Validation of a sequencing-based HBV genotyping assay with serological backup for DNA-suppressed patients

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Introduction

Hepatitis B Virus (HBV) genotyping traditionally relies on sequencing HBV DNA in serum/plasma. However, in clinical trials focused on achieving a functional cure, HBV DNA is often undetectable in individuals with virological suppression, which requires alternative measurement strategies. HBV RNA remains detectable in a subset of these patients, offering an opportunity for genotyping when HBV DNA is absent.

Aim

The goal of this study was to validate the clinical performance of the HBV RNA S/RT sequencing assay, based on Liu, et al 2020, using plasma and serum samples containing both HBV RNA and DNA. Furthermore, a serological backup method was implemented to ensure genotype determination for samples that failed amplification.

Method

Part of the clinical validation included testing the 1st WHO International Reference Panel for HBV from the Paul Ehrlich institute (PEI code 5086/08)

For the primary assay, reverse transcription was performed on extracted nucleic acid, containing both HBV DNA and RNA. Followed by outer and nested PCR amplification of a fragment spanning the S-gene and RT-domain (Figure 1 and Figure 2). Libraries were prepared from the PCR products and sequenced using Illumina short-read technology on an Illumina Nextseq1000 sequencer. Genotypes were assigned through phylogenetic analysis against reference sequences.

The second assay, used as a serological backup method for genotyping when amplification fails during clinical trials, was performed using the IMMUNIS HBV genotype enzyme immunoassay (EIA), which detects genotype-specific PreS2 epitopes to classify genotypes A–D.

Figure 1: Outline of HBV RNA/DNA S/RT gene sequencing method

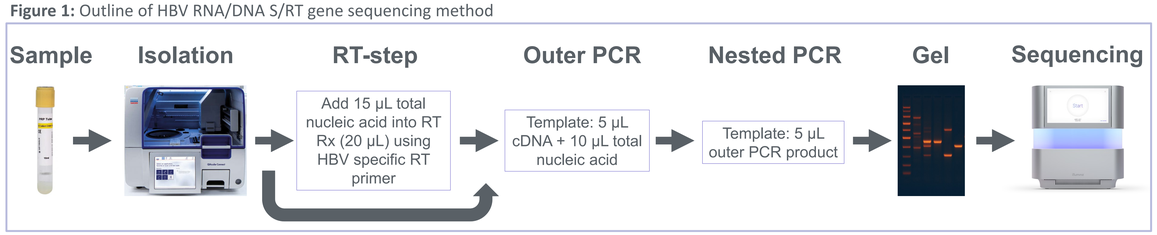
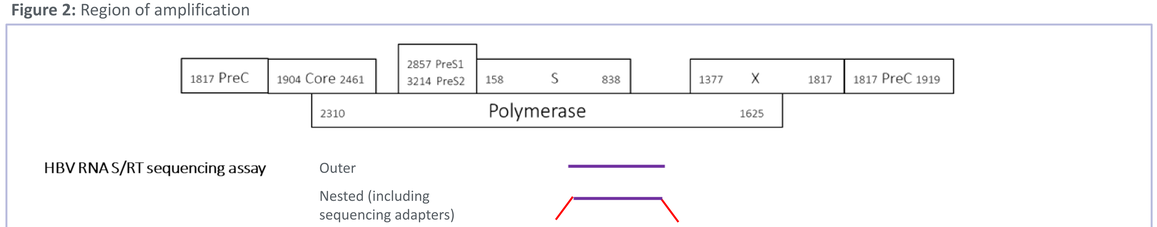


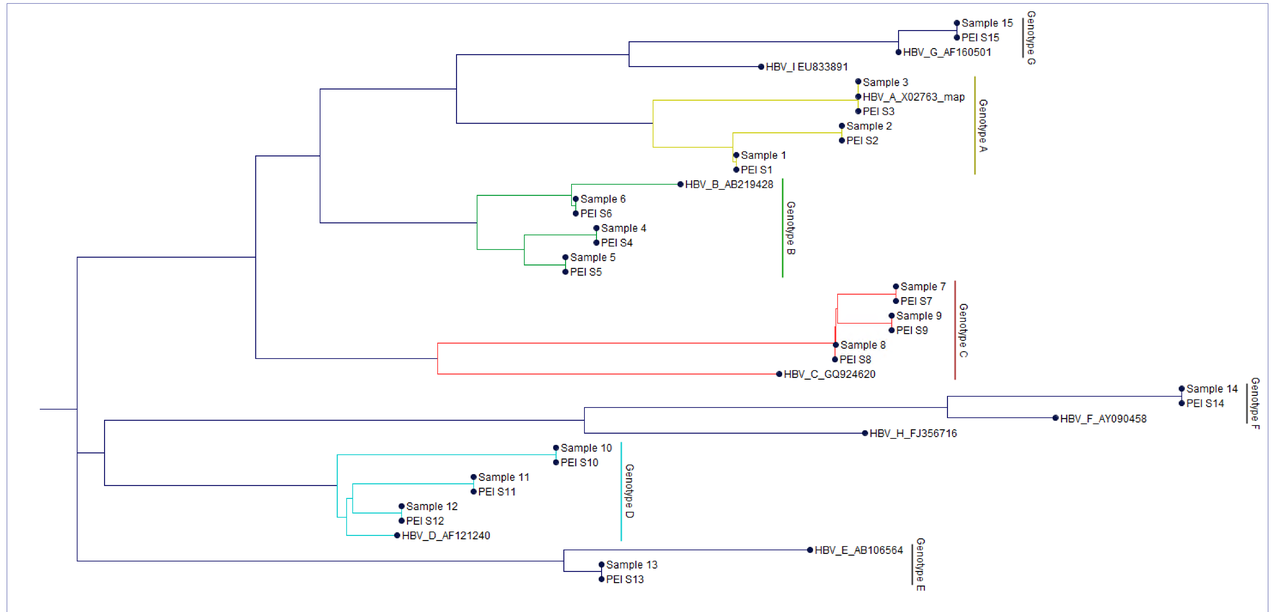
Figure 2: Region of amplification



Results

The primary sequencing assay resulted in a sensitivity of **79 copies/mL of HBV RNA** and **6 copies/mL of HBV DNA**. Sequencing was successful on the panel of 15 samples containing genotypes A-G, see Figure 3.

Figure 3: Phylogenetic tree of 25% consensus sequence from the S/RT assay and the sequences of the PEI panel.



IMMUNIS HBV Genotype EIA provided accurate results for genotypes A–D. Genotypes E and G were classified as D, and F was unclassified, due to kit limitations. The sensitivity was 3 IU/mL of Hepatitis B Surface Antigen, as stated in the kit insert.

Conclusions

The combined approach of sequencing and serological backup provides a robust solution for HBV genotyping in virologically suppressed individuals. By using HBV RNA for sequencing and IMMUNIS HBV Genotype EIA when amplification fails, this workflow ensures reliable genotype determination across diverse clinical scenarios. This strategy supports accurate monitoring of HBV genotypes in trials evaluating new HBV-related therapeutic options aimed at achieving functional cure.

References

Liu, Yang, Lindsey May, Xinan Liu, Ross Martin, Evguenia Svarovskaia, Anuj Gaggar, Hongmei Mo, and Becket Feierbach. "Developing a sensitive HBV genotyping assay for HBV DNA suppressed patients using both DNA and RNA sequencing." Journal of Medical Virology 92, no. 12 (2020): 3420- 3425.