Comparison of Four HCV Viral Load Assays at High Viral Load

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Background

Hepatitis C virus (HCV) load is the most important surrogate marker for HCV replication. The level of viral load before starting antiviral therapy with direct acting agents (DAA) can be predictive for treatment success. The combination of sofosbuvir, an inhibitor of hepatitis C virus NS5B polymerase and ledipasvir, a HCV NS5A inhibitor has proven to be highly effective in treatment of chronic HCV infection. While standard treatment duration of this combination is 12 weeks, in case of viral load below 6 Mio IU/ml and genotype 1, the treatment duration can be reduced to 8 weeks [1] without inferiority in percentage of sustained virological response (SVR), reducing adverse effects and lower the price of this highly effective but costly treatment option. While in clinical trials leading to approval of this drug the Roche high pure/Cobas Taqman (HPS/CTM) assay was used, this assay is usually not available in clinical routine. If an assay of Roche is used in clinical route it is in most of the cases the Cobas Ampliprep/TaqMan v2 (CAP/CTM) assay, due to its higher level of automation. As part of a bigger evaluation trial assessing assay performance and workflow between the Abbott RealTime HCV (ART) and the Hologic Aptima HCV (APT) assay (>1500 tests performed) we observed that in high viral load quantitative results were higher in the APT (Figure 1). As it was previously described by other groups that the HPS/CTM also shows higher quantitative results we additionally compared the CAP/CTM and the HPS/CTM assays with selected high viral load samples.

1 Kowdley KV et al. Ledipasvir and sofosbuvir for 8 or 12 weeks for chronic HCV without cirrhosis. N Engl J Med 2014; 370: 1879–1888

Methods

Seven leftover samples with extra high viral loads and different genotypes (2x 1a, 1b, 2b, 3a, 4d and 4p) where preliminary tested with ART and then diluted to a target concentration between 500 000 IU/ml and 3 000 000 IU/ml reaching the therapeutic decision area mentioned above with an expected difference in quantification between ART and HPS/CTM of 0.46 log IU/ml as described by Cloherty et. al. [2]. All samples were stored in aliquots at minus 80°C for shipping and prior testing. The tests were performed in five replicates for each assay, resulting in overall 140 viral load measurements. The HPS/CTM assay with the manual extraction of viral RNA with the high pure viral RNA extraction kit (Roche) was performed at Prof. Enders & Partners laboratory in Stuttgart, Germany; CAP/CTM assay was performed in the institute for Medical Virology Universitätsklinikum Frankfurt, Germany; and ART as well as APT were operated in the MIB, Berlin Germany. Mean viral loads and coefficients of variation were compared for each subtype between all four assays.

2 G. Cloherty et. al. Antiviral Therapy 2015; 20:177–183

Table 1: Comparison of HCV viral loads in log IU/ml for seven clinical samples with different genotypes tested in 5 replicates with the four quantification assays

genotype	1a				1a				1b				2b			
log IU/mL	ART	APT	HPS/CTM	CAP/CTM	ART	APT	HPS/CTM	CAP/CTM	ART	APT	HPS/CTM	CAP/CTM	ART	APT	HPS/CTM	CAP/CTM
Average	6.33	6.72	6.56	6.31	6.47	6.78	6.60	6.39	6.22	6.61	5.99	6.11	5.96	6.32	6.32	6.13
SD	0.05	0.03	0.03	0.02	0.03	0.07	0.08	0.08	0.03	0.02	0.11	0.04	0.03	0.05	0.05	0.02
%CV	0.87	0.49	0.52	0.32	0.46	0.97	1.21	1.23	0.43	0.37	1.84	0.64	0.43	0.84	0.78	0.29

genotype	3a				4d				4p			
log IU/mL	ART	APT	HPS/CTM	CAP/CTM	ART	APT	HPS/CTM	CAP/CTM	ART	APT	HPS/CTM	CAP/CTM
Average	6.12	6.41	6.28	5.91	5.87	6.49	6.21	5.90	5.81	6.23	5.48	5.66
SD	0.03	0.04	0.06	0.02	0.02	0.03	0.04	0.02	0.04	0.02	0.02	0.04
%CV	0.52	0.55	0.92	0.41	0.37	0.45	0.60	0,36	0,64	0,36	0.41	0.76

ART = Abbott RealTime
APT = Hologic Aptima HCV
HPS/CTM = Roche high pure/Cobas Taqman v2
CAP/CTM = Cobas Amplipreр/ Taqman v2

Figure 1:

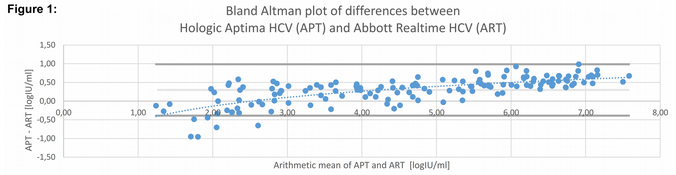
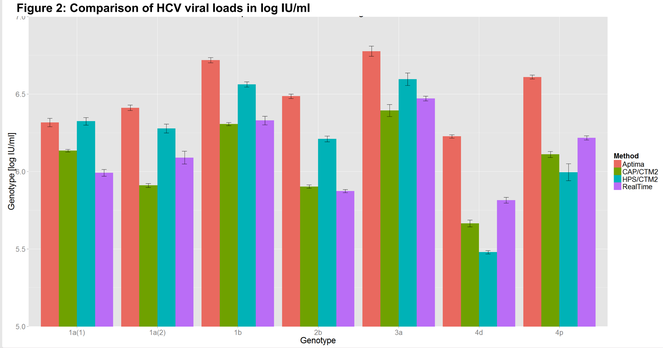


Figure 2: Comparison of HCV viral loads in log IU/ml



Results

As expected for the high viral load used for testing, the coefficients of variation (CV) were low for all assays. Calculated on the logarithmic values CV were between 0.29% and 1.84% (s. Tab. 1). Comparing viral load levels HPS/CTM results were 1.8 times higher than ART results for genotype 1 samples what is clearly less than 0.46 log. CAP/CTM results for the same samples were in mean not different to ART results. APT reached the highest values for all genotypes and quantified in mean 1.3 times higher than HPS/CTM the genotype 1 samples. APT results for all genotypes, except genotype 4, were quantified in mean 1.4 times higher than in HPS/CTM. Genotype 4 was obviously under quantified by HPS/CTM and CAP/CTM. ART results (genotype 4 excluded) were 1.2 times higher than CAP/CTM).

The relatively small differences in logarithmic results shown in Figure 2., smaller than 0.5 log IU/ml in genotype 1 for example, are more impressive regarding the absolute results the physicians normally have to handle with: e.g. genotype 1b mean IU/ml ART 2.15 million, APT 5.24 million, HPS/CTM 3.65 million, CAP/CTM 2.02 million.

Whereas APT and ART differed with nearly a constant factor for all tested samples the HPS/CTM and CAP/CTM assays showed varying discrepancies. Variation in differences between genotypes and other assays, between HPS/CTM and CAP/CTM themselves, and even within one genotype (Figure 2: 1a).

Conclusions

Despite the calibration on international standards, the assays show significantly different quantitative results in this high viral load range. Therefore. results obtained with assays used in clinical trials, cannot be easily translated to clinical routine. The Hologic Aptima HCV assay showed in genotype 1 the closest correlation to the HPS/CTM used in clinical trials. While the CAP/CTM for the tested samples quantified lower than HPS/CTM and on comparable level as ART did, it shows better performance on genotype 4 than HPS/CTM, but detection in genotype 4 is still better with APT and ART. Therapy thresholds based on HCV viral loads should be interpreted carefully having in mind differences between quantification assays and HCV genotypes.