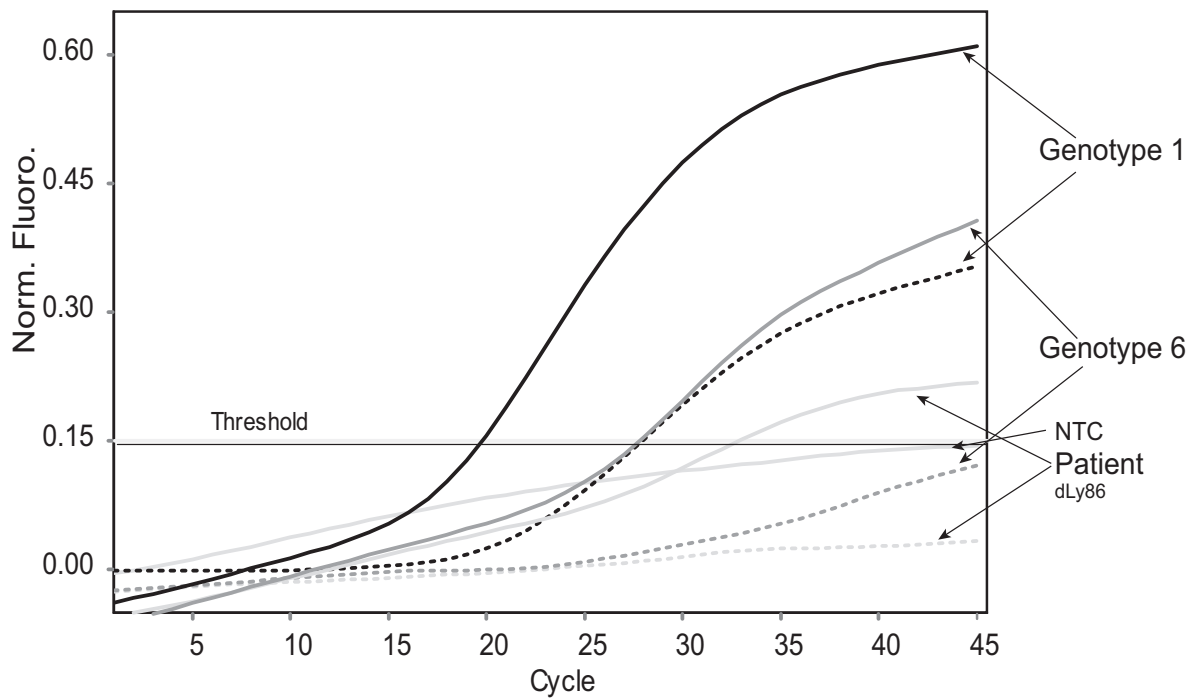


## Supplementary data

### **RTqPCR procedure:**

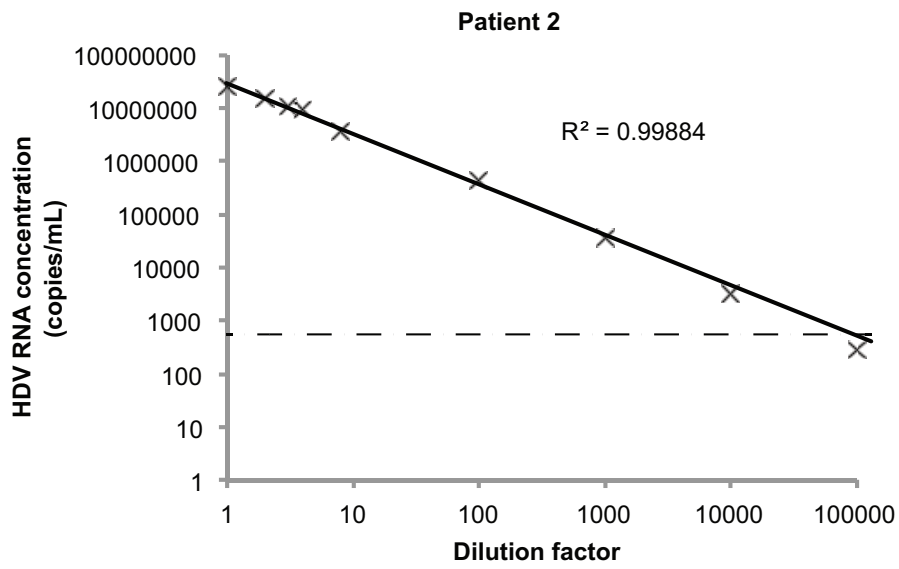
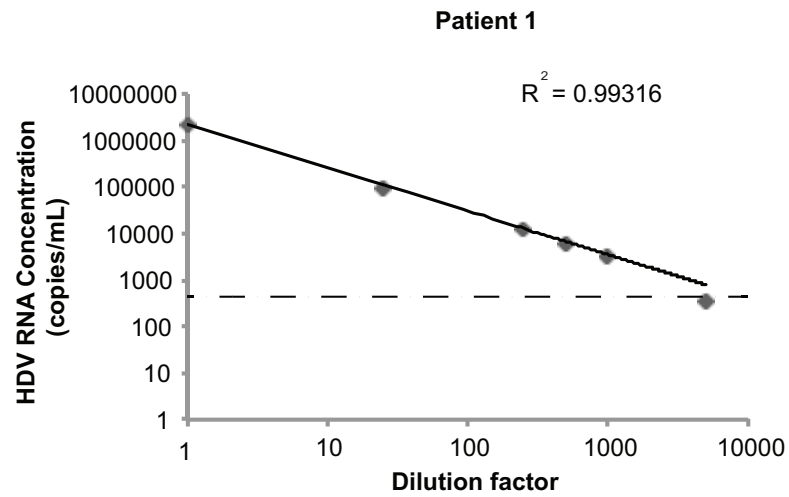
For each quantification the following material was used: 4µl of QuantiTect Virus Master Mix 5x, 0.2µl of QuantiTect Virus RT Mix 100x, 1µl of the 20x primer-probe mix and 0.4µL of the IC primer-probe mix. The final concentration of primers and probe were the following: 0.4µM AgD-F1, 0.4µM AgD-F2, 0.4µM AgD-R, 0.2µM probe. 8µl of transcript RNA or extracted product was added to the mix in a final volume of 20µl. Basematrix<sup>®</sup> diluent (SeraCare Life Sciences, USA) followed the whole process of extraction and amplification and was used as negative control. RT and amplification of both target and IC were simultaneously performed in a single tube. First, the RNA was transcribed by RT to cDNA at 50°C for 20 minutes, followed by denaturation at 95°C for 5 minutes. Afterwards, 45 cycles were performed each with denaturation at 95°C for 15 seconds and extension at 60°C for 45 seconds. Fluorescence signals were detected following each cycle at a temperature of 60°C for 2 seconds.



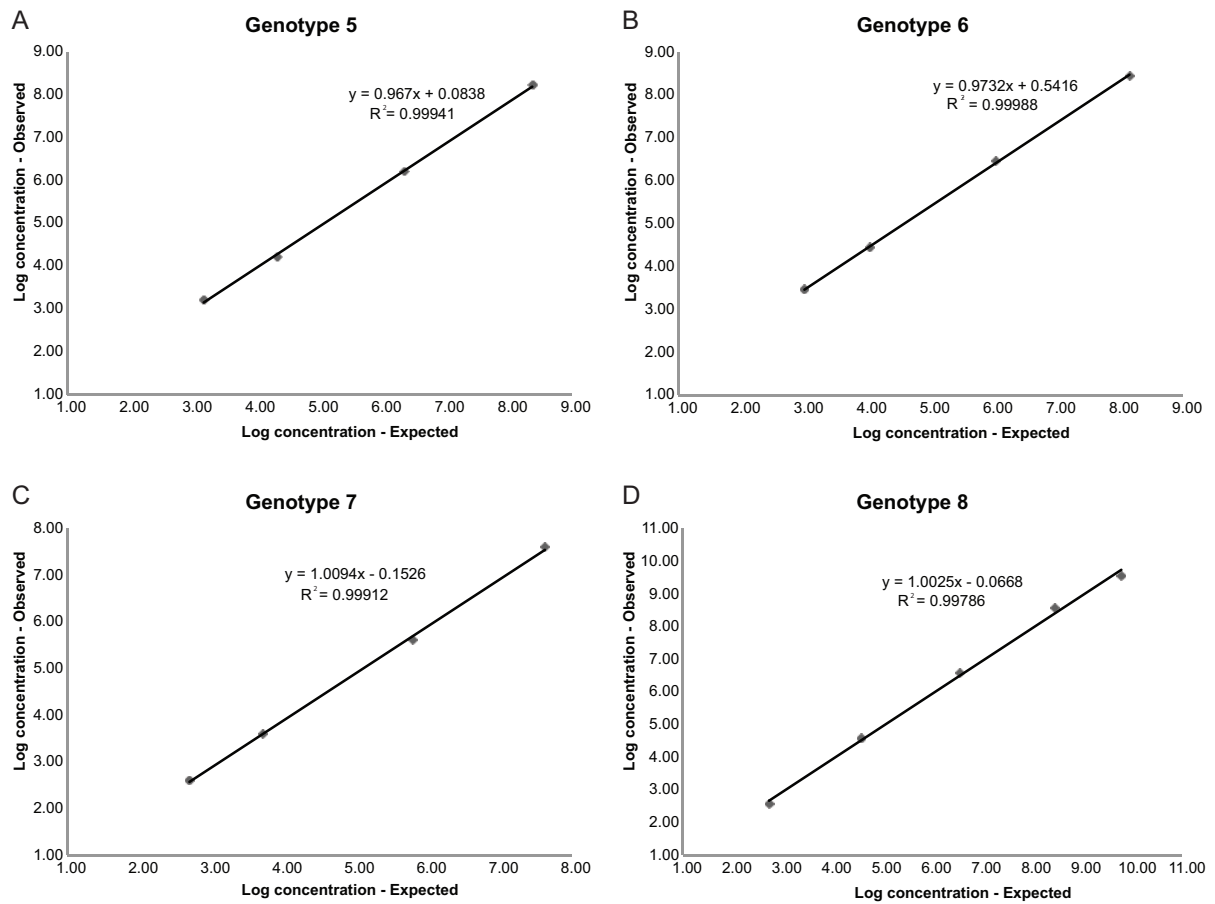
**FigS1: Comparison of the TaqMan<sup>®</sup>-MGB probe versus the LNA<sup>®</sup>-BHQ1 probe for HDV RNA quantification.** Fifty patients' samples and transcripts of genotypes 1, 5, 6, 7 and 8 were amplified and screened using either TaqMan<sup>®</sup>-MGB probe (dashed lines) or LNA<sup>®</sup> probe (solid lines). Only three representative samples are shown on the graph. Y axis = normalized fluorescence signal; X axis = cycle number. Black lines: genotype 1 transcript; dark gray lines: genotype 6 transcript; light gray lines: patient dLy86. NTC: no template control.

Standard curves obtained with the LNA<sup>®</sup> probe became positive  $7.2 \pm 0.7$  Ct earlier compared to that obtained with the TaqMan<sup>®</sup> probe. The sensitivity of the assay was thus significantly increased by 2 logs when using the LNA<sup>®</sup> probe. For the majority of the patients and for HDV-1, HDV-5 and HDV-8 transcripts, quantitative results obtained with the TaqMan probe were almost equivalent: mean difference was  $0.13 \pm 0.07 \log_{10}$  (copies/mL). On the other hand, quantitative results differed significantly for HDV-6 and HDV-7 transcripts: compared to the LNA<sup>®</sup> probe, TaqMan<sup>®</sup>-MGB probe assay under- and mis-quantified HDV-6 and HDV-7

samples (between 2 to 4 logs difference). Furthermore, 3 samples from patients of African origin were detected using the LNA<sup>®</sup> probe but not using the TaqMan<sup>®</sup>-MGB probe (although their concentrations were over the LOD of this probe). Sequencing of these HDV genomes in the *R0* region (nt 890-1288) highlighted mutations in the probe-binding region (Table 1). Although these mismatches concern both probes, they impaired the binding of the TaqMan<sup>®</sup> probe, whereas the LNA<sup>®</sup> probe could still hybridize on the target sequence. Finally, the LNA<sup>®</sup> probe had a higher fluorescence ratio facilitating the interpretation.



**FigS2: Linearity of the assay controlled using two patients' sera serially diluted before extraction. Dashed line represents the lower limit of quantification.**



**FigS3: HDV genotypes 5, 6, 7 and 8 amplification:** correlation between the copy number determined using the qPCR (Observed) and the theoretical calculated copy number (Expected) of Genotype 5 (panel A), 6 (panel B), 7 (panel C) and 8 (panel D) transcripts. Concentration is expressed as  $\log_{10}$  (copies/mL). Correlation coefficients ( $R^2$ ) and equations of the regression lines are displayed in the respective graphs.