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

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Introduction and Purpose:

Hologic's Aptima HCV Quant Dx assay is a HCV RNA 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 RealTime assay was performed. Special focus with clinical samples was put on linearity, reproducibility, viremia near the limit of detection, different genotypes, and in monitoring of treatment efficacy.

Methods

Fresh (n=173), frozen (n=130; from 30 patients therapy monitoring) and diluted (n=450) patient samples spread over the clinical relevant range were tested. Analytical sensitivity of the Aptima assay was assessed using dilutions of the AcroMetrix HCV standard (SKU963003) run in replicates of at least 10/dilution. Linearity of both assays was tested by dilution series of patient samples with HCV genotypes 1b, 3a and 4p from 6.78 to 2.78 log IU/mL in replicates of 5. Intra- and inter-assay variation was calculated by testing 30/20 samples in three dilution steps of genotypes 1a, 1b, 4a/3a, respectively.

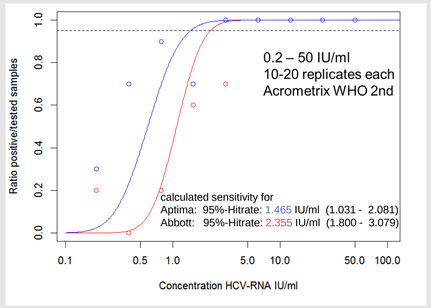

Fig. 1: Sensitivity

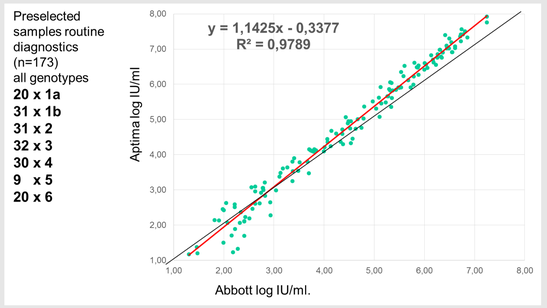

Fig. 2: Correlation of clinical samples

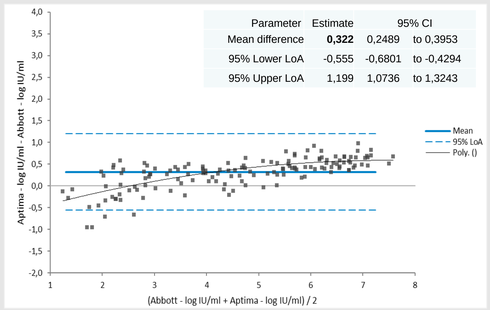

Fig. 3: Bland-Altman Analysis

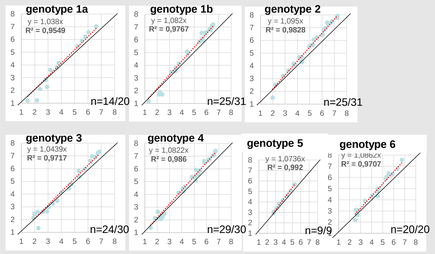

Fig. 4: Correlation of clinical samples by genotype

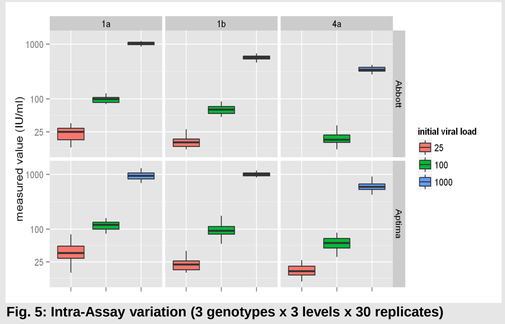

Fig. 5: Intra-Assay variation (3 genotypes x 3 levels x 30 replicates)

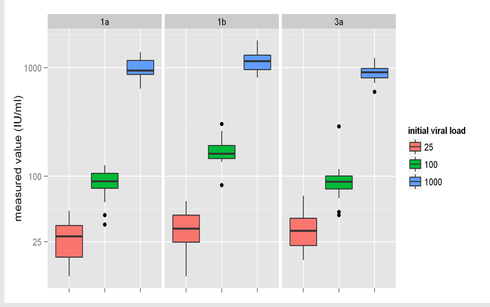

Fig. 6: Inter-Assay variation (Aptima only) **(3 genotypes x 3 levels x 20 days)**

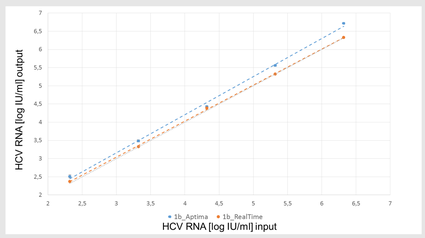

Fig. 7a: Linearity genotype 1b

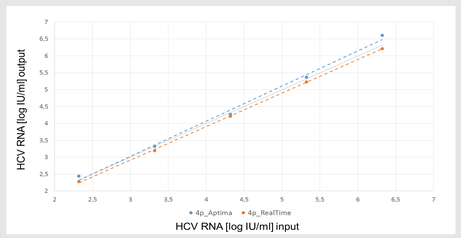

Fig. 7b: Linearity genotype 4p

Results

Aptima HCV Quant Dx assay showed excellent performance in high throughput routine, with a lower limit of quantification (LLOQ) of 10 IU/mL and a lower limit of detection (LLOD) of 4.3 IU/mL (plasma). Regression models demonstrated high concordance between the two assays for all genotypes. In the correlation analyses for all tested samples (1a I/II, 1b, 2, 3, 4, 5 and 6) the slope was 1.14 with an intercept of 0.34 and R2 of 0.98. Bland Altman plots (Aptima minus RealTime) showed a mean difference of 0.322 with a trend to higher quantification in the upper viral load range and lower quantification in the low end for the Aptima compared to RealTime. Linearity was proofed by serial dilution from 6.23log IU/mL to 2.23log IU/mL also with higher results for Aptima in the upper range. Intra- and inter-assay variation was low and comparable to RealTime with intra-assay %CV ranging from 2.5% for samples with a viral load of 3.0log IU/mL to 9.6% with 1.4log IU/mL In monitoring of treatment efficacy at weeks 4, 8, 12 and SVR 12 of 30 DAA treated patients Aptima showed less often detectable results than RealTime in patients with successful treatment outcome.

Conclusions

The Aptima HCV Quant Dx assay showed good correlation with RealTime with higher sensitivity, linearity and accuracy for all tested HCV genotypes. The higher sensitivity of the Aptima assay could be drawn by the target capture technology used in RNA isolation. The phenomenon of higher results compared to RealTime in the upper end of viral loads was shown for Roche HPS/CTM as well (Cloherty et al. 2014). With random access and time to first result of about 150 minutes this assay is a major improvement in the viral load monitoring of HCV infection.

Tab 1: DAA treated patients - treatment course until SVR12

Nr.	GT	Therapy	Week 2		Week 4		Week 6		Week 8		Week 10		Week 12		Week 16		Week 24		>Week 24		Clinical Endpoint
			Aptima	m2000	Aptima	m2000	Aptima	m2000	Aptima	m2000	Aptima	m2000	Aptima	m2000	Aptima	m2000	Aptima	m2000	Aptima	m2000	
1	1a	LDP+SOF			104	39							17	detec	nd	nd					
2	1a	LDP+SOF+riba 12w			217	647					29	32			nd	detec	nd	nd			SVR12
3	1a	LDP+SOF			37	60			62	detec							detec	nd			
4	1a	LDP+SOF			22	46			13	detec			62	detec			nd	nd			
5	1a	SOF+DKL 12wo			163	40			85	detec					nd	nd	nd	nd			SVR12
6	1a	SOF+DKL 24wo	16	51	detec	20			nd	nd					nd	nd	nd	nd			SVR12
7	1a	LDP+SOF+riba 12w	68	74	142	34			nd	detec			nd	nd							eot
8	1a	NS3 NS5A ?	30	111	nd	detec	nd	detec							nd	nd	nd	nd			
9	1a	LDP+SOF 12w			46	68			detec	28	nd	dect					nd	nd			SVR12
10	1a	SOF+DKL 24wo			nd	17			nd	nd			nd	nd	nd	nd			detec	nd	SVR12
11	1a	LDP+SOF			12	63			nd	detec			nd	nd			nd	nd			
12	1a	LDP+SOF			13	56			nd	30	nd	dect			nd	detec			nd	nd	
13	1a	?			nd	detec							nd	nd			nd	nd			
14	1a	SOF+DKL 24wo	370	485	15	110			nd	detec							nd	nd	nd	nd	SVR12t
15	1a	LDP+SOF 24wo	25	35	detec	nd			nd	nd							nd	nd	nd	nd	SVR16t
16	1a	LDP+SOF 8Wo	76	126	detec	15			nd	nd					nd	nd					SVR6
17	1a	SOF+DKL 20wo	16	70	detec	43			nd	detec									nd	nd	SVR12
18	1a	SIM+SOF 12wo			12	29							nd	detec	nd	detec			nd	nd	SVR20
19	1a	SOF+DKL			nd	detec			nd	detec			nd	nd			nd	nd			SVR24
20	1a	LDP+SOF			nd	nd							nd	nd			nd	nd			
21	1a	LDP+SOF			21	81							nd	nd			nd	nd			
22	4d	LDP+SOF			70	127	detec	22	nd	detec			nd	detec					nd	nd	svr24
23	1a	SIM+SOF 12wo			detec	24			detec	detec			nd	nd					nd	nd	svr24
24	1b	LDP+SOF+riba 12w	detec	17	nd	12					nd	nd					nd	nd			SVR12
25	3a	LDP+SOF 24wo			detec	26			nd	nd			nd	nd			nd	nd	nd	nd	SVR12
26	3a	LDP+SOF 24wo	detec	16			nd	12					nd	nd			nd	nd	nd	nd	svr4
27	3a	LDP+SOF+riba 12w	detec	18	nd	nd									nd	nd	nd	nd	nd	nd	SVR12
28	3a	LDP+SOF+riba 12w	37	108	detec	50			nd	16			nd	nd			nd	nd			SVR12
29	4a	viekri+riba	18	24	nd	detec	nd	detec					nd	nd			nd	nd			SVR12
30	4d	LDP+SOF	21	180			nd	12					nd	detec			nd	nd			SVR12