Comparison of An Automated Molecular Diagnostic Assay for the Quantitative Determination of Cytomegalovirus (CMV)

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Abstract

We developed and evaluated an in vitro, real-time polymerase chain reaction (PCR) human Cytomegalovirus (CMV) assay for the quantitative determination of CMV nucleic acid in human plasma using the Beckman Coulter Molecular diagnostics system (BCI MDx, in development). The BCI MDx instrument is a fully automated sample-to-result system for the quantitative/qualitative analysis of molecular targets. The system will integrate sample introduction, nucleic acid extraction, reaction setup, real-time PCR amplification/detection, and results interpretation. The CMV assay is being developed for use in conjunction with clinical presentation and other laboratory markers as an aid in monitoring CMV viral load in immunocompromised individuals at risk of CMV infections for the detection of virus reactivation and disease as well as for patient management. The CMV assay will provide quantitative results in the range 175 IU/mL to 10,000,000 IU/mL, with a limit of detection (LoD) of 60 IU/mL.

Objectives

Design evaluation of the BCI MDx CMV assay was performed on the BCI MDx engineering prototype instrument to demonstrate the assay performance of analytical sensitivity, measuring interval and precision. A comparison study was conducted between the BCI MDx CMV Assay and the COBAS AmpliPrep/COBAS® TaqMan® CMV Test by running a CMV panel, which was value assigned using the Roche CMV assay, on the BCI MDx instrument.

Methods

Each BCI MDx CMV test was performed with 250 uL 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 was performed on the BCI MDx engineering prototype instrument. A generic extraction and purification (E/P) cartridge was used to extract and purify nucleic acids, and a CMV-specific Assay Reagent Pack (ARP) was used for PCR amplification/detection. Two levels of calibrators, which are traceable to the 1st WHO standard for CMV (NIBSC 09/162), were used to generate an ARP lot-specific calibration curve. The CMV viral load in IU/mL was manually calculated using this curve. A set of three CMV daily controls, including a negative control, a low positive control and a high positive control, were run at the beginning and the end of each run for run validation.

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Results

Assay Sensitivity (LoD): Eight levels of CMV virus (AD169 strain, traceable to WHO standard) from 15 IU/mL to 300 IU/mL were assayed with two lots each of DNA E/P and CMV ARP reagents, for a total of four reagent lot combinations on two BCI MDx engineering prototypes. A minimum of twelve replicates were assayed under each condition. The LoD calculated by Probit analysis for the combined assay condition is 33 IU/mL, with 95% confidence interval from 28-39 IU/mL.

Table 1. LoD of the BCI MDx CMV Assay

BCI MDx Instrument	DNA E/P Reagent Lot	CMV ARP Reagent Lot	LoD (IU/mL)	95% Confidence Interval (IU/mL)
1	1	1	46	33-68
2	1	1	50	36-72
1	2	2	36	25-56
2	2	2	27	17-45
1	1	2	27	17-45
2	1	2	33	22-52
1	2	1	16	8-34
2	2	1	17	8-35
Combined	Combined	Combined	33	28-39

Assay Measuring Interval: A nine-level CMV panel from 100 IU/mL to $2x10^7$ IU/mL was used to evaluate the linear range of the BCI MDx CMV Assay. Twelve replicates of each level were extracted on one BCI MDx engineering prototype with two combinations of E/P and ARP reagents on two days. It was determined that the linear model was the best fit for the BCI MDx CMV Assay from 100 IU/mL to $2x10^7$ IU/mL. The maximum bias of the test result was within 0.26 Log IU/mL of the nominal input for all CMV concentration levels tested.

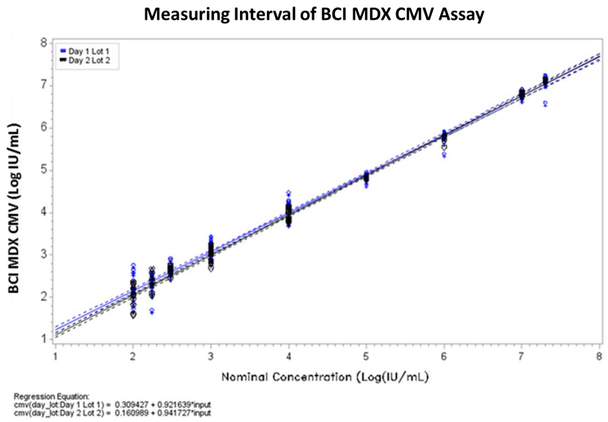

Figure 1. Measuring interval of the BCI MDx CMV Assay

Precision : Three levels of CMV at 175 IU/mL, 10^4 IU/mL and 10^7 IU/mL were run using four E/P and ARP reagent lot combinations on three BCI MDx engineering prototypes across 5 days. The total Assay SD for all three CMV levels were less than 0.21 Log IU/mL.

Table 2. Precision of the BCI MDx CMV Assay

Nominal Input (Log IU/mL)	Sample Size	Within-Run/Day SD (Log IU/mL)	Between-Run/Day SD (Log IU/mL)	Between-Reagent Lot SD (Log IU/mL)	Between Instrument/Operator SD (Log IU/mL)	Total Assay SD (Log IU/mL)
2.24	240	0.21	0.03	0.02	0.02	0.21
4.00	240	0.09	0.01	0.02	0.10	0.14
7.00	238	0.06	0.01	0.01	0.05	0.08

Method Comparison: A six-level CMV linearity panel, from 160 IU/mL to $2.65x10^6$ IU/mL, was assayed using the BCI MDx CMV assay with two E/P and ARP reagent lot combinations on one BCI MDx engineering prototype with a total 4-6 replicates per level. The panel members were value assigned using Roche COBAS Ampliprep/COBAS Taqman CMV assay at SeraCare Life Sciences. The linear relationship between the two assays was evaluated using Deming regression. The bias of CMV results from the two assays was less than 0.5 Log IU/mL for all CMV levels tested.

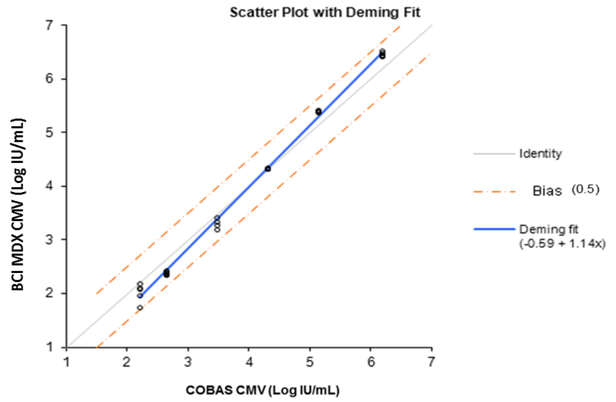

Figure 2. Deming regression: the BCI MDx CMV Assay compared to COBAS AmpliPrep/COBAS® TaqMan® CMV Test

Conclusion

We have demonstrated that the BCI MDx CMV assay on the BCI MDx engineering prototype instrument has an analytical sensitivity down to 33 IU/mL and is linear from 100 IU/mL to $2x10^7$ IU/mL. The precision of the assay was found to be within a total SD of 0.21 Log IU/mL and bias of results from the COBAS AmpliPrep/COBAS® TaqMan® CMV Test is less than 0.5 Log IU/mL across a 5 log range.

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