Title
Comparison of the Digene Hybrid Capture® System CMV DNA (version 2.0), Roche CMV UL54 Analyte Specific Reagent, and Qiagen RealArt™ CMV LC PCR Reagent Tests using AcroMetrix OptiQuant™ CMV DNA Quantification Panels and Specimens from Allogeneic Stem Cell Transplant Recipients

Running title
Comparison of three molecular diagnostic tests for CMV

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Abstract

The Digene Hybrid Capture® System CMV DNA (version 2.0), Roche CMV UL54 Analyte Specific Reagent, and Qiagen RealArt™ CMV LC PCR Reagent Tests were compared using whole virus standards and plasma specimens collected from allogeneic stem cell transplant recipients. PCR assays showed better speed, sensitivity, and specificity.

Use of in-house developed PCR-based assays for the quantification of CMV DNA in clinical specimens is hampered by variability in specimen type, nucleic acid purification, target sequence, and detection method (3). The Roche CMV UL54 Analyte Specific Reagent (Roche Diagnostics, Indianapolis, IN) and Qiagen RealArt™ CMV LC PCR Reagent (Qiagen, Germantown, MD) are two standardized real-time PCR tests that have yet to be clinically validated. Therefore, using whole virus standards and clinical specimens, we compared these new analyte specific reagents (ASRs) with our current method, the Digene Hybrid Capture® System CMV DNA Test (version 2.0) (Digene Corporation, Gaithersburg, MD).

The Hybrid Capture (HC) test was performed with 3.5 mL of whole blood according to the manufacturer’s instructions (1). For PCR analyses, nucleic acid
was purified with the MagNA Pure LC Total Nucleic Acid Isolation Kit (Roche Applied Science, Mannheim, Germany) on the MagNA Pure instrument (Roche Diagnostics). 200 µL of plasma was concentrated to a 100 µL eluate and sequential aliquots used for both PCR assays on the same LightCycler® 1.5 instrument. The Roche ASR is designed to detect a 240 bp fragment of the CMV DNA polymerase gene (UL54). A 20 µL reaction volume (5 µL eluate plus 15 µL LightCycler® FastStart DNA MasterPLUS mix) was used for PCR analysis. Recovery template from Roche was spiked into the lysis buffer before nucleic acid extraction to serve as a qualitative extraction and amplification control. The Qiagen ASR targeted a 105 bp fragment of the CMV IE gene. The reaction volume was 25 µL (10 µL eluate plus 15 µL Qiagen master mix with internal control).

All three assays were tested using OptiQuant™ CMV DNA panels (AcroMetrix Corp.) that contained 4 concentrations of CMV strain AD169 spiked into normal human plasma (Fig. 1). Eight additional dilutions from 63 to 2500 copies/mL were prepared from the panel with NAT Dilution Matrix (AcroMetrix) for analytic sensitivity analyses. After consent was obtained, EDTA whole blood was collected prospectively from adult and pediatric stem cell transplant (SCT) recipients and medical records were reviewed.

PROBIT analysis was used to determine the lower limit of detection with 95% confidence interval (CI) for each assay. Pearson correlation coefficients were applied for viral load comparisons; agreement between assays was assessed using the kappa statistic and bias plots. For three-way assay
comparisons, the Friedman repeated measures test was used for continuous
variables and the Cochran Mantel-Haenszel test for nominal variables. All P
values and 95% CIs were two-tailed.

The CMV DNA panels generated reproducible results (SD range 0.3 to 0.4
log_{10} copies/mL). The PCR tests performed better at lower viral loads (Fig. 1),
but only the Qiagen appeared log-linear across the range of CMV DNA panel
concentrations. The lower limits of detection in copies/mL (95% CI) of each
assay were HC, 2600 (1756 to 5869), Roche, 340 (178 to 3005), and Qiagen, 70
(not computable).

In 556 samples from 50 SCT recipients, CMV DNA was detected more
often (P<0.001) by Qiagen (79/556; 14.2%) compared with the Roche (47/556;
8.5%) and HC (34/556; 6.1%). The number of patients with detectable CMV
DNA was similar between the 3 assays (P = 0.13); however, 10 of 23 patients
had single, positive results by HC only (likely false-positive). Four patients had
CMV detected by PCR only (median viral load in copies/mL [range] was 423 [113
to 3218] by Roche and 33 [10 to 4778] by Qiagen). None of these patients were
treated with CMV-specific antiviral therapy and none developed recognized
sequelae of CMV infection.

There was moderate-to-substantial agreement among the 3 assays with
clinical samples (Table 1). The Roche and Qiagen assays had the strongest
correlation (r = 0.79, P < 0.0001), followed by HC versus Roche (r = 0.48, P <
0.0001), and HC versus Qiagen (r = 0.46, P < 0.0001). CMV viral loads with
Qiagen tended to be lower than both the Roche (log_{10} bias -0.4 [95% CI, -0.6 to -
and HC methods ($\log_{10}$ bias -0.6 [95% CI, -0.6 to -0.3]). Roche viral loads also were lower than HC (log bias -0.3 [95% CI, 0.0 to -0.6]). PCR assays detected CMV DNA earlier post-transplant than HC ($P = 0.001$). Median time (range) to first positive was 42.5 days (18 to 171) by HC, 39.5 (12 to 171) by Roche, and 32 (12 to 171) by Qiagen. The median (range) duration of CMV viremia on therapy was 7 days by HC (4 to 28), 19 by Roche (4 to 28), and 21 by Qiagen (11 to 35). CMV viral load by PCR increased (2 to 25-fold) during the initial 1 to 2 weeks of treatment in 4/17 (23.5%) of patients, before declining to undetectable levels. This phenomenon was not observed with HC. Increases in PCR viral load on therapy ranged from 0.2 to 1.3 $\log_{10}$ and was not associated with treatment failure.

Commercially available CMV reagents have the potential to promote standardization across laboratories and to enable better correlation with clinical trial results. The Roche and Qiagen ASRs were more sensitive than HC, detected CMV DNA earlier after transplant, and remained positive longer once antiviral treatment was initiated. CMV viral loads tended to be higher by HC as compared with PCR, in accordance with previous observations of higher viral loads in whole blood compared with plasma (2). Qiagen was the more sensitive of the two PCRs (0.7 $\log_{10}$ difference) and performed better at the lower end of the dynamic range of standards, which may be related to the manufacturer’s instructions to use 10 µL of purified DNA in the PCR reaction as compared with Roche’s 5 µL.
Quantitative real-time PCR assays are likely to become the standard method for assessing CMV viral load owing to speed, sensitivity, specificity, and improved work flow for the laboratory. Standardized CMV PCR tests should enable refined preemptive treatment strategies based on multi-center clinical trials.

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References


**FIGURE 1**

Digene Hybrid Capture® System (HC), Roche CMV UL54 Analyte Specific Reagent, and Qiagen RealArt™ CMV LC PCR Reagent test comparisons using OptiQuant™ CMV DNA Quantification Panels.

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| Expected OptiQuant viral load results: standard #1 | 2.7 log_{10} (500 copies/mL), #2 | 3.7 log_{10} (5000 copies/mL), #3 | 4.7 log_{10} (50000 copies/mL), and #4 | 5.7 log_{10} (500000 copies/mL) |
TABLE 1

Concordance and kappa coefficients for the agreement between the Digene Hybrid Capture® System (HC), Roche CMV UL54 Analyte Specific Reagent, and Qiagen RealArt™ CMV LC PCR Reagent tests using clinical specimens.

<table>
<thead>
<tr>
<th>Test comparison</th>
<th>Concordance (%)</th>
<th>Kappa coefficient</th>
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<tbody>
<tr>
<td>HC versus Roche PCR</td>
<td>513/556 (92.3)</td>
<td>0.43</td>
</tr>
<tr>
<td>HC versus Qiagen PCR</td>
<td>487/556 (87.6)</td>
<td>0.33</td>
</tr>
<tr>
<td>Roche PCR versus Qiagen PCR</td>
<td>526/556 (94.6)</td>
<td>0.64</td>
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