# Verification and validation data of the AltoStar® HDV RT-PCR Kit 1.5* for monitoring HDV RNA viral load in plasma and serum samples.

AJ. Castillo, K. Rottengatter, P. Rosenstock, A. Jurado; altona Diagnostics GmbH

## Background

Next to precise and reliable HDV RNA detection and quantification automation covering extraction of the nucleic acid to amplification of the specific HDV target are pivotal for diagnosis and monitoring of response to newly approved treatment in HDV diagnostics.

Under the *In Vitro* Diagnostic Regulation (EU) 2017/746 (IVDR), devices intended for the quantification of Hepatitis D virus (HDV) RNA are classified in the highest risk category and a comprehensive verification and validation is required.

## Aim

Demonstration of an excerpt of verification and validation data of the AltoStar® HDV RT-PCR Kit 1.5* (CE)

## Material and Method

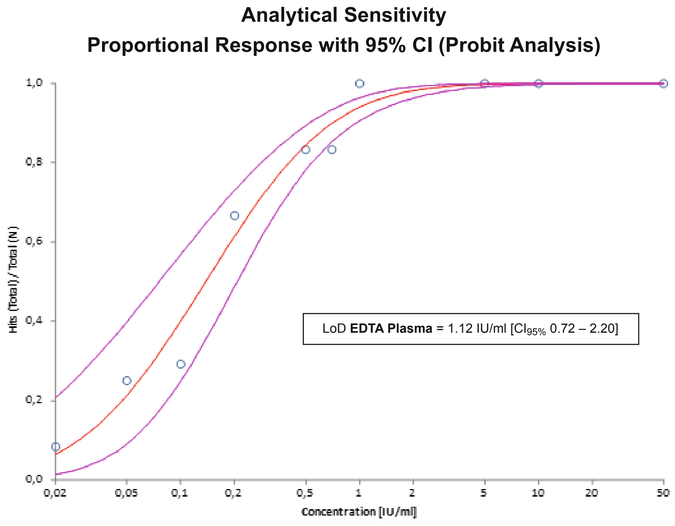
Next to clinical samples the WHO IS[1] for HDV was used.

All samples were processed using the AltoStar® Automation System AM16 (Hamilton) and analysed with the AltoStar® HDV RT-PCR Kit 1.5.

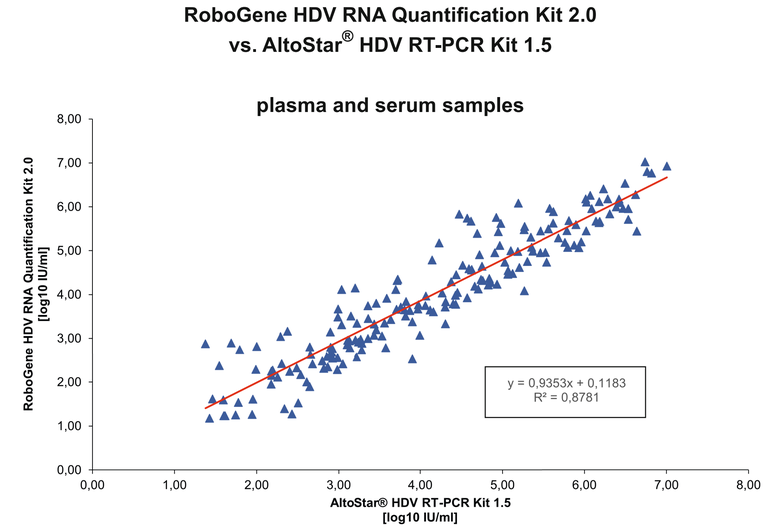
Next to data shown in graphs/table, trueness to WHO Standard (≥ ± 0.10 $log_{10}$ IU/ml) and inter-laboratory precision were analysed (all CV% ≤5%). Proficiency panel data confirmed correct qualitative and quantita-tive testing. Data for repeatability (CV% ≤5%) and within laboratory precision (CV% ≤5%) not shown.

## Conclusion

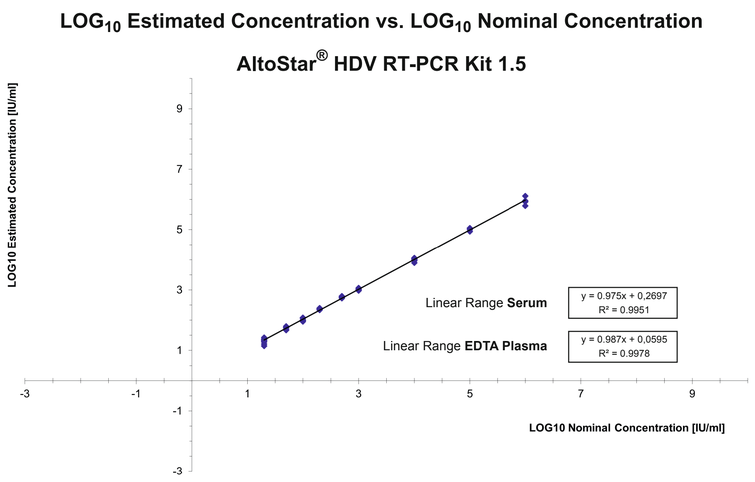
Accurate measurement of molecular targets, such as HDV RNA is essential for correct disease diagnosis, monitoring, and treatment decision-making. Standardization by automation minimizes variability across different laboratories, enhancing the comparability of results and ensuring consistency in patient care.



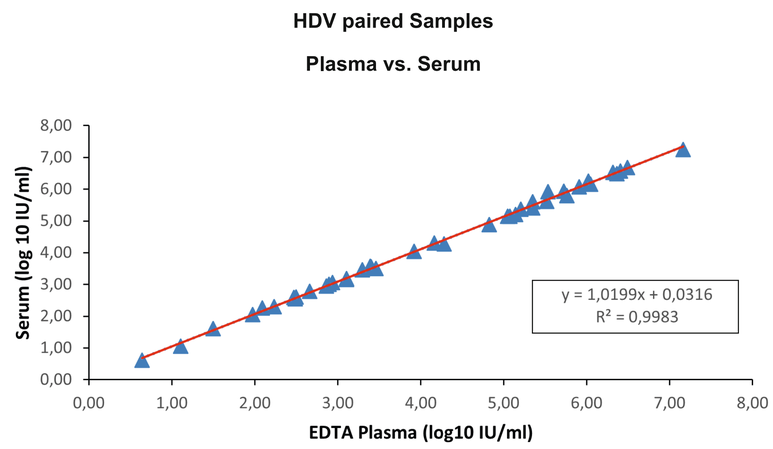

The LoD for **serum** and the **HDV genotypes 2–8** was confirmed for at least 1.12 IU/ml. Tested according to CLSI guideline EP17-A2 *(Evaluation of Linearity of Quantitative Measurement Procedures).*



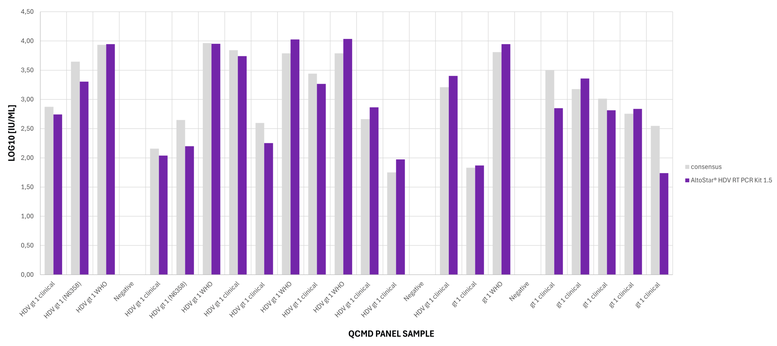

The diagnostic sensitivity and specificity of the AltoStar® HDV RT-PCR Kit 1.5 compared to the RoboGene HDV RNA Quantification Kit 2.0 were **100% (CI: 98.13%–100.0%)** and **100% (CI: 97.2%–100.0%)**, respectively.




The **Linear Range** was defined with 2.00E+01–1.00E+06 IU/ml *(extended to 1.00E+08 in 2025).* Tested according to CLSI guideline EP06 *(Evaluation of Linearity of Quantitative Measurement Procedures).*




**Paired sample** testing: Correlation of 40 HDV plasma and serum samples.




**QCMD proficiency panel results** over time. Mean difference of the AltoStar® HDV RT-PCR Kit 1.5 to consensus = 0.08 $log_{10}$ IU/ml (Consensus result = mean quantification result of all participants using commercial assays).

**Site-to-site reproducibility for the AltoStar® HDV RT-PCR Kit 1.5 in EDTA plasma**

All samples tested at 3 x LoD (low positive samples) were detected positive in all laboratories.

| | | Site-to-site reproducibility | |
|---|---|---|---|
| Sample | Mean quantification (log10 IU/ml) | SD (log10 IU/ml) | CV % |
| High positive | 4.32 | 0.03 | 0.77 |
| Medium positive | 2.18 | 0.05 | 2.11 |
| | Mean ($C_q$ values) | SD ($C_q$ values) | CV % |
| Negative | 28.65 | 0.36 | 1.25 |

**Notice:** *The AltoStar® HDV RT-PCR Kit 1.5 is a CE-marked product according to Regulation (EU) 2017/746 on *in vitro* diagnostic medical devices. [1]

1st WHO International Standard for Hepatitis D Virus RNA (PEI code 7657/12)

powered by integrity