Comparison of the recently launched Hologic Aptima HBV Quant assay with the established Abbott RealTi*me* HBV assay in viral load measurement

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Background:

Hologic's Aptima HBV Quant assay is a HBV DNA quantitative assay based on real-time Transcription Mediated Amplification (TMA) that runs on the fully automated Panther system with random access. A comparison with the Abbott m2000 RealTi*me* assay was performed. Special focus with clinical samples was put on reproducibility, linearity, sensitivity and performance in different genotypes.

Methods

Fresh (n=450), frozen (n=174; from 28 known genotypes) and diluted (n=618) patient samples spread over the clinical relevant range were tested. Analytical sensitivity of the Aptima assay was assessed using dilutions of the AcroMetrix HBV standard (SKU963003) run in replicates of 10/dilution. Linearity of both assays was tested by dilution series of patient samples with HBV genotypes A-F from 8.0 to 2.0 log IU/mL in replicates of 3. Intra- and inter-assay variation was calculated by testing 30 samples in three dilution steps of genotypes A, D and one unspecified in both systems. Inter-assay variation for the Hologic Aptima system was assessed testing replicates of clinical samples with genotype A, D and one sample with undetermined genotype in three dilution steps on 22 different days. Discrepant samples with a difference in viral load greater than 0.5 log IU/ml were retested with the Roche CAP/CTM HBV assay.

Pre-selected by viral load (Abbott) (n=175)
unkonwn genotypes
n= VL [IU/mL]
10 x nd (2x detected)
19 x detec (6x nd,1q)
38 x log 1-2 (19x nq)
39 x log 2-3
39 x log 3-4
11 x log 4-5
6 x log 5-6
1 x log 6-7
5 x log 7-8
8 x log 8-9

nd= not detected
q= quantificated
nq= not quantificated

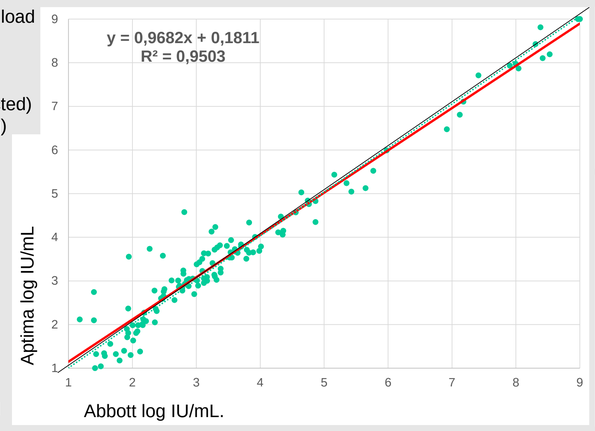


Figure 1a: Correlation of clinical samples preselected by VL unknown genotypes n=175

Aptima

RealTime	Neg	<LLOQ	Quant	>ULQ	Total
Neg	8	2	0	0	10
<LLOQ	6	12	1	0	19
Quant	1	18	137	1	157
>ULQ	0	0	1	2	3
Total	15	32	139	3	189

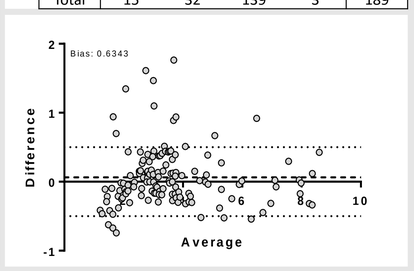


Figure 1b: Bland Altman
137 quantificated samples

Results

Aptima HBV Quant Dx assay showed excellent performance in high throughput routine. The calculated lower limit of detection (LLOD) using the AcroMetrix standard was 2.02 IU/mL (plasma, package insert: 5.58 IU/mL). Regression models demonstrated high concordance between the two assays for all genotypes. In the correlation analyses for all tested samples the slope was 0.97 with an intercept of 0.17 and R² of 0.94. Bland Altman plots (Aptima minus RealTi*me*) showed a mean difference of 0.045 with no change in bias over the complete range from 10 IU/mL up to 650,000,000 IU/mL. Linearity was proofed by serial dilution from 8 log IU/mL to 2 log IU/mL showing no difference between the two assays. Intra- and inter-assay variation was low and comparable to RealTi*me* with intra-assay %CV ranging from 1.9% for samples with a viral load of 3.0 log IU/mL to 16.6% with 1.3 log IU/mL 44 samples with a differende of greater than 0.5 log IU/mL were retested. Most of the discrepant samples showed higher values in the Aptima assay as compared to the Abbott assay. This was supported by the Roche CAP/CTM, which also showed higher values than the Abbott assay, though not as high as the Aptima assay.

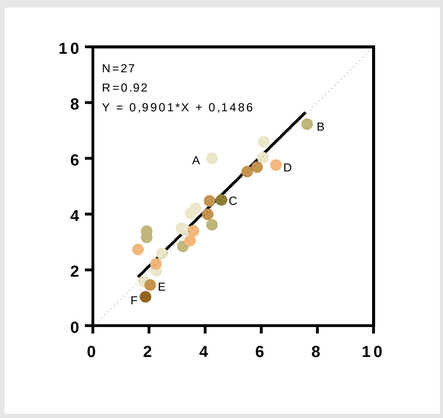
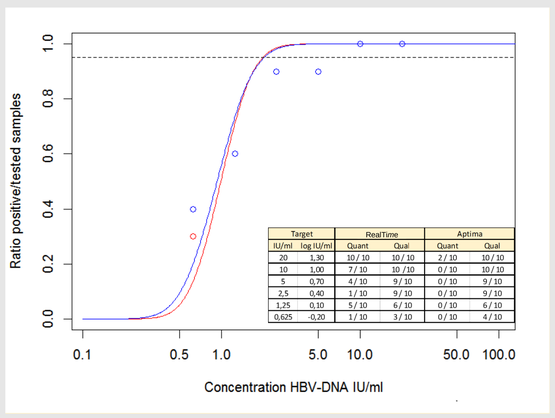


Figure 2: Correlation preselected by genotype
27 frozen preselected samples



Target		RealTime		Aptima	
IU/ml	log IU/ml	Quant	Qual	Quant	Qual
20	1,30	10 / 10	10 / 10	2 / 10	10 / 10
10	1,00	7 / 10	10 /10	0 / 10	10 / 10
5	0,70	4 / 10	9 / 10	0 / 10	9 / 10
2,5	0,40	1 / 10	9 / 10	0 / 10	9 / 10
1,25	0,10	5 / 10	6 / 10	0 / 10	6 / 10
0,625	-0,20	1 / 10	3 / 10	0 / 10	4 / 10

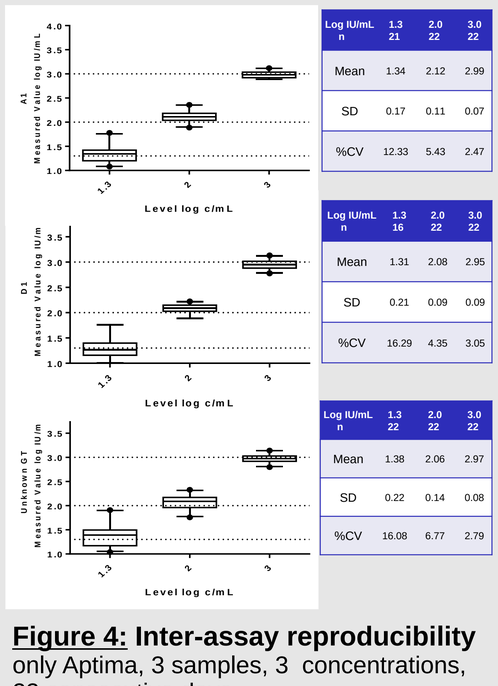
Figure 3: Sensitivity
Acrometrix standard: Dilutions from 20 to 0.6 IU/ml
Calculated for Aptima: 95%-Hitrate: 2.028 IU/ml (1.408 - 2.922)
Calculated for RealTi*me*: 95%-Hitrate: 1.982 IU/ml (1.417 - 2.771)

Table 1: Correlation of fresh clinical samples
Not preselected fresh clinical routine samples (n=98)
80 of 98 results agree, no difference in sensitivity

		Aptima			Total
		Nd	Detec	quant	
RealTi*me*	Nd	31	9	0	40
	Detec	4	9	2	15
	Quant	1	2	40	43
Total		36	20	42	98

Table 2: Intra-assay variance
Coefficients of variation (3 genotypes x 3 levels x 30 samples
no significant difference

Viral load IU/ml	Assay	GT A2	GT D2	GT undet.
20 / log 1,30	Aptima	13,27%	16,64%	13,12%
	RealTi*me*	13,12%	5,54%	5,12%
100 / log 2,0	Aptima	4,67%	6,16%	5,55%
	RealTi*me*	4,99%	2,55%	3,05%
1000 / log 3,0	Aptima	1,93%	2,64%	2,43%
	RealTi*me*	3,82%	1,20%	1,18%



Log IU/mL n	1.3 21	2.0 22	3.0 22
Mean	1.34	2.12	2.99
SD	0.17	0.11	0.07
%CV	12.33	5.43	2.47

Log IU/mL n	1.3 16	2.0 22	3.0 22
Mean	1.31	2.08	2.95
SD	0.21	0.09	0.09
%CV	16.29	4.35	3.05

Log IU/mL n	1.3 22	2.0 22	3.0 22
Mean	1.38	2.06	2.97
SD	0.22	0.14	0.08
%CV	16.08	6.77	2.79

Figure 4: Inter-assay reproducibility
only Aptima, 3 samples, 3 concentrations, 22 consecutive days

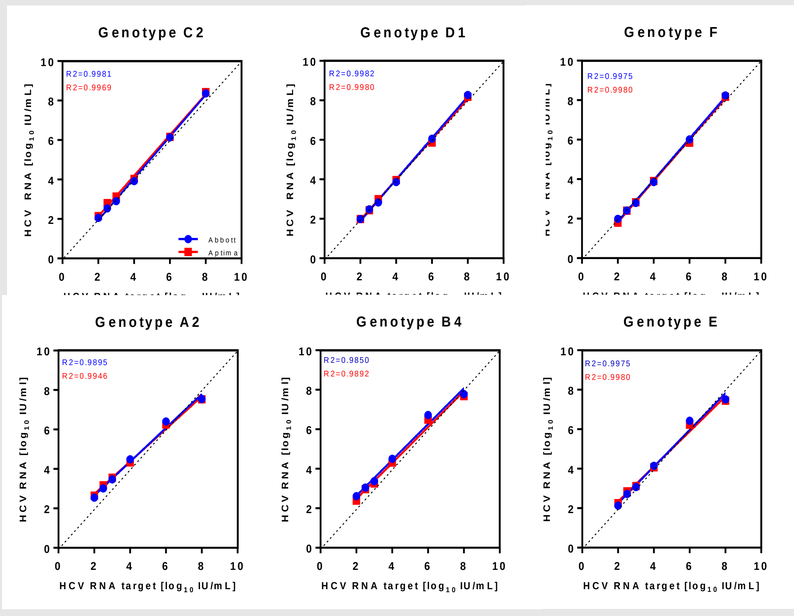


Figure 5: Linearity
clinical samples diluted based on Abbott result

Table 3: Clinical specificity
patients solitaire positive for HBc antibody, archived samples

101 samples patients only anti HBc positive		Aptima		
		not detected	detected	quantified
RealTi*me*	not detected	94	0	0
	detected	2	0	0
	quantified	0	4	1

Conclusions

The Aptima HBV Quant assay showed good correlation with Abbott RealTi*me* with the same high sensitivity, linearity and accuracy for all tested HBV genotypes. In this large comparison study only a small amount of samples showed discrepant results. These were mainly in the low to intermediate viral load range and showed al higher quantification in the Aptima assay, what was supported by the results of retesting those samples with the Roche CAP/CTM assay. With random access and time to first result of 150 minutes this assay is a major improvement in the viral load monitoring of HBV infection.