# SUMMARY OF SAFETY AND EFFECTIVENESS DATA (SSED)

## I. GENERAL INFORMATION

| | |
|---|---|
| Device Generic Name: | *In vitro* real-time polymerase chain reaction (PCR) based assay for CMV viral load measurement in human plasma |
| Device Trade Name: | COBAS® AmpliPrep/COBAS® TaqMan® CMV Test |
| Device Procode: | PAB |
| Applicant's Name and Address: | Roche Molecular Systems, Inc.<br>4300 Hacienda Drive<br>Pleasanton, CA 94588 |
| Date(s) of Panel Recommendation: | None |
| Premarket Approval Application (PMA) Number: | P110037 |
| Date of FDA Notice of Approval: | July 5, 2012 |

Expedited: Not applicable

## II. INDICATIONS FOR USE

The COBAS® AmpliPrep/COBAS® TaqMan® CMV Test is an *in vitro* nucleic acid amplification test for the quantitative measurement of cytomegalovirus (CMV) DNA in human EDTA plasma using the COBAS® AmpliPrep Instrument for automated specimen processing and the COBAS® TaqMan® Analyzer or the COBAS® TaqMan® 48 Analyzer for automated amplification and detection.

The COBAS® AmpliPrep/COBAS® TaqMan® CMV Test is intended for use as an aid in the management of solid-organ transplant patients who are undergoing anti-CMV therapy. In this population serial DNA measurements can be used to assess virological response to antiviral treatment. The results from the COBAS® AmpliPrep/COBAS® TaqMan® CMV Test must be interpreted within the context of all relevant clinical and laboratory findings.

The COBAS® AmpliPrep/COBAS® TaqMan® CMV Test is not intended for use as a screening test for the presence of CMV DNA in blood or blood products.

## III. CONTRAINDICATIONS

None

## IV. WARNINGS AND PRECAUTIONS

The warnings and precautions can be found in the COBAS® AmpliPrep/COBAS® TaqMan® CMV Test labeling.

## V. DEVICE DESCRIPTION

The COBAS® AmpliPrep/COBAS® TaqMan® CMV Test (CAP/CTM CMV Test) is an *in vitro* nucleic acid amplification test for the quantitative measurement of cytomegalovirus (CMV) DNA in human EDTA plasma using the COBAS® AmpliPrep Instrument for automated specimen processing and the COBAS® TaqMan® Analyzer or the COBAS® TaqMan® 48 Analyzer for automated amplification and detection. The CAP/CTM CMV Test is intended for use as an aid in the management of solid-organ transplant patients who have been diagnosed with CMV disease and are undergoing antiviral therapy.

The CAP/CTM CMV Test is based on two major processes: (1) specimen preparation to isolate CMV DNA and (2) simultaneous PCR amplification of target DNA and detection of cleaved dual-labeled oligonucleotide detection probe specific to the target. The CAP/CTM CMV Test permits automated specimen preparation followed by PCR amplification and detection of CMV target DNA and CMV Quantitation Standard (QS) DNA. The Master Mix reagent contains primers and probes specific for both CMV DNA and CMV QS DNA. The detection of amplified DNA is performed using target-specific and QS-specific dual-labeled oligonucleotide probes that permit independent identification of CMV amplicon and CMV QS amplicon. The quantitation of CMV viral DNA is performed using the CMV QS. It compensates for effects of inhibition and controls the preparation and amplification processes, allowing a more accurate quantitation of CMV DNA in each specimen.

The CMV QS is a non-infectious DNA construct that contains CMV sequences with identical primer binding sites as the CMV target DNA and a unique probe binding region that allows CMV QS amplicon to be distinguished from CMV target amplicon. The CMV QS is added to each specimen at a known copy number and is carried through the subsequent steps of specimen preparation and simultaneous PCR amplification and detection of cleaved dual-labeled oligonucleotide detection probes. The COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer calculates the CMV DNA concentration in the test specimens by comparing the CMV signal to the CMV QS signal for each specimen and control.

**Target Selection**

The CMV target for this test is a highly-conserved, non-drug target region of the CMV DNA polymerase (UL54) gene. Generic silica-based specimen preparation is used to capture the CMV DNA and CMV QS DNA, and defined oligonucleotides are used as primers in amplification of the CMV DNA and CMV QS DNA. A target-specific and a QS-specific dual-labeled oligonucleotide probe permit independent identification of the CMV amplicon and of the CMV QS amplicon. The CAP/CTM CMV Test uses two amplification primers for PCR. A fluorescent, signal-generating probe, modified with a 5' fluorochrome (FAM) and a 3' quencher, hybridizes to one of the two strands and is cleaved by Z05 DNA polymerase during extension of the primers.

**Specimen Preparation**

The CAP/CTM CMV Test utilizes automated specimen preparation on the COBAS® AmpliPrep Instrument by a generic silica-based capture technique. The procedure requires a sample input volume of 500 µL, 350 µL of which is processed by the COBAS® AmpliPrep Instrument. The CMV virus particles are lysed by incubation at elevated temperature with a protease and chaotropic lysis/binding buffer that releases nucleic acids and protects the released CMV DNA from DNAses in plasma. Protease and a known number of CMV QS DNA molecules are introduced into each specimen along with the lysis reagent and magnetic glass particles. Subsequently, the mixture is incubated and the CMV DNA and CMV QS DNA are bound to the surface of the magnetic glass particles. Unbound substances, such as salts, proteins, and other cellular impurities, are removed by washing the magnetic glass particles. After separating the magnetic glass particles and completing the washing steps, the adsorbed nucleic acids are eluted at an elevated temperature with an aqueous solution. The processed specimen, containing the released CMV DNA and CMV QS DNA, is added to the amplification mixture and transferred to the COBAS® TaqMan® Analyzer or the COBAS® TaqMan® 48 Analyzer.

**PCR Amplification**

The PCR amplification reaction is performed with the thermo stable recombinant DNA Polymerase enzyme (Z05) from Thermus species Z05. In the presence of magnesium ($Mg^{2+}$) and under the appropriate buffer conditions, Z05 has DNA polymerase activity. This allows PCR amplification to occur together with real-time detection of the amplicon. Processed specimens are added to the amplification mixture in amplification tubes (K-tubes) in which PCR amplification occurs. In the presence of $Mg^{2+}$ and excess deoxynucleotide triphosphates (dNTPs), including deoxyadenosine, deoxyguanosine, deoxycytidine, deoxyuridine and deoxythymidine triphosphates (dATP, dGTP, dCTP, dUTP, and dTTP), Z05 polymerase extends the annealed primers forming double-stranded DNA.

**Target Amplification**

The Thermal Cycler in the COBAS® TaqMan® Analyzer or the COBAS® TaqMan® 48 Analyzer heats the reaction mixture to denature the double-stranded DNA and to expose the specific primer target sequences. As the mixture cools, the primers anneal to the target DNA. Z05, in the presence of $Mg^{2+}$ and excess deoxynucleotide triphosphates (dNTPs), extends the annealed primers along the target template to produce double-stranded DNA molecules termed an amplicon. The COBAS® TaqMan® Analyzer or the COBAS® TaqMan® 48 Analyzer automatically repeats this process for a designated number of cycles, with each cycle intended to double the amount of amplicon DNA. The required number of cycles is preprogrammed into the COBAS® TaqMan® Analyzer or the COBAS® TaqMan® 48 Analyzer. Amplification occurs only in the region of the CMV genome between the primers; the entire CMV genome is not amplified.

**Target Detection**

The CAP/CTM CMV Test utilizes real-time PCR technology. The use of fluorescently-labeled hydrolysis probes allows for real-time detection of PCR product accumulation by monitoring of the emission intensity of fluorescent reporter dyes released during the amplification process. The probes consist of CMV target and CMV QS-specific oligonucleotide probes with a reporter dye and a quencher dye. In the CAP/CTM CMV Test the CMV target and CMV QS probes are labeled with different fluorescent reporter dyes. When these probes are intact, the fluorescence of the reporter dye is suppressed by the proximity of the quencher dye due to Förster-type energy transfer effects. During PCR, the probe hybridizes to a target sequence and is cleaved by the 5' → 3' nuclease activity of the thermo stable Z05 DNA polymerase during the extension phase of the PCR cycle. Once the reporter and quencher dyes are released and separated, quenching no longer occurs, and the fluorescent activity of the reporter dye is increased. The amplification of CMV DNA and CMV QS DNA are measured independently at different wavelengths. This process is repeated for a designated number of cycles, each cycle effectively increasing the emission intensity of the individual reporter dyes, permitting independent identification of CMV DNA and CMV QS DNA. The PCR cycle where a growth curve starts exponential growth is related to the amount of starting material at the beginning of the PCR.

**Selective Amplification**

Selective amplification of target nucleic acid from the specimen is achieved in the CAP/CTM CMV Test by the use of AmpErase (uracil-N-glycosylase) enzyme and deoxyuridine triphosphate (dUTP). The AmpErase enzyme recognizes and catalyzes the destruction of DNA strands containing deoxyuridine, but not DNA containing deoxythymidine. Deoxyuridine is not present in naturally occurring DNA, but is always present in amplicon due to the use of deoxyuridine triphosphate as one of the dNTPs in the Master Mix reagent; therefore, only amplicon contains deoxyuridine. Deoxyuridine renders contaminating amplicon susceptible to destruction by the AmpErase enzyme prior to amplification of the target DNA. Also, any nonspecific product formed after initial activation of the Master Mix by magnesium is destroyed by the AmpErase

enzyme. The AmpErase enzyme, which is included in the Master Mix reagent, catalyzes the cleavage of deoxyuridine-containing DNA at the deoxyuridine residues by opening the deoxyribose chain at the C1-position. When heated in the first thermal cycling step, the amplicon DNA chain breaks at the position of the deoxyuridine, thereby rendering the DNA non-amplifiable. The AmpErase enzyme remains inactive for a prolonged period of time once exposed to temperatures above 55°C, i.e., throughout the thermal cycling steps, and therefore does not destroy target amplicon formed during amplification.

**CMV DNA Quantitation**

The CAP/CTM CMV Test quantifies CMV viral DNA by analyzing the difference between the CMV target and QS Ct values. The CMV QS is a non-infectious DNA construct, containing fragments of CMV sequences with primer binding regions identical to those of the CMV target sequence. The CMV QS contains CMV primer binding regions and generates an amplification product of the same length and base composition as the CMV target DNA. The detection probe binding region of the CMV QS has been modified to differentiate CMV QS amplicon from CMV target amplicon. During the extension phase of the PCR in the COBAS® TaqMan® Analyzer or the COBAS® TaqMan® 48 Analyzer, the specimens are illuminated and excited by filtered light and filtered emission fluorescence data are collected for each specimen. The readings from each specimen are then corrected for instrumental fluctuations. These fluorescence readings are sent by the instrument to the AMPLILINK software and stored in a database. Pre-Checks are used to determine if the CMV DNA target and CMV QS DNA data represent sets that are valid, and flags are generated when the data lie outside the preset limits. After all Pre-Checks are completed and passed, the fluorescence readings are processed to generate Ct values for the CMV DNA target and the CMV QS DNA. The lot-specific calibration constants provided with the CAP/CTM CMV Test are used to calculate the titer value for the specimens and controls based on both the CMV DNA target and CMV QS DNA Ct values. Titer results are reported in International Units/mL (IU/mL).

**Quality Control Information**

One replicate each of the COBAS® TaqMan® Negative Control, the CMV Low Positive Control, and the CMV High Positive Control must be included in each test batch. The batch is valid if no flags appear for any of the controls. The user is instructed to check the run printout for flags and comments to ensure that the batch is valid.

- Negative Control: The CMV negative control must yield a "Target Not Detected" result. If the CMV negative control is flagged as invalid, then the entire batch is invalid.
- Positive Controls: The acceptable titer ranges for CMV low positive control and CMV high positive control are provided on the CAP/CTM CMV Test reagent cassette barcodes. The CMV DNA IU/mL for CMV high and low positive

controls should fall within their acceptable titer ranges. If one or both of the positive controls are flagged as invalid, then the entire batch is invalid.

- Run Validation — AMPLILINK version 3.3 Series: The user is instructed to check AMPLILINK software results window or printout for flags and comments to ensure that the batch is valid. For control orders, a check is made to determine if the IU/mL value for the control is within its fixed range. If the IU/mL value for the control lies outside of its range, a FLAG is generated to show the control has failed.

The batch is valid if no flags appear for any of the controls. The batch is not valid if any of the following flags appear:

Negative Control

| Flag | Result | Interpretation |
|---|---|---|
| NC_INVALID | Invalid | An invalid result or a "valid" result that was not negative for CMV target. |

CMV Low Positive Control

| Flag | Result | Interpretation |
|---|---|---|
| LPC_INVALID | Invalid | An invalid result or a control out of range. |

CMV High Positive Control

| Flag | Result | Interpretation |
|---|---|---|
| HPC_INVALID | Invalid | An invalid result or a control out of range. |

**Interpretation of Results**

The COBAS® TaqMan® Analyzer or the COBAS® TaqMan® 48 Analyzer automatically determines the CMV DNA concentration for the specimens and controls. The CMV DNA concentration is expressed in IU/mL.

A valid batch may include both valid and invalid specimen results, depending on whether flags and/or comments are obtained for the individual specimens.

Specimen results are interpreted as follows:

| Titer Result | Interpretation |
|---|---|
| Target Not Detected | Report results as "CMV DNA not detected". |
| <1.37E+02 IU/mL | Calculated IU/mL results are below the Lower Limit of Quantitation (LLoQ) of the test. Report results as "CMV DNA detected, less than 137 CMV DNA IU/mL." |
| ≥1.37E+02 IU/mL and ≤9.10E+06 IU/mL | Calculated results greater than or equal to 137 CMV DNA IU/mL and less than or equal to 9.10E+06 CMV DNA IU/mL are within the Linear Range of the test. |

| | |
|---|---|
| >9.10E+06 IU/mL | Calculated IU/mL results are above the Upper Limit Of Quantitation (ULoQ) of the test. Report results as "greater than 9.10E+06 CMV DNA IU/mL." Or if quantitative results are desired, the original specimen should be diluted with CMV-negative human EDTA-plasma and the test should be repeated. Multiply the obtained result by the dilution factor. |
| Failed | Specimen was not correctly processed. Test should be repeated with another aliquot of the original sample. |
| Invalid | An invalid result. Test should be repeated with another aliquot of the original sample. |

**Note:** Samples above the ULoQ of the test that produce an invalid result with a flag "QS_INVALID" should not be reported as >9.10E+06 IU/mL. The original sample should be diluted with CMV-negative EDTA plasma and the test should be repeated. Multiply the obtained result by the dilution factor.

**Note:** One International Unit (IU) (as reported by the CAP/CTM CMV Test) based on the 1st WHO International Standard for Human Cytomegalovirus (HCMV) for Nucleic Acid Amplification (NAT)-based Assays (NIBSC 09/162) is equivalent to 1.1 copy (cp) CMV DNA as defined by the CAP/CTM CMV Test.

**Note:** The test can quantitate CMV DNA over the range of 1.37E+02 to 9.10E+06 IU/mL.

**Note:** The analytical measurement range of analyte values that can be directly measured for a specimen with a maximum dilution of one to one hundred using the CAP/CTM CMV Test is 1.37E+02 to 9.10E+08 IU/mL.

**Kit Configuration and Components**

The CAP/CTM CMV Test utilizes two kits for the detection of CMV DNA in human plasma using automated specimen preparation on the COBAS® AmpliPrep Instrument and automated amplification/detection on the COBAS® TaqMan® Analyzer. All reagents and controls required for sample preparation, amplification and detection are provided in the CAP/CTM CMV Test kit and are packaged in one of four barcoded reagent cassettes, which are loaded directly onto the COBAS® AmpliPrep instrument along with specimens and controls.

**Materials Provided:**

A. COBAS® AmpliPrep/COBAS® TaqMan® CMV Test (For 72 Tests)

- CMV CS1 (CMV Magnetic Glass Particles Reagent Cassette)
- CMV CS2 (CMV Lysis Reagent Cassette)
- CMV CS3 (CMV Multi-Reagent Cassette)
- CMV CS4 (CMV Test-Specific Reagent Cassette)
- CMV H(+)C (CMV High Positive Control)



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- CMV L(+)C (CMV Low Positive Control)
- CTM (-)C [COBAS TaqMan Negative Control (Human Plasma)]
- CMV H(+)C Clip (High Positive Control Barcode Clip)
- CMV L(+)C Clip (Low Positive Control Barcode Clip)
- CMV (-)C Clip (Negative Control Barcode Clip)

B. COBAS® AmpliPrep/COBAS® TaqMan® Wash Reagent

**Materials Required but Not Provided:**

**Instrumentation and Software**

- COBAS® AmpliPrep Instrument
- COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer
- Optional: Docking Station
- Optional: cobas p 630 Instrument
- AMPLILINK Software, Version 3.3 Series
- Data Station for the AMPLILINK software, with printer.
- AMPLILINK Software v3.3 Series Manuals:
  - COBAS® AmpliPrep Instrument Manual. For use with the COBAS® TaqMan® Analyzer, COBAS® TaqMan® 48 Analyzer, COBAS® AMPLICOR® Analyzer, or cobas p 630 Instrument, and the AMPLILINK software, Version 3.2 and 3.3 Series
  - COBAS® TaqMan® Analyzer (plus optional docking station) Instrument Manual For use with the AMPLILINK software, Version 3.2 and 3.3 Series Application Manual
  - COBAS® TaqMan® 48 Analyzer Instrument Manual For use with the AMPLILINK software, Version 3.2 and 3.3 series Application Manual
  - AMPLILINK software Version 3.3 Series Application Manual. For use with the COBAS® AmpliPrep Instrument, COBAS® TaqMan® Analyzer, COBAS® TaqMan® 48 Analyzer, COBAS® AMPLICOR® Analyzer, and cobas p 630 Instrument.
  - Optional: cobas p 630 Instrument Operator's Manual Software Version 2.2

**Disposables**

- Sample processing units (SPUs)
- Sample input tubes (S-tubes) with barcode clips
- Racks of K-tips
- Racks of K-tubes

**Other Materials Required but Not Provided:**

- Sample Rack (SK 24 rack)
- Reagent Rack

- SPU rack
- K-carrier
- K-carrier Transporter
- K-carrier rack (required for use with COBAS® TaqMan® 48 Analyzer only)
- Pipettors with aerosol barrier or positive displacement DNase-free tips (capacity 1000 µL)
- Disposable gloves, powderless
- Vortex mixer

## VI. ALTERNATIVE PRACTICES AND PROCEDURES

To date, there is no FDA cleared or approved *in vitro* nucleic acid amplification test for the quantitative measurement of Cytomegalovirus (CMV) DNA in either human blood or plasma. Current quantitative CMV DNA testing is based on non-FDA-approved laboratory developed tests and practices established by transplant centers and associated laboratories.

The pp65 antigenemia assay is an alternative to measurement of CMV viral load that has been used in transplant centers. The test is a fluorescent assay based on detection of infected cells in peripheral blood. The test is comparable in sensitivity to laboratory-developed CMV amplification-based assays but has largely been supplanted by CMV PCR assays due to greater reliability and technical ease of the latter assays.

## VII. MARKETING HISTORY

The CAP/CTM CMV Test received CE certification and was launched on March 31, 2011 outside of the United States, under the list number 4902068190.

The following countries receive the CAP/CTM CMV Test: Australia, Austria, Belgium, Croatia, Czech Republic, Denmark, Estonia, Finland, France, Germany, Hong Kong, Italy, Kuwait, Malaysia, Netherlands, New Zealand, Norway, Poland, Portugal, Republic of Korea, Saudi Arabia, Slovakia, Slovenia, South Africa, Spain, Sweden, Switzerland, Taiwan, Turkey, and the United Kingdom.

This product has not been withdrawn from the market from any country for reasons related to safety or effectiveness, or for any other reason.

## VIII. POTENTIAL ADVERSE EFFECTS OF THE DEVICE ON HEALTH

To aid in the management of solid-organ transplant patients who are undergoing anti-CMV drug therapy, the results from the CAP/CTM CMV Test must be interpreted in the context of all relevant clinical and laboratory findings. Failure of the CAP/CTM CMV Test to perform as indicated, or human error in the use of the test or the interpretation of the test result, may result in an incorrect test result that is too low or too high. An erroneous low test result may lead to inappropriate patient management

decisions, a delay or lack of treatment, or may instill a false sense of security in a patient or clinician. An erroneous high test result may contribute to unnecessary treatment or create anxiety in the patient.

## IX. SUMMARY OF PRECLINICAL STUDIES

### A. Laboratory Studies

***Traceability to the 1st WHO International Standard for Human Cytomegalovirus (HCMV) for Nucleic Acid Amplification (NAA)-based Assays***

Several Standards and Controls have been used during development of the CAP/CTM CMV Test to provide traceability to the 1st WHO International Standard for Human Cytomegalovirus (HCMV) for Nucleic Acid Amplification (NAT)-based Assays (NIBSC 09/162) [1] ( 1st CMV WHO Standard). The standards used during development of the test include the 1st CMV WHO Standard, RMS CMV Secondary Standard, RMS CMV Secondary Standard Source Material, and RMS CMV Calibration Panel (Lambda CMA1.2).

The calibration of the CAP/CTM CMV Test is traceable to the 1st WHO International Standard for HCMV through the use of the RMS CMV Secondary Standard (SS). Due to the limited amount of the available primary standard, the 1st WHO International Standard for HCMV, use of the RMS CMV SS, which is traceable to the CMV WHO International Standard, is the only practical option for use in routine manufacturing and calibration of the CAP/CTM CMV Test. Each lot of the CAP/CTM CMV Test is assigned a set of lot-specific calibration coefficients as follows:

(1) The RMS CMV SS and a 5-member calibration panel, consisting of lambda packaged CMV DNA in Negative Human Plasma at the nominal concentrations of 1.00E+07 copy/mL, 3.16E+06 copy/mL, 3.16E+04 copy/mL, 1.00E+03 copy/mL, and 5.62E+02 copy/mL, are tested in multiple replicates in each of six batches.
(2) A validated Internal Calibration Software (ICS) is used to pool data from all six batches and to screen out outliers (at the level of significance of 0.05) based on an Extreme Studentized Deviate (ESD) test. A dose-response curve between the log-transformed nominal titers of the calibrators and the observed delta Ct values (which are obtained by subtracting Ct value of the CMV target from the Ct value of the Quantitation Standard) is established using a second order polynomial regression. The three "a", "b", and "c" coefficients of the regression are the tentative calibration coefficients.
(3) Based on the observed delta Ct values for the RMS CMV SS, the mean observed titer value for the RMS CMV SS for the run is calculated using the tentative calibration coefficients obtained in the step (2).

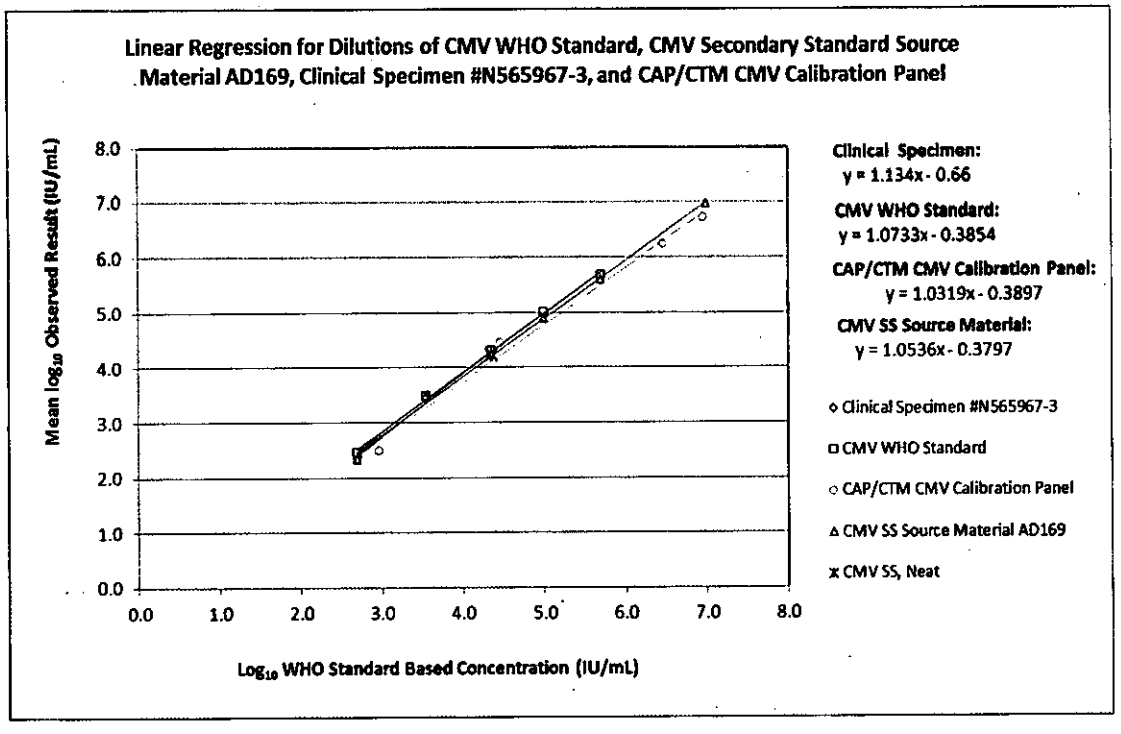
(4) A Calibration Adjustment Factor (CAF), a ratio of the observed titer value to the assigned (fixed) titer value (in IU/mL) of the RMS CMV SS, is calculated. The resulting CAF value is then applied to adjust all titers including the expected titers of the calibrators.
(5) A new dose-response curve between the new expected titers of the calibrators and their observed delta Ct values for the calibration run is established using a second order polynomial regression. The three coefficients in the second order polynomial equation, "a", "b", and "c", are the lot-specific calibration coefficients.

This process confers a lot-specific adjustment in calibration and anchors the calibration to the RMS CMV SS in a way that can be traced to the CMV WHO International Standard.

The Standards, the Calibration Panel and an independent CMV clinical specimen were tested at similar levels in the "Traceability" study. The concentration range tested for the 1st CMV WHO Standard was from 5.00E+02 IU/mL to 5.00E+05 IU/mL (2.70 – 5.70 $\log_{10}$ IU/mL), the RMS CMV Secondary Standard Source Material was tested from 5.00E+02 IU/mL to 1.00E+07 IU/mL (2.70 – 7.00 $\log_{10}$ IU/mL), the RMS CMV Calibration Panel was tested from 5.23E+02 to 9.30E+06 IU/mL (2.72 – 6.97 $\log_{10}$ IU/mL), and the independent CMV clinical specimen was tested from 5.00E+02 IU/mL to 2.27E+04 IU/mL (2.70 – 4.36 $\log_{10}$ IU/mL).

The results indicate that the calibration and standardization process of the CAP/CTM CMV Test provide quantitation values for the calibration panel, the clinical sample, the source material for the RMS CMV Secondary Standard, and the CMV WHO Standard that are similar to the expected values with deviation of no more than 0.28 $\log_{10}$ IU/mL. The maximum deviation was obtained at the test LLoQ using the regression analyses for the Calibration Panel and the CMV WHO Standard.

Comparison of the 1st CMV WHO Standard with the RMS CMV Secondary Standard Source Material, CAP/CTM CMV Calibration Panel (Lambda CMA1.2), and an independent clinical sample:




***Limit of Detection and Lower Limit of Quantitation using the 1st WHO International Standard for Human Cytomegalovirus***

The Limit of Detection (LoD) and lower limit of quantitation (LLoQ) of the CAP/CTM CMV Test were determined according to CLSI Guideline EP17-A, Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline, by analysis of six low level CMV DNA positive panels. The six independent low level CMV DNA panels were prepared using the 1st WHO International Standard for Human Cytomegalovirus (HCMV) (NIBSC 09/162, Merlin strain, genotype 1 based on the glycoprotein B gene UL55), and six independent pools of EDTA plasma as diluents. Each panel consisted of six CMV concentrations (46, 91, 137, 182, 270, and 364 IU/mL) and a CMV DNA-negative sample (blank). In addition, the limit of blank (LoB) was confirmed to be 0 IU/mL by analysis of blank samples from six unique pools of CMV DNA negative EDTA plasma, which were also used as the diluents for the low level CMV DNA positive panels.

Testing for this study was carried out using three CAP/CTM CMV Test reagent lots and three CAP/CTM systems over nine days. Two operators were employed in this study. At least 223 valid results per concentration level were obtained from 75 valid runs over nine days, which were split across three reagent lots and three CAP/CTM systems.

LoB Confirmation

CMV DNA-negative EDTA plasma ("blank") samples from six different panels yielded 100% "Target Not Detected" results out of a total of 225 valid results. Since no blank replicates reported a titer value, the determined LoB is confirmed to be 0 IU/mL.

WHO CMV Standard LoD Study — LoB Results

| Negative EDTA Plasma Panel # | Number of Positives | Number of Results | % Positive |
|---|---|---|---|
| 1 | 0 | 39 | 0% |
| 2 | 0 | 36 | 0% |
| 3 | 0 | 36 | 0% |
| 4 | 0 | 36 | 0% |
| 5 | 0 | 36 | 0% |
| 6 | 0 | 42 | 0% |
| Total | 0 | 225 | 0% |

LoD – Hit Rate Analysis

Limit of Detection (LoD), as defined by hit rate analysis, is the lowest concentration level that has a hit rate that is greater than or equal to 95% and for which all higher concentrations tested also have hit rates greater than or equal to 95%. When analyzed lot-wise, the LoD is 46 IU/mL for lot 16578B (hit rate =100%) and 91 IU/mL for lot 16580B (hit rate =100%) and lot P11496 (hit rate = 100%). The LoD for the test when all three reagent lots are combined is 46 IU/mL (hit rate = 96.4%).

WHO CMV Standard LoD Study — LoD Hit Rate Analysis Results by Reagent Lot

| Kit Lot | Nominal Concentration (IU/mL) | N | Total Positive | Hit Rate |
|---|---|---|---|---|
| All 3 Lots combined | 0 | 225 | 0 | 0% |
| | 46 | 224 | 216 | 96.4% |
| | 91 | 225 | 224 | 99.6% |
| | 137 | 223 | 223 | 100% |
| | 182 | 224 | 224 | 100% |
| | 273 | 224 | 224 | 100% |
| | 364 | 224 | 224 | 100% |
| Lot 16578B | 0 | 75 | 0 | 0% |
| | 46 | 75 | 75 | 100% |
| | 91 | 75 | 74 | 98.7% |
| | 137 | 75 | 75 | 100% |
| | 182 | 75 | 75 | 100% |

| | | | | |
|---|---|---|---|---|
| | 273 | 74 | 74 | 100% |
| | 364 | 75 | 75 | 100% |
| Lot 16580B | 0 | 78 | 0 | 0% |
| | 46 | 77 | 73 | 94.8% |
| | 91 | 78 | 78 | 100.0% |
| | 137 | 76 | 76 | 100% |
| | 182 | 77 | 77 | 100% |
| | 273 | 78 | 78 | 100% |
| | 364 | 78 | 78 | 100% |
| Lot P11496 | 0 | 72 | 0 | 0% |
| | 46 | 72 | 68 | 94.4% |
| | 91 | 72 | 72 | 100.0% |
| | 137 | 72 | 72 | 100% |
| | 182 | 72 | 72 | 100% |
| | 273 | 72 | 72 | 100% |
| | 364 | 71 | 71 | 100% |

LoD — Geometric Mean Analysis

Geometric means of the observed titers obtained at the LoD level determined by the hit rate analysis were calculated. In general, the geometric mean values were in agreement with the nominal concentrations. The geometric mean titer at the hit rate based LoD level ranged from 55.6 to 111.7 IU/mL for individual lots, and was 50.8 IU/mL for all three reagent lots combined.

WHO CMV Standard LoD Study — Geometric Mean Titers at the Hit Rate Based LoD Level

| Kit Lot | LoD (Nominal Concentration in IU/mL) | N Positive | Geometric Mean Titer (IU/mL) |
|---|---|---|---|
| ALL LOTS | 46 | 216 | 50.8 |
| LOT 16578B | 46 | 75 | 55.6 |
| LOT 16580B | 91 | 78 | 84.1 |
| LOT P11496 | 91 | 72 | 111.7 |

**Based on the analyses above, the LoD of the CAP/CTM CMV Test using the 1st WHO International Standard for Human Cytomegalovirus (HCMV) (NIBSC 09/162, Merlin strain, genotype 1 based on the glycoprotein B gene UL55) is determined to be 91 IU/mL, with the geometric mean of the observed titer at the LoD level of 111.7 IU/mL.**

LLoQ Analysis

LLoQ is defined as the lowest level of CMV DNA that can be reliably detected (i.e., percent of detected greater than 95%) and at which the total error for accuracy is less than or equal to 1.0 $\log_{10}$, where total error (TAE) is calculated as |bias| + 2 x standard deviations (SDs) per CLSI EP-17A guideline, and TAE is also calculated as SQRT(2) x 2 x SDs based on the "difference between two measurements" approach.

WHO CMV Standard LoD Study — LLoQ Summary by Reagent Lot

| Kit Lot | Nominal CMV Concentration (IU/mL) | $\log_{10}$ Nominal (IU/mL) | N | AVG $\log_{10}$ Titer (IU/mL) | SD $\log_{10}$ Titer (IU/mL) | Bias | TAE = \|Bias\| + 2 x SD | TAE = SQRT(2) x 2 x SD |
|---|---|---|---|---|---|---|---|---|
| All 3 Lots combined | 46 | 1.66 | 224 | 1.71 | 0.37 | 0.05 | 0.79 | 1.05 |
| | 91 | 1.96 | 225 | 1.99 | 0.31 | 0.04 | 0.66 | 0.88 |
| | 137 | 2.14 | 223 | 2.20 | 0.29 | 0.06 | 0.64 | 0.82 |
| | 182 | 2.26 | 224 | 2.34 | 0.27 | 0.08 | 0.62 | 0.76 |
| | 273 | 2.44 | 224 | 2.55 | 0.26 | 0.11 | 0.63 | 0.74 |
| | 364 | 2.56 | 224 | 2.60 | 0.27 | 0.04 | 0.58 | 0.76 |
| Lot 16578B | 46 | 1.66 | 75 | 1.74 | 0.39 | 0.09 | 0.87 | 1.10 |
| | 91 | 1.96 | 75 | 2.02 | 0.32 | 0.06 | 0.70 | 0.90 |
| | 137 | 2.14 | 75 | 2.23 | 0.26 | 0.10 | 0.62 | 0.74 |
| | 182 | 2.26 | 75 | 2.37 | 0.24 | 0.11 | 0.59 | 0.68 |
| | 273 | 2.44 | 74 | 2.58 | 0.25 | 0.14 | 0.64 | 0.71 |
| | 364 | 2.56 | 75 | 2.63 | 0.29 | 0.07 | 0.65 | 0.82 |
| Lot 16580B | 46 | 1.66 | 77 | 1.63 | 0.34 | -0.03 | 0.71 | 0.96 |
| | 91 | 1.96 | 78 | 1.92 | 0.33 | -0.03 | 0.69 | 0.93 |
| | 137 | 2.14 | 76 | 2.13 | 0.33 | 0.00 | 0.66 | 0.93 |
| | 182 | 2.26 | 77 | 2.28 | 0.29 | 0.02 | 0.60 | 0.82 |
| | 273 | 2.44 | 78 | 2.49 | 0.25 | 0.05 | 0.55 | 0.71 |
| | 364 | 2.56 | 78 | 2.55 | 0.27 | -0.01 | 0.55 | 0.76 |
| Lot P11496 | 46 | 1.66 | 72 | 1.75 | 0.36 | 0.09 | 0.81 | 1.02 |
| | 91 | 1.96 | 72 | 2.05 | 0.28 | 0.09 | 0.65 | 0.79 |
| | 137 | 2.14 | 72 | 2.23 | 0.27 | 0.09 | 0.63 | 0.76 |
| | 182 | 2.26 | 72 | 2.37 | 0.25 | 0.11 | 0.61 | 0.71 |
| | 273 | 2.44 | 72 | 2.58 | 0.28 | 0.14 | 0.70 | 0.79 |
| | 364 | 2.56 | 71 | 2.64 | 0.24 | 0.08 | 0.56 | 0.68 |

**Based on the analyses above, the LLoQ of the CAP/CTM CMV Test using the 1st WHO International Standard for Human Cytomegalovirus (HCMV) (NIBSC 09/162, Merlin strain, genotype 1 based on the glycoprotein B gene UL55) is determined to be 91 IU/mL, with the mean of the observed titer of 111.7 IU/mL. The results of this study also support the claimed LLoQ of 137 IU/mL.**

***Limit of Detection and Lower Limit of Quantitation Using CMV Glycoprotein B (gB) Genotypes 2, 3, and 4 Specimens***

The Limit of Detection (LoD) and the Lower Limit of Quantitation (LLoQ) for the CAP/CTM CMV Test using CMV glycoprotein B (gB) genotypes 2-4 clinical specimens were evaluated by testing at least 49 valid replicates at 137 IU/mL, 91 IU/mL, and 27 IU/mL, respectively, for each gB genotype 2-4. The testing was completed with 25 valid runs tested across six days using two kit lots of reagents and two CAP/CTM instrument systems. Two operators participated in the execution of this study.

CMV clinical specimens of three different glycoprotein-B (gB) genotypes, genotype 2 (IMPACT Accession Number P915569), genotype 3 (IMPACT Accession Number M554408, and genotype 4 (IMPACT Accession Number P722647), were collected as part of the IMPACT clinical trial for the Roche drug valganciclovir (protocol NT18435/A). The gB genotype of the clinical specimens was determined by DNA sequencing analysis. The gB genotype specimen titers were assigned by normalizing the mean measured titer of a prepared dilution of each clinical specimen to the mean measured titer of the CMV Secondary Standard (Lot TRLOT03, RMD Pleasanton, CA.) tested at two levels (2.18E+04 IU/mL (neat) and 4.55E+02 IU/mL).

LoD – Hit Rate Analysis

Limit of Detection, as defined by hit rate analysis, is the lowest concentration level that has a hit rate that is greater than or equal to 95% and for which all higher concentrations tested also have hit rates greater than or equal to 95%. For all three genotypes, both the 137 IU/mL and 91 IU/mL levels gave 100% hit rates. At the level of 27 IU/mL, observed hit rates were less than 95% for all three genotypes (Genotype 2 = 88.0%, Genotype 3 = 78.0%, and Genotype 4 = 94.0%).

CMV gB Genotypes 2-4 LoD Study — Summary Results

| Sample ID | Nominal Concentration in IU/mL | Number of Observed Positive Hits | Number of Valid Replicates | Hit Rate |
|---|---|---|---|---|
| gB2 - 1 | 27 IU/mL | 44 | 50 | 88% |
| gB2 - 2 | 91 IU/mL | 50 | 50 | 100% |
| gB2 - 3 | 137 IU/mL | 49 | 49 | 100% |
| | | | | |
| gB3 - 1 | 27 IU/mL | 39 | 50 | 78% |
| gB3 - 2 | 91 IU/mL | 50 | 50 | 100% |
| gB3 - 3 | 137 IU/mL | 49 | 49 | 100% |
| | | | | |
| gB4 - 1 | 27 IU/mL | 47 | 50 | 94% |
| gB4 - 2 | 91 IU/mL | 50 | 50 | 100% |

| | | | | |
|---|---|---|---|---|
| gB4 - 3 | 137 IU/mL | 49 | 49 | 100% |

The observed Limit of Detection for each of the three genotypes in this study is 91 IU/mL, which is similar to the observed LoD performance of the predominant gB genotype 1 in the LoD study using the 1st WHO International Standard for Human Cytomegalovirus (HCMV) (NIBSC 09/162, Merlin strain, genotype 1 based on the glycoprotein B gene UL55).

**Based on the analyses above, the LoD of the CAP/CTM CMV Test using CMV Glycoprotein B (gB) Genotypes 2-4 clinical specimens is determined to be 91 IU/mL.**

LLoQ Analysis

LLoQ is defined as the lowest level of CMV DNA that can be reliably detected (i.e., percent of detected greater than 95%) and at which the total error for accuracy is less than or equal to 1.0 $\log_{10}$, where total error (TAE) is calculated as |bias| + 2 x standard deviations (SDs) per CLSI EP-17A guideline, and TAE is also calculated as SQRT(2) x 2 x SDs based on the "difference between two measurements" approach.

CMV gB Genotypes 2-4 LoD Study — LLoQ Summary Results

| Sample ID | Nominal Concentration in (IU/mL) | $\log_{10}$ Nominal (IU/mL) | N | AVG $\log_{10}$ Titer (IU/mL) | SD $\log_{10}$ Titer (IU/mL) | Bias | TAE = \|Bias\| + 2 x SD | TAE = SQRT(2) x 2 x SD |
|---|---|---|---|---|---|---|---|---|
| gB2 - 1 | 27 | 1.43 | 50 | 1.61 | 0.30 | 0.17 | 0.77 | 0.85 |
| gB2 - 2 | 91 | 1.96 | 50 | 2.04 | 0.22 | 0.08 | 0.52 | 0.62 |
| gB2 - 3 | 137 | 2.14 | 49 | 2.21 | 0.20 | 0.08 | 0.48 | 0.57 |
| | | | | | | | | |
| gB3 - 1 | 27 | 1.43 | 50 | 1.51 | 0.39 | 0.08 | 0.86 | 1.10 |
| gB3 - 2 | 91 | 1.96 | 50 | 1.93 | 0.27 | -0.03 | 0.57 | 0.62 |
| gB3 - 3 | 137 | 2.14 | 49 | 2.22 | 0.13 | 0.08 | 0.34 | 0.37 |
| | | | | | | | | |
| gB4 - 1 | 27 | 1.43 | 50 | 1.52 | 0.32 | 0.09 | 0.73 | 0.90 |
| gB4 - 2 | 91 | 1.96 | 50 | 2.03 | 0.22 | 0.07 | 0.51 | 0.62 |
| gB4 - 3 | 137 | 2.14 | 49 | 2.24 | 0.21 | 0.11 | 0.53 | 0.59 |

**Based on the analyses above, the LLoQ of the CAP/CTM CMV Test using CMV Glycoprotein B (gB) Genotypes 2-4 clinical specimens is determined to be 91 IU/mL, with the mean of the observed titer of 111.7 IU/mL. The results of this study also support the claimed LLoQ of 137 IU/mL.**

***Linear Range Using Cultured CMV AD169 Virus***

A 10-member panel was used to evaluate the linear range of the CAP/CTM CMV Test in accordance with CLSI Guideline EP06-A, Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline. The panel was prepared by diluting cultured CMV virus (strain AD169, genotype 2 based on the glycoprotein B gene UL55) using a pool of CMV DNA negative EDTA plasma as the diluent. Each panel member was tested with two replicates per run, with three runs per day, for a minimum of six days for a total of 36 replicates per panel member evenly distributed across three lots of CAP/CTM CMV Test kit reagents, and three CAP/CTM systems.

Cultured human CMV stock material (Advanced Biotechnology Inc.; Columbia, MD; M/N: 10-103-100, Lot: 7E0006-PV, strain AD169, genotype 2 based on the glycoprotein B gene UL55) was diluted into a pool of CMV DNA negative EDTA plasma to prepare the 10-level linearity panel. The cultured human CMV stock material (source material for the RMS CMV Secondary Standard, Lot TRLOT03) was value assigned as 7.28E+10 IU/mL based on the CMV Secondary Standard lot TRLOT03 (2.184E+04 IU/mL, n=62, COBAS® AMPLICOR® CMV MONITOR Test).

CMV AD169 Linearity Study — Test Panel Levels

| Level / Panel Member # | Nominal Titer (copy/mL) | Nominal Titer (IU/mL) | $Log_{10}$ Nominal Titer (IU/mL) | Description |
|---|---|---|---|---|
| 1 | 2.0E+07 | 1.82E+07 | 7.26 | > ULoQ |
| 2 | 1.0E+07 | 9.10E+06 | 6.96 | ULoQ |
| 3 | 1.0E+06 | 9.10E+05 | 5.96 | < ULoQ |
| 4 | 1.0E+05 | 9.10E+04 | 4.96 | Intermediate Level |
| 5 | 1.0E+04 | 9.10E+03 | 3.96 | Intermediate Level |
| 6 | 1.0E+03 | 9.10E+02 | 2.96 | Intermediate Level |
| 7 | 5.0E+02 | 4.55E+02 | 2.66 | > LLoQ |
| 8 | 2.5E+02 | 2.28E+02 | 2.36 | > LLoQ |
| 9 | 1.5E+02 | 1.37E+02 | 2.14 | LLoQ |
| 10 | 1.0E+02 | 9.10E+01 | 1.96 | < LLoQ |

Deviation from linearity and bias were assessed by evaluating mean $log_{10}$ observed values and the nominal values.

Acceptance criteria for the CMV AD169 Linearity Study were the following:

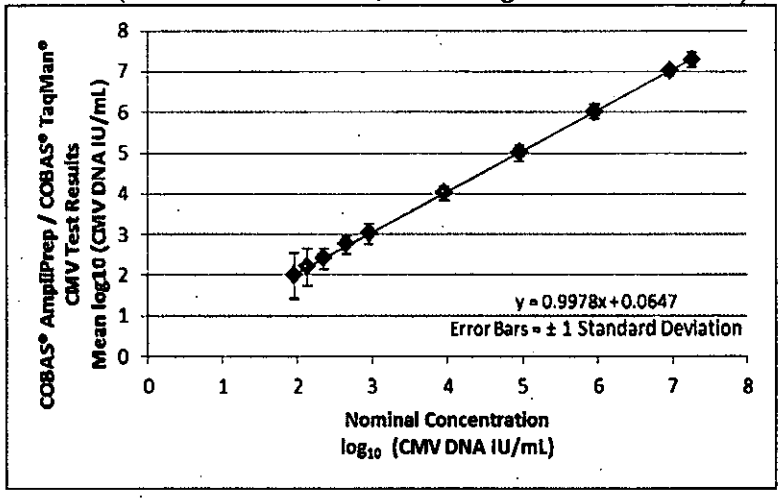
- Linearity range: Maximum deviation between linear regression and better fitting non-linear regression (i.e., deviation from linearity) should be $\leq 0.3$ $log_{10}$ for all tested concentration levels.

- Repeatability: $\leq 0.3$ $\log_{10}$ for all concentration levels tested pooled.

Out of the 377 valid results, two outliers were identified by the Extreme Studentized Deviate Test (alpha = 0.01). One outlier (4.38 $\log_{10}$ titer IU/mL) was identified from level 9.10E+01 IU/mL (expected titer = 1.96 $\log_{10}$) with ESD score of 4.1773, exceeding the lambda cutoff of 3.3561. A second outlier (3.91 $\log_{10}$ titer IU/mL) was identified from level 1.37E+02 IU/mL (expected titer = 2.14 $\log_{10}$) with ESD score of 3.8050, exceeding the lambda cutoff of 3.3561. Data was analyzed with and without the outliers.

Linear (1st order), quadratic (2nd order), and cubic (3rd order) polynomial models were fitted to the total data set (with and without the two outliers). It was determined that the linear (1st order) model was the best fit for each of the two data sets (with and without the two outliers). Linear regression analyses of the mean $\log_{10}$ observed titer vs. the nominal $\log_{10}$ titer with and without the two outliers are presented below.

CMV AD169 Linearity Study — Linearity of the CAP/CTM CMV Test
(All Data Combined, Including the Two Outliers)

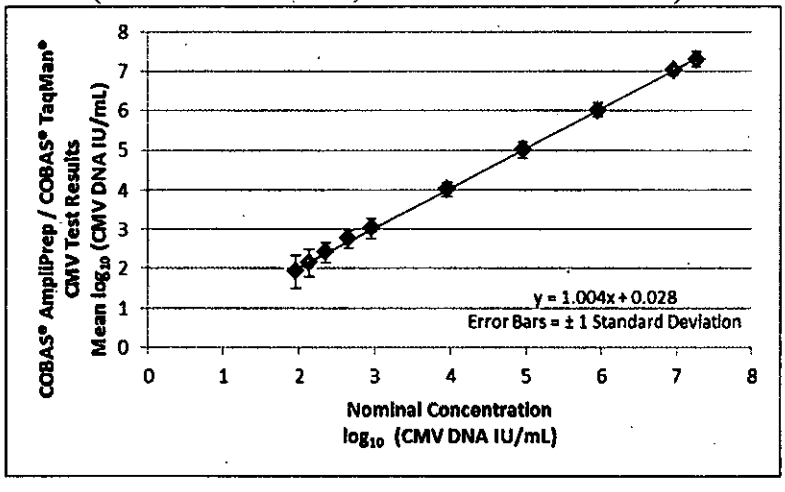



CMV AD169 Linearity Study — Observed Titer Summary
(All Data Combined, Including the Two Outliers)

| Nominal (IU/mL) | $\text{Log}_{10}$ Nominal (IU/mL) | N | Mean $\log_{10}$ Titer (IU/mL) | SD $\log_{10}$ Titer (IU/mL) | Bias | Predicted 1st-order | Deviation |
|---|---|---|---|---|---|---|---|
| 9.1E+01 | 1.96 | 38 | 1.99 | 0.57 | 0.03 | 2.02 | -0.030 |
| 1.4E+02 | 2.14 | 38 | 2.20 | 0.45 | 0.07 | 2.19 | 0.000 |
| 2.3E+02 | 2.36 | 38 | 2.41 | 0.25 | 0.05 | 2.42 | -0.010 |
| 4.6E+02 | 2.66 | 37 | 2.75 | 0.23 | 0.09 | 2.72 | 0.031 |
| 9.1E+02 | 2.96 | 38 | 3.02 | 0.25 | 0.06 | 3.02 | 0.002 |
| 9.1E+03 | 3.96 | 37 | 4.01 | 0.19 | 0.05 | 4.01 | -0.006 |
| 9.1E+04 | 4.96 | 37 | 5.01 | 0.20 | 0.05 | 5.01 | -0.004 |
| 9.1E+05 | 5.96 | 38 | 6.01 | 0.17 | 0.05 | 6.01 | -0.002 |
| 9.1E+06 | 6.96 | 38 | 7.01 | 0.13 | 0.05 | 7.01 | 0.001 |
| 1.8E+07 | 7.26 | 38 | 7.30 | 0.19 | 0.04 | 7.31 | -0.009 |

The bias of the observed mean $\log_{10}$ titer was within 0.03 $\log_{10}$ and 0.09 $\log_{10}$ of the $\log_{10}$ nominal titer for all the concentration levels tested, including the two outliers. The maximum deviation between the observed mean $\log_{10}$ titer and the best fitted 1st-order model (deviation from linearity) was < 0.04 $\log_{10}$ for each concentration level tested, including the two outliers.

CMV AD169 Linearity Study — Linearity of the CAP/CTM CMV Test
(All Data Combined, Both Outliers Removed)




CMV AD169 Linearity Study — Observed Titer Summary
(All Data Combined, Outliers Removed)

| Nominal (IU/mL) | $\text{Log}_{10}$ Nominal (IU/mL) | N | Mean $\log_{10}$ Titer (IU/mL) | SD $\log_{10}$ Titer (IU/mL) | Bias | Predicted 1st-order | Deviation |
|---|---|---|---|---|---|---|---|
| 9.1E+01 | 1.96 | 37 | 1.92 | 0.42 | -0.04 | 2.00 | -0.076 |
| 1.4E+02 | 2.14 | 37 | 2.15 | 0.35 | 0.02 | 2.17 | -0.027 |
| 2.3E+02 | 2.36 | 38 | 2.41 | 0.25 | 0.05 | 2.39 | 0.013 |
| 4.6E+02 | 2.66 | 37 | 2.75 | 0.23 | 0.09 | 2.70 | 0.051 |
| 9.1E+02 | 2.96 | 38 | 3.02 | 0.25 | 0.06 | 3.00 | 0.020 |
| 9.1E+03 | 3.96 | 37 | 4.01 | 0.19 | 0.05 | 4.00 | 0.006 |
| 9.1E+04 | 4.96 | 37 | 5.01 | 0.20 | 0.05 | 5.01 | 0.002 |
| 9.1E+05 | 5.96 | 38 | 6.01 | 0.17 | 0.05 | 6.01 | -0.002 |
| 9.1E+06 | 6.96 | 38 | 7.01 | 0.13 | 0.05 | 7.01 | -0.006 |
| 1.8E+07 | 7.26 | 38 | 7.30 | 0.19 | 0.04 | 7.32 | -0.017 |

The bias of the observed mean $\log_{10}$ titer was within -0.04 $\log_{10}$ and 0.09 $\log_{10}$ of the $\log_{10}$ nominal titer for all the concentration levels tested, with the two outliers removed. The maximum deviation between the observed mean $\log_{10}$ titer and the best fitted 1st-order model (deviation from linearity) was < 0.08 $\log_{10}$ for each concentration level tested, with the two outliers removed.

**Based on the analyses above, the CAP/CTM CMV Test was found in the CMV AD169 Linearity Study to give a linear response from 9.1E+01 ($\log_{10}$ = 1.96) CMV DNA IU/mL to at least 9.1E+06 ($\log_{10}$ = 6.96) CMV DNA IU/mL, with maximum deviation from linearity of less than or equal to 0.3 $\log_{10}$ in this interval. The results of this study support the claimed LLoQ of 1.37E+02 IU/mL and the claimed linear range of 1.37E+02 to 9.1E+06 IU/mL.**

***Linear Range Using CMV Glycoprotein B (gB) Genotypes 1 – 4 Specimens***

Four 5-member panels were used to verify the linear range of the CAP/CTM CMV Test for CMV glycoprotein B (gB) genotypes 1, 2, 3, and 4 in accordance with CLSI Guideline EP6-A, Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline. Each of the four panels was prepared by diluting the four gB plasmids to 9.10E+06, 3.35E+05, 1.24E+04, 4.55E+02, and 1.37E+02 IU/mL using a pool of CMV DNA negative EDTA plasma as the diluent.

Each of the gB genotype samples used in this study were full-length UL54 gene plasmid clones. Plasmid clones for CMV gB genotypes 1 and 2 were prepared using CMV Merlin and CMV AD169, respectively. Plasmid clones for CMV gB genotypes 3 and 4 were prepared using clinical specimens. Each of the four glycoprotein B genotype plasmid stocks was assigned a concentration by the Calibrator Bracketing Method using CMV Secondary Standard (Lot TRLOT03) which is traceable to the 1st WHO International Standard for Human Cytomegalovirus (NIBSC code 09/162). Four 5-member panels were then prepared (one panel for each glycoprotein B genotype) by serial dilution using a pool of negative CMV DNA EDTA plasma (see the table below).

CMV gB Genotypes Linearity Study — Panel Members

| Nominal Titer (IU/mL) | Nominal Titer (copy/mL) | $Log_{10}$ Nominal Titer (IU/mL) | Description |
|---|---|---|---|
| 9.10E+06 | 1.000E+07 | 6.96 | ULoQ |
| 3.35E+05 | 3.684E+05 | 5.53 | Intermediate level |
| 1.24E+04 | 1.360E+04 | 4.09 | Intermediate level |
| 4.55E+02 | 5.000E+02 | 2.66 | 3.3x LLoQ |
| 1.37E+02 | 1.500E+02 | 2.14 | 1x LLoQ |

One run was comprised of one replicate of each genotype panel member. A total of 16 runs were completed in four days for a total of 16 replicates per genotype panel member evenly distributed among two lots of CAP/CTM CMV Test kit reagents and two CAP/CTM systems. Eight replicates were run for each level for each lot of CAP/CTM CMV Test kit reagents; a minimum of seven valid replicates per lot for each level were required for data analysis.

In addition, bias was assessed by evaluation of mean $log_{10}$ differences between the observed and nominal value.

Acceptance criteria for the linearity study are the following:

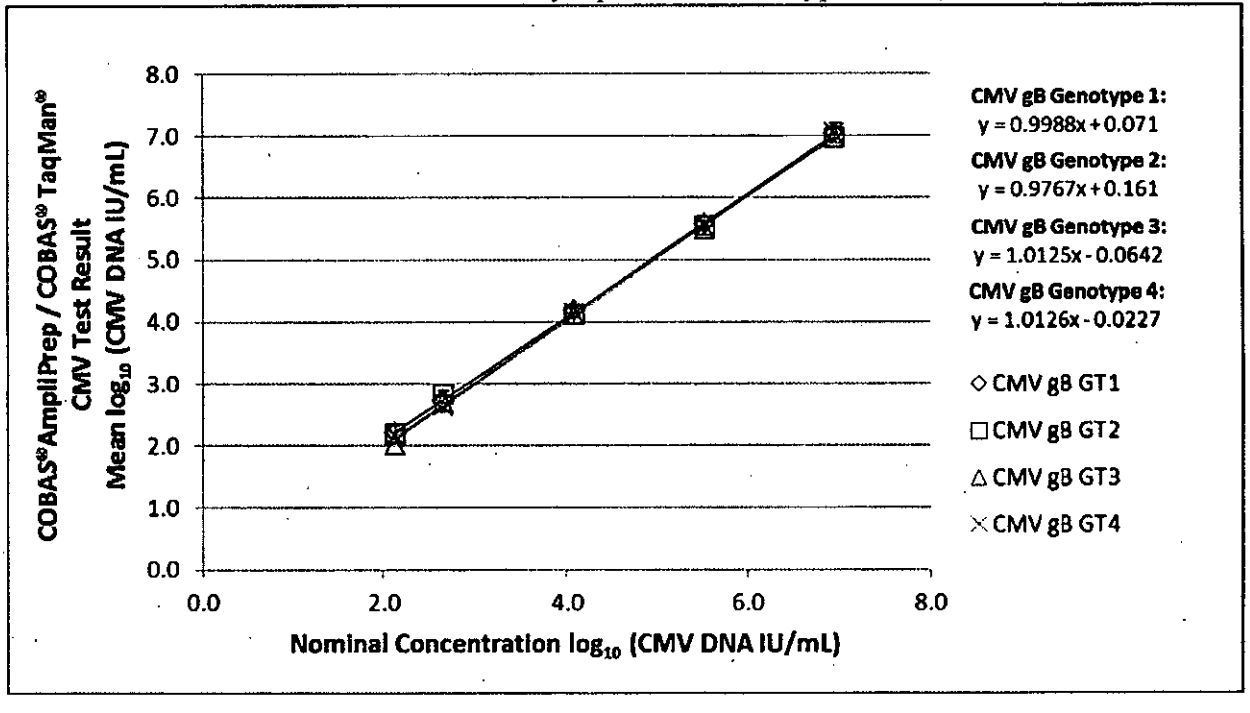
Linearity range: Maximum deviation between linear regression and better fitting non-linear regression (i.e., deviation from linearity) should be $\leq 0.3$ $log_{10}$ for each concentration level tested.

Repeatability: ≤ 0.3 $\log_{10}$ for each genotype tested (all concentration levels pooled).

There were no invalid results in this study. No outlier was identified by the Extreme Studentized Deviate Test (alpha = 0.01). The linear (1st order), quadratic (2nd order), and cubic (3rd order) polynomial models were fitted to the dataset for each gB genotype 1-4. It was determined that for each of the four gB genotypes, the linear (1st order) model was the best fit.

Linear regression analyses of the mean $\log_{10}$ observed titer vs. the nominal $\log_{10}$ titer for each of the gB genotype linearity panels is shown for gB genotypes 1 - 4 in the following figure: .

CMV gB Genotypes Linearity Study — Linearity of the CAP/CTM CMV Test for CMV Glycoprotein B Genotypes 1 to 4




The accuracy of the observed mean $\log_{10}$ titer was within 0.08 $\log_{10}$, 0.18 $\log_{10}$, 0.12 $\log_{10}$ and 0.09 $\log_{10}$ of the nominal $\log_{10}$ titer for all the concentration levels tested with gB genotypes 1 to 4, respectively. The maximum deviation between the observed mean $\log_{10}$ titer and the best fitted 1st-order model (deviation from linearity) was ≤ 0.03 $\log_{10}$, ≤ 0.08 $\log_{10}$, ≤ 0.08 $\log_{10}$, and ≤ 0.03 $\log_{10}$ for each concentration level tested with gB genotypes 1 to 4, respectively.

CMV gB Genotypes Linearity Study — Observed Titer Summary for CMV Glycoprotein B Genotype 1

| Nominal Concentration (IU/mL) | Log10 Nominal (IU/mL) | N | AVG Titer (IU/mL) | SD Titer (IU/mL) | AVG $log_{10}$ Titer (IU/mL) | SD $log_{10}$ Titer (IU/mL) | Bias | Predicted 1st-order | Deviation |
|---|---|---|---|---|---|---|---|---|---|
| 1.37E+02 | 2.14 | 16 | 1.71E+02 | 6.87E+01 | 2.21 | 0.15 | 0.07 | 2.20 | 0.01 |
| 4.55E+02 | 2.66 | 16 | 5.70E+02 | 2.12E+02 | 2.73 | 0.16 | 0.07 | 2.73 | 0.00 |
| 1.24E+04 | 4.09 | 16 | 1.48E+04 | 2.30E+03 | 4.16 | 0.07 | 0.07 | 4.16 | 0.00 |
| 3.35E+05 | 5.53 | 16 | 3.70E+05 | 5.68E+04 | 5.56 | 0.07 | 0.04 | 5.59 | -0.03 |
| 9.10E+06 | 6.96 | 16 | 1.17E+07 | 4.93E+06 | 7.04 | 0.16 | 0.08 | 7.02 | 0.02 |

CMV gB Genotypes Linearity Study — Observed Titer Summary for CMV Glycoprotein B Genotype 2

| Nominal Concentration (IU/mL) | Log10 Nominal (IU/mL) | N | AVG Titer (IU/mL) | SD Titer (IU/mL) | AVG $log_{10}$ Titer (IU/mL) | SD $log_{10}$ Titer (IU/mL) | Bias | Predicted 1st-order | Deviation |
|---|---|---|---|---|---|---|---|---|---|
| 1.37E+02 | 2.14 | 16 | 1.71E+02 | 7.48E+01 | 2.19 | 0.19 | 0.06 | 2.25 | -0.06 |
| 4.55E+02 | 2.66 | 16 | 7.00E+02 | 1.29E+02 | 2.84 | 0.08 | 0.18 | 2.76 | 0.08 |
| 1.24E+04 | 4.09 | 16 | 1.36E+04 | 1.66E+03 | 4.13 | 0.05 | 0.04 | 4.16 | -0.03 |
| 3.35E+05 | 5.53 | 16 | 3.61E+05 | 6.39E+04 | 5.55 | 0.08 | 0.03 | 5.56 | -0.01 |
| 9.10E+06 | 6.96 | 16 | 9.35E+06 | 1.55E+06 | 6.96 | 0.07 | 0.01 | 6.96 | 0.00 |

CMV gB Genotypes Linearity Study — Observed Titer Summary for CMV Glycoprotein B Genotype 3

| Nominal Concentration (IU/mL) | Log10 Nominal (IU/mL) | N | AVG Titer (IU/mL) | SD Titer (IU/mL) | AVG $log_{10}$ Titer (IU/mL) | SD $log_{10}$ Titer (IU/mL) | Bias | Predicted 1st-order | Deviation |
|---|---|---|---|---|---|---|---|---|---|
| 1.37E+02 | 2.14 | 16 | 1.23E+02 | 6.71E+01 | 2.02 | 0.29 | -0.12 | 2.10 | -0.08 |
| 4.55E+02 | 2.66 | 16 | 5.29E+02 | 1.45E+02 | 2.71 | 0.11 | 0.05 | 2.63 | 0.08 |
| 1.24E+04 | 4.09 | 16 | 1.30E+04 | 9.54E+02 | 4.11 | 0.03 | 0.02 | 4.08 | 0.03 |
| 3.35E+05 | 5.53 | 16 | 3.11E+05 | 3.40E+04 | 5.49 | 0.05 | -0.03 | 5.53 | -0.04 |
| 9.10E+06 | 6.96 | 16 | 9.81E+06 | 1.77E+06 | 6.99 | 0.07 | 0.03 | 6.98 | 0.01 |

CMV gB Genotypes Linearity Study — Observed Titer Summary for CMV Glycoprotein B Genotype 4

| Nominal Concentration (IU/mL) | Log10 Nominal (IU/mL) | N | AVG Titer (IU/mL) | SD Titer (IU/mL) | AVG $log_{10}$ Titer (IU/mL) | SD $log_{10}$ Titer (IU/mL) | Bias | Predicted 1st-order | Deviation |
|---|---|---|---|---|---|---|---|---|---|
| 1.37E+02 | 2.14 | 16 | 1.58E+02 | 5.64E+01 | 2.17 | 0.18 | 0.03 | 2.14 | **0.03** |
| 4.55E+02 | 2.66 | 16 | 4.64E+02 | 1.50E+02 | 2.65 | 0.14 | -0.01 | 2.67 | -0.02 |
| 1.24E+04 | 4.09 | 16 | 1.36E+04 | 1.44E+03 | 4.13 | 0.05 | 0.04 | 4.12 | 0.01 |
| 3.35E+05 | 5.53 | 16 | 3.45E+05 | 3.13E+04 | 5.54 | 0.04 | 0.01 | 5.57 | -0.03 |
| 9.10E+06 | 6.96 | 16 | 1.22E+07 | 7.19E+06 | 7.05 | 0.16 | **0.09** | 7.02 | 0.03 |

**Based on these analyses above, the CAP/CTM CMV Test was found in the CMV gB Genotypes Linearity Study to give a linear response from 1.37E+02 ($log_{10}$ = 2.14) CMV DNA IU/mL to 9.1E+06 ($log_{10}$ = 6.96) CMV DNA IU/mL, with maximum deviation from linearity of less than or equal to 0.3 $log_{10}$ in this interval. The results of this study support the claimed LLoQ of 1.37E+02 IU/mL and the claimed linear range of 1.37E+02 to 9.1E+06 IU/mL.**

In addition, the linearity performance of the CAP/CTM CMV Test detecting CMV gB genotypes 1 to 4 was also assessed by determining the maximum difference between gB1 and the other three gB genotypes (i.e., gB 2, 3, and 4) as the following:

CAP/CTM CMV Test linearity by CMV gB Genotype

| CMV gB Genotype | Linear Equation in gB Genotype Linearity Study | Maximum Difference[a] Between gB1 and Corresponding gB Genotype (log IU/mL) |
|---|---|---|
| 1 | y = 0.9988x + 0.071 | n/a |
| 2 | y = 0.9767x + 0.161 | 0.06 |
| 3 | y = 1.0125x - 0.0642 | 0.11 |
| 4 | y = 1.0126x -0.0227 | 0.06 |

[a] The maximum difference was obtained at the assay ULoQ or LLoQ

**This analysis demonstrated that the CAP/CTM CMV Test is able to quantitate different CMV genotypes across the linear range with deviation of not more 0.11 $\log_{10}$ IU/mL.**

***Precision***

The precision of the CAP/CTM CMV Test was determined according to CLSI guideline EP05-A2, Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline — Second Edition by analysis of an 8-member panel. The panel was prepared using a CMV DNA-positive clinical specimen for the lower end of the dynamic range and by diluting cultured CMV (strain AD169) for the mid and high end of the dynamic range. Both source materials were diluted in CMV-negative EDTA plasma. The 8-member panel covered a range from 1.82E+02 CMV DNA IU/mL to 9.10E+06 CMV DNA IU/mL.

Cultured human CMV stock material (Advanced Biotechnology Inc.; Columbia, MD; M/N: 10-103-100, Lot: 7E0006-PV, strain AD169, genotype 2 based on the glycoprotein B gene UL55) was diluted into a pool of CMV DNA negative EDTA plasma to prepare a part of the 8-level linearity panel. The cultured human CMV stock material (source material for the RMS CMV Secondary Standard, Lot TRLOT03) was value assigned at 7.28E+10 IU/mL based on the CMV Secondary Standard lot TRLOT03 (2.184E+04 IU/mL, n=62, COBAS® AMPLICOR® CMV MONITOR Test).

A CMV DNA positive clinical specimen (Roche IMPAACT study, Accession #: T439708-3) was diluted into a pool of CMV DNA negative EDTA plasma to prepare the rest of the 8-level linearity panel. The clinical specimen was value assigned at 7.30E+05 IU/mL by the CAP/CTM CMV Test by normalizing the mean measured titer (n=12) of a prepared dilution of the clinical specimen to the mean measured titer (n=12) of the CMV Secondary Standard (Lot TRLOT03) tested at two levels (2.184E+04

IU/mL (neat) and diluted to 4.55E+02 IU/mL), which bracketed the level of the prepared clinical specimen.

Precision Panel Levels

| Level # | Nominal Titer (IU/mL) | Nominal $Log_{10}$ Titer ($Log_{10}$ IU/mL) | Description | CMV DNA Source |
|---|---|---|---|---|
| 8 | 9.10E+06 | 6.96 | ULoQ | Cultured Virus |
| 7 | 9.10E+05 | 5.96 | <ULoQ | Cultured Virus |
| 6 | 9.10E+04 | 4.96 | Intermediate Level | Cultured Virus |
| 5 | 1.82E+04 | 4.26 | Intermediate Level | Cultured Virus |
| 4 | 4.55E+02 | 2.66 | Intermediate Level | Cultured Virus |
| 3 | 9.10E+02 | 2.96 | LLoQ minimum product requirement | Clinical Specimen |
| 2 | 2.91E+02 | 2.46 | ~LLoQ | Clinical Specimen |
| 1 | 1.82E+02 | 2.26 | LLoQ target product requirement | Clinical Specimen |

Each panel member was tested with two replicates per run, with two runs per day, for 12 days, and for each of the two workflows (COBAS® AmpliPrep docked to a COBAS® TaqMan® and COBAS® AmpliPrep linked to a COBAS® TaqMan® 48) for a total 96 replicates per panel member, with replicates evenly distributed across three reagent kit lots, four COBAS® AmpliPrep/COBAS® TaqMan® systems, and at least two operators.

All results, without any exclusion of outliers, were included in the analysis. Each sample was carried through the entire CAP/CTM CMV Test procedure, including specimen preparation, amplification, and detection. Therefore, the precision reported below represents all aspects of the test procedure.

Precision of the CAP/CTM CMV Test (EDTA-Plasma in IU/mL)

| Level | Average Observed CMV DNA Titer (IU/mL) | Total No. Replicates | Within- Run %CV | Between-Run/Operator* %CV | Between-Lot %CV | Between-Workflow %CV | Between Instrument %CV | Between Day %CV | Total %CV |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 8.29E+01 | 96 | 50% | 0% | 18% | 0% | 6% | 0% | 55% |
| 2 | 1.66E+02 | 96 | 33% | 12% | 14% | 0% | 14% | 0% | 41% |
| 3 | 2.83E+02 | 96 | 54% | 0% | 0% | 0% | 15% | 18% | 60% |
| 4 | 5.20E+02 | 96 | 19% | 16% | 14% | 3% | 12% | 0% | 32% |
| 5 | 1.49E+04 | 96 | 29% | 10% | 4% | 3% | 3% | 0% | 31% |
| 6 | 8.00E+04 | 96 | 17% | 13% | 10% | 0% | 7% | 5% | 25% |
| 7 | 8.05E+05 | 96 | 15% | 0% | 11% | 0% | 12% | 6% | 23% |
| 8 | 7.62E+06 | 96 | 20% | 0% | 18% | 3% | 15% | 7% | 32% |

*Between-Run is confounded with Between-Operator and therefore, presented as Between Run/Operator.

Precision of the CAP/CTM CMV Test (EDTA-Plasma in $\log_{10}$ IU/mL)

| Level | Average Observed CMV DNA Titer ($\log_{10}$ IU/mL) | Total No. Replicates | Within-Run SD | Between-Run/Operator* SD | Between-Lot SD | Between-Workflow SD | Between Instrument SD | Between Day SD | Total SD |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 1.92 | 96 | 0.21 | 0.00 | 0.08 | 0.00 | 0.03 | 0.00 | 0.22 |
| 2 | 2.22 | 96 | 0.14 | 0.05 | 0.06 | 0.00 | 0.06 | 0.00 | 0.17 |
| 3 | 2.45 | 96 | 0.22 | 0.00 | 0.00 | 0.00 | 0.06 | 0.08 | 0.24 |
| 4 | 2.72 | 96 | 0.08 | 0.07 | 0.06 | 0.01 | 0.05 | 0.00 | 0.14 |
| 5 | 4.17 | 96 | 0.12 | 0.04 | 0.02 | 0.01 | 0.01 | 0.00 | 0.13 |
| 6 | 4.90 | 96 | 0.07 | 0.06 | 0.04 | 0.00 | 0.03 | 0.02 | 0.11 |
| 7 | 5.91 | 96 | 0.07 | 0.00 | 0.05 | 0.00 | 0.05 | 0.03 | 0.10 |
| 8 | 6.88 | 96 | 0.08 | 0.00 | 0.08 | 0.01 | 0.06 | 0.03 | 0.13 |

*Between-Run is confounded with Between-Operator and therefore, presented as Between Run/Operator.

***Performance with CMV DNA-Negative Samples***

The performance of the CAP/CTM CMV Test with CMV DNA-negative samples was determined by testing 227 anti-CMV IgG seronegative EDTA plasma specimens obtained from an FDA-registered Donor Testing Laboratory. The 227 anti-CMV IgG seronegative EDTA plasma specimens were from de-identified EDTA plasma specimens from patients under routine diagnostic care.

For CMV IgG seronegative specimens, all 227 specimens tested negative for CMV DNA by the CAP/CTM CMV Test, yielding a 100% negativity rate with 95% CI: 98.3% to 100%.

***Analytical Specificity (Cross-reactivity)***

Various pathogenic organisms that may be present in patient specimens were evaluated for cross-reactivity with the CAP/CTM CMV Test by adding cultured organisms (viruses, bacteria, fungi) or positive clinical specimens at 1.0E+06 particles/mL input concentration into CMV DNA-negative human EDTA plasma and into CMV DNA-positive EDTA plasma at 6.82E+02 IU/mL CMV. Each sample was tested in triplicates using the CAP/CTM CMV Test.

Analytical Specificity Specimens

| **Human Herpesviruses** | **Other Viruses** |
|---|---|
| Herpes simplex virus types 1 and 2<br>Varicella-Zoster virus<br>Epstein-Barr virus<br>Human herpesvirus 6, 7, and 8 | BK Polyomavirus<br>JC Polyomavirus<br>Hepatitis virus A, B, and C<br>HIV type 1<br>Adenovirus 5<br>Parvovirus B19 |
| **Bacteria** | **Fungi** |
| *Mycoplasma pneumoniae*<br>*Propionibacterium acnes*<br>*Salmonella typhimurium*<br>*Staphylococcus aureus*<br>*Streptococcus pneumoniae* | *Aspergillus niger*<br>*Candida albicans*<br>*Cryptococcus neoformans* |

Analytical Specificity Specimens – Method of Quantitation

| Specimen # | Description | Unit | Method of Quantitation | Specimen # | Description | Unit | Method of Quantitation |
|---|---|---|---|---|---|---|---|
| S1. | Adenovirus 5 | PFU/mL | Plaque Assay | S13. | Human immunodeficiency virus type 1 | PFU/mL | Plaque Assay |
| S2. | *Aspergillus niger* | CFU/mL | Plate Count | S14. | Herpes simplex virus type 1 | PFU/mL | Plaque Assay |
| S3. | BK Polyomavirus | copies/mL | Real-Time PCR using Roche LightCycler® | S15. | Herpes simplex virus type 2 | PFU/mL | Plaque Assay |
| S4. | *Candida albicans* | CFU/mL | Plate Count | S16. | JC Polyomavirus | copies/ mL | Real-Time PCR using Roche LightCycler® |
| S5. | *Cryptococcus neoformans* | CFU/mL | Plate Count | S17. | *Mycoplasma pneumoniae* | CFU/mL | Plate Count |
| S6. | Epstein-Barr virus | copies/mL | Real-Time PCR using Roche LightCycler® | S18. | Parvovirus B19 | IU/mL | cobas® TaqScreen DPX Test |
| S7. | Hepatitis A virus | copies/mL | RT-qPCR | S19. | *Propionibacterium acnes* | CFU/mL | Plate Count |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| S8. | Hepatitis B virus | IU/mL | High Pure System / COBAS® TaqMan® HBV Test, US-IVD | S20. | *Salmonella typhimurium* | CFU/mL | Plate Count |
| S9. | Hepatitis C virus | IU/mL | COBAS® AmpliPrep / COBAS® TaqMan® HCV Test, US-IVD | S21. | *Staphylococcus aureus* | CFU/mL | Plate Count |
| S10. | Human Herpesvirus type 6 | vp/mL | Transmission Electron Microscopy (TEM) | S22. | *Streptococcus pneumoniae* | CFU/mL | Plate Count |
| S11. | Human Herpesvirus type 7 | copies/mL | Real-Time PCR using Roche LightCycler® | S23. | Varicella-Zoster Virus | PFU/mL | Plaque Assay |
| S12. | Human Herpesvirus type 8 | copies/mL | Real-Time PCR using Roche LightCycler® | | | | |

None of the organisms at the concentrations tested showed cross-reactivity with the CAP/CTM CMV Test. CMV-positive specimens returned titer results that were within ± 0.3 $\log_{10}$ from a CMV-positive control without the potentially cross-reactive organism spiked.

***Interfering Substances (Exogenous)***

A total of 21 exogenous substances (i.e., pharmaceutical drugs) that may be present in patient specimens were evaluated for potential interference with the CAP/CTM CMV Test by adding the drugs at levels recommended in CLSI guideline EP07-A2, Interference Testing in Clinical Chemistry; Approved Guideline – 2nd Edition, or three times the peak plasma drug concentration level ($C_{max}$) (see footnote in the table below), whichever was greater, into CMV DNA-negative human EDTA plasma, and into CMV DNA-positive EDTA plasma at 6.82E+02 IU/mL CMV. Each sample was tested in triplicate using the CAP/CTM CMV Test.

These 21 exogenous substances, described in the table below, were shown not to interfere with the CAP/CTM CMV Test when tested at the levels mentioned above.

The mean $\log_{10}$ titer of the positive CMV specimens containing a potential interfering substance was within ± 0.3 $\log_{10}$ of the mean $\log_{10}$ titer of the positive CMV specimens without the potential interfering substance. In addition, specimens negative for CMV DNA, regardless of containing potential interfering substance, had "Target Not Detected" as a result.

Potentially Interfering Exogenous Substance

| ID | Drug | | Function | CLSI Test Concentration (ug/mL) | 1 x $C_{max}$ (ug/mL) | 3 x $C_{max}$ (ug/mL) |
|---|---|---|---|---|---|---|
| 1. | Azathioprine (Imuran)† | | Immunosuppressant | 3.0 | 1.0 | 3.0 |
| 2. | Sulfamethoxazole† | | Antibiotic | 399.8 | 68 | 204 |
| 3. | Trimethoprim† | | Antibiotic | 40.1 | 2 | 6 |
| 4. | Cefotetan (Cefotan) † | | Antibiotic | NA | 237 | 711 |
| 5. | Cidofovir (Vistide)† | | Anti-CMV | NA | 19.6 | 58.8 |
| 6. | Cyclosporine (Genraf, Neoral, Sandimmune) † | | Immunosuppressant | NA | 1.8 | 5.4 |
| 7. | Everolimus (Afinitor)‡ | | Immunosuppressant | NA | 4.0 | 12.0 |
| 8. | Fluconazole (Diflucan or Trican)† | | Anti-fungal | 75.0 | 14.1 | 42.3 |
| 9. | Foscarnet (Foscavir, Sodium phosphonoformate tribasic hexahydrate)† | | Anti-CMV | NA | 187 | 561 |
| 10. | Ganciclovir (Cytovene)† | | Anti-CMV | NA | 9.0 | 27.0 |
| 11. | Mycophenolate mofetil (CellCept)† | | Immunosuppressant | NA | 26.0 | 78.0 |
| 12. | Mycophenolate sodium (Myfortic)† | | Immunosuppressant | NA | 37 | 111 |
| 13. | Zosyn | Piperacillin† | Antibiotic | NA | 298 | 894 |
| 14. | | Tazobactam sodium† | Antibiotic | NA | 34 | 102 |
| 15. | Prednisone‡ | | Immunosuppressant | 0.3 | 12.0 | 36.0 |
| 16. | Sirolimus (Rapamune or rapamycin)† | | Immunosuppressant | NA | 0.035 | 0.105 |
| 17. | Tacrolimus (FK506 or Prograf)† | | Immunosuppressant | 0.040 | 0.069 | 0.207 |
| 18. | Timentin | Clavulanate potassium† | Antibiotic | 7.0 | 8.0 | 24.0 |
| 19. | | Ticarcillin disodium† | Antibiotic | NA | 330.0 | 990.0 |
| 20. | Valganciclovir (Valcyte)† | | Anti-CMV | NA | 9.5 | 28.5 |
| 21. | Vancomycin† | | Antibiotic | 100.0 | 63 | 189 |

* Test levels recommended in the CLSI Guideline EP07-A2 (Interference Testing in Clinical Chemistry; Approved Guideline-Second Edition)

† Levels of ~1x$C_{max}$ found at www.drugs.com/pro/

‡ Levels calculated according to CLSI guidelines as 1 and 3 times the maximum dose in 5L of blood. Doses used for calculations were 20 mg and 60 mg for Everolimus and Prednisone, respectively (ref. www.drugs.com/pro/ for dose information)

***Interfering Substances (Endogenous)***

A list of physiologically occurring substances that may be present in patient specimens were evaluated for potential interference with the CAP/CTM CMV Test by adding the endogenous substances at levels recommended in CLSI guideline EP07-A2, Interference Testing in Clinical Chemistry; Approved Guideline – 2nd Edition, into CMV DNA-negative human EDTA plasma, and into CMV DNA-positive EDTA

plasma at 6.82E+02 IU/mL CMV. Each sample was tested in duplicate using the CAP/CTM CMV Test.

The physiologically occurring substances shown in the table below, when tested at the test levels recommended in CLSI guideline EP07-A2, were shown not to interfere with the CAP/CTM CMV Test.

Potentially Interfering Endogenous Substances

| Potential Interfering Substance | Reference Interval | Test Level |
|---|---|---|
| Conjugated bilirubin (Ditaurobilirubin) | 0 – 0.2 mg/dL | ≥ 20 mg/dL* |
| Un-conjugated Bilirubin | 0.3 – 1.2 mg/dL | ≥ 20 mg/dL* |
| Hemoglobin | 100 - 200 mg/dL | ≥ 200 mg/dL* |
| Human DNA | N/A | 0.4 mg/dL |
| Human albumin | 3,900 – 5,100 mg/dL | ≥ 6,000 mg/dL* |
| Triglycerides | 30 – 330 mg/dL | ≥ 3,300 mg/dL* |
| Systemic Lupus Erythematosus | Natural specimens obtained from 10 different patients (per disease) diagnosed with one of the three auto-immune diseases. | |
| Rheumatoid Factor | | |
| Antinuclear antibody | | |

* Test levels recommended in the CLSI Guideline EP07-A2

The mean $\log_{10}$ titer of the positive CMV specimens containing a potential endogenous interfering substance were all within ± 0.3 $\log_{10}$ of the mean $\log_{10}$ titer of the CMV positive control specimens without the potential interfering substance. In addition, specimens negative for CMV DNA and containing elevated levels of potential interfering substance all tested as "Target Not Detected."

***Sample to Sample Cross Contamination (Carryover)***

The potential for sample to sample carryover contamination was evaluated for the CAP/CTM CMV Test by performing five runs, with each run comprised of three full SK-24 racks of alternating high positive samples (at a concentration of 9.10E+06 IU/mL) and negative specimens. Out of 165 CMV negative specimens tested, 0% were positive for CMV DNA when tested with the CAP/CTM CMV Test (95% CI: 0% to 2.3%).

***Robustness — Whole System Failure***

A study was conducted to assess the whole system failure rate when the entire process is performed as prescribed by the manufacturer. The whole system failure rate is defined as the number of false negative results observed in a minimum of 100 replicates at a titer level less than or equal to approximately three times the LoD.

For this study, a low CMV DNA positive sample was prepared by diluting a CMV DNA positive clinical specimen (IMPACT Study Accession No: T439708-3) with a pool of CMV DNA negative EDTA plasma to a final concentration of 2.72E+02 IU/mL, which is approximately 3 x LoD. Five SK24 racks were run on an individual COBAS® AmpliPrep/COBAS® TaqMan® system and another five SK24 racks were run on an individual COBAS® AmpliPrep/COBAS® TaqMan® 48 system. One kit lot of reagents was used for this study. Each SK24 rack contained three controls (one Negative Control, one Low Positive Control, and one High Positive Control) and 20 replicates of the CMV specimen at 2.72E+02 IU/mL (3x LoD).

The results from this study showed that 100 replicates of a CMV specimen at a concentration of 3x LoD reported 100% positivity rate for both the CAP/CTM and the CAP/CTM 48 workflows (95% CI: 96.3% to 100%), meeting the minimum requirement for Whole System Failure.

***Unprocessed Specimen Stability***

Unprocessed specimen stability was determined by examining the $\log_{10}$ titers results of 10 unique CMV DNA negative clinical specimens spiked with a CMV DNA positive clinical specimen to 5x LLoQ and two CMV DNA negative clinical specimens. All 12 specimens were stored at conditions and lengths of times that simulated the maximum intended limits for specimen handling (e.g., plasma separated from whole blood within six hours), transportation (no more than six hours at 25°C), storage (2-8°C or frozen), and specimen testing (on the COBAS® AmpliPrep instrument for six hours prior to processing).

For the unprocessed specimens to be considered stable, the following criteria must be met:

- For CMV (+) specimens at 5 x LLoQ, the difference between the mean measured and Day 0 mean measured $\log_{10}$ titer results must be within ±0.3 $\log_{10}$ for each condition at each time point to be considered stable. In addition, all replicates must have a positive result for each condition at each time point to be considered stable.
- For CMV (-) specimens, all replicates must have a "CMV Target Not Detected" result for each condition at each time point to be considered stable.

The mean $\log_{10}$ titer was within ±0.3 $\log_{10}$ of the Day 0 mean $\log_{10}$ titer for each time point up through seven days at 2-8°C and through six weeks at -20°C. The observed $\log_{10}$ differences in titer across the time points ranged from -0.28 to 0.36.

Unprocessed Specimen Stability Summary

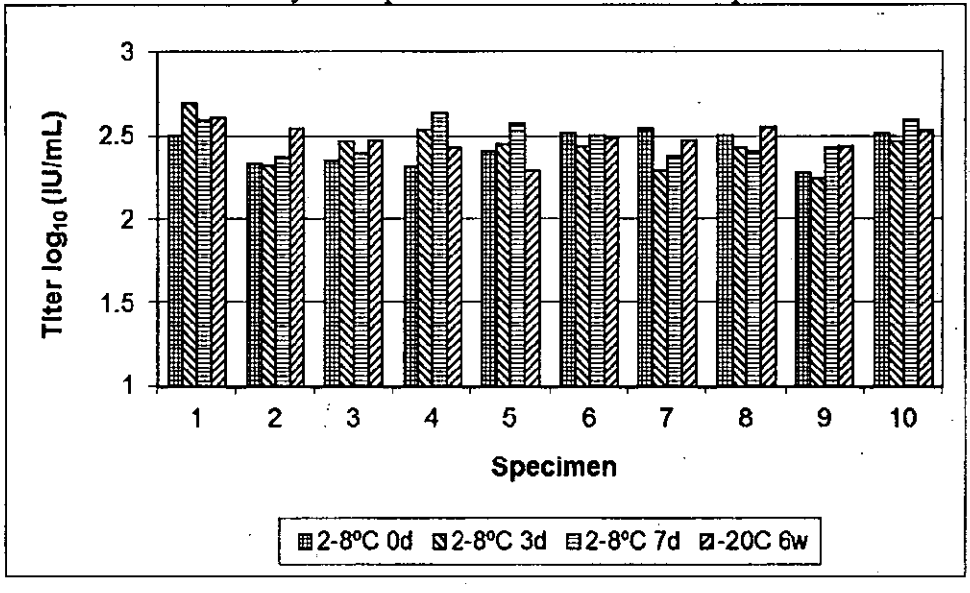
| Time Point | Mean $\log_{10}$ Titer | |
|---|---|---|
| | 4°C | -20°C |
| D0 | 2.66 | 2.66 |
| D3 | 2.67* | N/A |

| | | |
|---|---|---|
| D7 | 2.73* | N/A |
| W6 | N/A | 2.77#* |

* Includes 6 hours On-board Stability (38°C)
\# Includes 1, 2, and 3 Freeze/Thaw Cycles

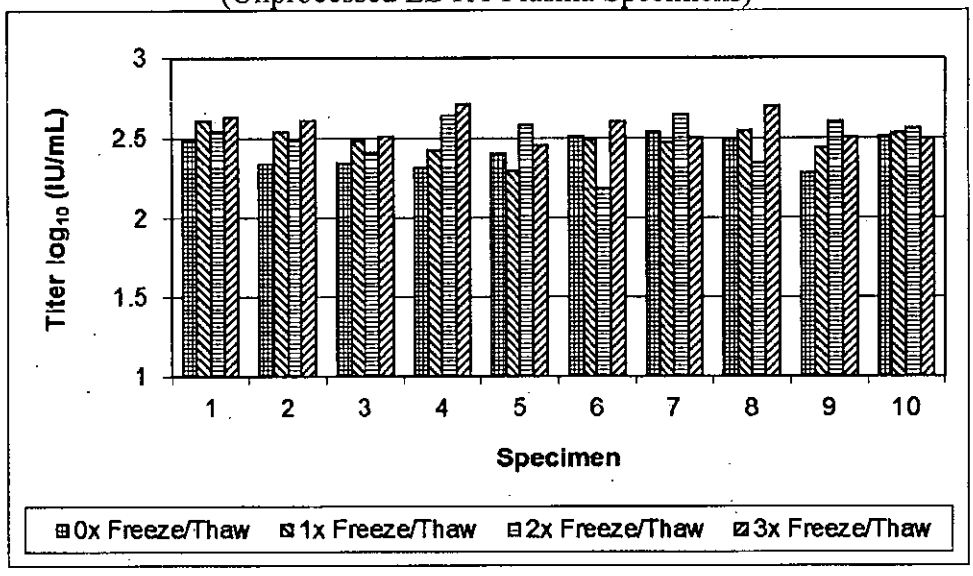
The average $\log_{10}$ titer was also within ±0.3 $\log_{10}$ of the Day 0 average $\log_{10}$ titer for the 6 week time point for 1, 2, and 3 freeze/thaw cycles. All 12 CMV specimens tested met the criteria describe above after storage at 2-8°C for seven days and at -20°C for six weeks. The figure below shows the $\log_{10}$ titer result for each of the 10 low positive specimens over time.

CMV Stability in Unprocessed EDTA-Plasma Specimens




In addition, all 12 CMV specimens stored frozen at -20°C for six weeks also met the criteria described above after three freeze/thaw cycles. The figure below shows the $\log_{10}$ titer result for each of the 10 low positive specimens over multiple freeze/thaw cycles. The observed $\log_{10}$ differences in titer across the freeze/thaw conditions ranged from -0.33 to 0.32.

CMV Results after up to Three Freeze/Thaw Cycles
(Unprocessed EDTA-Plasma Specimens)




**Based on the results of this functional stability testing, the following unprocessed specimen stability statements in the product package insert are substantiated:**

- **EDTA whole blood may be stored or transported at room temperature (25°C) for up to six hours. Plasma should be separated from whole blood within six hours of collection.**
- **Unprocessed EDTA-plasma CMV specimens are stable for up to seven days at 2-8°C and up to six weeks at -20°C.**
- **Unprocessed EDTA-plasma CMV specimens are stable for up to three freeze/thaw cycles.**
- **EDTA-plasma CMV specimens are stable on-board the COBAS® AmpliPrep Instrument (38°C) for up to six hours.**

***Prepared Specimen Stability (PCR Mixture)***

To determine the stability of the PCR mixture (35µL CMV MMx, 15µL $MgCl_2$, and 50µL processed sample) on-board the COBAS® AmpliPrep Instrument (CAP) prior to amplification/ detection on the COBAS® TaqMan® Analyzer (CTM), CAP/CTM CMV Test kit controls (HPC, LPC, and NC) and CMV samples at four concentration levels (1.37E+02 IU/mL, 4.55E+03 IU/mL, 2.18E+04 IU/mL, and 9.10E+06 IU/mL) were processed on the CAP instrument and the resulting PCR Mixtures were amplified/detected on the CTM after 0, 120, 180, and 240 minutes incubation at 40°C in the dark. (Note: 38°C is defined as the upper temperature limit for the CAP environment area).

For the prepared specimens (PCR mixtures) to be considered stable, the following criteria must be met:

- For 9.10E+06 IU/mL, 2.18E+04 IU/mL, and 4.55E+03 IU/mL samples, the mean $\log_{10}$ titer of the replicates must be within ± 0.3 $\log_{10}$ titer units of the mean $\log_{10}$ titer for Time Point 0.
- For 1.37E+02 IU/mL samples, the upper bound of 95% confidence interval for the observed positive hit rate should be greater than or equal to 99% (based on Clopper-Pearson confidence intervals). For example, 9 out of 10 results must be positive (90% hit rate with upper bound of 95% confidence interval of 99.8). If repeat testing occurs, 19 out of 20 trials must be positive (95% hit rate with upper 95% confidence interval of 99.9%).
- All heat stressed CAP/CTM CMV Controls must be valid.

All kit controls evaluated for PCR mixture stability were valid up to 240 minutes.

For 4.55E+03 IU/mL, 2.18E+04 IU/mL, and 9.10E+06 IU/mL PCR mixtures, the difference in mean $\log_{10}$ titers at each time point and Time 0 were within ±0.12 $\log_{10}$ at all time points. For 1.37E+02 IU/mL PCR mixtures, the upper bound of 95% confidence interval was 100% (10 positives/10 replicates) at 0, 120, and 180 minutes and was 99.8% (9 positives/10 replicates) at 240 minutes. Based on this data, the PCR mixtures remained within specification when stored at 40°C in the dark for up to 240 minutes.

After the eluate is mixed with activated MMx for the first sample of the batch, each subsequent sample in the batch takes approximately 4 minutes to complete. With a maximum batch size of 24, the PCR mixture for the first sample would be sitting onboard for ~92 minutes at the completion of the batch. When calculating the allowable time between the completion of the specimen and control preparation and the start of the amplification/detection, at least 92 minutes should be subtracted from the total stability time of 240 minutes. To be conservative, the COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer run must be started within 120 minutes following completion of specimen and control preparation.

**Based on the results of this functional stability testing, the following processed specimen stability (PCR mixture) claim in the product package insert is substantiated:**

- **The COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer run must be started within 120 minutes following completion of specimen and control preparation.**

***Open Vial Reagent Stability***

Onboard Reagent and Open Vial Stability for the CAP/CTM CMV Test was verified at CMV DNA concentrations of 1.37E+02 IU/mL, 9.10E+04 IU/mL, and 9.10E+06 IU/mL. Stability of the test was verified by simulating onboard storage of cap-punctured bottles for up to six runs (Note: 1 kit, consisting of 72 tests, is configured to

allow for six runs of 12 tests). In between runs, opened reagents were stored at 2-8°C for two weeks.

Test samples were prepared using CMV cultured strain AD169 diluted in a pool of CMV DNA negative EDTA plasma at concentrations of 1.37E+02 IU/mL, 9.10E+04 IU/mL and 9.10E+06 IU/mL. Twenty-seven (27) replicates of each of the three concentrations were tested at time 0 to establish a baseline. Nine replicates of each of the three concentrations were tested at each subsequent time point for a total of six time points (including baseline).

Each on-board reagent time point consisted of one and a half hours run on the CAP, followed by incubation at 28°C (except MGPs that were kept at 38°C) for 18.5 hours for a total cumulative time of 20 hours. Following 28°C incubation (or 38°C for MGPs), open vials were stored at 2-8°C uncovered until the next time point. Refer to the tables below for the testing schedule for each time point.

Open Vial / On-board Reagent Stability Study — Study Time Points

| Time Point | Onboard Stability (Hours) | Open Vial Stability (Days) |
|---|---|---|
| 0 | 0 | 0 |
| 1 | 20 | 14 |
| 2 | 40 | 28 |
| 3 | 60 | 42 |
| 4 | 80 | 56 |
| 5 | 100 | 70 |

Open Vial / On-board Reagent Stability Study —
Example Schedule of Activity for Each Time Point

| Time Point (Days) | Onboard Time (Hours) | Time | Description of Activity |
|---|---|---|---|
| 0, 14, 28, 42, 56, 70 | 0.0 | 1:00 PM | Start CAP run |
| | 0.5 | 1:30 PM | ↓ |
| | 1.0 | 2:00 PM | ↓ |
| | 1.5 | 2:30 PM | End run, Reagents into 28°C (or 38°C for MGPs) storage |
| | ↓ | ↓ | ↓ |
| | ↓ | ↓ | ↓ |
| | 20.0 | 9:00 AM | Reagents out of 28°C (or 38°C for MGPs) storage, Reagents into 2-8°C storage |

One lot of CAP/CTM CMV Test Kit reagents was used for this study.

The acceptance criterion for claimed time points is within ±0.3 $\log_{10}$ titer units of the mean $\log_{10}$ titer from that of Time 0.

Upon completion of the study, the target requirements were met for onboard and for open vial stability:

- The mean $\log_{10}$ titer for the 1.37E+02 IU/mL sample was within ± 0.3 $\log_{10}$ titer units of the mean $\log_{10}$ titer time 0 result for time points Day 14 and 20 onboard hours, Day 28 and 40 onboard hours, Day 56 and 80 onboard hours, and Day 70 and 100 onboard hours.
- The mean $\log_{10}$ titer for the 9.10E+04 IU/mL sample was within ± 0.3 $\log_{10}$ titer units of the mean $\log_{10}$ titer time 0 result for all the time points.
- The mean $\log_{10}$ titer for the 9.10E+06 IU/mL sample was within ± 0.3 $\log_{10}$ titer units of the mean $\log_{10}$ titer time 0 result for all the time points.

**Based on the results of this Onboard Reagent and Open Vial Stability testing, the following onboard reagent and open vial stability claims in the product package insert are substantiated:**

- **Onboard stability with cap punctured bottles is stable for 100 hours at 28°C for reagents, 37°C for MGPs, with in-between storage at 2-8°C.**
- **Open vial stability with punctured reagent vials stored at 2-8°C is stable for 70 days (or until the expiration date).**

***Whole Kit and Controls Stability***

The real-time stability of the CAP/CTM CMV Test was assessed using three Standardized Lots (SL) of CAP/CTM CMV Test Kits (SL1, SL2, SL3) run on one CAP/CTM system. In total, 10 replicates of CMV Secondary Standard, 22 replicates of CMV Secondary Standard diluted to 4.55E+02 IU/mL, four replicates of CMV Negative Control, six replicates CMV Low Positive Control, and six replicates of CMV High Positive Control were tested at each time point. Limit of detection, percent of "Target Not Detected" results, accuracy (bias and precision), and precision were examined to assess stability.

To assess the functional stability of the CAP/CTM CMV Test, three Standardized Lots (SL) of CAP/CTM CMV Test Kits (SL1, SL2, and SL3) were tested at each time point, using a total of two racks per run on one CAP/CTM system. Each of the two racks in a run utilized a 500µL sample input volume (into the S tube), and was comprised of five replicates of CMV Secondary Standard (Lot TRLOT03, DOM: 03JUN2009, 2.184E+04 IU/mL, RMD Pleasanton, CA) to assess accuracy and precision; 11 replicates of CMV Secondary Standard diluted with negative EDTA plasma to 4.55E+02 IU/mL to assess limit of detection; two replicates of Negative Control to assess percent of "Target Not Detected" results; and three replicates of Low Positive Control and three replicates of High Positive Control to assess accuracy and precision. The table below summarizes the testing schedule.

Real-time Whole Kit Stability Study (including Controls) — Testing Schedule

| Year | Month | Interval (in Months) |
|---|---|---|
| 2011 | Jun | 6 |
| 2011 | Sep | 9 |
| 2011 | Dec | 12 |
| 2012 | Apr | 16 |
| 2012 | Jul | 19 |
| 2012 | Sep | 21 |
| 2013 | Jan | 25 |

Acceptance criteria for the study are:

- Precision — At each time point, standard deviation of the mean for $\log_{10}$ titers must meet the following criteria:
  - HPC$\leq$ 0.24 $\log_{10}$
  - LPC$\leq$ 0.36 $\log_{10}$
  - CMV Secondary Standard (Neat)$\leq$ 0.36 $\log_{10}$
- Limit of detection — Hit Rate at 4.55E+02 IU/mL — The observed positive hit rate for the CMV Secondary Standard (diluted to 4.55E+02 IU/mL) must be greater than or equal to 95% to be considered stable for that condition at that time point.
- Accuracy — Stability — Secondary Standard (Neat), HPC, LPC — The difference between the mean $\log_{10}$ measured titer and Day 0 mean $\log_{10}$ measured titer for the CMV Secondary Standard (neat), HPC and LPC must be less than $\pm$0.3 $\log_{10}$ at each time point to be considered stable. All valid replicates of the Low Positive and High Positive Controls must be within the acceptable range for each condition at each time point to be considered stable for that condition at that time point.
- Percent of "Target Not Detected" results — 100% of valid Negative Control results must be interpreted as "Target Not Detected" for each condition at each time point to be considered stable for that condition at that time point.

**The results of the real-time whole reagent kit and controls study to date have met all the acceptance criteria for limit of detection, percent of "Target Not Detected" results, accuracy, and precision described above, and support a shelf life of 11 months for the CAP/CTM CMV Test when stored at 4°C.**

***Analytical Studies Verifying the Performance of the CAP/CTM CMV Test with the Pre-analytical cobas p 630 Pipettor***

Analytical studies were carried out to verify the performance of the CAP/CTM CMV Test with the cobas p 630 pipettor. The cobas p 630 pipettor is an optional automated pre-analytical instrument for primary sample tube handling (e.g., de-capping and capping of the sample tubes, pipetting test controls from control tubes to sample tubes,

and pipetting samples from primary tubes to sample tubes, etc.) that may be used with the CAP/CTM CMV Test. Four Analytical Studies were carried out: 1) Limit of Detection 2) Linearity 3) Precision and 4) Cross-contamination (Carryover).

Data from the LoD, Linearity, Precision, and Cross-contamination studies using the cobas p 630 pipettor in the CAP/CTM CMV workflow demonstrated that the performance of the CAP/CTM CMV Test with the cobas p 630 pre-analytical pipettor is comparable to that of the CAP/CTM CMV Test without using the cobas p 630 pre-analytical pipettor in the workflow.

## X. SUMMARY OF CLINICAL STUDIES

### A. Clinical Reproducibility Study

Reproducibility of the CAP/CTM CMV Test was evaluated across kit reagent lot, site/instrument, operator, day, run, and within run at three test sites, each of which was equipped with either the COBAS® AmpliPrep/COBAS® TaqMan® Analyzer system with docked workflow or the COBAS® AmpliPrep/COBAS® TaqMan 48 Analyzer system workflow.

Reproducibility was evaluated with coded 7-member panels tested in triplicate. The concentrations of the CMV panel members covered the linear range of the test, included medical decision points, and are expressed in $\log_{10}$ international units/mL (IU/mL). A CMV-negative panel member was also included to assess the reproducibility of negative results. Two operators at each of three sites tested two runs per day for three days for each of three lots for a total of 108 runs.

The placement of panel members within a run was randomized and blinded to site staff. The following analyses were performed:

- Valid runs and valid tests were determined. The numbers of valid and invalid runs and reasons for invalid runs were summarized by lot, site/instrument, and operator. The numbers of valid and invalid tests from valid runs were summarized by lot, site/instrument, operator, and by expected CMV DNA concentration.
- Precision was evaluated by using a random effects model with terms for (a) lot, (b) site/instrument, (c) operator nested within site/instrument, (d) day nested within lot, site/instrument, and operator, (e) run nested within lot, site/instrument, operator and day, and (f) aliquot within-run components, using $\log_{10}$ transformed results. The percentage of variability due to each component and coefficient of variation of the $\log_{10}$ transformed CMV DNA concentration were calculated.
- The detectable difference in viral load between 2 test results for each expected $\log_{10}$ CMV DNA concentration was estimated by using the total variance and

was calculated as the antilog of the 95% confidence limit for the standard deviation of the difference between two measurements.

- The reproducibility of the test was also evaluated by calculating the negative percent agreement across the aforementioned factors in the negative panel member.

During the clinical reproducibility study, 114 runs were valid out of the total of 122 runs performed during the course of the study. The remaining eight invalid runs (run invalid rate: 6.5%; 95% CI: 3.4% to 12.4%) were due to instrument error (seven runs) or human error (one run). Overall, 2,268 tests were performed in valid runs and 1/2,268 test was invalid (invalid rate: 0.04%; 95% CI: 0.008% to 0.25%).

The table below summarizes the attributable percentage of total variance and total precision standard deviation as determined by the expected $\log_{10}$ CMV DNA concentration.

Attributable Percentage of Total Variance and Precision Standard Deviation by Nominal $\text{Log}_{10}$ CMV DNA Concentration (IU/mL)

| CMV DNA Concentration | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| $\text{Log}_{10}$IU/mL | | | Contribution to Total Variance (Standard Deviation [SD]) | | | | | | Total Precision | |
| Nominal | Observed (Average) | No. of Valid Tests | Lot | Site | Oper-ator | Day | Run | Within-Run | SD | Log- normal CV % |
| 2.135 | 1.924 | 323* | 24% (0.125) | 1% (0.027) | 0% (0.000) | 2% (0.032) | 3% (0.042) | 71% (0.218) | 0.258 | 65 |
| 2.699 | 2.453 | 324** | 37% (0.109) | 7% (0.046) | 0% (0.000) | 3% (0.033) | 1% (0.019) | 52% (0.130) | 0.180 | 43 |
| 3.260 | 3.095 | 324 | 32% (0.076) | 5% (0.030) | 0% (0.000) | 3% (0.024) | 10% (0.043) | 50% (0.096) | 0.136 | 32 |
| 4.260 | 4.197 | 324 | 3% (0.028) | 2% (0.023) | 0% (0.000) | 8% (0.043) | 0% (0.000) | 87% (0.145) | 0.156 | 37 |
| 4.658 | 4.605 | 324 | 7% (0.033) | 5% (0.027) | 2% (0.017) | 4% (0.023) | 2% (0.017) | 81% (0.111) | 0.123 | 29 |
| 6.658 | 6.602 | 324*** | 2% (0.015) | 15% (0.039) | 0% (0.000) | 2% (0.014) | 8% (0.028) | 72% (0.084) | 0.098 | 23 |

Note: Results with detectable viral load are included in this table.
Note: Results <1.37E+2 or >9.10E+6 IU/mL were recalculated based on extrapolation of the calibration curve.
*261 of 323 test results were <1.37E+2 IU/mL and were recalculated based on extrapolation of the calibration curve.
**10 of 324 test results were <1.37E+2 IU/mL and were recalculated based on extrapolation of the calibration curve.
***1 of 324 test results were >9.10E+6 IU/mL and were recalculated based on extrapolation of the calibration curve.

The detectable fold difference is a clinically informative concept when serially assessing a patient's viral load for statistically significant changes. Variations between measurements that are within the detectable fold difference could be due to variability in the test's reproducibility. The following table shows the estimated maximum total variation and 95% confidence limits one would theoretically expect for a change between two consecutive CMV DNA determinations in a single patient at various nominal $\log_{10}$ CMV DNA concentrations.

Detectable Viral Load Difference by Nominal $Log_{10}$ CMV DNA Concentration (IU/mL)

| CMV DNA Concentration ($Log_{10}$IU/mL) | | Result | | | | |
|---|---|---|---|---|---|---|
| Nominal | Mean (Observed) | No. of Tests | Total Precision Standard Deviation (in logs) | Standard Deviation of Difference Between Two Measurements | 95% Confidence Limit[1] ($\pm log_{10}$) | Fold Detectable Difference[2] |
| 2.135 | 1.924 | 323 | 0.26 | 0.36 | 0.72 | 5.19 |
| 2.699 | 2.453 | 324 | 0.18 | 0.25 | 0.50 | 3.15 |
| 3.260 | 3.095 | 324 | 0.14 | 0.19 | 0.38 | 2.38 |
| 4.260 | 4.197 | 324 | 0.16 | 0.22 | 0.43 | 2.71 |
| 4.658 | 4.605 | 324 | 0.12 | 0.17 | 0.34 | 2.19 |
| 6.658 | 6.602 | 324 | 0.10 | 0.14 | 0.27 | 1.87 |

[1] The 95% confidence limit about the difference between 2 measurements of CMV DNA in the same subject. These measurements do not include within-subject biologic variation and they could be from the same sample tested at different times with different lots, testing sites and/or operators.
[2] The antilog of the 95% confidence limit for the SD of the difference between 2 measurements (eg. 10**0.7151 = 5.20)

Negative agreement of the CAP/CTM CMV Test with the negative panel member was 100% (324/324, 95% CI 98.8%-100%) indicating the reproducibility of a negative sample across lot, site/instrument, operator, day, run, and within run.

## B. Clinical Usefulness Study

***Study Design***

This clinical usefulness study is a retrospective, longitudinal cohort study of 211 kidney transplant recipients diagnosed with CMV disease or CMV syndrome (referred to collectively as CMV disease below) and treated with anti-CMV drugs (ganciclovir or valganciclovir). The overall objective of this study was to assess whether CMV viral load measured with the CAP/CTM CMV Test is informative in aiding in the management of CMV disease in kidney transplant recipients with active CMV disease undergoing anti-CMV drug treatment. The usefulness of this test in this clinical setting was assessed on the basis of its performance in predicting resolution of CMV disease when measured at Baseline and in assessing virological response to treatment when measured at subsequent time points. This study is designed to assess the association of time to resolution of CMV disease after the initiation of treatment with baseline viral load results as measured with the CAP/CTM CMV Test. It is also designed to determine the usefulness of CMV viral load in managing patients using the CAP/CTM CMV Test by analyzing the association between viral load results as measured by the CAP/CTM CMV Test and the time to resolution of CMV disease after the initiation of treatment.

The original study, the VICTOR Study [2] was a randomized controlled clinical trial comparing the efficacy of intravenous (IV) versus oral anti-CMV therapy for 21 days followed by oral therapy for an additional 28 days in solid organ transplant recipients diagnosed with CMV disease. Briefly, the VICTOR Study was designed to show non-inferiority between oral valganciclovir and IV ganciclovir for antiviral treatment

induction in transplant patients with confirmed CMV disease. Subjects were enrolled if they had undergone solid organ transplant, had confirmed CMV disease, and did not have a life-threatening illness[1,2]. Patients were randomized to receive either drug for 21 days followed by oral ganciclovir until Day 49. Subsequent follow up for 12 months was conducted on outpatients among those well enough to be discharged. Patients were not excluded if they had received prior anti-CMV treatment or prophylaxis. The principal finding of the study was that IV ganciclovir is noninferior to Oral valganciclovir in terms of virological and disease resolution.

In the original study, patients were stratified by organ transplant type; however, due to the limited number of non-renal solid organ transplant recipients enrolled in the VICTOR Study, this clinical usefulness study design focused only on kidney

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1 VICTOR Study Inclusion Criteria:

Inclusion criteria were as follow:

- Age ≥ 18 years.
- Renal function of >10 mL/min as estimated by the Cockcroft-Gault formula.
- Patients eligible for oral treatment according to the investigator's judgment.
- Patient is able and willing to give written informed consent and willingness to participate and to comply with the study.
- Female patients must either be not of child-bearing potential (e.g., surgically sterilized or postmenopausal), or if of child-bearing potential, have a negative pregnancy test at screening and willingness to utilize an effective method of contraception throughout the study period and for 90 Days following discontinuation of the Study Drugs.

2 VICTOR Study Exclusion Criteria:

Exclusion criteria were as follows:

- Patients with life-threatening CMV disease according to the investigator's judgment.
- Patients with a history of significant adverse reaction to acyclovir, valacyclovir, ganciclovir, or Valganciclovir, or with a proven ganciclovir resistance.
- Patients who have received an investigational new drug within the last 30 Days (90 days in UK).
- Patients with a history of a psychological illness or condition such as to interfere with the patient's ability to understand the requirements of the study.
- Patients who have participated in this study before.
- Patients requiring treatment with any of the following medications during the treatment period (due to possible drug interactions with the study medications): Oral or IV acyclovir, valacyclovir, famciclovir, cidofovir, leflunomide, interferons, oral ganciclovir, CMV hyperimmune globulin, foscarnet, lobucavir, lamivudine, HBIg, probenecid, and any investigational drug, including everolimus, FTY-720 and FK-778, and mycophenolate sodium. All these drugs (except investigational drugs) are allowed prior to Day 0.
- Lactating females

Both test medications, oral valganciclovir and IV ganciclovir, in treatment doses were not allowed to be used for treatment of the present specific CMV disease prior to Day 0, even though they both may have been used for treatment of earlier anti-CMV episodes or CMV prophylaxis. Acyclovir, valacyclovir, or famciclovir may however be used for up to 14 days, at the dose specified in the drug package insert, for treatment of clinically indicated acute herpes simplex or herpes zoster infection following Day 49.

transplant recipients enrolled in the VICTOR Study. Specimens in the clinical usefulness study were from patients who had undergone kidney transplantation and enrolled in the VICTOR Study. In addition, (1) specimens were required to be of an adequate volume for testing, (2) adequate clinical information was available to support the diagnosis of CMV disease and/or CMV syndrome, and (3) adequate clinical information was also available to support the determination of resolution of CMV disease and/or CMV syndrome. Patients had to have been followed prospectively for at least 7 weeks after onset of drug dosing, with frozen plasma from weekly samples available for analysis. All patients were required to have received anti-CMV treatment with intravenous ganciclovir or oral valganciclovir during the course of the VICTOR Study.

CMV disease in this clinical usefulness study is defined and classified as either tissue-invasive or CMV viral syndrome, characterized by:

- The presence of CMV in the blood by nucleic acid detection, shell vial culture, or pp65 antigenemia at study Screening or at Baseline, and
  - For CMV syndrome: one or more of the following signs or symptoms:
    - body temperature ≥38.0°C;
    - new or increased significant malaise;
    - leucopenia (WBC <3,500/μL);
    - atypical lymphocytosis ≥5%;
    - thrombocytopenia (<100,000/μL)
  - For tissue-invasive disease: the manifestations and diagnosis of CMV disease is dependent upon the end-organ that is infected by CMV. The clinical diagnosis of tissue-invasive CMV disease is based on the following:
    - the presence of symptoms or signs of end-organ damage;
    - laboratory evidence of end-organ damage; or
    - evidence of localized CMV infection (inclusion cells or *in situ* detection of CMV antigen or DNA) on biopsy or other tissue specimen

Resolution of CMV disease in this clinical usefulness study is defined as either resolution of tissue-invasive disease or resolution of CMV viral syndrome:

- Resolution of CMV viral syndrome is defined by any or all of the following:
  - Site physician's assessment of clinical resolution
  - Reduction in body temperature to <38.0°C
  - Resolution of malaise
  - Normalization of leucopenia (WBC >3,500/μL)
  - Resolution of atypical lymphocytosis to <5%
  - Resolution of thrombocytopenia to platelet count to >100,000/μL
- Resolution of tissue-invasive CMV disease is based upon either:
  - Site physician's assessment,

- Resolution of the initial organ-specific signs or symptoms upon repeated clinical examination, or
- Loss of CMV-specific findings on biopsy or other tissue specimen when applicable.

***Methodology***

Stored VICTOR Study specimens with sufficient sample volume were tested using the CAP/CTM CMV Test at available time points, i.e., Day 0 (Baseline), Day 7, Day 14, Day 21, Day 28 post-treatment initiation, and Day 49 (End of Treatment). Viral load data generated by the CAP/CTM CMV Test was analyzed alongside available clinical data generated from the VICTOR Study according to the clinical usefulness study design, controlling for relevant baseline clinical characteristics.

Study samples were acquired from storage and each sample was assigned a unique identification number. Samples were prepared, amplified, and detected according to the CAP/CTM CMV Test Instructions for Use. Each sample was tested at one of three clinical sites in a blinded fashion. If a sample had volume <500 µL, it was diluted in CMV-negative (IgG-, IgM-, and DNA-negative) human EDTA plasma to bring the sample volume up to 600 µL. Titers obtained from diluted samples were adjusted according to the dilution factor. The samples with test results that were above the upper limit of quantitation for the CAP/CTM CMV Test were diluted and re-tested to achieve a reportable quantitative result. All dilution procedures were performed in accordance with the detailed instructions in the protocol. Individual samples with invalid test results were re-tested in singleton if the remaining sample volume was sufficient for re-testing undiluted, or if sufficient volume was available to perform a dilution in CMV-negative human EDTA plasma to bring sample volume up to 600 µL. If a sample yielded an invalid result upon re-testing, the result was reported as invalid.

Study samples from the same patient across multiple time points in the VICTOR Study (i.e., Day 0 [Baseline], Day 7, Day 14, Day 21, and Day 28 post-treatment initiation, and Day 49 [End of Treatment]) were tested at the same randomly assigned test site in this clinical usefulness study. Sites were blinded to the previous viral load results and clinical outcomes.

Clinical data (e.g., demographics, CMV disease type upon entry, vital status, physical exam findings, biopsy/tissue sample findings, CMV disease status, clinical laboratory results [CBC, creatinine clearance], randomized treatment received, etc.) were extracted from the VICTOR Study database for applicable descriptive summaries or statistical analyses and linked to specimen CAP/CTM CMV Test results using the unique identification number.

Based on existing literature, a baseline CMV viremia of 20,000 copies/mL (18,200 IU/mL as measured by the CAP/CTM CMV Test) [3], and a 1.5 $\log_{10}$ IU/mL decline in CMV viremia from baseline to Day 14 [4] were assessed. Virological suppression

below the test's LLoQ (including target not detected results) at Day 7, Day 14, and Day 21 were also analyzed for the association with the time to resolution of CMV disease [4][5]. Because of the performance of any quantitative assay below its lower limit of quantitation, viral suppression was defined as results <LLoQ and "target not detected" results.

The primary clinical outcome measure for this study was the time to CMV disease resolution defined by the number of days from onset of antiviral treatment (Baseline) to clinical resolution of CMV disease. Regular clinical assessments for CMV disease (resolution of symptoms of viral syndrome and/or signs of end-organ damage) took place on study Days 3, 7, 10, 14, 17, 21, 28, 35, 42, and 49. Baseline clinical characteristics (i.e., baseline covariants) included demographics, randomized treatment received, recipient CMV serostatus, previous anti-CMV strategy, previous anti-CMV therapy, prior immunosuppressive regimen, CMV drug resistant status, CMV Glycoprotein B (gB) genotype (if available), and organ donor CMV serostatus (if available).

Time to resolution of CMV disease after the initiation of treatment was assessed for association with viral load results as measured with the CAP/CTM CMV Test at Baseline. The time to resolution of CMV disease after the initiation of treatment was also analyzed with the change in the viral load between Baseline and the respective days of viral load measurement to determine whether there was a correlation between change in viral load and CMV disease resolution. Furthermore, virological suppression below the assay's LLoQ (including target not detected results) at Day 7, Day 14, and Day 21 post-treatment initiation was also analyzed in relation to the time to resolution of CMV disease. Univariate Cox Proportional Hazards Models was used to assess the relationship between the time to resolution of CMV disease and each of the viral load-based variables of interest and each of the relevant baseline covariates described above. Cox Multivariate Proportional Hazards Models were used to examine the relationship between the time to resolution of CMV disease and each of the viral load-based variables of interest, adjusting for relevant baseline covariates (age, sex, race/ethnicity, recipient CMV serostatus, previous anti-CMV therapy, randomization to ganciclovir or valganciclovir arms, and previous immunosuppressive regimen). In addition, Cox Multivariate Proportional Hazard analysis was performed for the subjects with available donor serostatus and CMV gB genotype results.

***Statistical Methods***

In the VICTOR Study, there were 237 subjects with kidney transplants and 211 subjects were included in the clinical usefulness study. Because 11% (26/237) of the VICTOR Study subjects were not included in the clinical usefulness study, demographic and clinical characteristics were summarized and compared for all kidney transplant participants from the VICTOR Study and those evaluable participants with sufficient specimen volume at Baseline for inclusion in this clinical usefulness study (overall and by CMV disease type).

Mean $\log_{10}$-transformed CMV viral load ($\log_{10}$ IU/mL) with 95% confidence intervals (CIs) and the percentage of study participants with viral suppression below the LLoQ of the CAP/CTM CMV Test were summarized graphically.

Kaplan-Meier survival plots were used to assess differences between the time (days) to resolution of CMV disease stratified by the following variables:

- Baseline CMV viral load (<18,200 IU/mL, ≥18,200 IU/mL)
- Viral Suppression by Day 7 (<LLoQ, ≥LLoQ)
- Viral Suppression by Day 14 (<LLoQ, ≥LLoQ)
- Viral Suppression by Day 21 (<LLoQ, ≥LLoQ)

Censoring information, estimates of median time to disease resolution (95% CIs), the log-rank test statistic and associated P-value, and the hazard ratio (HR) from a Univariate Cox proportional hazards model were calculated for each Kaplan-Meier survival plot.

Univariate Cox Proportional Hazards Models were used to assess the relationship between the time to resolution of CMV disease and each of the viral load-based variables of interest, as well as the relationship between the time to resolution of CMV disease and each of the relevant baseline covariates.

Cox Multivariate Proportional Hazards Models were used to examine the relationship between the time to resolution of CMV disease and each of the viral load-based variables of interest listed above, adjusting for relevant baseline covariates. For categorical covariates (e.g., organ recipient CMV serostatus), adjusted hazard ratios were calculated. For the continuous covariate of the participant's age at randomization, the adjusted hazard was calculated

***Study Results***

During the clinical usefulness study, 100% of runs (63 of 63 runs) were valid, from which 12/1,235 test results were invalid (invalid rate: 0.97%; 95% CI: 0.51% to 1.69%).

The distributions of demographic characteristics were similar between VICTOR Study kidney transplant recipients and evaluable study participants for this clinical usefulness study.

Demographic Characteristics of VICTOR Study Kidney Transplant Patients and Evaluable Participants in the Clinical Usefulness Study

| | VICTOR Study Kidney Transplant Patient Set (N=237) | Evaluable Participants for the Clinical Usefulness Study[a] | | |
|---|---|---|---|---|
| | | CMV Disease Type | | All CMV Disease(N=211) |
| | | CMV Syndrome(N=113) | Tissue-Invasive CMV Disease (N=98) | |

| | | | | | |
|---|---|---|---|---|---|
| Sex | Male | 149 ( 62.9) | 75 ( 66.4) | 54 ( 55.1) | 129 ( 61.1) |
| | Female | 88 ( 37.1) | 38 ( 33.6) | 44 ( 44.9) | 82 ( 38.9) |
| Age Category | 18-29 | 47 ( 19.8) | 23 ( 20.4) | 22 ( 22.4) | 45 ( 21.3) |
| | 30-39 | 52 ( 21.9) | 27 ( 23.9) | 21 ( 21.4) | 48 ( 22.7) |
| | 40-49 | 46 ( 19.4) | 18 ( 15.9) | 20 ( 20.4) | 38 ( 18.0) |
| | 50-59 | 56 ( 23.6) | 26 ( 23.0) | 26 ( 26.5) | 52 ( 24.6) |
| | ≥60 | 36 ( 15.2) | 19 ( 16.8) | 9 ( 9.2) | 28 ( 13.3) |
| Race/Ethnicity | Caucasian/White | 172 ( 72.6) | 91 ( 80.5) | 60 ( 61.2) | 151 ( 71.6) |
| | Black | 8 ( 3.4) | 3 ( 2.7) | 2 ( 2.0) | 5 ( 2.4) |
| | Asian | 27 ( 11.4) | 10 ( 8.8) | 15 ( 15.3) | 25 ( 11.8) |
| | Hispanic | 18 ( 7.6) | 3 ( 2.7) | 15 ( 15.3) | 18 ( 8.5) |
| | Other | 12 ( 5.1) | 6 ( 5.3) | 6 ( 6.1) | 12 ( 5.7) |
| Region | Asia-Pacific | 77 ( 32.5) | 50 ( 44.2) | 21 ( 21.4) | 71 ( 33.6) |
| | Europe | 88 ( 37.1) | 29 ( 25.7) | 47 ( 48.0) | 76 ( 36.0) |
| | North America | 14 ( 5.9) | 8 ( 7.1) | 5 ( 5.1) | 13 ( 6.2) |
| | South America | 58 ( 24.5) | 26 ( 23.0) | 25 ( 25.5) | 51 ( 24.2) |

[a] Numbers are counts (with percentages) within each column.

Likewise, similar distributions of clinical characteristics were observed between VICTOR Study kidney transplant recipients and the evaluable study participants for this clinical usefulness study:

Clinical Characteristics of VICTOR Study Kidney Transplant Patients and Evaluable Participants in the Clinical Usefulness Study

| | | VICTOR Study Kidney Transplant Patient Set (N=237) | Evaluable Participants for the Clinical Usefulness Study[a] | | |
|---|---|---|---|---|---|
| | | | CMV Disease Type | | All CMV Disease (N=211) |
| | | | CMV Syndrome (N=113) | Tissue- Invasive CMV Disease (N=98) | |
| Organ Donor(D)/ Recipient(R) CMV Serostatus | Missing | 70 ( 29.5) | 39 ( 34.5) | 23 ( 23.5) | 62 ( 29.4) |
| | D-/R- | 13 ( 5.5) | 5 ( 4.4) | 6 ( 6.1) | 11 ( 5.2) |
| | D-/R+ | 16 ( 6.8) | 7 ( 6.2) | 8 ( 8.2) | 15 ( 7.1) |
| | D+/R- | 37 ( 15.6) | 11 ( 9.7) | 24 ( 24.5) | 35 ( 16.6) |
| | D+/R+ | 101 ( 42.6) | 51 ( 45.1) | 37 ( 37.8) | 88 ( 41.7) |
| Recipient(R) CMV Serostatus | Missing | 5 ( 2.1) | 3 ( 2.7) | 1 ( 1.0) | 4 ( 1.9) |
| | R+ | 172 ( 72.6) | 93 ( 82.3) | 59 ( 60.2) | 152 ( 72.0) |
| | R- | 60 ( 25.3) | 17 ( 15.0) | 38 ( 38.8) | 55 ( 26.1) |
| Previous Anti-CMV Strategy | Prophylactic | 80 ( 33.8) | 50 ( 44.2) | 25 ( 25.5) | 75 ( 35.5) |
| | Pre-Emptive | 1 ( 0.4) | 1 ( 0.9) | 0 ( 0.0) | 1 ( 0.5) |
| | Disease Treatment | 25 ( 10.5) | 4 ( 3.5) | 15 ( 15.3) | 19 ( 9.0) |
| | None | 136 ( 57.4) | 58 ( 51.3) | 62 ( 63.3) | 120 ( 56.9) |
| Previous Anti-CMV | Acyclovir | 43 ( 18.1) | 33 ( 29.2) | 7 ( 7.1) | 40 ( 19.0) |

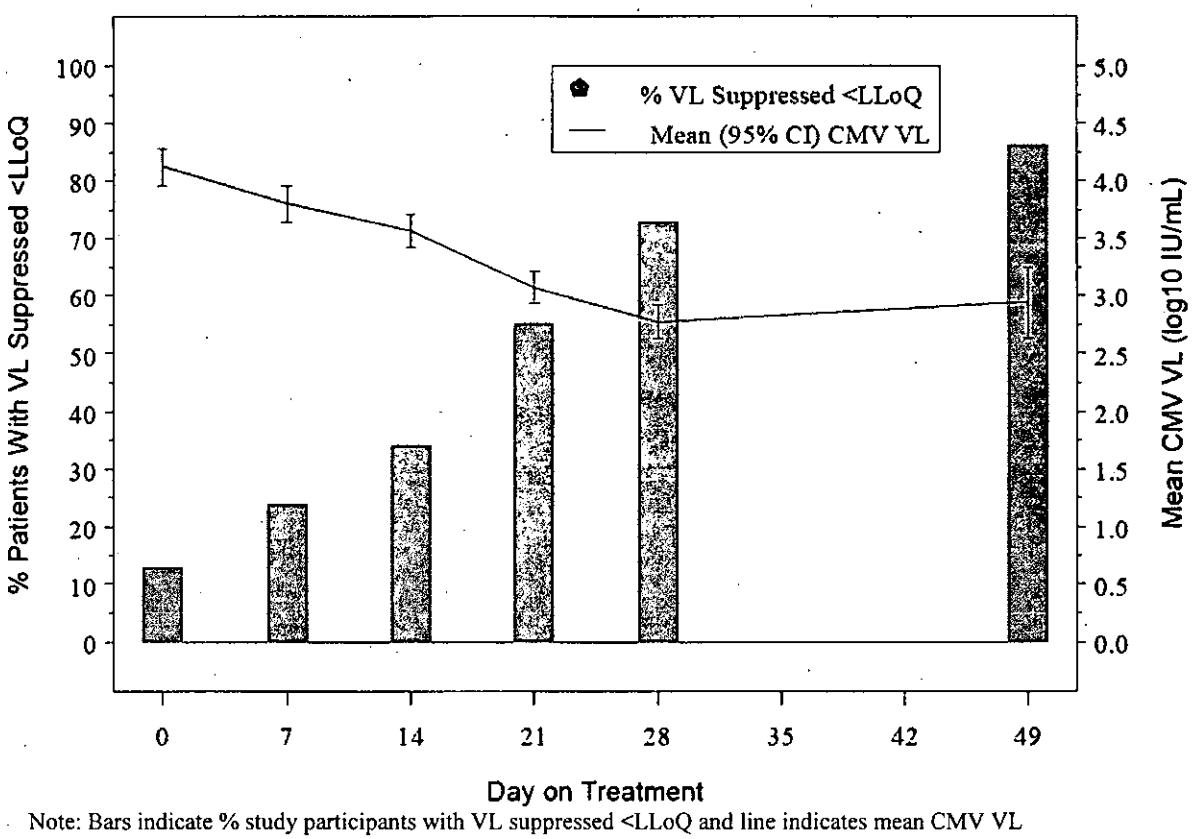
| | | | | | |
|---|---|---|---|---|---|
| | Ganciclovir | 47 ( 19.8) | 19 ( 16.8) | 22 ( 22.4) | 41 ( 19.4) |
| | Valaciclovir | 4 ( 1.7) | 3 ( 2.7) | 1 ( 1.0) | 4 ( 1.9) |
| | Valganciclovir | 33 ( 13.9) | 12 ( 10.6) | 17 ( 17.3) | 29 ( 13.7) |
| | None | 136 ( 57.4) | 58 ( 51.3) | 62 ( 63.3) | 120 ( 56.9) |
| Randomized Treatment Received | Ganciclovir | 115 ( 48.5) | 61 ( 54.0) | 43 ( 43.9) | 104 ( 49.3) |
| | Valganciclovir | 122 ( 51.5) | 52 ( 46.0) | 55 ( 56.1) | 107 ( 50.7) |
| CMV DiseaseSub-Category[b] | CMV Syndrome | 124 ( 52.3) | 113 (100.0) | 0 ( 0.0) | 113 ( 53.6) |
| | TI CMV: CMV GI | 19 ( 8.0) | 0 ( 0.0) | 18 ( 18.4) | 18 ( 8.5) |
| | TI CMV: Hepatitis | 7 ( 3.0) | 0 ( 0.0) | 7 ( 7.1) | 7 ( 3.3) |
| | TI CMV: Nephritis | 80 ( 33.8) | 0 ( 0.0) | 70 ( 71.4) | 70 ( 33.2) |
| | TI CMV: Pneumonia | 0 ( 0.0) | 0 ( 0.0) | 0 ( 0.0) | 0 ( 0.0) |
| | TI CMV: Retinitis | 0 ( 0.0) | 0 ( 0.0) | 0 ( 0.0) | 0 ( 0.0) |
| | Other TI CMV | 4 ( 1.7) | 0 ( 0.0) | 3 ( 3.1) | 3 ( 1.4) |
| | No CMV in Blood | 3 ( 1.3) | 0 ( 0.0) | 0 ( 0.0) | 0 ( 0.0) |
| Previous Immuno suppressive Therapy | ATG | 26 ( 11.0) | 13 ( 11.5) | 9 ( 9.2) | 22 ( 10.4) |
| | Azathioprine | 25 ( 10.5) | 12 ( 10.6) | 9 ( 9.2) | 21 ( 10.0) |
| | Basiliximab | 17 ( 7.2) | 4 ( 3.5) | 9 ( 9.2) | 13 ( 6.2) |
| | Cyclosporine A | 128 ( 54.0) | 68 ( 60.2) | 45 ( 45.9) | 113 ( 53.6) |
| | Daclizumab | 10 ( 4.2) | 4 ( 3.5) | 6 ( 6.1) | 10 ( 4.7) |
| | Methylprednisolone | 68 ( 28.7) | 29 ( 25.7) | 29 ( 29.6) | 58 ( 27.5) |
| | Mycophenolate mofetil | 173 ( 73.0) | 80 ( 70.8) | 73 ( 74.5) | 153 ( 72.5) |
| | OKT 3 | 1 ( 0.4) | 1 ( 0.9) | 0 ( 0.0) | 1 ( 0.5) |
| | Prednisolone | 85 ( 35.9) | 45 ( 39.8) | 28 ( 28.6) | 73 ( 34.6) |
| | Prednisone | 109 ( 46.0) | 51 ( 45.1) | 50 ( 51.0) | 101 ( 47.9) |
| | Sirolimus | 20 ( 8.4) | 7 ( 6.2) | 10 ( 10.2) | 17 ( 8.1) |
| | Tacrolimus | 75 ( 31.6) | 35 ( 31.0) | 35 ( 35.7) | 70 ( 33.2) |
| | Other | 18 ( 7.6) | 13 ( 11.5) | 4 ( 4.1) | 17 ( 8.1) |
| | Not provided | 11 ( 4.6) | 6 ( 5.3) | 4 ( 4.1) | 10 ( 4.7) |
| CMV Genotype | Not Done[c] | 103 ( 43.5) | 51 ( 45.1) | 36 ( 36.7) | 87 ( 41.2) |
| | Genotype 1 | 43 ( 18.1) | 16 ( 14.2) | 25 ( 25.5) | 41 ( 19.4) |
| | Genotype 2 | 30 ( 12.7) | 16 ( 14.2) | 12 ( 12.2) | 28 ( 13.3) |
| | Genotype 3 | 30 ( 12.7) | 16 ( 14.2) | 10 ( 10.2) | 26 ( 12.3) |
| | Genotype 4 | 13 ( 5.5) | 3 ( 2.7) | 8 ( 8.2) | 11 ( 5.2) |
| | Mixed Infection | 18 ( 7.6) | 11 ( 9.7) | 7 ( 7.1) | 18 ( 8.5) |
| UL54 Resistant | Not Done* | 52 ( 21.9) | 18 ( 15.9) | 23 ( 23.5) | 41 ( 19.4) |
| | Yes | 0 ( 0.0) | 0 ( 0.0) | 0 ( 0.0) | 0 ( 0.0) |
| | No | 185 ( 78.1) | 95 ( 84.1) | 75 ( 76.5) | 170 ( 80.6) |
| UL97 Resistant | Not Done* | 52 ( 21.9) | 18 ( 15.9) | 23 ( 23.5) | 41 ( 19.4) |
| | Yes | 1 ( 0.4) | 0 ( 0.0) | 1 ( 1.0) | 1 ( 0.5) |
| | No | 184 ( 77.6) | 95 ( 84.1) | 74 ( 75.5) | 169 ( 80.1) |

[a] Numbers are counts (with percentages) within each column.

[b] CMV GI = CMV esophagitis, gastroenteritis, or colitis; TI-CMV = Tissue Invasive CMV Disease; No CMV in Blood = No CMV in Blood (Screening/Baseline).
[c] 'Not Done' due to low sample volume and/or low viral load.

The figure below shows a comparison of the mean (with 95% CI) CMV viral load ($log_{10}$ IU/mL) and the percentage of study participants with CMV viral load suppressed <LLoQ (including "Target Not Detected" results) by the day on treatment. The percentage of study participants with viral suppression <LLoQ increased with the day on treatment. Mean CMV viral load generally decreased with day on treatment from Baseline to Day 49 among those study participants who still had quantifiable viral load titers.

Comparison of CMV Viral Load Suppression and Mean CMV Viral Load (VL) by Day on Treatment




Note: Bars indicate % study participants with VL suppressed <LLoQ and line indicates mean CMV VL

The summary results of the statistical analyses that assess the relationship between the time to resolution of CMV disease and each of the viral load-based variables of interest and each of the individual relevant baseline covariates using the Univariate Cox Proportional Hazards Model are presented below:

Univariate Cox Models for the Time to Resolution for Each Covariate/Variable
—All Evaluable Participants

| Covariate/Variable | Category | Total N | DF{a} | Parameter Estimate | Standard Error | Wald Chi-Square | P-value | Hazard Ratio (HR) | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | Estimate | 95% CI |
| Baseline CAP/CTM CMV Test Result | <18,200 IU/mL | 211 | 1 | 0.406 | 0.148 | 7.5941 | 0.006 | 1.50 | (1.12, 2.00) |
| | ≥18,200 IU/mL | | 0 | (0.000) | | | | (1.00) | |
| On-Treatment Viral Load Decline | >=1.5 $\log_{10}$ IU/mL | 209 | 1 | -0.106 | 0.147 | 0.5218 | 0.470 | 0.90 | (0.67, 1.20) |
| | < 1.5 $\log_{10}$ IU/mL | | 0 | (0.000) | | | | (1.00) | |
| Viral Suppression By Day 7 | <LLoQ | 205 | 1 | 0.396 | 0.169 | 5.4767 | 0.019 | 1.49 | (1.07, 2.07) |
| | ≥LLoQ | | 0 | (0.000) | | | | (1.00) | |
| Viral Suppression By Day 14 | < LLoQ | 209 | 1 | 0.547 | 0.150 | 13.271 | <.001 | 1.73 | (1.29, 2.32) |
| | >=LLoQ | | 0 | (0.000) | | | | (1.00) | |
| Viral Suppression By Day 21 | < LLoQ | 210 | 1 | 0.390 | 0.144 | 7.3282 | 0.007 | 1.48 | (1.11, 1.96) |
| | >=LLoQ | | 0 | (0.000) | | | | (1.00) | |
| Age (Years) | n/a | 211 | 1 | -0.011 | 0.005 | 4.1983 | 0.040 | 0.99 | (0.98, 1.00) |
| Age Category | 18-29 | 211 | 1 | 0.512 | 0.248 | 4.2461 | 0.039 | 1.67 | (1.03, 2.72) |
| | 30-39 | | 1 | 0.339 | 0.247 | 1.8792 | 0.170 | 1.40 | (0.86, 2.28) |
| | 40-49 | | 1 | 0.343 | 0.255 | 1.8014 | 0.180 | 1.41 | (0.85, 2.32) |
| | 50-59 | | 1 | 0.249 | 0.239 | 1.0882 | 0.297 | 1.28 | (0.80, 2.05) |
| | >= 60 | | 0 | (0.000) | | | | (1.00) | |
| Sex | Male | 211 | 1 | -0.059 | 0.147 | 0.1642 | 0.685 | 0.94 | (0.71, 1.26) |
| | Female | | 0 | (0.000) | | | | (1.00) | |
| Race/Ethnicity | Black | 211 | 1 | 1.720 | 0.470 | 13.371 | <.001 | 5.59 | (2.22, 14.0) |
| | Asian | | 1 | -0.701 | 0.231 | 9.2060 | 0.002 | 0.50 | (0.32, 0.78) |
| | Hispanic | | 1 | 0.287 | 0.260 | 1.2248 | 0.268 | 1.33 | (0.80, 2.22) |
| | Other | | 1 | -0.244 | 0.301 | 0.6543 | 0.419 | 0.78 | (0.43, 1.41) |
| | Caucasian/White | | 0 | (0.000) | | | | (1.00) | |
| Randomized Treatment Received | Ganciclovir | 211 | 1 | -0.002 | 0.143 | 0.0003 | 0.986 | 1.00 | (0.75, 1.32) |
| | Valganciclovir | | 0 | (0.000) | | | | (1.00) | |
| Organ Donor(D)/ Recipient(R) CMV Serostatus | D+/R+ | 149 | 1 | 0.739 | 0.326 | 5.1431 | 0.023 | 2.09 | (1.11, 3.96) |
| | D-/R+ | | 1 | 0.311 | 0.404 | 0.5934 | 0.441 | 1.37 | (0.62, 3.01) |
| | D+/R- | | 1 | 0.449 | 0.352 | 1.6322 | 0.201 | 1.57 | (0.79, 3.12) |
| | D-/R- | | 0 | (0.000) | | | | (1.00) | |
| Recipient(R) CMV Serostatus | R+ | 207 | 1 | 0.343 | 0.163 | 4.3979 | 0.036 | 1.41 | (1.02, 1.94) |
| | R- | | 0 | (0.000) | | | | (1.00) | |
| Previous Anti-CMV Strategy{b} | Prophylactic | 211 | 1 | 0.112 | 0.149 | 0.5663 | 0.452 | 1.12 | (0.84, 1.50) |
| | Pre-Emptive | 211 | 1 | 0.244 | 1.004 | 0.0588 | 0.808 | 1.28 | (0.18, 9.13) |
| | Disease Treatment | 211 | 1 | 0.420 | 0.250 | 2.8100 | 0.094 | 1.52 | (0.93, 2.49) |
| | None | 211 | 1 | -0.219 | 0.144 | 2.3174 | 0.128 | 0.80 | (0.61, 1.07) |
| Previous Anti-CMV Therapy{b} | Acyclovir | 211 | 1 | 0.038 | 0.184 | 0.0423 | 0.837 | 1.04 | (0.72, 1.49) |
| | Ganciclovir | 211 | 1 | 0.461 | 0.178 | 6.7176 | 0.010 | 1.59 | (1.12, 2.25) |
| | Valaciclovir | 211 | 1 | 0.343 | 0.507 | 0.4585 | 0.498 | 1.41 | (0.52, 3.81) |
| | Valganciclovir | 211 | 1 | -0.041 | 0.208 | 0.0381 | 0.845 | 0.96 | (0.64, 1.44) |
| | None | 211 | 1 | -0.219 | 0.144 | 2.3174 | 0.128 | 0.80 | (0.61, 1.07) |
| Previous Immunosuppressive Therapy{b} | ATG | 211 | 1 | 0.269 | 0.237 | 1.2954 | 0.255 | 1.31 | (0.82, 2.08) |

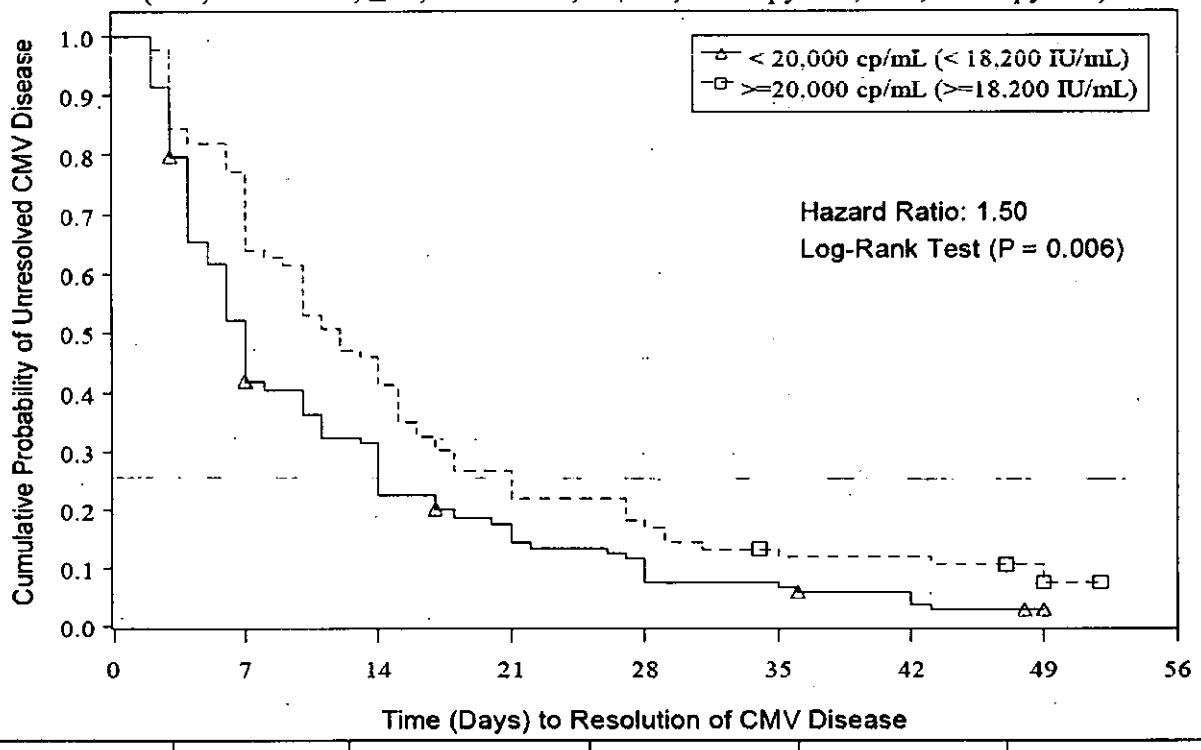
| Covariate/Variable | Category | Total N | DF{a} | Parameter Estimate | Standard Error | Wald Chi-Square | P-value | Hazard Ratio (HR) | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | Estimate | 95% CI |
| | Azathioprine | 211 | 1 | -0.044 | 0.232 | 0.0363 | 0.849 | 0.96 | (0.61, 1.51) |
| | Basiliximab | 211 | 1 | -0.312 | 0.325 | 0.9261 | 0.336 | 0.73 | (0.39, 1.38) |
| | Cyclosporine A | 211 | 1 | -0.529 | 0.148 | 12.743 | <.001 | 0.59 | (0.44, 0.79) |
| | Daclizumab | 211 | 1 | 0.171 | 0.327 | 0.2739 | 0.601 | 1.19 | (0.63, 2.25) |
| | Methylprednisolone | 211 | 1 | -0.135 | 0.160 | 0.7138 | 0.398 | 0.87 | (0.64, 1.19) |
| | Mycophenolate mofetil | 211 | 1 | -0.083 | 0.160 | 0.2686 | 0.604 | 0.92 | (0.67, 1.26) |
| | OKT 3 | 211 | 1 | -0.323 | 1.003 | 0.1035 | 0.748 | 0.72 | (0.10, 5.17) |
| | Prednisolone | 211 | 1 | -0.257 | 0.153 | 2.8188 | 0.093 | 0.77 | (0.57, 1.04) |
| | Prednisone | 211 | 1 | 0.253 | 0.145 | 3.0500 | 0.081 | 1.29 | (0.97, 1.71) |
| | Sirolimus | 211 | 1 | 0.798 | 0.264 | 9.1576 | 0.002 | 2.22 | (1.32, 3.73) |
| | Tacrolimus | 211 | 1 | 0.303 | 0.153 | 3.9268 | 0.048 | 1.35 | (1.00, 1.83) |
| | Other | 211 | 1 | 0.348 | 0.255 | 1.8657 | 0.172 | 1.42 | (0.86, 2.33) |
| | Not Provided | 211 | 1 | 0.774 | 0.347 | 4.9848 | 0.026 | 2.17 | ( 1.10, 4.28) |
| Previous Immunosuppressive Therapy Category{b} | T-cell Suppressors{c} | 211 | 1 | 0.506 | 0.149 | 11.528 | <.001 | 1.66 | (1.24, 2.22) |
| | Corticosteroids{d} | 211 | 1 | -0.144 | 0.227 | 0.4008 | 0.527 | 0.87 | (0.56, 1.35) |
| CMV gB Genotype | gB 2 | 199 | 1 | -0.156 | 0.252 | 0.3799 | 0.538 | 0.86 | (0.52, 1.40) |
| | gB 3 | | 1 | 0.338 | 0.260 | 1.6907 | 0.194 | 1.40 | (0.84, 2.33) |
| | gB 4 | | 1 | -0.157 | 0.371 | 0.1795 | 0.672 | 0.85 | (0.41, 1.77) |
| | Mixed Infection | | 1 | -0.288 | 0.314 | 0.8428 | 0.359 | 0.75 | (0.41, 1.39) |
| | Unknown | | 1 | 0.469 | 0.201 | 5.4238 | 0.020 | 1.60 | (1.08, 2.37) |
| | gB 1 | | 0 | (0.000) | | | | (1.00) | |
| UL54 Resistant | Yes | 170 | 0 | 0.000 | | | | | |
| | No | | 0 | (0.000) | | | | (1.00) | |
| UL97 Resistant | Yes | 170 | 1 | 0.004 | 1.005 | 0.0000 | 0.997 | 1.00 | (0.14, 7.19) |
| | No | | 0 | (0.000) | | | | (1.00) | |

Note: Only evaluable participants are included in this table. DF = degrees of freedom, CI = confidence interval.
{a} Rows with degrees of freedom of zero and parameter/hazard ratio estimates within parentheses indicate reference categories.
{b} Indicates covariates with non-mutually exclusive categories that were each modeled separately.
{c} 'T-cell Suppressors' was defined as participants with previous immunosuppressive therapies Sirolimus and/or Tacrolimus.
{d} 'Corticosteroids' was defined as participants with previous immunosuppressive therapies Methylprednisolone, Prednisolone, and/or Prednisone.

### *Baseline Viral Load and Clinical Resolution of CMV Disease*

A Kaplan-Meier survival plot for the time (days) to resolution of CMV disease stratified by baseline CMV viral load (<18,200 IU/mL, ≥18,200 IU/mL; or <20,000 copy/mL, ≥20,000 copy/mL) is shown in the figure below. There is a separation between the survival curves, with shorter times to resolution of CMV disease for participants with baseline CMV viral load <18,200 IU/mL (unadjusted hazard ratio [HR] = 1.50; 95% CI of 1.12 to 2.00; Log-Rank Test, P = 0.006).

Kaplan-Meier Survival Plot of Time to Resolution of CMV Disease
by Baseline CAP/CTM CMV Test Result
(<18,200 IU/mL, ≥18,200 IU/mL; or <20,000 copy/mL, ≥20,000 copy/mL)



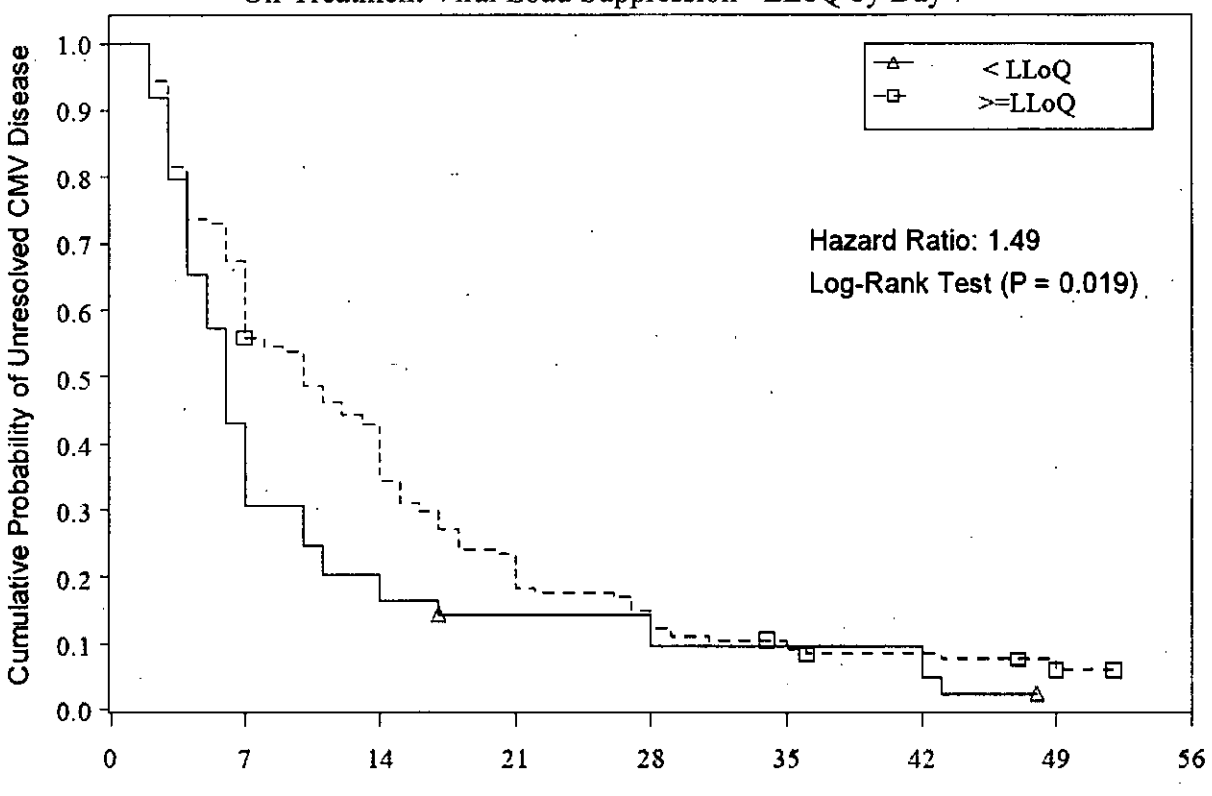

| Baseline CAP/CTM CMV Test Result | No. of Participants | Resolved n (%) | Censored n (%) | Median Time to Resolution in Days (95% CI) | Hazard Ratio(HR) |
|---|---|---|---|---|---|
| <20,000 cp/mL (<18,200 IU/mL) | 128 | 121 ( 94.5) | 7 ( 5.5) | 7.0 ( 6.0, 8.0) | 1.50 |
| ≥20,000 cp/mL (≥18,200 IU/mL) | 83 | 76 ( 91.6) | 7 ( 8.4) | 12.0 (10.0,15.0) | |
| Total | 211 | 197 | 14 | | |

Multivariate Cox Proportional Hazards Model analysis for the relationship between the time to resolution of CMV disease and baseline CMV viral load shows that the HR for baseline CMV viral load was 1.46 (95% CI = 1.08 to 1.99; P = 0.015), indicating a 46% higher chance of resolution of CMV disease at any point in time among participants with baseline CMV viral load <18,200 IU/mL (< 20,000 cp/mL) compared to those with baseline CMV viral load ≥18,200 IU/mL (≥20,000 cp/mL), after adjusting for relevant baseline covariates, i.e., age (continuous), sex, ethnicity/race, randomized treatment received, recipient (R) CMV serostatus, previous anti-CMV therapy, and prior immunosuppressive regimen (Cyclosporine A, T-Cell Suppressors, and/or corticosteroids).

***Day 7, Day 14, and Day 21 Viral Load Suppression (<LLoQ) and Clinical Resolution of CMV Disease***

The Kaplan-Meier survival plot for the time to resolution of CMV disease stratified by viral suppression <LLoQ by Day 7 shows wide separation between the survival curves from days 4 to 27. The unadjusted HR for the association of viral suppression at Day 7 and the time to resolution of CMV disease was 1.49 (95% CI of 1.07 to 2.07; Log-Rank Test P = 0.019).

Kaplan-Meier Survival Plot of Time to Resolution of CMV Disease by On-Treatment Viral Load Suppression <LLoQ by Day 7

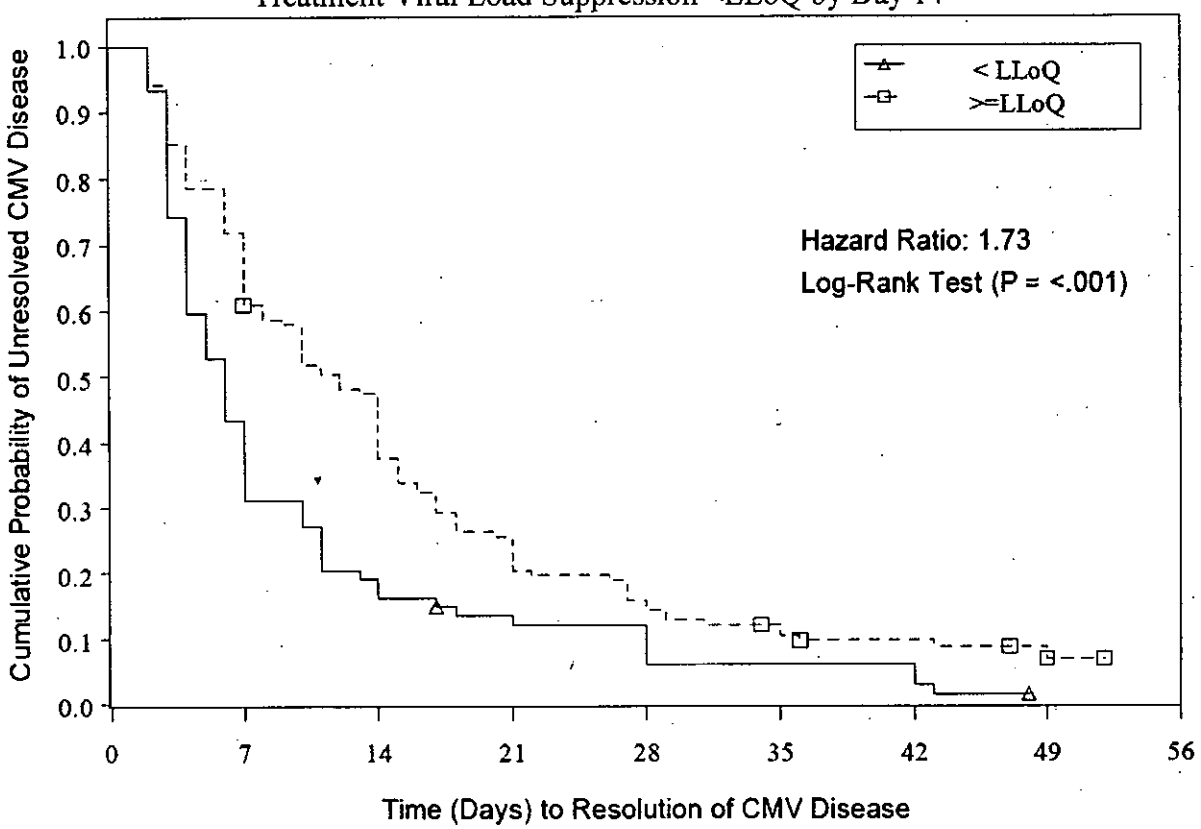



| Viral Suppression by Day 7 | No. of Participants | Resolved n (%) | Censored n (%) | Median Time to Resolution in Days (95% CI) | Hazard Ratio(HR) |
|---|---|---|---|---|---|
| <LLoQ | 49 | 47 ( 95.9) | 2 ( 4.1) | 6.0 ( 5.0, 7.0) | 1.49 |
| ≥LLoQ | 156 | 145 ( 92.9) | 11 ( 7.1) | 10.0 ( 7.0,14.0) | |
| Total | 205 | 192 | 13 | | |

After adjusting for relevant baseline covariates, i.e., age (continuous), sex, ethnicity/race, randomized treatment received, recipient (R) CMV serostatus, previous anti-CMV therapy, and prior immunosuppressive regimen (Cyclosporine A, T-Cell Suppressors, and/or corticosteroids), the HR for viral suppression by Day 7 was 1.62 (95% CI = 1.12 to 2.35; P = 0.010) from the Multivariate Cox Proportional Hazards Model. This result indicates that kidney transplant recipients with CMV disease and receiving anti-CMV therapy with a suppressed viral load by Day 7 have more rapid resolution of their CMV disease.

Shorter times to resolution of CMV disease were also observed for participants who exhibited viral suppression <LLoQ by Day 14. The log-rank test indicates a statistically significant difference between the survival curves (unadjusted hazard ratio [HR] = 1.73 with 95% CI of 1.29 to 2.32; Log-Rank Test P < 0. 001).

Kaplan-Meier Survival Plot of Time to Resolution of CMV Disease by On-Treatment Viral Load Suppression <LLoQ by Day 14

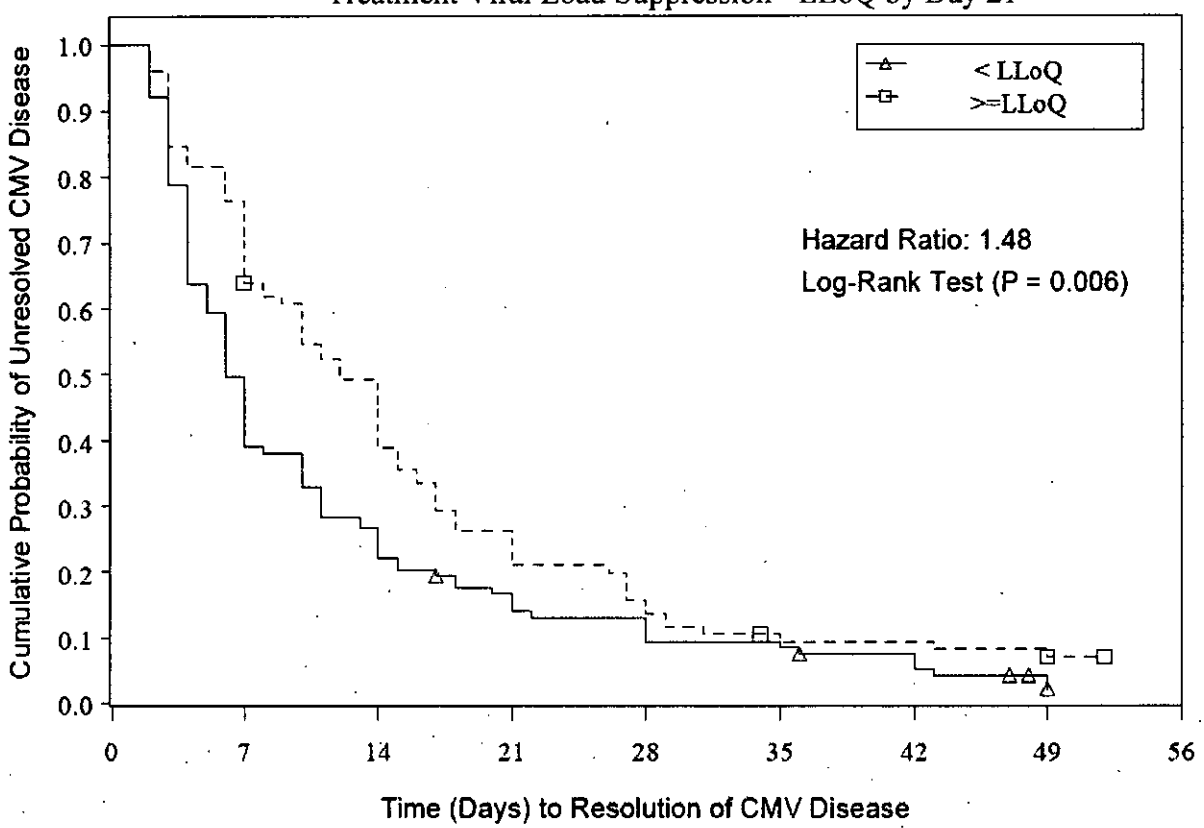



| Viral Suppression by Day 14 | No. of Participants | Resolved n (%) | Censored n (%) | Median Time to Resolution in Days (95% CI) | Hazard Ratio(HR) |
|---|---|---|---|---|---|
| <LLoQ | 74 | 72 ( 97.3) | 2 (2.7) | 6.0 ( 4.0, 7.0) | 1.73 |
| ≥LLoQ | 135 | 124 ( 91.9) | 11 (8.1) | 12.0 ( 8.0,14.0) | |
| Total | 209 | 196 | 13 | | |

Multivariate Cox Proportional Hazards Model analysis for the relationship between the time to resolution of CMV disease and viral suppression <LLoQ by Day 14 shows the HR of 1.83 (95% CI = 1.33 to 2.51; P < 0.001), indicates that there is an 83% greater chance of resolution of CMV disease at any point in time among participants with viral suppression <LLoQ by Day 14 compared to those with viral suppression ≥LLoQ by Day 14, after adjusting for relevant baseline covariates,i.e., age (continuous), sex, ethnicity/race, randomized treatment received, recipient (R) CMV serostatus, previous anti-CMV therapy, and prior immunosuppressive regimen (Cyclosporine A, T-Cell Suppressors, and/or corticosteroids).

The Kaplan-Meier survival plot for time to resolution of CMV disease stratified by those participants who do or do not exhibit viral suppression <LLoQ by Day 21 also shows separation between survival curves (unadjusted hazard ratio [HR] = 1.48 with 95% CI 1.11 to 1.96; Log-Rank Test P = 0. 006).

Kaplan-Meier Survival Plot of Time to Resolution of CMV Disease by On Treatment Viral Load Suppression <LLoQ by Day 21




| Viral Suppression by Day 21 | No. of Participants | Resolved n (%) | Censored n (%) | Median Time to Resolution (95% CI) | Hazard Ratio(HR) |
|---|---|---|---|---|---|
| <LLoQ | 113 | 108 (95.6) | 5 (4.4) | 6.0 ( 5.0, 7.0) | 1.48 |
| ≥LLoQ | 97 | 89 (91.8) | 8 (8.2) | 12.0 (10.0,14.0) | |
| Total | 210 | 197 | 13 | | |

After adjusting for relevant baseline covariates, i.e., age (continuous), sex, ethnicity/race, randomized treatment received, recipient (R) CMV serostatus, previous anti-CMV therapy, and prior immunosuppressive regimen (Cyclosporine A, T-Cell Suppressors, and/or corticosteroids), the HR for viral suppression by Day 21 was 1.44 (95% CI = 1.07 to 1.92; P = 0.015) from the Multivariate Cox Proportional Hazards Model. This result indicates that there is a 44% greater chance of resolution of disease at any point in time among participants with viral suppression <LLoQ by Day 21 compared to those with viral suppression ≥LLoQ by Day 21, after adjusting for relevant baseline covariates.

The analysis of 1.5 $\log_{10}$ IU/mL decline in viral load from Baseline at Day 14 calculated an unadjusted hazard ratio of 0.90 with 95% CI of 0.67 to 1.20 (Log-Rank Test P = 0.460). This finding did not change in multivariate analysis adjusting for baseline covariates, i.e., age (continuous), sex, ethnicity/race, randomized treatment received, recipient (R) CMV serostatus, previous anti-CMV therapy, and prior immunosuppressive regimen (Cyclosporine A, T-Cell Suppressors, and/or corticosteroids), indicating that a decline in viral load of greater than 1.5 $\log_{10}$ IU/mL was not associated with time to resolution of CMV disease. Other degrees of viral load decline ($\geq$1.0, $\geq$2.0, and $\geq$2.5 $\log_{10}$ IU/mL) were also evaluated and also not found to be predictive of time to resolution of CMV disease (data not shown).

The table below summarizes the findings from Multivariate Cox Proportional Hazards Models for the association between viral load based variables of interest (Baseline, 1.5 $\log_{10}$ decline at Day 14, and viral load suppression at Days 7, 14, and 21) and the time to CMV disease resolution. The multivariate models were adjusted for age, sex, race/ethnicity, recipient CMV serostatus, previous anti-CMV therapy, randomization to ganciclovir or valganciclovir arms, and prior immunosuppressive regimen. Due to incomplete data, donor serostatus and CMV gB genotype were not included in the Multivariate Cox Proportional Hazards Models.[3]

Adjusted Hazard Ratios for Viral Load-Based Variables of Interest from Multivariate Cox Proportional Hazards Model for the Time to Resolution of CMV Disease, Adjusted for Relevant Baseline Covariates

| | | | Adjusted Hazard Ratio[a] | | |
|---|---|---|---|---|---|
| **Viral Load-Based Variables of Interest** | **Viral Load Category** | **N** | **Estimate** | **95% CI** | **P-value** |
| Baseline CAP/CTM CMV Test Result | < 18,200 IU/mL | 207 | **1.46[c]** | **(1.08, 1.99)** | **0.015** |
| | ≥ 18,200 IU/mL | | (1.00) | | |

3 Of the 211 evaluable subjects, 88 (41.7%) had missing CMV gB genotyping results (most of these subjects had Day 3 results <2,000 copy/mL). Multivariate Cox Proportional Hazard analyses of 123 subjects with available CMV gB genotyping results that include all Viral Load-Based Variables of Interest, current baseline covariates, and CMV genotype did not show statistically significant HR for the Viral Load-Based Variables. Exclusion of the subjects without CMV gB genotyping results due to lower viral loads (i.e., lower viral loads resulted in the failure of obtaining CMV gB genotyping results by using the Sanger sequencing method) from the analyses can potentially bias the relationships between the viral load variables and time to clinical outcomes. Therefore, the results of these analyses cannot be generalized to the population similar to the VICTOR study population (kidney transplant recipients previously diagnosed with CMV disease and treated with anti-CMV (ganciclovir or valganciclovir). In addition, there were not sufficient data to evaluate the donor serostatus covariate in the multivariate models with statistical significance.

| | | | | | |
|---|---|---|---|---|---|
| On-Treatment Viral Load Decline at Day 14 | ≥ 1.5 log10 IU/mL | 205 | 0.93 | (0.68, 1.26) | 0.633 |
| | < 1.5 log10 IU/mL | | (1.00) | | |
| Viral Suppression by Day 7 | < LLoQ | 201 | **1.62** | **(1.12, 2.35)** | **0.010** |
| | ≥ LLoQ | | (1.00) | | |
| Viral Suppression by Day 14 | < LLoQ | 205 | **1.83** | **(1.33, 2.51)** | **<.001** |
| | ≥ LLoQ | | (1.00) | | |
| Viral Suppression by Day 21 | < LLoQ | 206 | **1.44** | **(1.07, 1.92)** | **0.015** |
| | ≥ LLoQ | | (1.00) | | |

[a] Adjusted for age (continuous), sex, ethnicity/race, randomized treatment received, recipient (R) CMV serostatus, previous anti-CMV therapy, and prior immunosuppressive regimen (Cyclosporine A, T-Cell Suppressors, and/or corticosteroids).
[b] CI = confidence interval.
[c] Note: Rows with hazard ratio estimates within parentheses indicate reference categories.

***Conclusion***

The CAP/CTM CMV Test provides clinical value for the baseline testing and assessing virological response of patients with CMV disease who are undergoing treatment with anti-CMV drugs (ganciclovir or valganciclovir). Clinicians managing such patients would benefit from knowing that patients with a baseline CMV viral load <18,200 IU/mL (20,000 copies/mL) are likely to resolve CMV disease more rapidly than those who have a higher baseline viral load. Data of this clinical study did not demonstrate that a decline in viral load of 1.5 $\log_{10}$ by Day 14 of treatment is informative to assess treatment response; however, viral load suppressions (defined as viral load below LLoQ) at Days 7, 14, and 21 are highly correlated with resolution of CMV disease.

## XI. SUMMARY OF SUPPLEMENTAL CLINICAL INFORMATION

***Guidelines for CMV DNA Testing in Clinical Practice***

Published guidelines and the medical literature support the importance of measuring CMV levels prior to treatment and at intervals during treatment [6][7]. The International Consensus Guidelines on the Management of Cytomegalovirus in Solid Organ Transplantation [8] state that laboratory monitoring of CMV should be applied weekly during the treatment phase with a quantitative nucleic acid test (QNAT) or antigenemia-based assay to monitor response and the possible development of resistance. Trends of serial monitoring are easier to interpret than an individual test result. Two consecutive negative samples (preferably sampled one week apart) have been recommended as a virological endpoint for treatment of acute CMV episodes. Periodic viral load monitoring should also be performed during secondary prophylaxis. This most recent document highlights the importance of serial testing of CMV DNA levels, and describes the issues associated with using a "universal" cutoff value for initiating therapy.

## XII. PANEL MEETING RECOMMENDATION AND FDA'S POST-PANEL ACTION

In accordance with the provisions of section 515(c)(2) of the act as amended by the Safe Medical Devices Act of 1990, this PMA was not referred to the Microbiology Advisory Panel, an FDA advisory committee, for review and recommendation because the information in the PMA duplicates information previously reviewed by this panel.

## XIII. CONCLUSIONS DRAWN FROM PRECLINICAL AND CLINICAL STUDIES

### A. Effectiveness Conclusions

The effectiveness of the CAP/CTM CMV Test has been demonstrated when used for the quantitation of cytomegalovirus (CMV) DNA in human EDTA plasma. A reasonable determination of effectiveness of the CAP/CTM CMV Test for aiding in the management of solid-organ transplant patients who are undergoing anti-CMV therapy, by serially measuring CMV DNA levels at baseline and during treatment to assess virological response to treatment, in conjunction with other laboratory results and clinical information, has been demonstrated.

### B. Safety Conclusions

Based on the results of the analytical and clinical laboratory studies, the CAP/CTM CMV Test, when used according to the provided directions and in conjunction with other laboratory results and clinical information, should be safe and pose minimal risk to the patient due to false test results.

### C. Benefit-Risk Conclusions

The probable benefits of the device are also based on data collected in a clinical study and in the analytical studies conducted to support PMA approval as described above. When used for the intended use, benefits to the clinicians and patients include: 1) an assessment of both the likely time to symptom resolution and the likely time to a decrease in CMV viral load below LLoQ in patients post-renal transplantation being treated for symptomatic CMV infection, and 2) confirmation that CMV viral load is responding to treatment as anticipated. The risks for the intended use are relatively low. When the assay is used as a predictive assay, i.e., for predicting rapidity of response, the assay would not be used to alter treatment and would therefore essentially pose no risk. The risk is greater when assessing response, i.e., an inaccurate result could lead to either initiating an alternative treatment (i.e., falsely assuming the patient is not responding) or prematurely stopping treatment (i.e., falsely assuming that the patient is below the test LLoQ). These risks, however, are substantially mitigated by recommendations for serial measurement of CMV viral load.

In conclusion, given the available information above, the data support that for the CAP/CTM CMV Test the probable benefits outweigh the probable risks.

### D. Overall Conclusions

The data in this application support the reasonable assurance of safety and effectiveness of this device when used in accordance with the indications for use. The data from the preclinical studies demonstrated acceptable analytical sensitivity, traceability, linearity, precision, and analytical specificity of the CAP/CTM CMV Test when used according to the instructions for use as stated in the labeling, the warnings and precautions, and limitations sections of the labeling. The clinical usefulness study and the statistical analysis of clinical data in this application has shown that serial CMV DNA levels measured with the CAP/CTM CMV Test are informative for assessing the virological response to treatment in solid organ transplant patients who are undergoing anti-CMV drug therapy, and that the test is safe and effective when used according to the directions for use in the labeling.

## XIV. CDRH DECISION

CDRH issued an approval order on July 5, 2012. The final conditions of approval cited in the approval order are described below.

The applicant's manufacturing facilities were inspected and found to be in compliance with the device Quality System (QS) regulation (21 CFR 820).

## XV. APPROVAL SPECIFICATIONS

Directions for use: See device labeling.

Hazards to Health from Use of the Device: See Indications, Contraindications, Warnings, Precautions, and Adverse Events in the device labeling.

Post-approval Requirements and Restrictions: See approval order.

## XVI. REFERENCES

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