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Poster for you

Diagnostic performances of different HDV RNA quantification assays used in clinical practice in Italy: results from a national quality control multicenter study

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Introduction and Aim

- A reliable quantification of serum hepatitis D virus (HDV) RNA is of paramount importance for a proper monitoring of patients under antiviral therapy. This has emerged as a hot topic particularly after the introduction of the novel anti-HDV drug bulevirtide.
- Different HDV-RNA quantification assays are currently available but information on their diagnostic performances is still limited.

This quality control National study aimed at comparing the diagnostic performances of different quantitative HDV-RNA assays, used in clinical practice, across laboratories in Italy.

Methods

- 30 participating centers (Vironet C network) coordinated by University of Roma "Tor Vergata".

- 6 commercial assays and 3 in-house HDV-RNA quantitative assays were used in the different centers

HDV-RNA Quantitative Assays	Centers (N)
RoboGene HDV RNA Quant. Kit 2.0 – Biosense	9
EurobioPlex HDV qRT-PCR on Elitech InGenius	7
RealStar® HDV RT-PCR Kit 1.0 – Altona	4
on AltoStar® Automation System AM16	1
Bosphore HDV Real-Time PCR Kit – Anatolia Geneworks	3
HDV Quant. Real-Time PCR kit – DIA.PRO	2
HDV Real TM Quant - Nuclear Laser Medicine	1
Home Made ddPCR or Real-Time PCR	3

- Each center received 41 samples: 20 for panel A and 21 for panel B and performed 165 HDV-RNA quantifications

Panel A WHO HDV Standard Genotype 1		Panel B 21 clinical samples from 9 pts		
log IU/ml	Vials (N)	log IU/ml		
5.0	1	6.7	3.0	0.8
4.0	1	6.0	2.6	0.5
3.0	3	5.5	2.3	0.0
2.5	3	5.0	2.0	0.0
2.0	3	4.3	1.6	0.0
1.5	3	4.0	1.3	
1.0	3	3.7	1.0	
0.5	3	3.5	0.9	

Diagnostic parameters defined for each assay:

- **Assay sensitivity - "Limit of detection" (LOD):** *Panel A samples was used to estimate LOD95%, defined as the lowest HDV-RNA load detected in ≥95% of replicates.*
- **Assay accuracy:** *Defined as the differences between observed and expected values at each tested HDV RNA load.*
- **Assay precision - coefficients of variation:** *Panel B samples up to lower limit of quantification (LLOQ) of each assay were used to define the intra-run and inter-run coefficient of variation (CV).*
- **Assay linearity:** *Results from both panels were used to define assay linearity (as R^2) by linear regression analysis.*

Results

ASSAYS SENSITIVITY

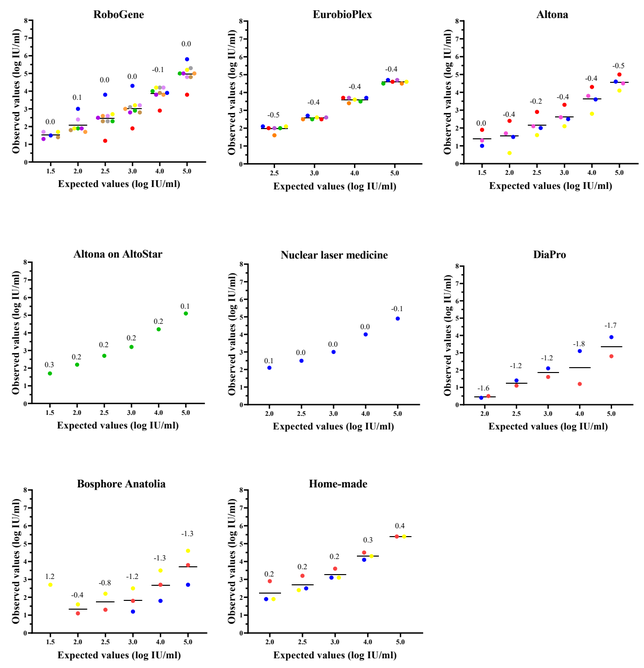
By analysing Panel A, 95% LOD varied across the assays highlighting different degrees of sensitivity across the assays. A heterogeneity in the sensitivity was also observed across the different laboratories using the same assay, raising the need of technical standardization

Assay	Limit of detection 95% (LOD 95%)	
	Median LOD 95% (IU/ml)[a]	LOD 95% Range MIN-MAX (IU/ml)
RoboGene	31	3-316
EurobioPlex on Elitech InGenius	100	100-316
Altona	10	3-316
on AltoStar AM16	-	3
Anatolia	316	31-316
DIA.PRO	-	316-1,000
Nuclear Laser Medicine	-	31
Home Made	-	31-316

[a] Median 95% Limit of Detection (LOD95%) is calculated by using the LOD95% observed for each center

ASSAYS ACCURACY

Overall, six assays show <0.5 logIU/ml mean difference between observed and expected HDV-RNA values for all tested dilutions of Panel A



The graphs report for each center (as dot) the observed values of HDV-RNA at each expected concentration. The black line represents, for each expected concentration, the mean of observed values across centers. The numbers indicate the differences between the observed and expected HDV-RNA. The X axis reports expected values in the range between the first tested dilution below LLOQ of each assay and 5.0 logIU/ml. For Elitech assay, the HDV-RNA loads <100 IU/ml have not been included because detected values are < LLOQ. For Anatolia assay, the HDV-RNA loads <131 IU/ml have not been included because detected values are < LLOQ

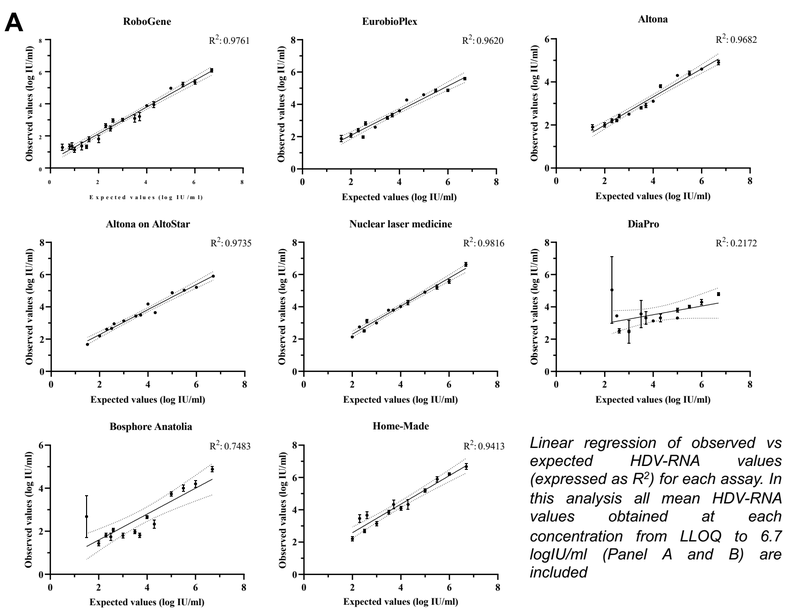
ASSAYS PRECISION

Mean intra-run CV was ~10% for EurobioPlex and Altona and ~15% for Robogene and Nuclear Laser Medicine. Higher mean intra-run CVs was observed for other assays, with intra-run CV up to 60% for specific HDV-RNA loads. As expected, inter-run CVs were higher than intra-run CVs for all assays. Only Altona, Nuclear Laser Medicine and EurobioPlex maintained a mean inter-run CV <25%.

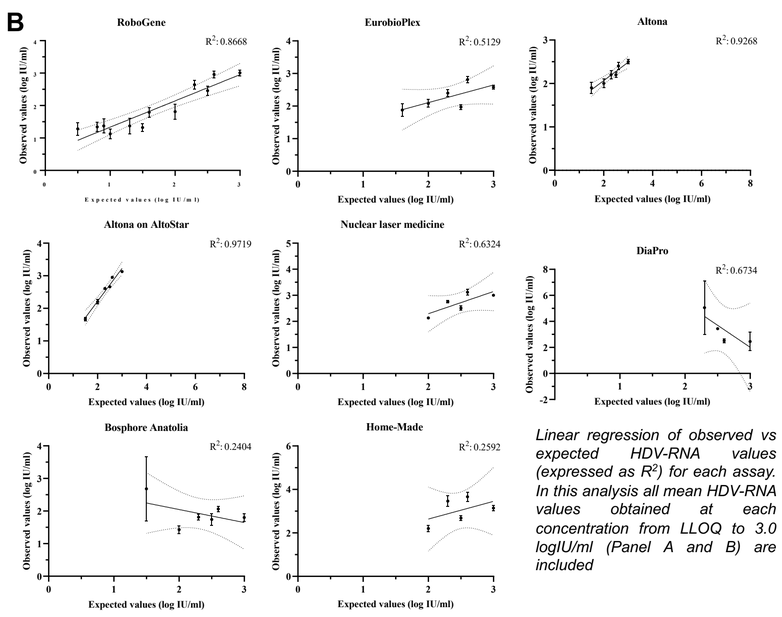
Exected logIU/ml	RoboGene		EurobioPlex		Altona		Altona on AltoStar		Bosphore Anatolia		DiaPro		Home-made		Nuclear laser medicine	
	Intra-run	Inter-run	Intra-run	Inter-run	Intra-run	Inter-run	Intra-run	Inter-run	Intra-run	Inter-run	Intra-run	Inter-run	Intra-run	Inter-run	Intra-run	Inter-run
1.6	25.8	25.5	8.3	43.8	-	-	-	34.4	7.3	33.5	-	-	-	-	-	-
2	32.1	34.7	15.7	26.5	10.6	25.6	-	24.3	36.3	53.8	-	-	33.6	78.2	2.8	31.6
2.3	19.9	29.9	11.7	23.8	34.2	39.7	-	1.3	12.9	32.8	-	-	30	40.6	21.1	11.9
2.6	17.8	19.9	6.5	23.5	9.4	19.2	-	-	42.4	31.6	-	-	32.9	58.8	15.4	22.6
3	17.6	46.4	8.6	15.9	5.7	8.6	-	4.2	27.6	45.8	-	-	36.7	67.5	20.8	22.1
3.5	15	42.2	5.3	23.6	17.5	7.3	-	10.6	19.8	36.8	-	-	11.2	42.4	18.9	0.8
3.7	13	47.4	11.6	23.8	12.1	19.6	-	8.1	24.8	41.6	-	-	27.7	49.1	10.1	8.5
4.3	12.3	36.8	6.3	15.5	9.2	10.4	-	2.5	21.9	45.7	53	54.1	25.4	69	27	31.9
5	11.3	28.6	7.5	22.5	13	9.3	-	0.9	7.5	29.4	18.3	26.4	6.5	35.4	-	30.2
5.5	9.9	31.6	8.9	22.6	6.3	15.7	-	0.3	9.8	28.3	24.7	19.1	8.4	27.7	-	30.3
6	12.1	29.6	6.5	21	6.9	13.8	-	-	6.7	34.9	23.3	37	13.9	29.8	-	31.3
6.7	12.9	23.8	6.4	19.9	4.9	21	-	4.8	4.7	29.3	2.7	27	12.2	62.8	-	16.7
Mean	**16.3**	**33.0**	**8.6**	**23.5**	**11.8**	**17.3**	**-**	**9.1**	**18.5**	**36.9**	**24.4**	**32.7**	**21.7**	**51**	**16.6**	**21.6**

ASSAYS LINEARITY

Six assays are characterized by a good linearity (R2 > 0.90) in the range between their LLOQ and 6.7 log IU/ml (A), while focusing on HDV-RNA <1000 IU/ml, a decreased linearity was observed across the different assays (B).



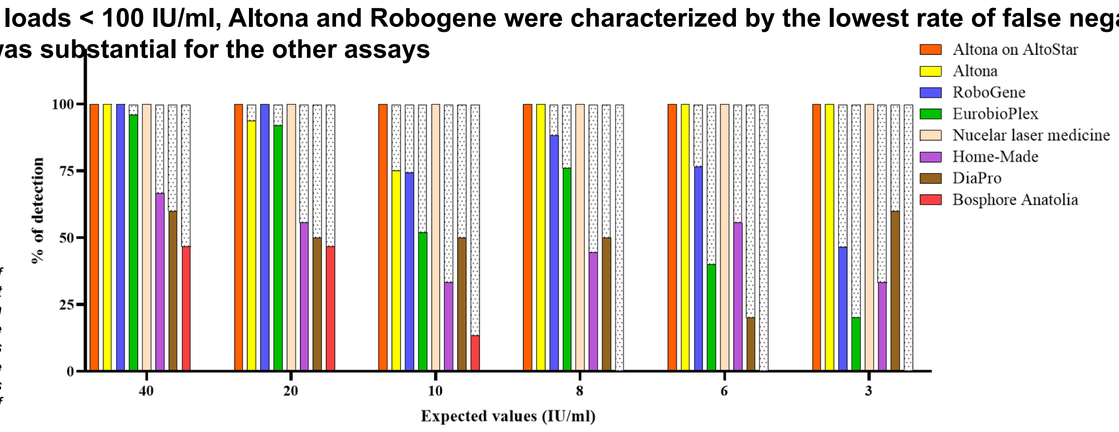
Linear regression of observed vs expected HDV-RNA values (expressed as R^2) for each assay. In this analysis all mean HDV-RNA values obtained at each concentration from LLOQ to 6.7 logIU/ml (Panel A and B) are included



Linear regression of observed vs expected HDV-RNA values (expressed as R^2) for each assay. In this analysis all mean HDV-RNA values obtained at each concentration from LLOQ to 3.0 logIU/ml (Panel A and B) are included

FALSE POSITIVE RATE

Focusing on HDV-RNA loads < 100 IU/ml, Altona and Robogene were characterized by the lowest rate of false negative results, while false negativity was substantial for the other assays



The histogram reports the percentage of HDV-RNA detection of each assay at each expected value. Each column represent a single assay. The percentage of detection of each assay is calculated by dividing the sum of the replicates in which HDV-RNA is detected for the total number of replicates of each expected value.

Conclusion

- This National validation study underlines a substantial heterogeneity in term of sensitivities across the different assays for HDV-RNA quantification.
- The heterogeneity in the sensitivity is also observed across the different laboratories using the same assay, rising the need to standardize technical procedures.
- For HDV-RNA <1,000IU/ml, a drop in linearity was observed for most assays, highlighting the need to optimize the quantification of low levels of HDV-RNA.
- Moreover, the rate of false negative results increases with the reduction of HDV-RNA concentrations with relevant implication for optimizing therapeutical approaches aimed at achieving HDV cure

Overall, these results should be carefully taken into account for the proper monitoring of virological response to anti-HDV drugs

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