Cytomegalovirus (CMV) Viral Load Assay for the VERIS MDx System

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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 assay menu includes the VERIS Cytomegalovirus (CMV) Assay**, which is intended for use in conjunction with clinical presentation and other laboratory markers as an aid in monitoring CMV viral load and for the detection of virus reactivation.

The objective of this study was to test and report performance of the VERIS CMV Assay in key analytical and clinical measures.

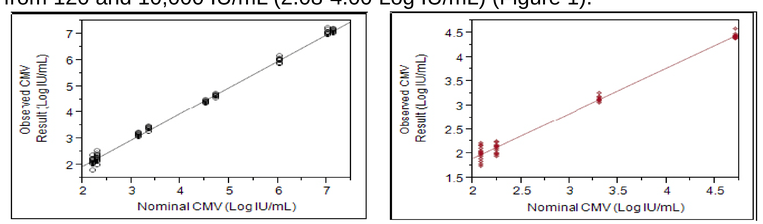
Methods

Verification and clinical validation of the VERIS CMV Assay were performed. All data were collected from K_2-EDTA plasma samples.

- Limit of Detection (LoD) was tested using a protocol based on CLSI EP17-A2 by analysis of a panel of 8 low-level samples (5-120 IU/mL).
- The linear range of the VERIS CMV Assay was tested in accordance with CLSI EP6-A.
- A precision study was conducted at a single Beckman Coulter Internal site using CLSI EP5-A2 as a guideline to evaluate precision of the VERIS CMV Assay under general use over 22 days (2 runs/day), using 4 reagent lot combinations.
- 137 paired samples tested on both the VERIS CMV Assay and the Roche COBAS AmpliPrep®/COBAS TaqMan® CMV Test were used to demonstrate Method Comparison in accordance with CLSI EP9-A2.
- 287 specimens collected from apparently healthy, CMV IgG antibody and DNA negative blood donors were tested to demonstrate the clinical specificity of the VERIS CMV Assay.

Results

Assay Measuring Interval: The VERIS CMV Assay is linear for human CMV with a lower limit of quantitation (LLQ) of 120 IU/mL and an upper limit of quantitation (ULQ) of 10,000,000 IU/mL. A nine member panel of the CMV AD169 reference strain demonstrated a linear range of 159 to 13,400,000 IU/mL (2.20-7.13 Log IU/mL). A 4-member panel of the 1st WHO International Standard for Human Cytomegalovirus (HCMV) (NIBSC 09/162) demonstrated a linear range from 120 and 10,000 IU/mL (2.08-4.00 Log IU/mL) (Figure 1).



Linear Fit Analyte Type = "AD169"

Linear Fit Analyte Type = "CMV WHO"

Figure 1. Linear Fits of observed VERIS CMV Assay results against expected WHO input by analyte type (AD169 and WHO).

Results

Assay Sensitivity (LoD): The VERIS CMV Assay has a Limit of Detection (LoD) of 30 IU/mL (1.48 Log IU/mL) across all subtypes tested (Table 1-Table 3).

Table 1. Hit rate analysis of CMV AD169 reference strain. 95% percent hit rate and the confidence intervals were determined by Probit analysis.

Lot	Nominal Input Level (IU/mL)	Total Samples Tested	Total Positive	Hit Rate (%)	Estimate At 95%	95% CI	
						Lower	Upper
All	0	60	N/A	N/A	15	12	23
	5	59	44	75			
	10	60	50	83			
	20	61	59	97			
	30	60	60	100			
	45	60	60	100			
	60	61	61	100			
	90	60	60	100			
	120	60	60	100			

Table 2. Hit rate analysis of 1st WHO International Standard for Human Cytomegalovirus (HCMV) (NIBSC 09/162) (Merlin strain). 95% percent hit rate and the confidence intervals were determined by Probit analysis.

Lot	Nominal Input Level	Total Samples Tested	Total Positive	Hit Rate (%)	Estimate At 95%	95% CI	
						Lower	Upper
All	5	60	32	53	19	15	27
	10	58	45	78			
	20	58	56	97			
	30	60	60	100			
	45	59	58	98			
	60	60	60	100			
	90	57	57	100			
	120	60	60	100			

Table 3. Probit analyses of other CMV subtypes tested at 8 levels (5 – 120 IU/mL).

CMV Subtype	Estimate at 95%	Lower 95% CI	Upper 95% CI
VR-2356 RC256	16	12	30
VR-977 Towne	11	8	25
VR-1578 UL32-EGFP-HCMV-TB40	N/A[1]		
Caen 1140	5	5	5
79/90	5	5	5
NL-MG	13	9	28
E545	5	N/A[2]	
Bichat 7175	N/A[3]		
VR-807 Davis	N/A[4]		

1 LoD estimate and 95% CI not computable by Probit model fit as a result of extreme hit rates (<0.10 and/or >0.95). 97% hit at 60 IU/mL and 100% hit rate at all other levels down to 5 IU/mL.
2 95% CI not computable by Probit model fit as a result of extreme hit rates (<0.10 and/or >0.95). 77% hit at 5 IU/mL and 100% hit rate at all other levels down to 10 IU/mL.
3 LoD estimate and 95% CI not computable by Probit model fit as a result of extreme hit rates (<0.10 and/or >0.95). 100% hit rate at all levels down to 5 IU/mL.
4 Not evaluated. CMV VR-807 strain contain the same DNA sequence in the primer/probe target region as two other CMV strain strains: VR-977 Towne strain and VR-2356 RC256 strain.

Precision: The VERIS CMV Assay demonstrated a total (within-run and between-run) standard deviation of less than 0.15 across its linear range (Table 4).

Table 4. Precision was evaluated based on CLSI guideline EP5-A2 by calculated (values were Log base 10 transformed) coefficient of variation (CV) for total precision. Within run, between lot and day CVs are reported. Units for mean and SD are log IU/mL.

Level	N	Mean Log (IU/mL)	Within Run Std.	%CV	Between ARP Std.	%CV	Between EP Std.	%CV	Between Day Std.	%CV	Total Std.	%CV
120	88	1.99	0.14	6.77	0.00	0.00	0.00	0.00	0.07	3.55	**0.15**	7.65
10000	87	4.13	0.03	0.69	0.03	0.65	0.00	0.00	0.02	0.48	**0.05**	1.23
10000000	86	6.84	0.03	0.44	0.00	0.00	0.04	0.52	0.04	0.56	**0.06**	0.91

Results

Method Comparison: VERIS CMV Assay using the VERIS MDx System performs comparably to the Roche COBAS AmpliPrep®/COBAS TaqMan® CMV Test (CE Marked). Correlation between the VERIS and COBAS CMV Assays was analyzed by fitting log-transformed measurements of paired samples using Passing-Bablok regression model. The regression plot containing the identity line, regression line, and the 95% confidence bands around the regression line is shown in Figure 2 .

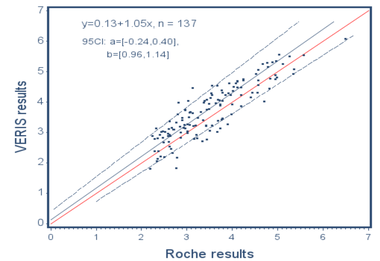


Figure 2. Recoveries are plotted as log IU/mL on both the x- (Roche) and y-axis (VERIS MDx System).

Clinical Specificity (Performance with CMV IgG and DNA negative Samples): The VERIS CMV Assay has a clinical specificity of 100% and a lower bound two-sided 95% confidence interval of 98.7% (Table 4).

Table 4. Specificity analysis results of CMV IgG and DNA negative samples.

Viral load	n	Percent	Proportion	95% Confidence Interval*	
				Lower CL	Upper CL
Not detected	287	100.0	1.0	98.7	100.0
*Confidence intervals calculated using Wilson Score method					

Conclusions

Based on these data, the VERIS CMV Assay is a rapid, automated molecular test for the sensitive, repeatable, and accurate viral load monitoring required for effective patient management of CMV 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-A2: Protocols for Determination of Limits of Detection and Limits of Quantitation

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