Hepatitis C Virus High Resolution Subtyping Using Next Generation Sequencing (NGS) Data.

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Background

- Compared to Sanger sequencing, NGS provides the ability to detect mutations present at low frequencies, some of which are potentially leading to antiviral treatment failure.
- Yet, NGS data comes at the cost of increased complexity. Additionally, as the number of new direct antiviral agents (DAAs) approved for the management of chronic HCV infection increases, reliable and well validated analysis software that can easily handle NGS data and be frequently updated with the most recent drug stratification information is in demand.
- We developed DeepChek-HCV (*Fig. 1*), a sequencing analysis system embedding well-validated methodologies for the management of HCV NGS and Sanger genomic data (high resolution subtyping).
- It also includes an HCV drug resistance module, which is in its initial versioning developed by ABL and will be further validated through evaluations and with additional clinical samples.

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

- DeepChek-HCV was developed as a secured and standardized software application in process of CE-IVD marking through a close collaboration with the Vall d'Hebron Institut of Research (VHIR-HUVH).
- Within minutes, the pipeline performs automated downstream analyses including high-resolution subtyping (HRS – *Fig. 2*).
- Part of the system, the HRS module for HCV quasispecies followed a strict methodology of development and validation embedding major filtering and data correction components adapted to the sequencing technology.
- The system currently supports two regions for HRS (5'UTR and NS5B) and will be adapted in the future to also perform variant calling and drug resistance interpretations from other regions (NS3, NS5A and NS5B).
- Many aspects of the analysis and reporting (*Fig. 3*) can be customized creating user profiles to assure the production of consistent reports over time.

DeepChek®-HCV High resolution subtyping

	Genotype		Subtype			
NS5B (Polymerase)	Name	Prevalence	Name	Prevalence	Number of reads	Confidence (*)
	3	46.85%	3a	46.85%	1554	100%
	1	53.15%	1b	34.43%	1142	100%
			1a	18.72%	621	100%

Fig. 2: Example of High Resolution Subtyping result obtained using DeepChek®-HCV.

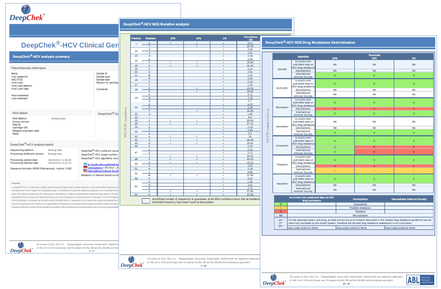

Fig. 3: Example of a DeepChek®-HCV clinical genotyping report.

Results

- We have analyzed 362 samples from 15 separate NGS-454 GS-Junior platform runs (*Fig. 4*).
- DeepChek-HCV took approximately 14 minutes to process a complete run for HRS and on average (*Fig. 4*).

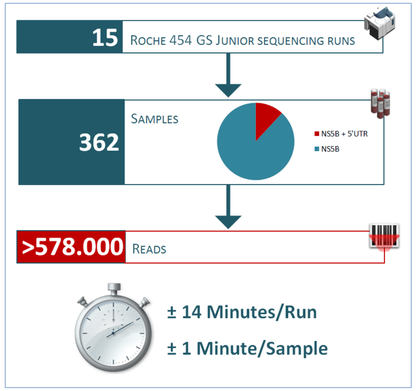

Fig. 4: Overview of the 454-HCV dataset.

- Sequences that have passed the filtering process are subtyped using sets of reference sequences including the Los Alamos database: http://hcv.lanl.gov/content/sequence/NEWALIGN/align.html/.
- At the end of a run, both a detailed CSV report with all the results and a clinical genotyping PDF report containing only the validated results are produced (Fig. 5).

Fig. 5: Types of analyses and reports produced by DeepChek-HCV

- Samples with low coverage for reliable subtype determination or insufficient coverage for confident detection of multiple infection, as well as subtypes found with a low confidence level will be flagged, providing warnings of interest to clinicians (*Fig. 6*).

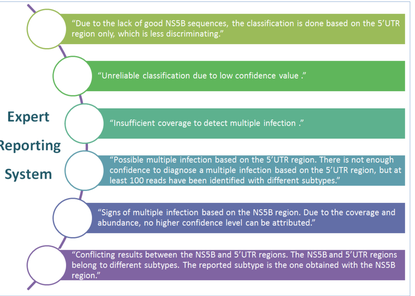

Fig. 6: Overview of the possible warnings attached of each HRS result and tailored to clinician understanding.

Conclusions

- This study illustrates the benefits of using well-validated downstream analysis software tailored for HRS of major and minor variants, for the management of patients infected with HCV in order to target optimal personalized medicine.

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