Quantitative Detection of Hepatitis B Virus (HBV) on the VERIS MDx System

J. Arter Williams, J. Rodriguez, Z. Wang, K. Lykstad, G. Donvito, J. Fung, C. Howell, V.V. Colinayo, E. Gustafson, T. Scholl (Beckman Coulter, Inc., Brea, CA, USA)*

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. The initial VERIS assay menu includes the Hepatitis B Virus (HBV) Assay*** intended for use in conjunction with clinical presentation and other laboratory markers as an aid in monitoring HBV viral load and for the detection of virus reactivation. The objective of this study was to test and report performance of the VERIS HBV Assay in key analytical and clinical measures.

Methods

Each VERIS HBV test was performed with 700 µL K_2EDTA plasma or serum sample. A process control (PC) was included in each test to monitor special cause variations. Fully automated sample introduction, nucleic acid extraction, real-time PCR reaction setup, and amplification/detection were performed on the VERIS instrument. An extraction and purification (E/P) cartridge was used to extract and purify nucleic acids, and an HBV-specific Assay Reagent Pack (ARP) was used for PCR amplification/detection. Two levels of calibrators, traceable to the 3rd WHO standard for HBV (NIBSC 10/264), were used to generate an ARP lot-specific calibration curve. The HBV viral load in IU/mL or log IU/mL was calculated using this curve. A set of three HBV daily controls, including a negative control, a low positive control and a high positive control, was run at the beginning and the end of each run for run validation.

Results

Assay Sensitivity (LOD): A proper Probit analysis cannot be employed for this assay and thus a detection rate analysis was completed. 20 replicates were run at 10 and 100 IU/mL, 59 replicates were run at 4 IU/mL and 60 replicates were run at 3 IU/mL.

The VERIS HBV Assay is able to detect 4 IU/mL (100% detection rate with replicates of 59) with HBV reference genotype A. The detection rate at 3 IU/mL is 92%. The detection rates at 10 and 100 IU/mL are each 100%.

Table 1. LOD of the VERIS HBV Assay

Nominal Input Level (IU/mL)	Total Samples Tested	Total Positive	Detection Rate (%)
3	60	55	92
4	59	59	100
10	20	20	100
100	20	20	100

Assay Measuring Interval (MI): Two calibration panel lots comprised of plasmid in TE-based matrix, contrived clinical samples in both plasma and serum, and the HBV WHO standard in plasma were assayed by the VERIS HBV Assay using two E/P and ARP reagent lots (excluding WHO) on two VERIS instruments with a total of 8 replicates per individual sample across the MI range 10 – 10^9 IU/mL. The equations of linear regression for the quantified viral load vs. nominal input have R^2 values between 0.995 and 0.999, and slopes between 0.958 and 1.018 for all targets.

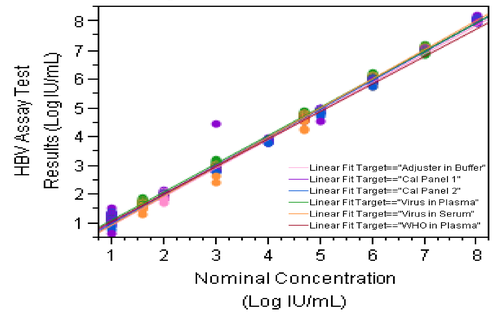

Figure 1. Measuring interval of the VERIS HBV Assay

Precision: The assay total standard deviations of within-run, between run and between lots on each of the instrument ranged from 0 to 0.24 Log IU/mL in the measuring interval ranging from 10 IU/mL to 10^8 IU/mL of HBV Calibration Panel (Table 2). The total SD observed with the WHO virus in plasma ranged from 0.03 to 0.11 Log IU/mL from 10 to $5x10^4$ IU/mL (data not shown).

Table 2. Precision of the VERIS HBV Assay

Level (Log IU/mL)	N	Mean (Log IU/mL)	Within SD (Log IU/mL)	% CV	Between SD (Log IU/mL)	% CV	Total SD (Log IU/mL)	% CV
1	72	1.00	0.21	21.49	0.11	11.04	0.24	24.10
2	72	2.01	0.06	2.94	0.03	1.60	0.07	3.39
3	72	3.00	0.19	6.43	0.00	0.00	0.19	6.43
4	72	3.99	0.04	0.88	0.03	0.63	0.04	1.08
5	71	5.00	0.06	1.14	0.02	0.48	0.06	1.24
6	72	6.03	0.05	0.75	0.03	0.43	0.05	0.86
7	71	7.01	0.02	0.34	0.02	0.23	0.03	0.40
8	72	7.98	0.04	0.53	0.03	0.35	0.05	0.64

Inclusivity-LOD: Initial evaluation of inclusivity was assessed using plasmids corresponding to HBV sub-genotypes: A1, A2, B2, B4, C2, D1, D3, E, F2, G and H; as well as SeraCare Worldwide HBV DNA Performance Panel (WWHD301) containing HBV virus genotypes A, B, C, D, E, F. All viral genotypes had a 100% detection rate at 10 IU/mL (data not shown).

Inclusivity-MI: The measuring interval of each HBV inclusivity sub-genotype was assessed by spiking into pooled negative K_2 EDTA plasma at levels from 10^1 to 10^9 IU/ml. Table 3 shows results from a subset of genotypes evaluated.

Table 3. MI linearity regression for subtypes A1, C2, D1 and H.

Target	Slope	R2	95% CI LCI - UCI	P-Value
A	1.026	0.995	1.00 – 1.05	<0.0001
C2	1.02	0.999	1.00 – 1.05	<0.0001
D1	1.02	0.998	1.01 – 1.03	<0.0001
H	1.02	0.999	0.99 – 1.03	<0.0001

Method Comparison: 58 clinical samples, 15 contrived samples and 5 levels of WHO standard dilutions (all in K2-EDTA plasma) with titers ranging from 1.2 Log IU/mL to 7.5 Log IU/mL, were assayed using the VERIS HBV Assay on VERIS instrument, and Roche COBAS Ampliprep/COBAS Taqman HBV 2.0 assay at ARUP Laboratories (Utah). A Deming Regression was completed to determine the bias at the medical decision points of 200, 2,000 and 20,000 IU/mL. Altman-Bland Analysis was used to determine the average bias across the titer range as -0.26 (95%CI: -0.37 to -0.15).

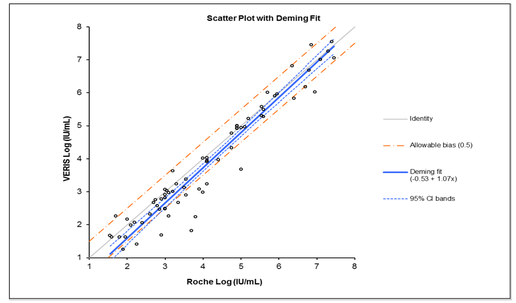

Figure 2. Method Comparison: the VERIS HBV Assay (VERIS) compared to COBAS AmpliPrep/COBAS® TaqMan® HBV v2.0 Test (Roche).

Sample Type Equivalence: A total of 56 matched serum and plasma samples were spiked with HBV virus at 14 input concentrations (range: 10 - 10^7 IU/mL). The overall mean difference (in log IU/mL) between serum and plasma matched samples was -0.03 (95% CI: -0.05 to 0.00).

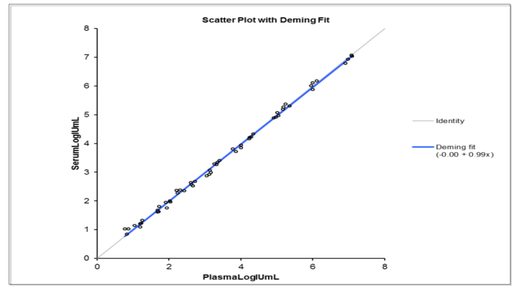

Figure 3. Sample type equivalence between K2 EDTA plasma vs. serum

Conclusion

Based on this data, the VERIS HBV Assay is a rapid, automated molecular test for the sensitive, repeatable, and accurate viral load monitoring required for effective patient management of HBV infection. Equivalent performance in plasma and serum was demonstrated.

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

*** In development