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HDV RNA Assays: Performance characteristics, clinical utility and challenges

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Working Group Overview

- Timeline
  - June 2022: launch of HDV working group at HBV Forum 8 meeting in London
  - August 2022 – January 2023: working group conference calls
  - January – June 2023: manuscript research, writing, revision
- Manuscript accepted for publication to *Hepatology*
  - June 2nd, 2023: manuscript submitted
  - June 14th, 2023: manuscript accepted

Full manuscript is confidentially shared with you via email.

In-person attendees have printed copies of Tables 1 – 4.

Key Points by Manuscript Chapter

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Manuscript Chapter	Key Point
1. Molecular epidemiology of HDV	Extensive HDV molecular diversity presents a challenge for commercial and research-based assays to properly detect or quantify HDV RNA across all genotypes/subgenotypes.
2. Role of HDV RNA in patients with CHD	Patient monitoring of HDV RNA levels in response to treatment is critical for clinical development, but the field lacks reliable, standardized, accurate assays. Table 1: Interpretation of HDV RNA levels according to lab report
3. HDV RNA assays: challenges from a virological perspective	Different RNA extraction methodologies, primer/probe design for NATs, lack of automation, and overall dearth of standardization contributes to variability in research-based and commercial assay performance characteristics.
4. Technical performance summary of NATs for HDV RNA quantitation	Table 2: Comprehensive list of published methods (LDTs) and RNA quantitation kits. Table 3: Detailed performance characteristics for assays and products calibrated to WHO IS, detect GT1-8, and used IC- for RNA extraction.
5. Future studies, perspectives, and recommendations	Table 4: Challenges and recommendations for the development of quantitative HDV RNA assays

Table 1. Interpretation of HDV RNA levels according to lab report.

As example, we considered a putative assay with LoD=10 and LLoQ=100 IU/mL			
HDV RNA levels	**HDV RNA limits**	**HDV RNA interpretation**	**Comments**
Below LLoQ	< 100 IU/ml	Low positive viremia (below 100 IU/mL but not quantifiable, i.e., HDV RNA target detected [TD]), or negative for viremia (i.e., HDV RNA target not detected [TND])	The LLoQ depends on the assay's performance characteristics. A result of below LLoQ includes both low level viremia and negative viremia test results
Below LoD	Interpreted as < 10 IU/ml	Low positive viremia (not quantifiable) or negative for viremia	Below LoD is not recognized by regulatory agencies as the assay cannot determine the concentration for samples < 100 IU/ml, i.e., the concentration could be < 10 IU/ml or between 10 and 100 IU/ml
TND	Undetectable	Undetectable	No virus (HDV RNA) detectable in sample (i.e., "negative"). This result is frequently/sometimes referred to HDV RNA <LLoQ TND

Table 2. Comprehensive list of published methods (LDTs) and RNA quantitation kits

Assay name	Manufacturer/Provider	Type	Source of data	Reference(s)	Technique	Calibrated to WHO International Standard
EurobioPlex HDV kit EBX 004	Eurobio Scientific	commercial kit	Le Gal (2017)	(1)	qPCR	yes
RoboGene HDV RNA Quantification Kit 2.0	Roboscreen GmbH	commercial kit	Wang (2018); IFU/website (2020)	(2, 3)	qPCR	yes
AltoStar® HDV RT-PCR Kit 1.5	altona Diagnostics GmbH	commercial kit	Supplier		qPCR	yes
RealStar® HDV RT-PCR Kit 1.0 RUO	altona Diagnostics GmbH	commercial kit	Supplier		qPCR	yes
AmpliSens® HDV-FRT	Federal Budget Institute of Sience	commercial kit	IFU/website		qPCR	yes
Fluorion HDV QNP 2.1 Real-Time PCR Kit	Iontec	commercial kit	IFU/website		qPCR	yes
cobas HDV	Roche	commercial kit	Dua (2023)	TBD	qPCR	yes
SYSTAAQ HDV Real Time PCR Kit	SYSTAAQ	commercial kit	IFU/website		qPCR	yes
LIPSGENE HDV Kit	VL-Diagnostics GmbH	commercial kit	IFU/website		qPCR	yes
Bosphore® HDV Quantification-Detection Kit v1	Anatolia Geneworks	commercial kit	IFU/website		qPCR	no
genesig Real-time PCR detection kit for HDV	Primerdesign	commercial kit	Supplier		qPCR	no
HDV Real-TM Qual Real Time PCR Test	Sacace Biotechnologies	commercial kit	IFU/website		qPCR	no
LightMix® Kit HDV[1]	TibMolBiol/Roche	commercial kit	IFU/website		qPCR	no
Hepatitis D Virus (HDV) Real Time RT-PCR Kit	Creative Biogene	commercial kit	IFU/website		qPCR	unknown
HDV Quantitation Real-Time PCR kit	Dia.Pro Diagnostic Bioprobes s.r.l	commercial kit	IFU/website		qPCR	unknown
ViroReal Kit HDV[1]	Ingenetix	commercial kit	IFU/website		qPCR	unknown
HDV Real Time RT-PCR Kit	liferiver	commercial kit	IFU/website		qPCR	unknown
Hepatitis Delta virus One-Step RT-qPCR Kit[1]	nzytech	commercial kit	IFU/website		qPCR	unknown
PCRmax LtdTM qPCR test Hepatitis Delta	PCRmax	commercial kit	IFU/website		qPCR	unknown
Hepatitis Delta Virus by Quantitative PCR	ARUP	LDT	Website		qPCR	yes

Assay name	Manufacturer/Provider	Type	Source of data	Reference(s)	Technique	Calibrated to WHO International Standard
Hepatitis D Virus RNA, Quantitative Real-Time PCR	Quest Diagnostics	LDT	Website/coauthor		qPCR	yes
NA (LDT)		LDT	LeGal (2005)	(5, 6)	qPCR	no
NA (LDT)		LDT	Tseng (2008)	(7)	qPCR	no
NA (LDT)		LDT	Hofmann (2010)	(8)	qPCR	no
NA (LDT)		LDT	Mederacke (2010)	(9)	qPCR	no
NA (LDT)		LDT	Schaper (2010)	(10)	qPCR	no
NA (LDT)		LDT	Ferns (2012)	(11)	qPCR	no
NA (LDT)		LDT	Scholtes (2012)	(12)	qPCR	no
NA (LDT)		LDT	Shang (2012)	(13)	qPCR	no
NA (LDT)		LDT	Katsoulidou (2013)	(14)	qPCR	no
NA (LDT)		LDT	Kodani (2013)	(15)	qPCR	no
NA (LDT)		LDT	Botelho-Souza (2014)	(16)	qPCR	no
NA (LDT)		LDT	Karataylı (2014)	(17)	qPCR	no
NA (LDT)		LDT	Coller (2018)	(18)	qPCR	yes
NA (LDT)		LDT	Pflüger (2021)	(19)	qPCR	yes
NA (LDT)		LDT	Olivero (2022)	(20)	ddPCR/qPCR	yes
NA (LDT)		LDT	Xu (2022)	(21)	ddPCR	

ddPCR, droplet digital polymerase chain reaction; IFU, instructions for use; LDT, laboratory-developed test; qPCR, quantitative polymerase chain reaction; WHO, World Health Organization.

[1]no longer available

NA: not applicable

Table 3. Detailed performance characteristics for assays and products calibrated to WHO IS, detect GT1-8 and used IC- for RNA extraction.

Assay name	Manufacturer/ Provider	Target site	LoD (IU/mL)	LLoQ (IU/mL)	ULoQ (IU/mL)	Regulation	RNA extraction method	Detection equipment (cycler)	Comments
EurobioPlex HDV kit EBX 004	Eurobio Scientific	HDAg	10	562	3.16E+08	CE-IVD	m2000sp	CFX96	
RoboGene HDV RNA Quantification Kit 2.0	Roboscreen GmbH	HDAg	6	60	1.00E+08	CE-IVD	Instand Virus RNA/DNA Kit	several options	
HDV QNP 2.1 Real-Time PCR Kit	Iontec	Proprietary	400	1000	1.00E+10	CE-IVD	Fluorion i12, i24/i12 Kit	several options	*no information on GT8
AltoStar® HDV RT-PCR Kit 1.5	altona Diagnostics	Proprietary	<10*	100*	1.00E+06	RUO**	AltoStar AM16r	CFX96	* still under verification. **The kit is CE-IVDR ready
RealStar® HDV RT-PCR Kit 1.0	altona Diagnostics	Proprietary				RUO	several options	several options	LoD depend on extraction / detection method
SYSTAAQ HDV Real Time PCR Kit	SYSTAAQ	Proprietary	10	10	8.00E+06	RUO	several options	not specified	LoD depend on extraction/detection method
Hepatitis D Virus RNA, Quantitative Real-Time PCR	Quest Diagnostics	Proprietary	5	40	1.00E+07	LDT	MagNA Pure 96	ABI 7500	
Pflüger (LDT)		Ribozyme	3.9	10	1.00E+08	LDT	cobas 6800	cobas 6800	
Olivero (LDT)		Ribozyme	9.2	10	1.00E+06	LDT	EZ1 Advanced XL	CFX96 QX200	

CE-IVD, conformité européenne in vitro diagnostic; GT, genotype; HDAg, hepatitis D antigen; IC, internal control; LDT, laboratory-developed test; LLoQ, lower limit of quantitation; LoD, limit of detection; QS, quantitation standard; RUO, research use only; ULoQ, upper limit of quantitation, WHO IS, World Health Organization International Standard.

Table 4: HDV RNA Sequence Variability

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Challenges	Recommendations
Primary sequence and secondary structures vary considerably between and within genotypes. There are replicative and defective quasispecies in clinical samples.	Primer/probe design for RT-PCR assays should focus on highly conserved regions, and assay validation should assess geographically and temporally diverse clinical isolates (e.g., at least 10-20 isolates), rather than cDNA or IVT RNA which do not have the same degree of secondary structure.
Sequence data of HDV are limited (especially non-HDV-1), complicating the primer/probe design for RT-PCR assays.	HDV sequencing should be performed consistently in clinical trials and epidemiological studies to increase the available sequence data
Long-term studies may be confounded by natural sequence variation, potentially impacting primer/probe binding affinity.	Longitudinal studies should assess the primer/probe binding regions over time to monitor for sequence changes.

Table 4: Assay Platforms and Validation

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Challenges	Recommendations
Different assays with different performance characteristics are used across laboratories and trials.	Assays should use the WHO international standard for validation, and an internal RNA control of known concentration at the RNA extraction stage, with primers/probe distinct from those used for HDV RNA. Clinical trials and patient management should use a central laboratory with a validated assay (FDA approved/CE marked if available).
Assay performance data in non-HDV-1 are limited and difficult to generate given scarcity of non-HDV-1 samples.	Non-HDV-1 in vitro transcribed RNA can be used for assay characterization, with the caveat that it lacks the secondary structures associated with viral RNA and therefore may have limited accuracy with respect to assay sensitivity and linearity.
Manual RNA extraction has been reported to be more sensitive than automated procedures but is prone to higher variability and is more labor-intensive.	Automated assays, ideally on standard platforms, should be developed/used as much as possible

Table 4: Data Interpretation

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Challenges	Recommendations
Use of assays with different performance characteristics complicates data interpretation.	Data should be reported in IU/mL. When selecting an assay platform, the sensitivity and specificity should be reported, and preferably these should be comparable to the best performing assays available.
Clinical relevance of undetectable vs detectable HDV RNA is uncertain.	Clinical studies are needed to assess threshold of HDV RNA for long-term clinical outcomes (suppressive therapy) and/or viral relapse (finite therapy).
Different ways to report HDV RNA values below LLoQ are used.	Use consistent nomenclature to report HDV RNA values below the quantitative range (below LLoQ): i.e., data should be reported as either below LLoQ, target detected or below LLoQ, target not detected. Use of reporting below LoD* should be avoided because it incorrectly implies virus absence.
For novel treatments, there is no clear guidance/consensus on frequency of HDV RNA testing during treatment and during follow-up.	Guidelines will need to be developed considering resource-limited regions.

Thank you to the authors!

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Open Discussion

Please consider future directions for the HDV Co-Infection Working Group.