PERFORMANCE EVALUATION OF THE NOVEL ALTOSTAR® HCV RT-PCR KIT 1.5 ON THE FULLY AUTOMATED ALTOSTAR® SYSTEM

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Background: For patients chronically infected with hepatitis C virus (HCV) RNA viral load monitoring is recommended by current guidelines to determine the efficacy of treatment. The goal of therapy is to reach a sustained virologic response, which is defined as undetectable HCV RNA plasma/serum concentration using a sensitive HCV RNA quantitation assay with a lower limit of quantification of ≤25 IU/ml. Several nucleic acid amplification tests are available, which fulfill this requirement. However, variations among assays at low HCV RNA concentrations have been observed. The AltoStar® HCV RT-PCR Kit 1.5* is a novel assay, which recently received CE IVD mark. The objective of this study was to evaluate the performance of this assay on the AltoStar® Automation System AM16.

Materials/Methods: We used the 5th WHO International Standard for HCV NAT (HCV genotype 1a) to determine the limit of detection (LoD). Probit analysis was performed to calculate the LoD.

Genotypes: For each genotype 59 to 60 replicates at the LoD value (11.1 IU/ml) were tested in different runs using AltoStar® Automation System instruments and CFX96™ Deep Well Real-Time PCR Detection System instruments.

The diagnostic performance of the AltoStar® Automation System was compared to the Abbott RealTi*me* HCV assay on the Abbott *m*2000 Sample Preparation System. In total, 460 samples from HCV-infected patients were analyzed. We assessed diagnostic sensitivity and specificity and compared quantitative results by linear regression analysis and Bland-Altman Plot.

Results: The Limit of Detection (LoD) of the AltoStar® workflow for the detection of HCV genotypes 1a, 1b and 2 to 6 in EDTA plasma was 11.1 IU/ml [95% confidence interval (CI): 7.8 – 18.5 IU/ml]. The analytical specificity was 100% as assessed on 100 HCV RNA negative samples. The diagnostic sensitivity and specificity of the AltoStar® assay was 96.5% [95%CI 92.9 – 98.6%] and 94.6% [95%CI 91.3 – 96.9%], respectively. 20 out of 21 samples giving a discordant result showed a very low viral load close to or below the LoD of both assays, suggesting random results in a statistical manner. There was very good correlation between quantitative results obtained with the AltoStar® Molecular Diagnostic Workflow and the Abbott system (correlation coefficient R = 0.95 (R^2 = 0.90).

Figure 1a and b: Linear regression of the quantitative results for HCV obtained with the Abbott RealTi*me* HCV assay (reference) and the AltoStar® HCV RT-PCR Kit 1.5.

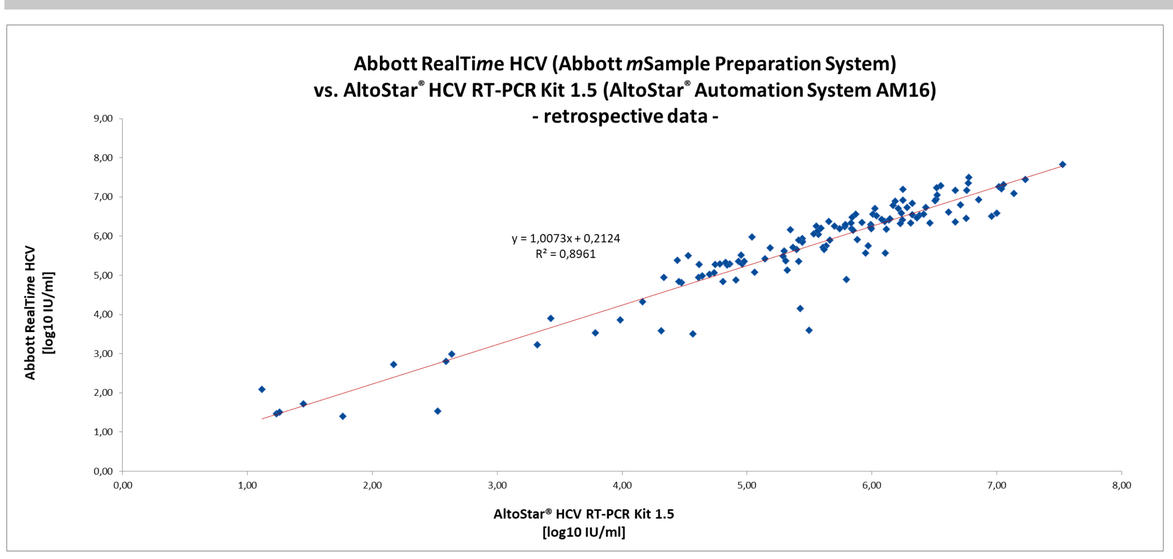


Figure 1a: retrospective data

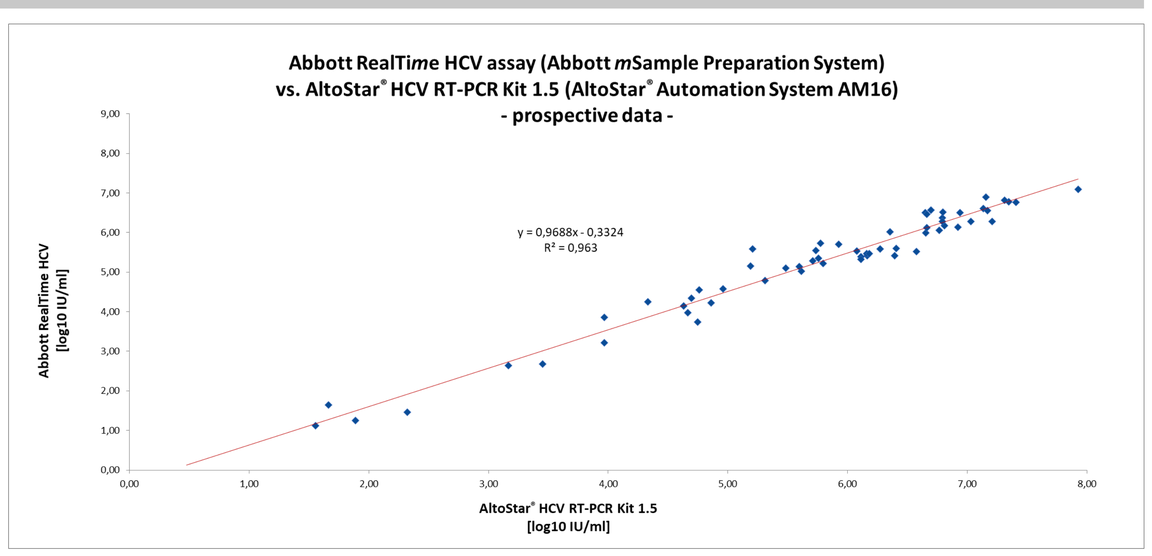


Figure 1b: prospective data

Figure 2a and b: Bland-Altman Plot for comparison of mean differences of quantitative results generated with the Abbott RealTi*me* HCV assay (reference) and the AltoStar® HCV RT-PCR Kit 1.5.

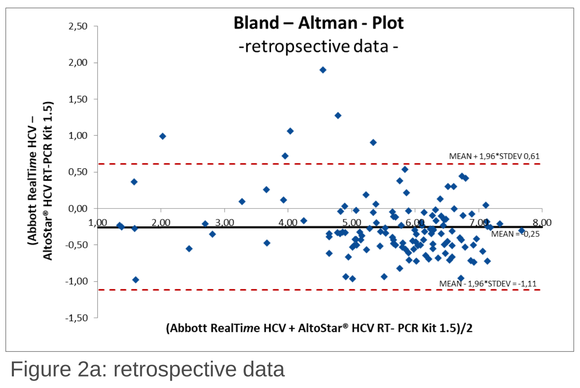


Figure 2a: retrospective data

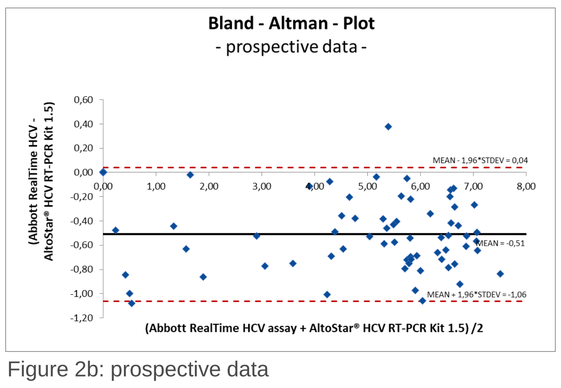
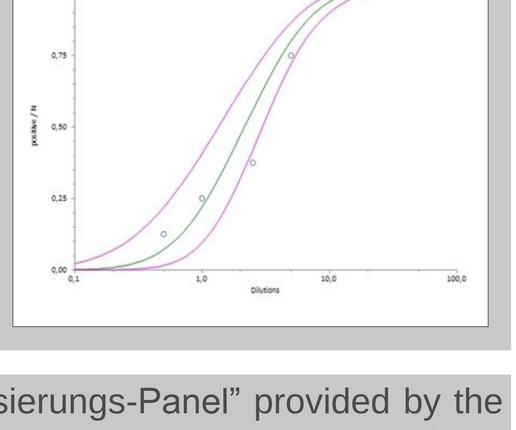


Figure 2b: prospective data

Limit of Detection: The LoD of 11.1 IU/ml [CI 7.84 – 18.54 IU/ml] for HCV genotype 1a was determined by testing serial dilutions of the 5th WHO International Standard for HCV NAT NIBSC (National Institute for Biological Standards and Control) code: 14/150; (genotype 1a).
The LoD for the HCV genotypes 1b, 2 to 6 was confirmed.



4 x 4 Table: In total, 480 samples from HCV-infected patients were analyzed.
20 out of 21 samples giving a discordant result showed a very low viral load close to or below the LoD of both assays, suggesting random results in a statistical manner.

Total number of samples: 460		Abbott RealTi*me* HCV assay	
		POSITIVE	NEGATIVE
AltoStar® HCV RT-PCR Kit 1.5	POSITIVE	195	14
	NEGATIVE	7	244

Genotype-Panel: The “HCV-Genotypisierungs-Panel” provided by the Universitätsklinikum Essen (Germany) and patient samples from *BocaBiolistics* (USA) were used for this test. One member from each genotype (1b, 2b, 3b, 4, 5a and 6a-1) was used and tested in several replicates at the LoD value (11.1 IU/ml) with the AltoStar® HCV RT-PCR 1.5 Kit. All replicates of each tested genotype were detected 100% positive.

Conclusions: The AltoStar® HCV RT-PCR 1.5 Kit in combination with the AltoStar® Automation System AM16 demonstrated an analytical and diagnostic performance comparable to that of a currently market-leading HCV assays. It may aid in clinical decision making of HCV infected patients.

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