Quantitative Detection of Hepatitis C Virus (HCV) on the VERIS MDx System.

R. Gilfillan , Z. Wang, Y. Xu, P. Brannen, J. Wood, C. Howell, E. Joseph, E. Gustafson, T. Scholl*

(Beckman Coulter, Inc., Danvers, US)

Introduction and Purpose

The Beckman Coulter VERIS MDx** system is a fully-automated, random-access, sample-to-answer system for the quantitative/qualitative analysis of molecular targets. VERIS incorporates the extraction, purification, quantification, and results interpretation of infectious disease nucleic acid targets using the polymerase chain reaction (PCR).

The VERIS Hepatitis C Virus (HCV) Assay*** is an *in vitro* nucleic acid test, combining reverse transcription and real-time polymerase chain reaction (RT-PCR) assay, for the quantitative determination of HCV RNA in human plasma samples. It is intended to be used in conjunction with clinical presentation and other laboratory markers as an aid in monitoring HCV viral load for the detection of virus reactivation and disease, as well as for the management of patient therapy. The purpose of this study was to test and report performance of the VERIS HCV Assay in key analytical and clinical measures.

Methods

Each VERIS HCV test was performed with 1000 µL K_2EDTA plasma. A process control (PC) was included in each test to monitor process variations. Fully automated sample introduction, nucleic acid extraction, real-time PCR reaction setup, and amplification/detection were performed on the VERIS Instrument. A generic extraction and purification (E/P) cartridge was used to extract and purify nucleic acids, and a HCV-specific Assay Reagent Pack (ARP) was used for PCR amplification/detection. Two levels of calibration adjusters, traceable to the 4th WHO standard for HCV (NIBSC 06/102), were used to generate an ARP lot-specific calibration curve. HCV viral loads were calculated in IU/mL or Log IU/mL using this curve. A set of three HCV daily controls, including a negative control, a low positive control and a high positive control, were run at the beginning and end of each run for run validation.

Results

Precision: Precision of 1E+03 IU/mL and 1E+05 IU/mL samples were tested at a single Beckman Coulter site using CLSI EP5-A2 as a guideline. Four E/P cartridge and HCV ARP reagent lot combinations and three VERIS Instruments were used across five days. The VERIS HCV Assay demonstrated a total standard deviation of 0.15.

Table 1. Precision of the VERIS HCV Assay. Within run, between reagent lot, and between day standard deviations are reported. Units for mean and Std are Log IU/mL.

Level	N	Mean Log (IU/mL)	Within Run Std.	Between ARP Std	Between EP Std.	Between Day Std.	Total Std.
1000	157	2.88	0.12	0.01	0.01	0.01	0.12
100000	157	4.90	0.15	0.01	0.02	0.02	0.15

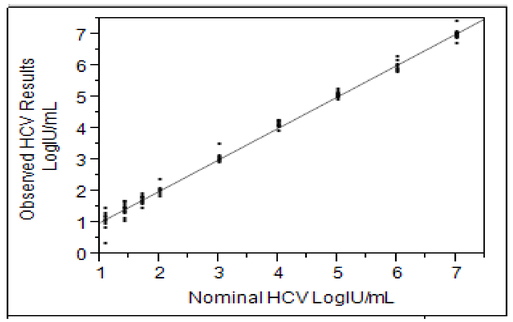
Analytical Sensitivity (LoD): A panel of 8 dilutions of the 4th WHO International Standard (IS) ranging from 1 IU/mL to 25 IU/mL were assayed using one lot of E/P cartridges, two lots of HCV ARP reagents, and two VERIS Instruments, with EP17-A as a guideline. For each of the remaining HCV genotypes (genotypes 2-6), seven levels ranging from 1 IU/mL to 48 IU/mL were tested with a minimum of 10 replicates per level using one lot of E/P cartridges and one lot of HCV ARP reagents on one VERIS Instrument. The LoD calculated by Probit analysis for the 4th WHO International Standard (IS) for HCV is 7.04 IU/mL, with a confidence interval of 5.14 – 9.64 IU/mL.

Table 2. Hit rate of 4th WHO International Standard for Hepatitis C Virus (HCV) (NIBSC 06/102). 95% percent point estimate and the confidence intervals were determined by Probit analysis.

Nominal Input Level (IU/mL)	Total Samples Tested	Total Positive	Hit Rate (%)	Estimate at 95%	95% CI	
					Lower	Upper
1	39	15	38.5	7.04	5.14	9.64
2	44	31	70.4			
3	43	36	83.7			
4	43	32	74.4			
8	45	43	95.6			
12	48	48	100			
16	42	42	100			
25	47	47	100			

Measuring Interval: Linear range was tested using a nine-level panel prepared from Acrometrix® HCV-Syntura panel from 12 IU/mL to 1E+07 IU/mL, using EP6-A as a guideline. A minimum of thirteen replicates of each level were extracted on one VERIS Instrument and two combinations of E/P cartridge and HCV ARP reagents over two days. The VERIS HCV Assay is linear for HCV with a lower limit of quantitation (LLQ) of 12 IU/mL and upper limit of quantitation (ULQ) of 1E+07 IU/mL. The maximum bias of the test result was within 0.16 Log IU/mL of the nominal input for all HCV concentration levels tested.

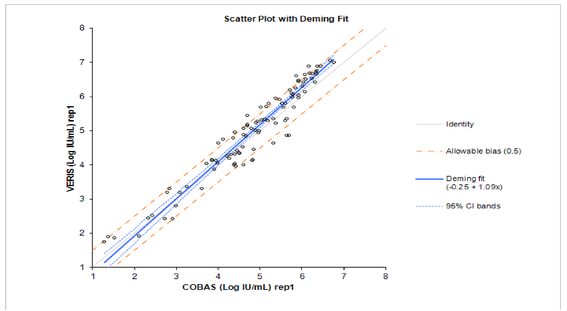
Figure 1. Measuring interval of the VERIS HCV Assay



Turn Around Time: Based on 496 observations, VERIS HCV Assay turn around time is 95.92 minutes (95.87 – 95.97, 95% CI).

Method Comparison: Method Comparison was performed using one replicate from each of 97 paired samples (representing 1.75-6.75 Log IU/mL) on both the VERIS HCV Assay and the Roche COBAS Ampliprep®/COBAS TaqMan® HCV Test v2.0 using CLSI EP9-A2 as a guideline. One E/P cartridge and HCV ARP reagent lot combination was used on one VERIS Instrument. Correlation between the VERIS HCV Assay and AmpliPrep/COBAS® TaqMan® HCV Test v2.0 was analyzed by fitting log-transformed measurements of paired samples using Deming regression. Average bias across the entire titer range is 0.17 (0.09 – 0.23, 95% CI). Bias at medical decision points 1000 IU/mL, 100 IU/mL and 12 IU/mL is 0.01, -0.08, and -0.16, respectively.

Figure 2. Deming regression: the VERIS HCV Assay compared to COBAS AmpliPrep/COBAS® TaqMan® HCV Test, v2.0



Conclusion

Based on these data, the VERIS HCV Assay is a rapid, automated molecular test for the sensitive, repeatable, and accurate viral load monitoring required for effective patient management of HCV infection.

References

EP5-A2:Evaluation of Precision Performance of Quantitative Measurement Methods

EP6-A: Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach

EP9-A2: Method comparison and bias estimation using patient samples; approved guideline—second edition.

EP17-A: Protocols for Determination of Limits of Detection and Limits of Quantitation

* **E-mail for contact: emjoseph@beckman.com**

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*** **In development**

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