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Poster for you

Novel anti-HDV therapies require reliable quantification of plasma HDV RNA: A European multicenter study

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Introduction

- Quantification of plasma HDV RNA is the essential tool for patient management during anti-HDV therapy. Results of HDV RNA vary depending on different test systems.
- The aim of this study was to estimate and improve the comparability of quantitative results reported by different laboratories using the new RoboGene HDV RNA Quantification Kit 3.0 - RUO with different nucleic acid (NA) extractions and qPCR devices and to compare with the RoboGene HDV RNA Quantification Kit 2.0.
- Undetectable HDV RNA is considered a desirable endpoint, but it still remains unclear if clinical outcome is improved by undetectable HDV RNA, or if suppressing to a low but still detectable level, provides a similar benefit.

Methods

The new RoboGene HDV RNA Quantification Kit 3.0 was used by seven laboratories for detection and quantification of HDV RNA in combination with different NA extraction platforms and qPCR devices. The study was accompanied by one additional site using the currently available RoboGene HDV RNA Quantification Kit 2.0 with a manual extraction. Characteristics of extraction platforms, extraction kits, input and elution volume, protocols, amplification platforms, PCR input volume, and kit version used by the different centers are shown in Table 1.

I. All combinations have been re-calibrated with a 0.5 log10 dilution series of the 1st WHO International Standard for HDV RNA (WHO IS HDV RNA; PEI code 7657/12), to determine correction factors (CF) which redefines the values of the quantification standards included in the kits. For all subsequent analyses, the test system-specific CF was applied.

II. The limit of detection (LOD) was determined by analyzing a dilution series of the WHO IS HDV RNA starting from 575 IU/mL followed by 8 additional 2-fold dilutions and 1 negative control (NC; HDV negative plasma). The whole procedure for determination of the LOD was performed three times, resulting in 24 results for each dilution and NC.

III. For accuracy testing, the Quality Control for Molecular Diagnostics (QCMD) 2023 Hepatitis D Virus EQA Program was used (https://www.qcmd.org/). The program consisted of 8 EQC panel members. Five of them, labeled with DS1and DS2, represented dilutions of two different clinical plasma samples, while another two contained a dilution of the WHO IS HDV RNA in plasma (code D1) and one EQC sample was negative for HDV RNA.

IV. Anonymized left-over samples obtained from female and male patients with HDV infection originally collected for clinical testing were pooled. Four HDV RNA positive clinical plasma sample pools, were analyzed (20 positive samples in total). Furthermore, two clinical plasma samples that had been tested negative for HDV antibodies were utilized. One of these two samples tested positive for hepatitis B surface antigen. Additionally, a 1:10 dilution of the WHO IS HDV RNA containing a nominal concentration of 57,500 IU/mL and a 1:10,000-dilution containing a nominal concentration of 57.5 IU/mL were tested.

Results

I. The CFs ranged from 14 to 10,000 depending on the test system used (**Table 1**).

II. The estimated LODs ranged from <2.2 (center H) to >575 IU/mL (center G, **Table 1**). The LOD of Center A using RoboGene HDV RNA Quantification Kit 2.0 was found to be 2.22 IU/ml.

III. The number of datasets reported from all participants for the accuracy testing and the sample relation are shown in Figure 1. Panel members DS1_2 and DS2_3 were not detected by center G. Both panel members containing the WHO IS HDV RNA could be quantified with all test systems. The panel member negative for HDV RNA was reported as "target not detected" by all centers. The maximum standard deviation calculated from all participants using RoboGene HDV RNA Quantification Kit 3.0 was found to be 0.37 log10 IU/mL. Box-blots of quantitative results of participants using RoboGene HDV RNA Quantification Kit 3.0 as well as the result for RoboGene HDV RNA Quantification Kit 2.0 (red cross) are shown in **Fig. 1**.

IV. Box-blots of clinical samples results of participants using RoboGene HDV RNA Quantification Kit 3.0 (and two dilutions of the WHO IS HDV RNA utilized as positive controls) as well as the result for RoboGene HDV RNA Quantification Kit 2.0 (red cross) and numbers of results are shown in **Fig. 2**. For clinical samples, the mean standard deviation of results returned from participants using RoboGene HDV RNA Quantification Kit 3.0 was found to be 0.31 log10 IU/ml, the maximum standard deviation was found to be 0.52 log10 IU/mL at a concentration of 280 IU/ml.

The dilution of the WHO International Standard showed a standard deviation for results of participants using RoboGene HDV RNA Quantification Kit 3.0 of 0.23 log10 IU/mL at 57,500 IU/ml and 0.40 log10 IU/ml at 57.5 IU/ml. Additionally, two HDV RNA negative sample pools were tested. For both negative sample pools, two centers reported one positive result out of a duplicate measurement, the rest was found to be negative.

Discussion

In this study we demonstrated how to calibrate different combinations of nucleic acid extraction methods with the PCR detection System. A CF and the LOD of each combination were determined. Quantitative results were comparable after calibration. The center with highest sensitivity could not detect sensitive samples in accuracy or clinical sample testing. Since the LODs shown in this study varied broadly, this aspect should be taken into account during patient management, especially with regard to possible endpoints or maintenance strategies for anti-HDV therapies. Even if we found lower values for the RoboGene HDV RNA Quantification Kit 2.0 during accuracy testing, we have verified performance during clinical testing. Here a better comparability to the new RoboGene HDV RNA Quantification Kit 3.0 could be shown.

Conclusion

To ensure reliability in quantification of HDV RNA, any modification of the extraction and amplification/detection protocol validated by the manufacturer requires revalidation. With the 1st WHO IS for HDV RNA, CF's and LOD's could easily be calculated in this study leading to harmonization of quantitative results and verification of sensitivity. Both are essential for accurate monitoring of viral response to existing anti-HDV treatment as well as for comparability of study results investigating novel anti-HDV drugs during treatment and follow up after end of treatment.

Table 1: Overview of the centers and the combination used, estimated Correction Factors CF and LODs

Center	Extraction platform	Extraction kit	Input volume	Elution volume	Protocol	Amplification platform	PCR input volume	RoboGene HDV RNA Quantification Kit version	CF	LOD (IU/mL, 95% confidence interval)
A	Manual	INSTANT Virus RNA/DNA Kit	400	60	Manual NA extraction	Rotor-Gene Q	5	2.0	17.5	2.22 (n.a.)
B	CyBio FeliX	INSTANT Virus RNA/DNA Kit FX2.0	400	60	No special protocol	LC480II	10	3.0	50.0	5.58 (3.86 – 23.07)
C	EZ1 Advanced XL	EZ1 DSP Virus Kit	400	60	No special protocol	CFX96	10	3.0	115.0	6.07 (4.37 – 15.22)
D	Manual	INSTANT Virus RNA/DNA Kit	400	60	Manual NA extraction	ABI7500Fast	10	3.0	16.0	2.32 (n.a.)
E	QiaSymphonie	DSP Virus/Pathogen Midi Kit	500	130	Cellfree500_V5_DSP	ABI7500Fast	10	3.0	350.0	75.55 (43.79 – 187.13)
F	QiaCube	QIAamp DNA Blood Mini	200	100	No special protocol	LC480II	10	3.0	1,000.0	120.63 (75.94 – 241.03)
G	EasyMag	NucliSens	200	50	Generic	LC480II	10	3.0	10,000.0	>575 (n.a.)
H	Manual	INSTANT Virus RNA/DNA Kit	400	60	Manual NA extraction	LC480II	10	3.0	14.0	< 2.20 (n.a.)

Figure 1 **Boxplot Accuracy testing, Red mark represents RoboGene HDV RNA Quantification Kit 2.0**

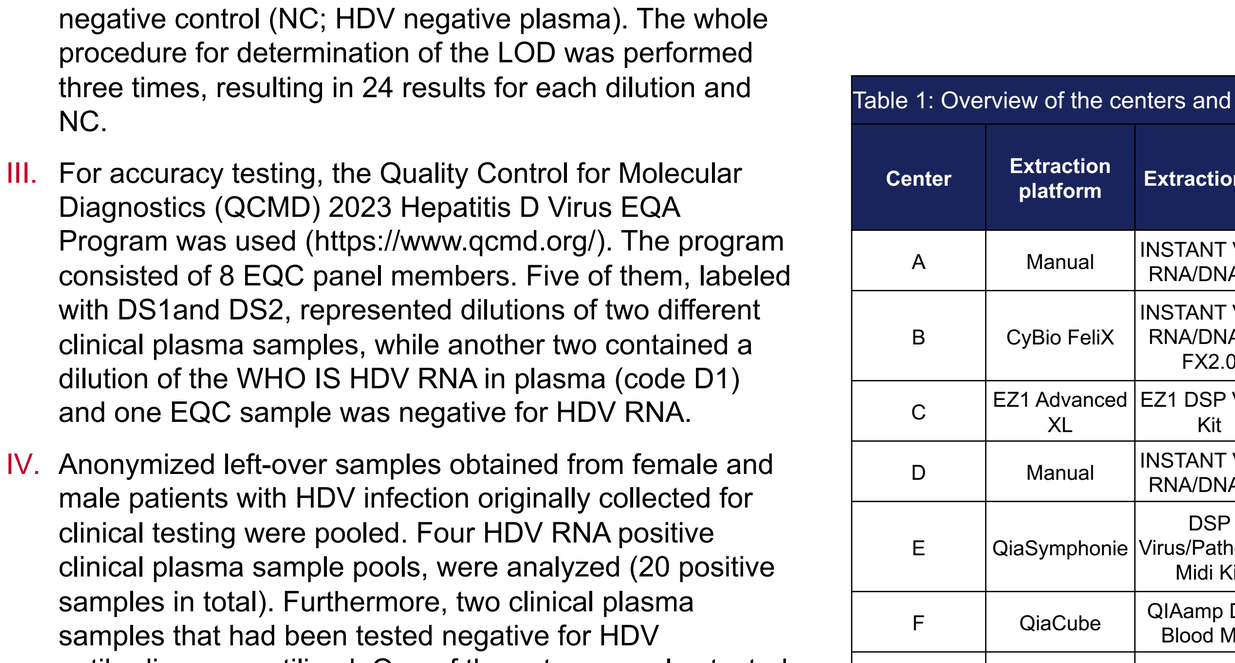

Figure 2 **Boxplot Clinical samples, Red mark represents RoboGene HDV RNA Quantification Kit 2.0**