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P6
Poster for you

The WHO HDV RNA International Standard does not reflect variability of real-world samples

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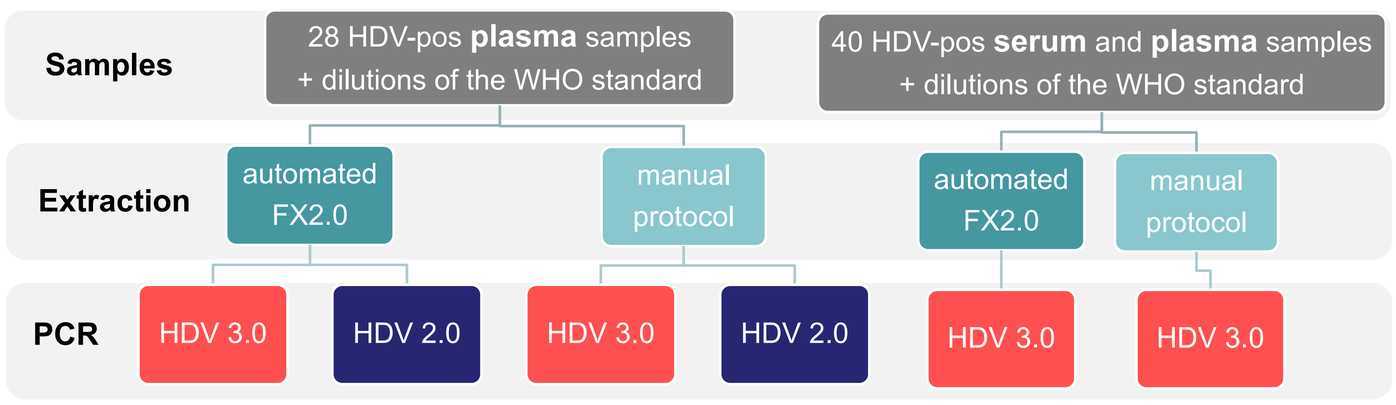
Background & Aim:

- Reliable detection and quantification of HDV RNA is the basis for initiating and guiding antiviral therapies in chronic HDV infection.
- The WHO HDV standard should allow standardization of quantitative HDV RNA values across different assays.
- However, comparison of different HDV RNA assays that all have been calibrated against the WHO standard revealed differences in quantitative HDV RNA values of real-world samples in several studies.

Methods:



Extraction methods:

- INSTANT Virus RNA/DNA Kit – FX 2.0 (automated extraction)
- INSTANT Virus RNA/DNA Kit (manual extraction)

Quantification methods:

- RoboGene HDV RNA Quantification Kit 3.0 for use with qTOWER³/ IRIS, CFX96 Real-Time PCR Detection Systems, QuantStudio™ real-time PCR systems, Rotor-Gene™ 3000/ 6000/ Q or LightCycler® 480II
- RoboGene HDV RNA Quantification Kit 2.0 for use with LightCycler® 480 II, 7500 FAST real-time PCR systems or Rotor-Gene™ 3000/6000/Q

Dilutions of the HDV WHO international standard were included

Results:

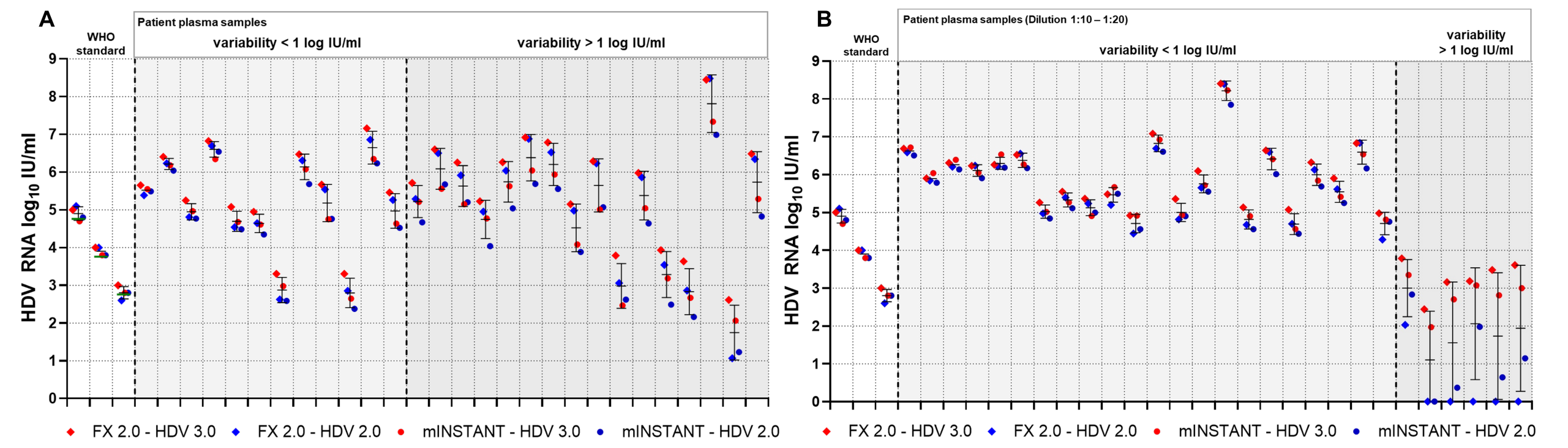


Figure 1: Comparison of HDV RNA levels quantified by different combinations of extraction and quantification assays of undiluted (A) and diluted (B) plasma samples. RoboGene HDV RNA Quantification Kit 2.0 (HDV 2.0) or the newly developed RoboGene HDV RNA Quantification Kit 3.0 (CE-IVD pending) (HDV 3.0) were combined with either automated (INSTANT Virus RNA/DNA Kit – FX 2.0) (FX 2.0) or manual (INSTANT Virus RNA/DNA Kit Protocol 2 or 3, as recommended) (mINSTANT) extraction. Clinical plasma samples with inter-assay variability < 1 log IU/ml are shown on light grey background, those with inter-assay variability > 1 log IU/ml are depicted on dark grey background. Error bars represent mean and standard deviation. The short green line represents the concentration of the WHO standard (4.76 log IU/ml, 3.76 log IU/ml, 2.76 log IU/ml).

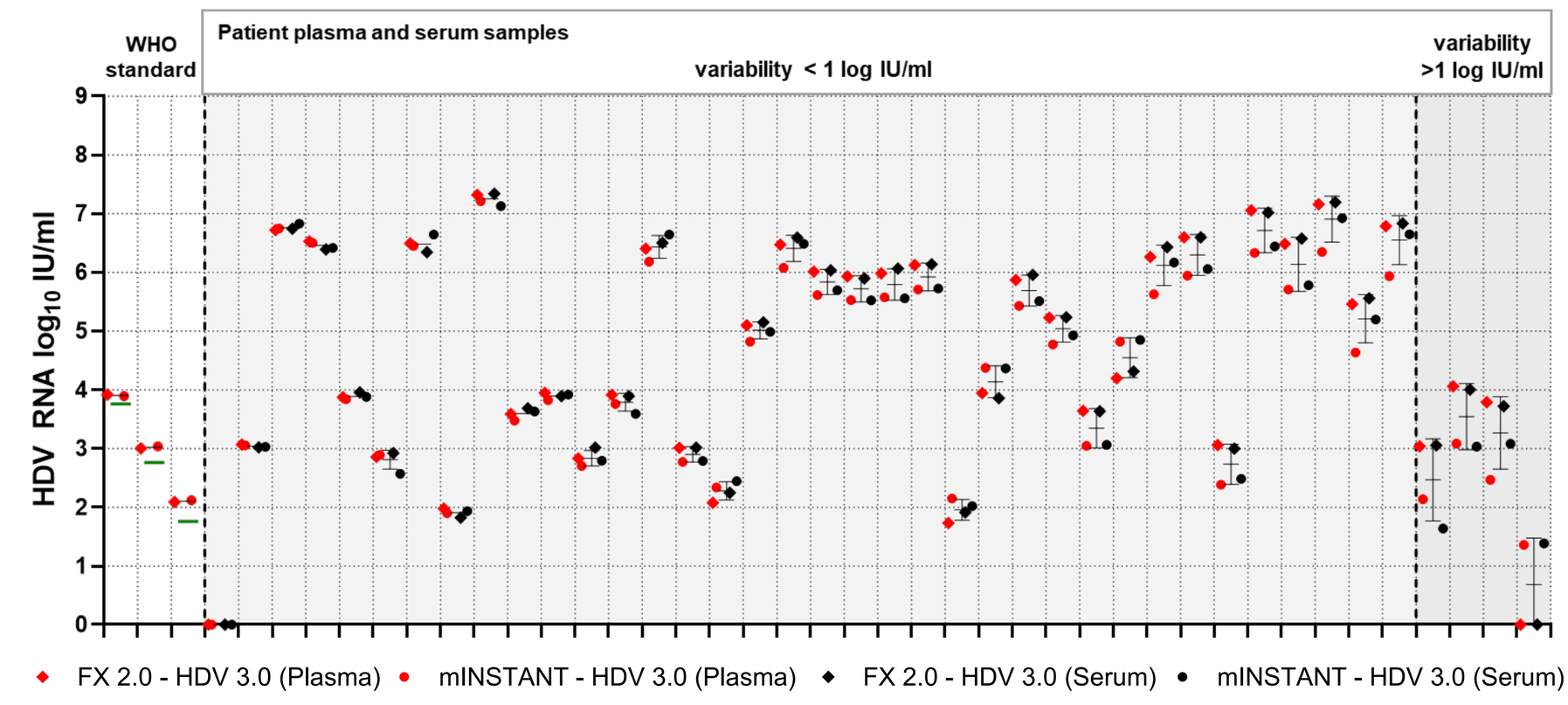


Figure 2: Comparison of HDV RNA levels from plasma and serum samples by using automated and manual extraction. RoboGene HDV RNA Quantification Kit 3.0 (CE-IVD pending) (HDV 3.0) was combined with either automated (INSTANT Virus RNA/DNA Kit – FX 2.0) (FX 2.0) or manual (INSTANT Virus RNA/DNA Kit) (mINSTANT) extraction. Clinical samples with inter-assay variability <1 log IU/ml are shown on light grey, those with inter-assay variability >1 log IU/ml are depicted on dark grey background. Error bars represent mean and standard deviation. The short green line represents the concentration of the WHO standard (3.76 log IU/ml, 2.76 log IU/ml, 1.76 log IU/ml).

Conclusion:

- HDV RNA quantification is comparable when using similar extraction methods, irrespective of sample material.
- In the tested clinical samples, manual extraction is not comparable to automated INSTANT FX 2.0 extraction regardless of the quantification kit.
- Only minor discrepancies were detected for the quantification of the WHO HDV RNA standard.
- Calibrating HDV RNA assays based on the WHO standard alone may miss substantial differences between assays that become evident only when clinical samples are tested.
- The reason for heterogeneity of samples requires further investigation.

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