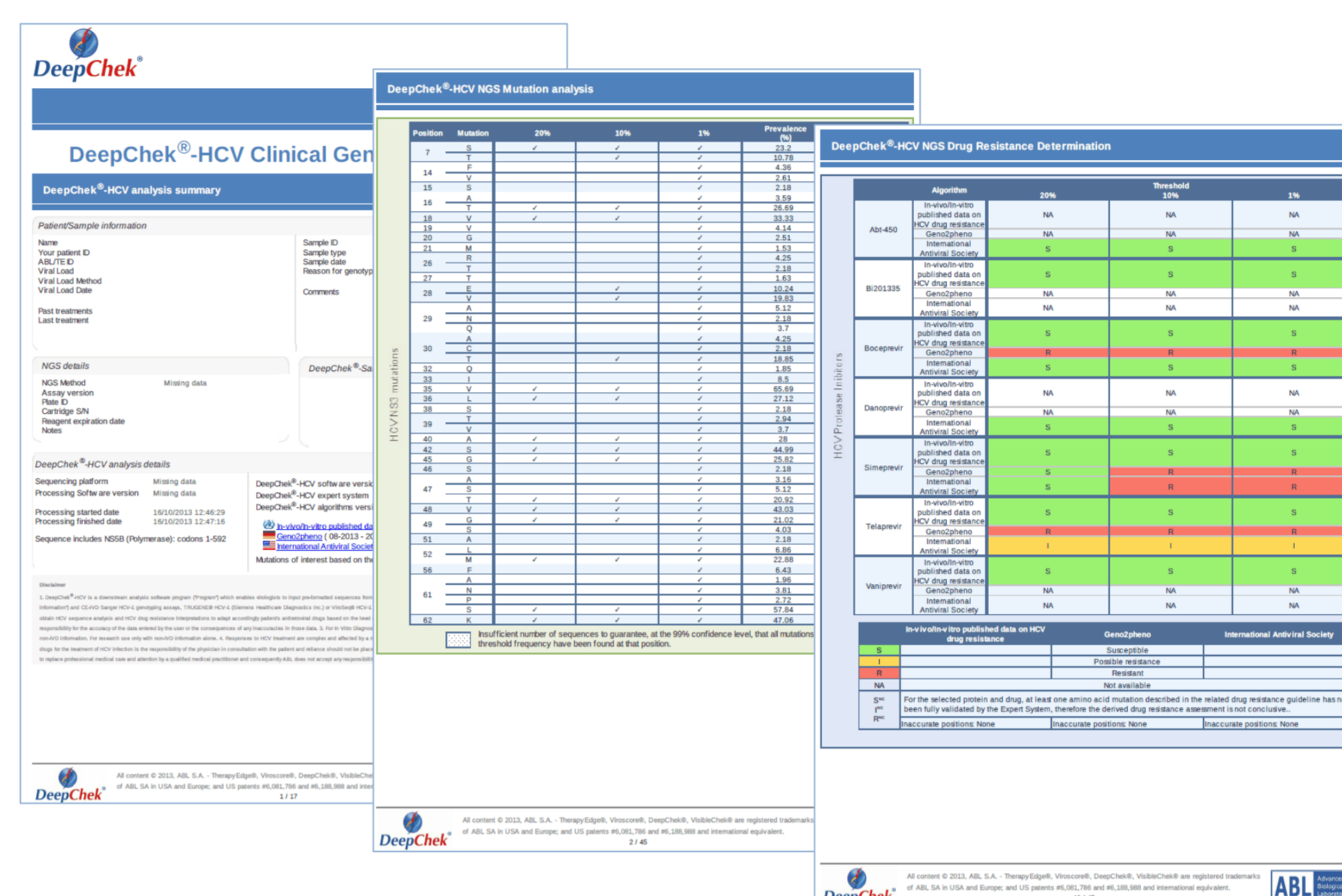


Fig. 2: Example of a DeepChek®-HCV clinical genotyping report.

DeepChek®-HCV High resolution subtyping						
Region	Genotype		Subtype		Number of reads	Confidence (*)
	Name	Prevalence	Name	Prevalence		
NS5B (Polymerase)	3	100%	3a	100%	777	100%
5'-UTR	3	99.87%	3a	99.87%	1575	100%
	1	0.13%	1b	0.13%	2	100%

Fig. 3: Example of High Resolution Subtyping result obtained using DeepChek®-HCV.

Results

- On a panel of 129 samples coming from 6 NGS runs and designed with 2 amplicons covering the 5'UTR and NS5B regions (170 sample/amplicon combinations), a total of 22126 unique haplotypes were analyzed by DeepChek®-HCV (Fig. 4).
- The high-resolution subtyping analysis classified all the reads within 30 seconds (Fig. 5) while the other method identified a subset of 297 haplotypes (representing 48% of the reads) in about 20 minutes.
- The majority of the sample/amplicon combinations were classified with only one subtype. Co-infection with heterogeneous quasispecies were observed on about 10% of the samples (17/170=10% for DeepChek-HCV and 13/170=7.6% for VHIR-HUVH) (Fig. 6).
- Among the 297 sequences which could be subtyped by both methodologies, the agreement of the classification reached 97.6% (Fig. 6). The majority of the differences were found with a low confidence level (<60%) and related to genotype 2 and/or to the 5'UTR region (less discriminative than NS5B - Fig 7).
- Increasing the list of genotype 2 reference strains will reduce the observed discrepancies.
- Genotyping and drug resistance assessments were also performed and the related clinical genotyping reports were produced for validation by a panel of expert who confirmed its usefulness.

6	ROCHE 454 GS JUNIOR SEQUENCING RUNS
129	PATIENTS
2	AMPLICONS (COVERING PART OF THE 5'UTR AND NS5B REGIONS)
22126	UNIQUE HAPLOTYPES
297	SUBSET OF UNIQUE HAPLOTYPES (REPRESENTING 48% OF THE READS)

Fig. 4: Overview of the panel of data used for the study.

TIME TO ANALYSE THE COMPLETE DATASET	PIPELINE VALL D'HEBRON
	20 MINUTES
	DEEPCHEK®-HCV
	30 SECONDS

Fig. 5: Processing time comparison

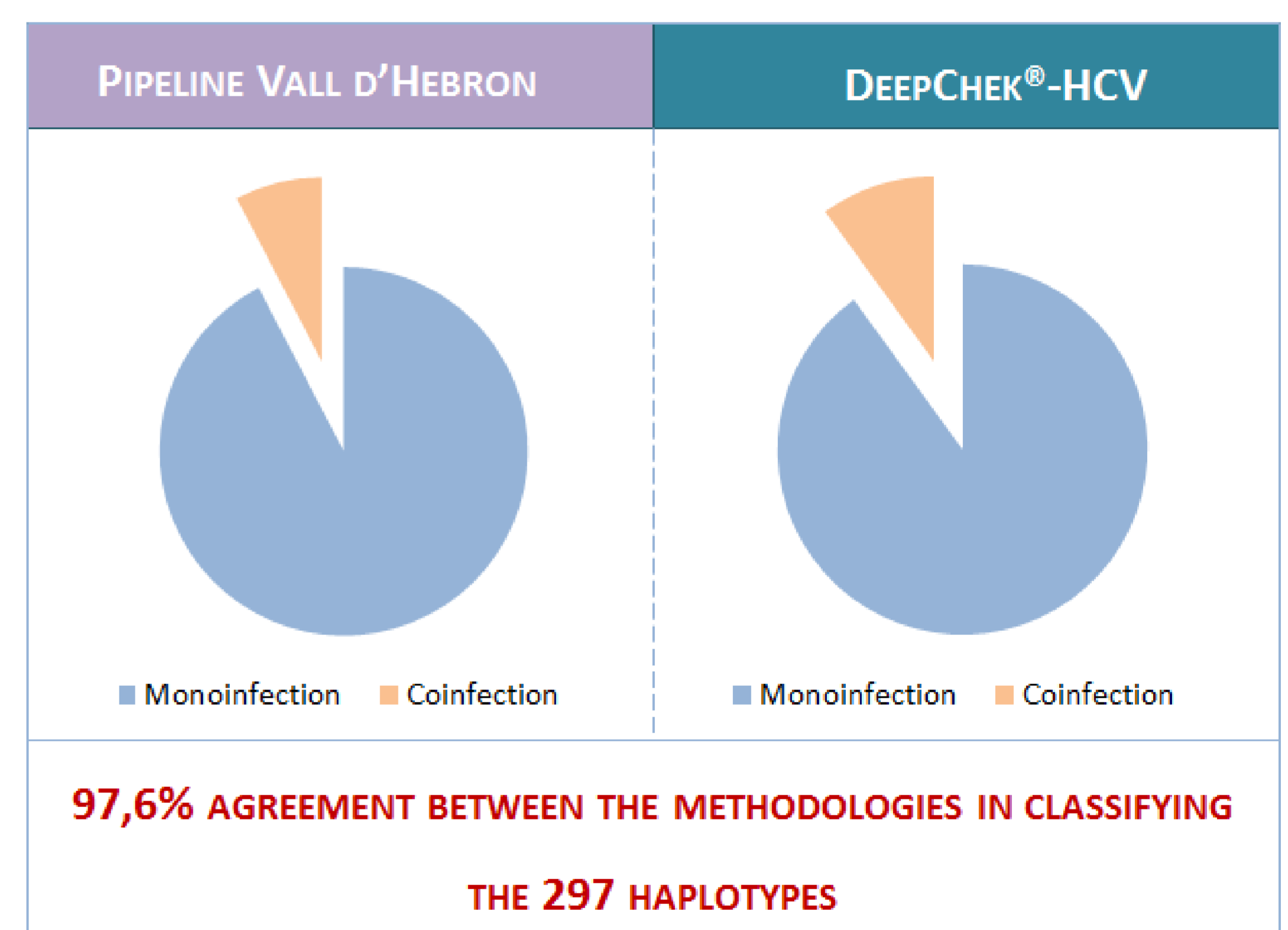


Fig. 6: Subtype classification obtained from DeepChek®-HCV and the Vall d'Hebron pipeline.

ID	Region	Reads	DeepChek®-HCV	Vall d'Hebron	Oxford
Sample A	UTR-core	11	2a	2c	2c
Sample A	UTR-core	2	2j	2k	2
Sample B	UTR-core	514	2a	2c	2c
Sample B	UTR-core	2	2j	2c	2c
Sample C	NS5B	1542	2i	2c	2
Sample D	NS5B	130	2j	2k	-
Sample E	UTR-core	834	4a	4c, 4c, 4p	4a

Fig. 7: Discrepant subtyping characterization results observed on 297 5'UTR and NS5B sequences.

Conclusions

- This study illustrates the benefits of using well-validated downstream analysis software combining high-resolution subtyping and drug resistance assessment of major and minor variants, for the management of patients infected with HCV in order to target personalized medicine.
- A simple access to clinically-meaningful information known to be relevant for the management of HCV infection (like a high resolution subtyping of the viral Hepatitis C strains) is a key factor to optimize patients suffering from chronic hepatitis C.

References

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