

## Background

- Despite the potent antiviral activity of approved therapeutic options available for the treatment of chronic hepatitis B (CHB), HBV still persists.
- Long durations of therapy are required and often lead to the emergence of drug resistance.
- New insight in HBV drug resistance is starting with the introduction of Next Generation Sequencing (NGS) which identifies major and minor variants within the HBV RT.
- Specific algorithms managing as well Hepatitis B Surface Antigen (HbSAg) mutants are required for proper disease management and prevention.
- Using DeepChek®-HBV, we performed an analysis of a panel of HBV samples to find minor variant populations and assess the impact of such viruses on the drugs susceptibility using various drug resistance guidelines.
- Beside drug resistance determination, we are also in the process of determining additional key markers for the general management of Hepatitis B including the detection of surface antigen mutations for vaccine escape interpretation or the determination of the relationship between genomic variations and risk of liver cancers using our expert SeqHepB knowledge database.

## Methods

- We used DeepChek®-HBV, a secured and well-validated software application, in process of CE-IVD marking, to perform within minutes automated downstream analysis and HBV resistance interpretation of any NGS and Sanger sequencing data.
- Several clinical guidelines including SeqHepB, the largest HBV drug resistance database, and Geno2pheno, assessed anti-HBV drugs sensitivity levels.
- Vaccine escape determination through the analysis of HBsAg mutants was included.
- The genotype of the HBV sequence submitted for analysis was determined by comparison with each of the reference standards using an efficient variant calling system tailored to NGS platforms.
- Through validation studies in Thailand and Turkey, we harvested 19 key HBV samples and processed them through Roche-454 GS-Junior and DeepChek®-HBV.
- Per-sample clinical genotyping reports (Fig. 1A) were inspected for the determination of key information related to minor variants population.
- Overall data visualization and querying are currently being performed through VisibleChek (Fig. 1B) to correlate genomic information with clinical outcomes.

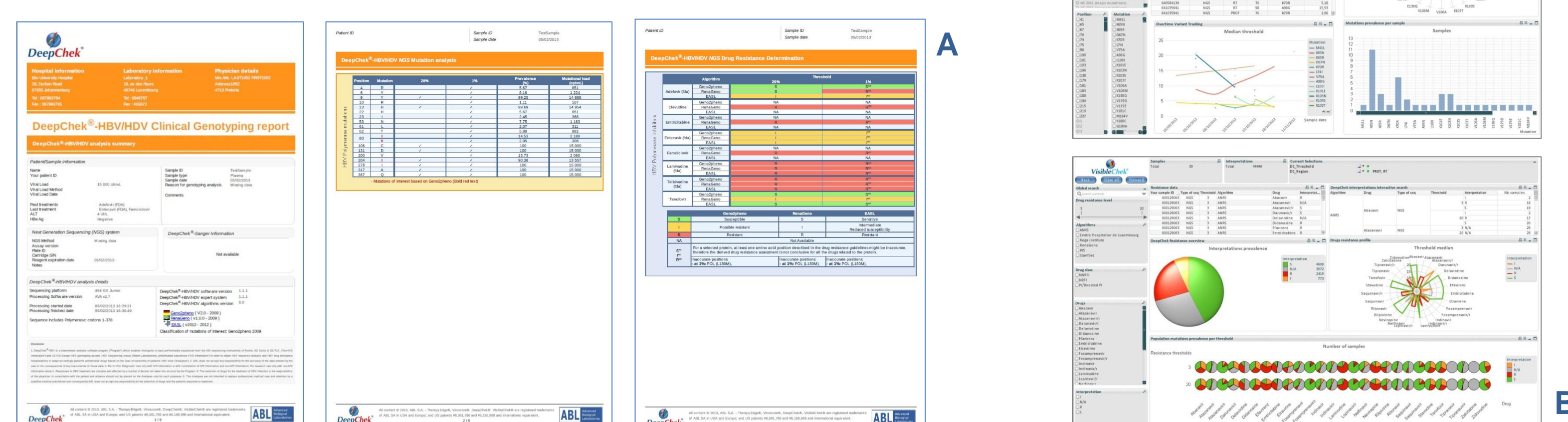


Fig. 1: Example of a DeepChek-HBV clinical genotyping report (A) and data visualization within VisibleChek (B).

## Results

- In a pool of 19 samples, DeepChek®-HBV identified 3 samples (15.8%) and 5 samples (26.3%) harboring known drug resistance mutations at 20% and 1% thresholds, respectively (Fig. 2).
- The number of DRMs comparing 20% (usually correlated with conventional Sanger sequencing) and 1% thresholds increased from 6 to 11 (Fig. 3 and 4).
- Drug resistance susceptibility reductions were observed in 1, 2, 2, 2, 0 samples for Adefovir, Entecavir, Lamivudine, Telbivudine and Tenofovir respectively (Fig. 3 and 4).

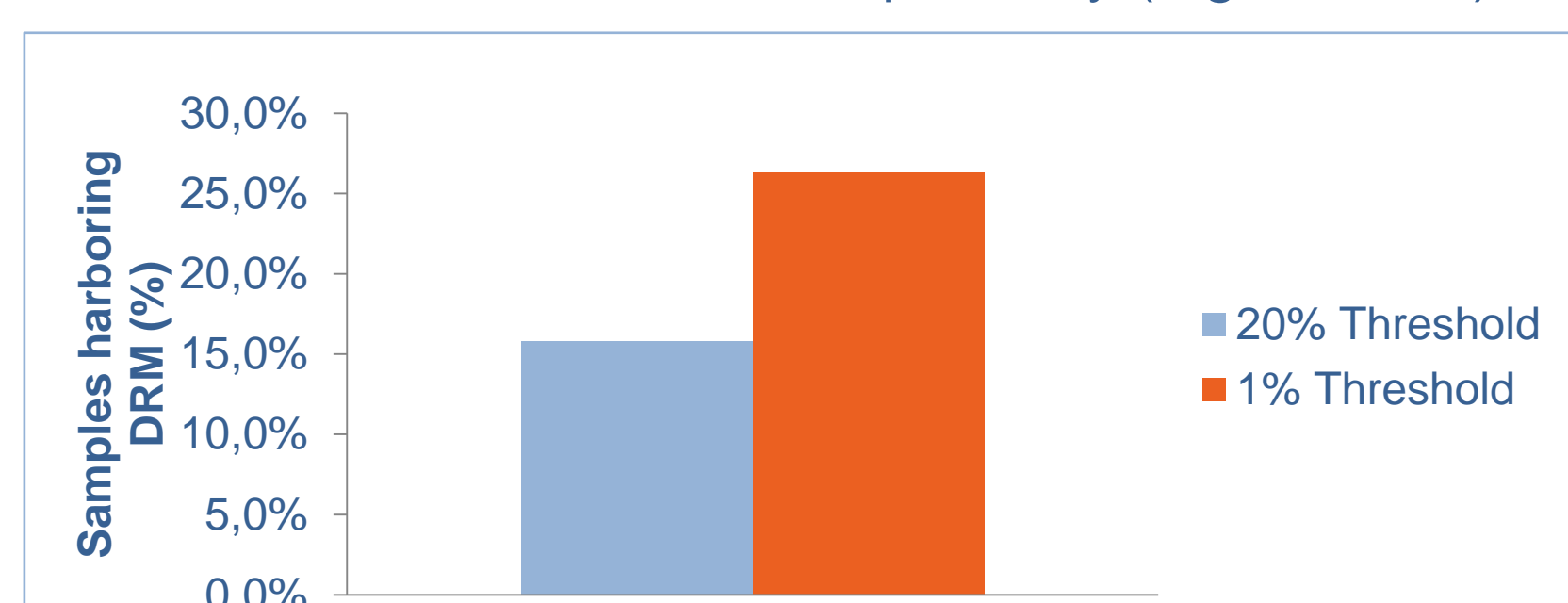


Fig. 2: percentage of samples harboring at least one DRM at 20% (blue) and 1% (red) thresholds.

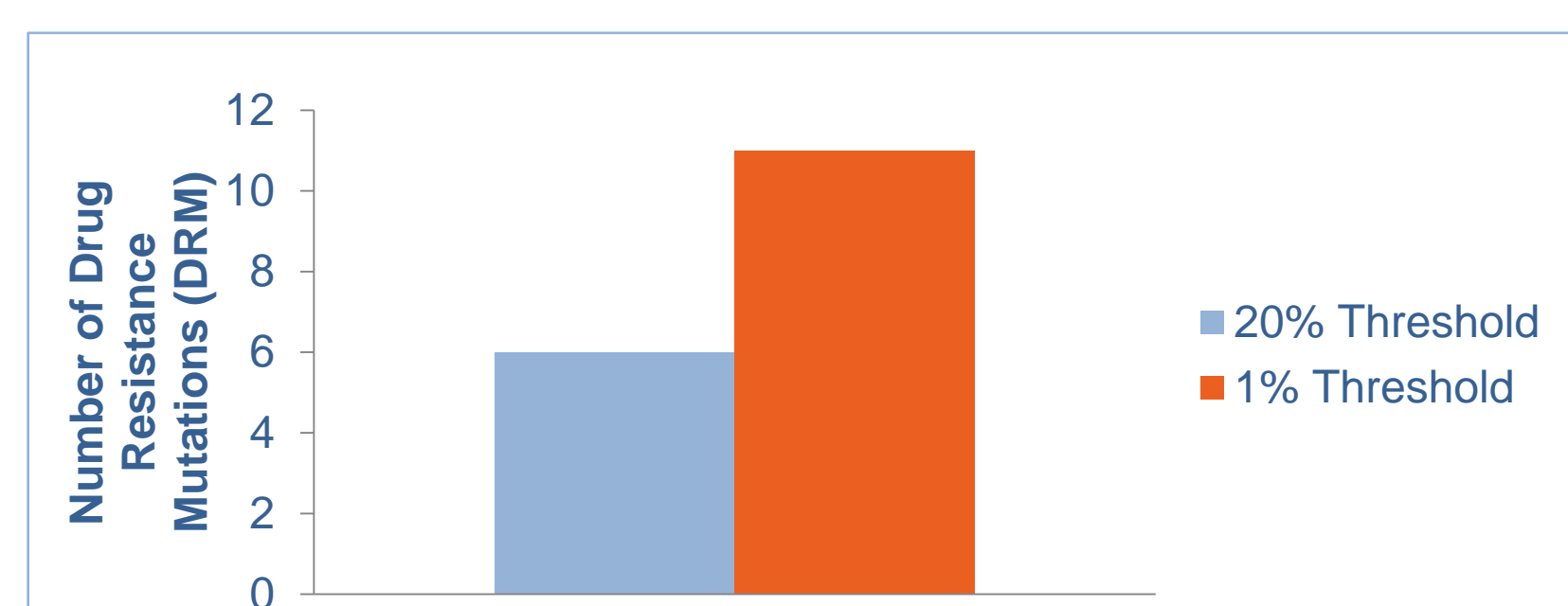


Fig. 3: Number of Drug Resistance Mutations found at 20% (conventional sequencing) and 1% (minor variants detection) thresholds.

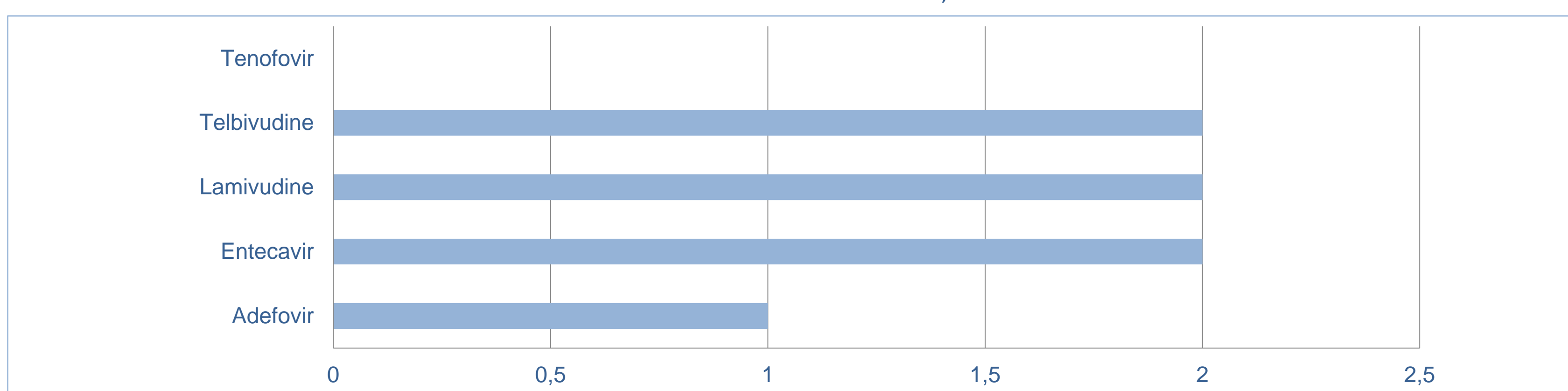


Fig. 3: Overview of the number of samples where an increase of the drug resistance has been observed per drug using the Geno2Pheno algorithm comparing interpretations given at 20 and 1%.

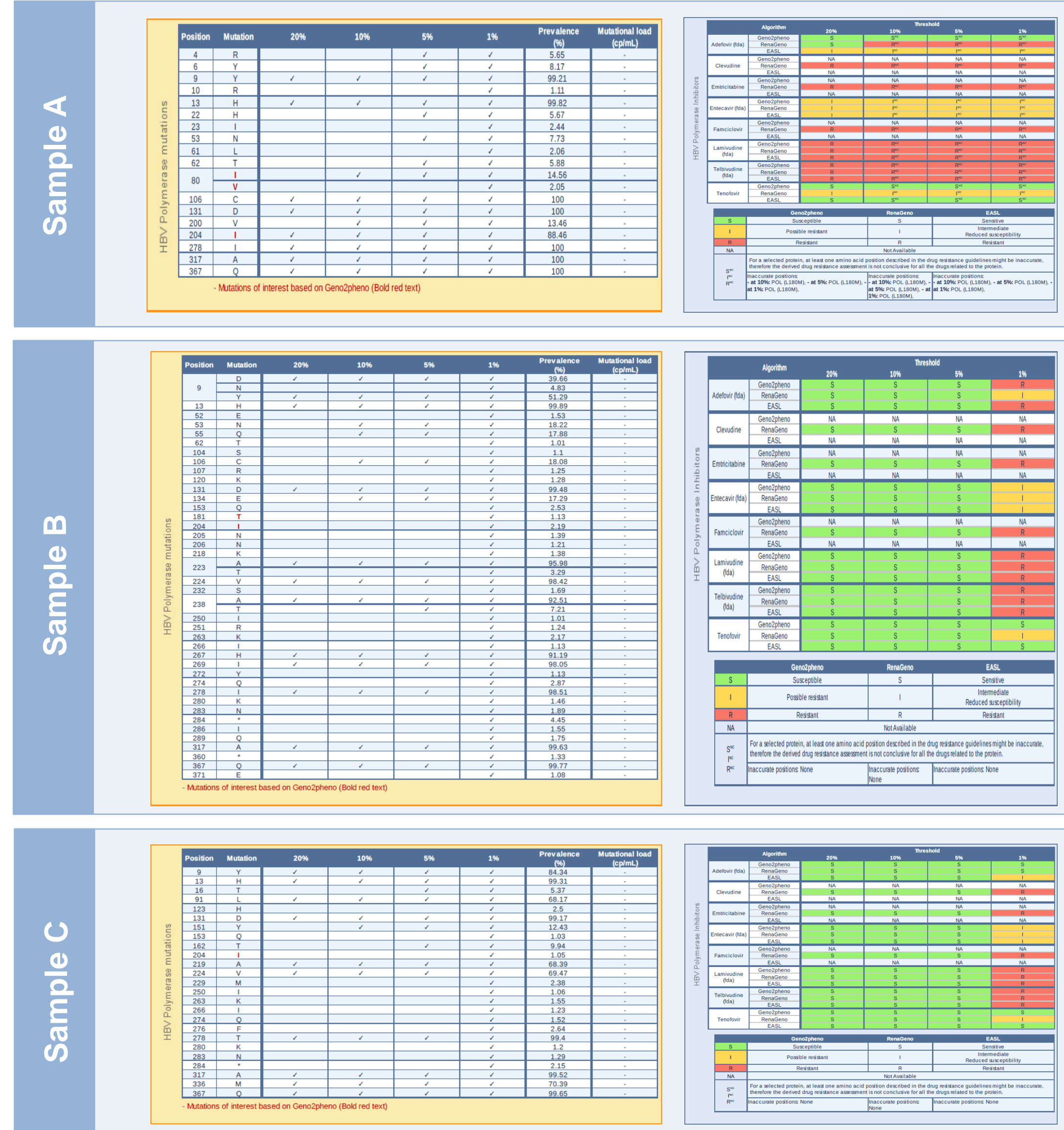


Fig. 4: DeepChek®-HBV reporting of HBV RT mutations and drug resistance interpretations for 3 samples harboring minor drug resistance mutations.

## Conclusions

- This study showed the impact of minority variants in drug resistance assessment which still needs to be correlated with clinical utilities (ongoing analysis with VisibleChek® - Fig. 5).
- It illustrated the benefits of combining well-validated downstream analysis software and updated knowledge database for managing HBV Sanger and NGS outputs.
- Such solution can be extended nationwide as an innovative service for the management of CHB and establishment of national HBV databases for personalized medicine.

Fig. 5: Overview of the integration of genomics information with clinical data through VisibleChek®.

