Journal of Clinical Virology 90 (2017) 18–25

Contents lists available at ScienceDirect

Journal of Clinical Virology

journal homepage: www.elsevier.com/locate/jcv

A European multicentre study on the comparison of HCV viral loads between VERIS HCV assay and COBAS® TaqMan® HCV Test and RealTime HCV Assay

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ARTICLE INFO

Article history:
Received 17 November 2016
Received in revised form 20 February 2017
Accepted 7 March 2017

Keywords:
HCV RNA quantification
Method comparison
VERIS HCV assay
COBAS® TaqMan® HCV test
RealTime HCV assay

ABSTRACT

Background: Beckman Coulter has developed the VERIS HCV Assay for use on the new fully automated DxN VERIS Molecular Diagnostic System¥ for HCV viral load monitoring.
Objectives: Evaluate the clinical performance of the new quantitative VERIS HCV Assay.
Study design: Comparison was performed on 279 plasma specimens from HCV infected patients tested with the VERIS HCV Assay and COBAS® Ampliprep/COBAS® Taqman® HCV Test and 369 specimens tested with the VERIS HCV Assay and RealTime HCV Assay. Patient monitoring sample results from four time points were also compared.
Results: The average bias between the VERIS HCV Assay and the COBAS® Ampliprep/COBAS® Taqman® HCV Test was 0.04 $\log_{10}$ IU/mL, while between the VERIS HCV Assay and the RealTime HCV Assay average bias was 0.21 $\log_{10}$ IU/mL. Bias, however, was not consistent across the measuring range. Analysis at the lower end of quantification levels 50, 100, and 1000 IU/mL showed a predicted bias for VERIS HCV Assay versus COBAS® Ampliprep/COBAS® Taqman® HCV Test between −0.42 and −0.22 $\log_{10}$ IU/mL and for VERIS HCV Assay versus RealTime HCV Assay between 0.00 and 0.13 $\log_{10}$ IU/mL. Patient monitoring of HCV viral load over time demonstrated similar levels between VERIS HCV Assay results and COBAS® Ampliprep/COBAS® Taqman® HCV Test (52 samples from 13 patients) and RealTime HCV Assay (112 samples from 28 patients).
Conclusions: VERIS HCV Assay for use on the DxN VERIS Molecular Diagnostic System represents a reliable new tool for easy sample to result HCV RNA viral load monitoring.

1. Background and objectives

HCV RNA viral load (VL) testing is indicated for patients prior to and during treatment with antivirals, as well as post treatment to evaluate achievement of sustained virologic response (SVR) [1–3]. Monitoring may also be used in patients with suspected antiviral resistance and as a measure of treatment adherence in groups at risk for poor compliance [1,4].

There are several assays currently available for the quantification of HCV based on real-time PCR quantification. These methods are recommended based on their excellent analytical sensitivity, specificity, accuracy, and broad dynamic range of linear quantification. Many systems are designed to use separate instruments for extraction and amplification steps. Batching of tests is often required to help keep costs at a moderate level. Both can negatively

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http://dx.doi.org/10.1016/j.jcv.2017.03.006
1386-6532/

affect the time to result. Beckman Coulter's DxN VERIS Molecular Diagnostic System (DxN VERIS System) is a new, fully automated system for the quantitative analysis of molecular targets. The system integrates sample introduction, nucleic acid extraction, reaction set-up, real-time PCR amplification and detection using TaqMan® chemistry, and results interpretation. The VERIS HCV Assay is a RNA-based quantitative nucleic acid amplification based assay for HCV, calibrated to the 4th WHO International HCV Standard (NIBSC 06/102).

The objective of this study was to compare the HCV VLs obtained with the VERIS HCV Assay (VERIS Assay) to those obtained with the Roche COBAS® AmpliPrep/COBAS® TaqMan® HCV Test (COBAS Test) and Abbott RealTime HCV Assay (RealTime Assay) from clinical patient samples at multiple sites in the European Union. Additionally, data from patients being monitored for HCV VL were also compared between VERIS Assay and the two comparators.

2. Study design

2.1. Patients and assays

Seven European sites participated in this evaluation, three using the COBAS Test for routine HCV VL monitoring and four using the RealTime Assay. Table 1 summarizes the assay characteristics and testing sites.

Each site had ethics approval prior to start either for the use of leftover patient samples, or a waiver for the use of such samples. All sites were accredited and followed the same protocol, DxN System HCV Assay Beta Study. The study design was based on Clinical and Laboratory Standards Institute (CLSI) EP09-A3 guideline [5]. Samples were provided by each site and were collected in K_2EDTA plasma tubes and tested first for routine HCV clinical testing and then with the VERIS Assay. In some cases testing was done on fresh samples, without prior freezing. In other cases, frozen stored aliquots were used, with thawed sample tested on both the comparator and VERIS instruments. Samples were stored at −70 °C or lower and for no more than 1 year prior to testing. For the patient monitoring study, three sites tested stored frozen aliquots collected at four consecutive time points from patients being monitored for HCV RNA levels. In some cases an aliquot was available for testing on both VERIS and comparator instruments, in other cases testing was only done on VERIS and the original comparator result was used.

An active calibration was required for all samples tested and was performed prior to start at each site and every 30 days if necessary. Each site used one lot of Assay Reagent Packs (ARPs) during the entire study. Quality controls (negative, low and high) were passed daily prior to start of testing. All experiments were performed by trained laboratory technicians.

The performance evaluation only DxN VERIS System with VERIS HCV Assay information for use (IFU) and system guide IFU were followed during testing. For comparator testing, laboratories followed appropriate instructions for use (IFU) and their internal standard operating procedures (SOPs).

2.2. Statistical analysis

All VL values were first transformed into $\log_{10}$ format prior to analysis. Correlation between the VERIS and each comparator was analyzed using Passing-Bablok regression to estimate the bias at the 25th, 50th, and 75th percentile and the lower end of quantitation levels of 50, 100, and 1000 IU/mL. The coefficient of determination, R^2 (Pearson correlation squared) was used to measure the overall correlation between two methods. Bland–Altman analysis was used to analyze the concordance and mean difference between assays. Overall agreement was measured by weighted Cohen's kappa coefficient (κ). Tests were two sided and p-values <0.05 were considered statistically significant. For patient monitoring analysis, HCV results in $\log_{10}$ IU/mL were plotted versus time point for visual comparison. If the result returned was detected, not quantified (below lower limit of quantification (LLOQ)), the value for LLOQ was used, whereas if the results returned was not detected (ND), 0.0 was used.

3. Results

3.1. VERIS Assay versus COBAS Test

3.1.1. Method comparison

A total of 279 paired plasma samples were tested by both methods at three sites. Of the 279 samples, 268 were quantified by both assays, four were below the LLOQ on both assays, two were quantified with VERIS Assay but below LLOQ with COBAS Test, and five were quantified with COBAS Test but below the LLOQ with VERIS Assay. Passing-Bablok linear regression analysis of the 268 samples that were quantified for both assays showed an overall correlation

Table 1
Summary of HCV assay characteristics.

	Assay		
	Beckman Coulter - VERIS HCV Assay (VERIS Assay)	**Roche** – COBAS® AmpliPrep/COBAS® TaqMan® HCV Test, v2.0 (COBAS Test)	**Abbott** – RealTime HCV Assay (RealTime Assay)
Standardized against	4th WHO Standard (NIBSC code 06/102)	3rd WHO Standard (NIBSC code 06/100)	2nd WHO Standard (NIBSC code 96/798)
Extraction and Amplification System	DxN VERIS System	Automated COBAS® AmpliPrep and COBAS® TaqMan®	Automated m2000sp and m2000rt
HCV target region	5′UTR	5′UTR	5′UTR
Amplification and detection method	Real-time PCR, fluorescent detection	Real-time PCR, fluorescent detection	Real-time PCR, fluorescent detection
Sample input volume*	1000 μL	650 μL	500 μL
Sample type claim	Plasma	Plasma and serum	Plasma and serum
Linear range	12 IU/mL to 10^8 IU/mL	15 IU/mL to 10^8 IU/mL	12 IU/mL to 10^8 IU/mL
Comparator sites testing	All	Toulouse, France Sheffield, UK Berlin, Germany	Bordeaux, France Aachen, Germany Milan, Italy (Ca Granda) Milan, Italy (Sacco)

* Plus dead volume depending on sample tube type.