SUMMARY OF SAFETY AND EFFECTIVENESS DATA (SSED)

I. GENERAL INFORMATION

Device Generic Name: Real-time polymerase chain reaction (PCR) based assay for CMV viral load measurement

Device Trade Name: **cobas**® CMV

Device Procode: PAB

Applicant's Name and Address: Roche Molecular Systems, Inc. (RMS)
4300 Hacienda Drive
Pleasanton, CA 94588-2722

Date(s) of Panel Recommendation: None

Premarket Approval Application (PMA) Number: P160041

Date of FDA Notice of Approval: June 1, 2017

II. INDICATIONS FOR USE

cobas® CMV is an in vitro nucleic acid amplification test for the quantitation of cytomegalovirus (CMV) DNA in human EDTA plasma.

cobas® CMV is intended for use as an aid in the management of CMV in solid organ transplant patients and in hematopoietic stem cell transplant patients. In patients receiving anti-CMV therapy, serial DNA measurements can be used to assess viral response to treatment.

The results from **cobas**® CMV must be interpreted within the context of all relevant clinical and laboratory findings.

cobas® CMV is not intended for use as a screening test for blood or blood products.

III. CONTRAINDICATIONS

There are no known contraindications.

IV. WARNINGS AND PRECAUTIONS

The warnings and precautions can be found in the **cobas** CMV product labeling.

V. DEVICE DESCRIPTION

The **cobas** CMV test is a quantitative nucleic acid amplification assay performed on either the **cobas** 6800 System or the **cobas** 8800 System. The **cobas** CMV test enables the detection and quantitation of CMV DNA in EDTA plasma from solid organ transplant patients (SOT) and from hematopoietic stem cell transplant (HSCT) patients. The test is intended for use as an aid in the management of SOT patients and HSCT patients who are undergoing CMV antiviral therapy.

The CMV viral load is quantified against a non-CMV DNA quantitation standard (DNA-QS), which is introduced into each specimen during sample preparation. The DNA-QS also functions as an internal control for sample preparation and the PCR amplification process. In addition, the test utilizes three external controls: a high titer positive, a low titer positive, and a negative control.

The **cobas** CMV test system consists of:

- **cobas** 6800/8800 Systems (including **cobas** 6800/8800 Systems Software)
- **cobas** CMV Assay Specific Analysis Package (ASAP) software
- **cobas** CMV test reagents in kit cassettes
- **cobas** CMV Control reagents (HPC and LPC) in kit cassettes
- **cobas** NHP Negative Control reagent in kit cassettes
- Specimen preparation reagents (**cobas** OMNI Reagents)

The **cobas** CMV test uses fully automated sample preparation (nucleic acid extraction and purification) followed by PCR amplification and detection. The **cobas** 6800/8800 Systems consist of the sample supply module, the transfer module, the processing module, and the analytic module. Automated data management is performed by the **cobas** 6800/8800 Systems Software which assigns test results for all tests. Results can be reviewed directly on the system screen, exported, or printed as a report.

Target Selection
Selective amplification of CMV target nucleic acid from the sample is achieved by the use of specific forward and reverse primers which are selected from highly-conserved regions of the CMV DNA polymerase (UL54) gene. A single probe is used to detect and quantify the CMV targets. Selective amplification of DNA-QS is achieved by the use of sequence-specific forward and reverse primers which are selected to have no homology with the CMV genome.

Sample Preparation (Nucleic Acid Extraction and Purification)
The **cobas** CMV test is intended to be used with plasma (EDTA) samples. Nucleic acid from patient samples, external controls and added DNA-QS molecules are extracted simultaneously. Viral nucleic acid is released by addition of proteinase and lysis reagent to the sample. The released nucleic acid binds to the silica surface of the added magnetic glass particles. Unbound substances and impurities, such as denatured proteins, cellular debris and potential PCR inhibitors are removed with subsequent wash reagent steps and

purified nucleic acid is eluted from the magnetic glass particles with elution buffer at elevated temperature.

Nucleic Acid Amplification and Target Detection
The **cobas** CMV master mix contains detection probes which are specific for the CMV target sequence and the DNA-QS nucleic acid, respectively. The specific CMV and DNA-QS detection probes are each labeled with one of two unique fluorescent dyes which act as a reporter. The two reporter dyes are measured at defined wavelengths, thus permitting simultaneous detection and discrimination of the CMV target and the DNA-QS amplification products generated by a thermostable DNA polymerase enzyme.

Each probe also has a second dye which acts as a quencher. When not bound to the target sequence, the fluorescent signals of the intact probes are suppressed by a quencher dye. During the PCR amplification step, hybridization of the probes to the specific single-stranded DNA template results in cleavage of the probe by the 5' to 3' exonuclease activity of the DNA polymerase resulting in separation of the reporter and quencher dyes and the generation of a fluorescent signal. With each PCR cycle, increasing amounts of cleaved probe is generated and the cumulative signal of the reporter dye increases concomitantly. Since the two specific reporter dyes are measured at defined wavelengths, simultaneous detection and discrimination of the amplified CMV target and the DNA-QS are possible.

The master mix also includes deoxyuridine triphosphate (dUTP), instead of deoxythimidine triphosphate (dTTP); the former is incorporated into the newly synthesized DNA (amplicon). Any contaminating amplicons from previous PCR runs are eliminated by the AmpErase enzyme, which is included in the PCR mix, during the first thermal cycling step. However, newly formed amplicons are not eliminated since the AmpErase enzyme is inactivated once exposed to temperatures above 55°C.

CMV DNA Quantitation
During the extension phase of the PCR process, the fluorescent signals of the cleaved CMV and DNA-QS probes are collected for each specimen. 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 **cobas** 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. CMV viral load results are reported in International Units/mL (IU/mL).

Controls
One replicate each of the **cobas** CMV Negative Control, the CMV Low Positive Control, and the CMV High Positive Control must be included in each run. The validity of the results for the controls as well as for the DNA-QS is determined by the assay specific analysis software package used by the **cobas** 6800/8800 instrument. The run is valid if no flags appear for any of the controls. Otherwise the **cobas** 6800/8800 software will

automatically invalidate the run and generate flags to provide further explanation to the user. The user should check for flags and their associated results in the **cobas** 6800/8800 software and/or report.

- 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 within the label of the **cobas** CMV Control kit. 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.

Instrumentation and Software

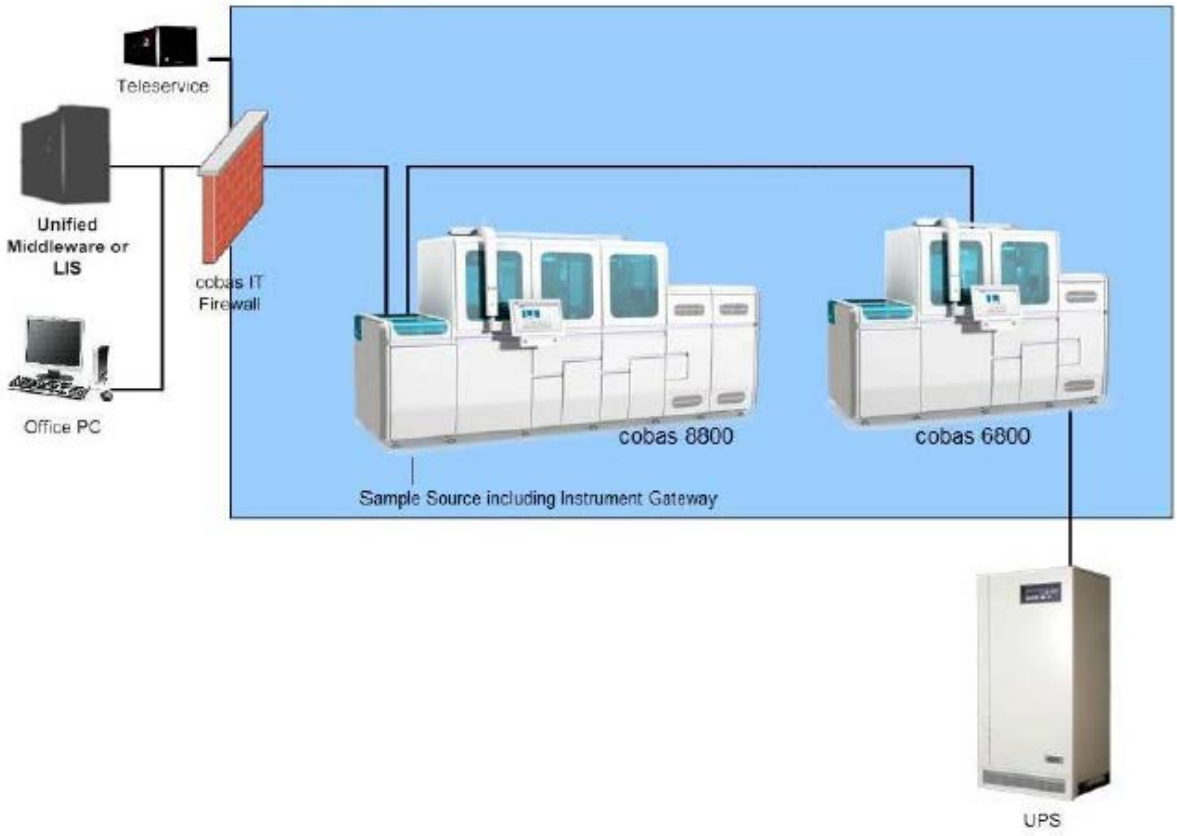
The **cobas** 6800/8800 platform consists of two instrument versions: the **cobas** 6800 System, and the **cobas** 8800 System. Each system is comprised of a **cobas** 6800 or **cobas** 8800 instrument, system software, Assay Specific Analysis Packages (ASAP), and a sample source unit, which can be connected to a conveyor system for automated transport of samples to and from the system. The test kits consist of assay-specific reagents and omni reagents (or common reagents) which can be used with any of the **cobas** assays, and on either the **cobas** 6800 or the **cobas** 8800 system.

In addition, the **cobas** omni (common) reagents and consumables, such as the P-plates, racks, AD-plates, waste bags, pipette tips, and secondary tubes, can be used with any of the **cobas** assays, and on either the **cobas** 6800 or the **cobas** 8800 systems.

Either system can be interfaced to an uninterruptible power supply (UPS), a Laboratory Information System (LIS) or middleware, and office PCs for remote monitoring functionalities.

Figure 1 below depicts the **cobas** 6800/8800 Platform.

Figure 1: cobas 6800/8800 Platform



Interpretation of Results

Results are determined automatically by the **cobas** software and are reported in IU/mL (scientific format). The calculated CMV concentration (in IU/mL) is only provided for samples within the linear range of the assay. Result reporting is shown in the following table:

Table 1: Result Interpretation

Results	Interpretation
Target Not Detected	CMV DNA not detected. Report results as "CMV not detected."
< Titer Min	Calculated titer is below the Lower Limit of Quantitation (LLoQ) of the assay. Report results as "CMV detected, less than (Titer Min)." Titer min = 34.5 IU/mL
Titer	Calculated titer is within the Linear Range of the assay – greater than or equal to Titer Min and less than or equal to Titer Max. Report results as "(Titer) of CMV detected".
> Titer Max[a]	Calculated titer is above the Upper Limit of Quantitation (ULoQ) of the assay. Report results as "CMV detected, greater than (Titer Max)." Titer max = 1.0E+07 IU/mL

a Sample result > Titer Max refers to CMV positive samples detected with titers above the upper limit of quantitation (ULoQ). If a quantitative result is desired, the original sample should be diluted with CMV-negative human EDTA plasma and the test should be repeated. Multiply the reported result by the dilution factor.

Kit Configuration and Components

cobas CMV Reagent Cassetts:

- Proteinase Solution
- DNA Quantitation Standard (DNA-QS)
- Elution Buffer (EB)
- Master Mix Reagent 1 (MMX-R1)
- CMV Master Mix Reagent 2 (CMV MMX-R2)

cobas CMV Control Kit:

- CMV Low Positive Control (CMV L(+)C)
- High Positive Control (CMV H(+)C)

cobas NHP Negative Control kit:

- Normal Human Plasma (NHP) Negative Control

cobas omni Reagents:

The **cobas** omni reagents are common sample preparation reagents that are used with other assays that are run on the **cobas** 6800/8800 Systems and are:

- **cobas** omni MGP Reagent (containing magnetic glass particles that bind nucleic acid)
- **cobas** omni Lysis Reagent
- **cobas** omni Specimen Diluent
- **cobas** omni Wash Reagent

VI. ALTERNATIVE PRACTICES AND PROCEDURES

There are currently two other alternatives for the quantitation of CMV in the management of transplant patients.

- COBAS AmpliPrep/COBAS TaqMan CMV Test (Roche Molecular Systems)
- *artus* CMV RGQ MDx Kit (QIAGEN)

Each alternative has its own advantages and disadvantages.

VII. MARKETING HISTORY

The **cobas** CMV test is currently available in the countries listed below. To date, there have been no adverse incidents or potentially-critical complaints reported for this test. The device has not been withdrawn from marketing for any reasons related to its safety or effectiveness.

Argentina	Austria	Belgium
Brazil	Bulgaria	Columbia
Croatia	Cyprus	Czech Republic
Denmark	Ecuador	Egypt
Estonia	Finland	France

Germany	Guatemala	Greece
Hungary	Iceland	India
Ireland	Italy	Latvia
Liechtenstein	Lithuania	Luxembourg
Malta	Netherlands	Norway
Panama	Pakistan	Peru
Poland	Portugal	Romania
Russia	Saudi Arabia	Singapore
Slovakia	Slovenia	Spain
Sweden	Switzerland	Thailand
Turkey	United Arab Emirates	United Kingdom

VIII. POTENTIAL ADVERSE EFFECTS OF THE DEVICE ON HEALTH

Failure of the **cobas** CMV test to perform as indicated, or human error in the use of the test, may result in an incorrect test result that is too low or too high. The following factors can impact the performance of the **cobas** CMV test:

- Mutations in the CMV UL54 gene have been observed. Mutant CMV variants may not be detectable or detectable with decreased efficiency if mutations in the primer or probe binding sites occur; such mutations consequently may result in the failure to detect CMV or in the underquantitation of CMV viral loads. The test design for the **cobas** CMV test mitigates, but does not entirely exclude, this risk. Additional testing should be performed if mutations are suspected.
- Invasive CMVdisease may not be reflected in detectable CMV viral DNA in peripheral blood. The results from the **cobas** CMV test must therefore be interpreted by a qualified healthcare professional.

Failure of the device to perform as expected or failure to correctly interpret test results in the context of all clinical findings may lead to improper patient management decisions. A test result that is erroneous negative or too low may lead to 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.

To mitigate these risks in the management of transplant patients the viral load results from the **cobas** CMV test must be interpreted in the context of all relevant clinical and laboratory findings by a medical professional who is adequately trained in the interpretation of CMV viral load results in the context of transplant patient management.

IX. SUMMARY OF NONCLINICAL STUDIES

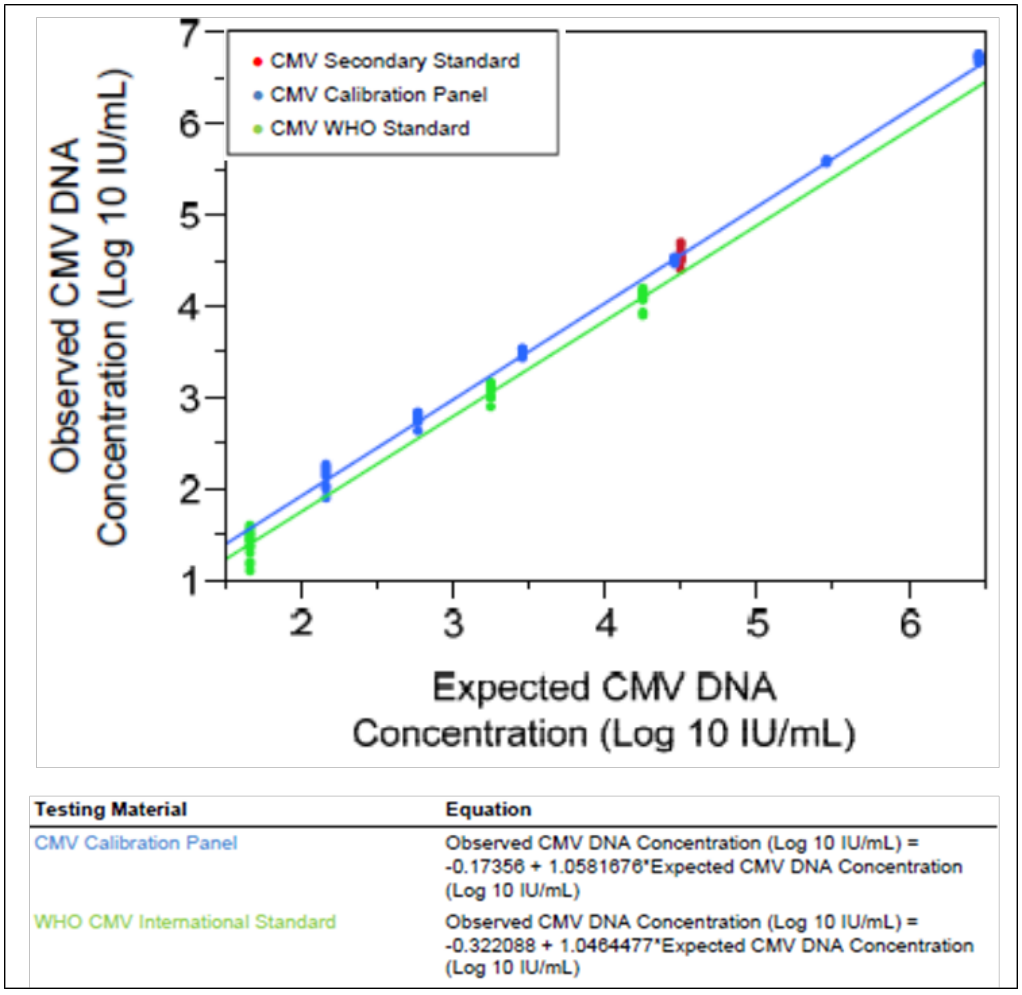
A. Laboratory Studies

1. Traceability to the 1st WHO International Standard for Human Cytomegalovirus for Nucleic Acid Amplification Techniques Based Assays

Several standards and controls have been used during development of the **cobas** CMV test to provide traceability to the WHO CMV Standard [1 st WHO International Standard for human Cytomegalovirus DNA for Nucleic Acid Amplification Techniques (NIBSC 09/162)]. The standards used during development of the test include the WHO CMV Standard, the Roche Molecular Systems (RMS) CMV Secondary Standard, and the RMS CMV Calibration Panel. The concentration range tested for the CMV WHO Standard was from 4.60E+01 IU/mL to 1.80E+04 IU/mL (1.66 – 4.26 $\log_{10}$ IU/mL), the RMS CMV Secondary Standard was tested at 3.16E+04 IU/mL (4.50 $\log_{10}$ IU/mL), and the RMS CMV Calibration Panel was tested from 1.47E+02 to 2.94E+06 IU/mL (2.17 - 6.47 $\log_{10}$ IU/mL).

All materials demonstrated co-linear dilution performance across the linear range of **cobas** CMV (Figure 2) albeit the calibration panel delivers slightly higher values for the **cobas** CMV test. Based on these results, the calibration and standardization process of **cobas** CMV provides quantitation values for the calibration panel, the RMS CMV Secondary Standard, and the CMV WHO Standard that are similar to the expected values with deviation of not more than 0.23 $\log_{10}$ IU/mL. The maximum deviation was obtained around the test LLOQ.

Figure 2: Traceability to HCMV WHO International Standard Using cobas CMV



Testing Material	Equation
CMV Calibration Panel	Observed CMV DNA Concentration (Log 10 IU/mL) = -0.17356 + 1.0581676*Expected CMV DNA Concentration (Log 10 IU/mL)
WHO CMV International Standard	Observed CMV DNA Concentration (Log 10 IU/mL) = -0.322088 + 1.0464477*Expected CMV DNA Concentration (Log 10 IU/mL)

2. Limit of Detection (LOD)

a. LOD Using the 1st WHO International Standard for Human Cytomegalovirus

The LOD of the **cobas** CMV test for the 1st WHO CMV Standard was determined by analysis of serial dilutions of the Standard in CMV-negative human EDTA plasma. Panels of eight concentration levels plus a blank were tested over three lots of **cobas** CMV test reagents and three instruments with multiple runs and operators over a period of 3 days. Each dilution was determined in 21 replicates per lot and day (n=63 total replicates per day).

The results from testing the WHO CMV Standard in EDTA plasma as well as the calculated LOD values are shown in Table 2. The LOD values in Table 2 were determined by Probit analysis which provided the concentration of CMV expected to give a 95% hit rate. The study demonstrated that the highest LOD, namely 30.7 IU/mL, was obtained with lot 2 as determined by Probit analysis. The lowest concentration of CMV that was experimentally observed to give a a hit rate ≥ 95% with lot 2 was 34.5 IU/mL; at this concentration, the observed hit rate was 98.4% (62/63). The claimed LoD value is 34.5 IU/mL and this concentration was used in studies for confirmation of the LoD.

Table 2: LOD with CMV DNA 1st WHO International Standard in EDTA Plasma

Kit Lot	Nominal Concentration (IU/mL)	Number of Valid Replicates	Number of Positive Replicates	Hit Rate [%]	LOD by Probit [95% CI]
Lot 1	92	63	63	100.0	14.7 IU/mL [11.7 – 20.0 IU/mL]
	46	63	63	100.0	
	34.5	62	62	100.0	
	23	63	62	98.4	
	11.5	63	57	90.5	
	5.8	63	45	71.4	
	2.9	63	26	41.3	
	1.4	63	11	17.5	
	0	63	0	0.0	
Lot 2	92	63	63	100.0	30.7 IU/mL [24.5 – 40.9 IU/mL]
	46	63	62	98.4	
	34.5	62	62	98.4	
	23	63	57	90.5	
	11.5	63	43	68.3	
	5.8	63	27	42.9	
	2.9	63	16	25.4	
	1.4	63	4	6.4	
	0	63	0	0.0	
Lot 3	92	63	63	100.0	14.6 IU/mL [11.6 – 19.9 IU/mL]
	46	63	63	100.0	
	34.5	62	63	100.0	
	23	63	62	98.4	
	11.5	63	58	92.1	
	5.8	63	45	71.4	
	2.9	63	24	38.1	
	1.4	63	13	20.6	
	0	63	0	0.0	
All lots combined	92	189	189	100.0	20.6 IU/mL [17.9 – 24.3 IU/mL]
	46	189	188	99.5	
	34.5	188	187	99.5	
	23	189	181	95.8	
	11.5	189	158	83.6	
	5.8	189	117	61.9	
	2.9	189	66	34.9	
	1.4	189	28	14.8	
	0	189	0	0.0	

b. Verification of the LOD for Glycoprotein B Genotypes gB-2, gB-3 and gB-4

The Limit of Detection (34.5 IU/mL) was verified for the **cobas** CMV test with all relevant CMV Glycoprotein B Genotypes (gB-2, gB-3 and gB-4) following the CLSI Guideline EP17-A2. CMV cell culture supernatants for the three different Glycoprotein B genotypes were diluted to three different concentration levels in CMV negative EDTA plasma. The hit rate determination was performed with 63 replicates for each level. Testing was conducted with three lots of **cobas** CMV reagents across three days of testing.

The results are shown in Table 3 (below) and verify that a hit rate of 95% or higher was observed at 34.5 IU/mL for each genotype. Thus, the observed hit rates verify that the LOD for each of the three genotypes is 34.5 IU/mL or lower.

Table 3: Verification of the LOD for Different Glycoprotein B (gB) Genotypes Across Three Lots.

gB Genotypes	Nominal Concentration (IU/mL)	Number of Valid Replicates	Number of Positive Replicates	Hit Rate [%]	LOD by Hit Rate
gB-2	17.25	63	61	96.8	17.25 IU/mL
	34.5	63	63	100.0	
	51.75	63	63	100.0	
gB-3	17.25	63	57	90.5	34.5 IU/mL
	34.5	63	63	100.0	
	51.75	63	63	100.0	
gB-4	17.25	63	55	87.3	34.5 IU/mL
	34.5	63	63	100.0	
	51.75	63	63	100.0	

c. Verification of the LOD for Different Drug Resistant CMV Isolates

The Limit of Detection (34.5 IU/mL) was verified for the **cobas** CMV test with cell culture supernatants from two different drug resistant CMV isolates (one isolate resistant against foscarnet and one isolate resistant against ganciclovir, valganciclovir and cidofovir). LOD verification followed the CLSI Guideline EP17-A2. Samples of drug resistant CMV were diluted to three different concentration levels in CMV negative EDTA plasma. Testing was conducted with three lots of **cobas** CMV reagents.The hit rate determination was performed with 63 replicates for each level (n=21 replicates per lot).

The results are shown in Table 4 (below) and verify that the observed LOD for each of the two drug resistant strains of CMV was 34.5 IU/mL or lower.

Table 4: Verification of the LOD for Drug Resistant CMV Isolates

gB Genotypes	Nominal Concentration (IU/mL)	Number of Valid Replicates	Number of Positive Replicates	Hit Rate [%]	LOD by Hit Rate
Forscarnet (E756Q)	17.25	63	58	92.1	34.5 IU/mL
	34.5	63	63	100.0	
	51.75	63	63	100.0	
Ganciclovir, Valganciclovi, Cidofovir (L545S)	17.25	63	59	93.7	34.5 IU/mL
	34.5	63	63	100.0	
	51.75	63	63	100.0	

The Limit of Detection (LOD) for the **cobas** CMV test across the tested genotypes and phenotypes (i.e., drug sensitive and drug resistant) is 34.5 IU/mL (1.54 $\log_{10}$ IU/mL).

3. Lower Limit of Quantitation (LLOQ)

The LLOQ was determined using data from the LOD study with the WHO CMV Standard (Section 2 above) for each of the lots using all concentration levels with a hit rate ≥ 95%.

The LLOQ is defined as the lowest level of CMV that can be reliably detected and at which the total analytical error (TAE) meets both of the following two criteria:

- The TAE, when calculated as |Bias| + 2SD, is ≤1.0 $\log_{10}$ IU/mL, and
- The TAE has to be such that the standard deviation for the difference between two measurements calculated as SQRT(2) x 2 x SD is ≤ 1.0 $\log_{10}$ IU/mL

Meeting the |Bias| + 2SD ≤ 1.0 $\log_{10}$ IU/mL criterion ensures that, for samples with assay values equal to the LLOQ, there is 95% or greater probability that the measured value will be within 1.0 $\log_{10}$ IU/mL of the true value. Meeting the SQRT(2) x 2 x SD ≤ 1.0 $\log_{10}$ IU/mL criterion ensures that, for samples with assay values equal to the LLOQ, a difference of more than 1.0 $\log_{10}$ IU/mL between two measurements is statistically significant (a true change is detected).

Table 5 below shows both criteria for the total analytical error (TAE = |Bias| + 2 x SD ≤ 1.0 $\log_{10}$ IU/mL and SQRT(2) x 2 x SD ≤ 1.0 $\log_{10}$ IU/mL).

Table 5: LLOQ - TAE and Difference between Measurements

Lot	Nominal Concentration (IU/mL)	Nominal Concentration (log_{10} IU/mL)	Mean Observed Concentration (log_{10} IU/mL)	SD (log_{10} IU/mL)	Absolute Bias (log_{10} IU/mL)	TAE (log_{10} IU/mL)	Difference Between Measurements (log_{10} IU/mL)
1	23	1.36	1.04	0.32	0.32	0.96	0.91
	34.5	1.54	1.28	0.29	0.26	0.83	0.81
	46	1.66	1.43	0.17	0.23	0.57	0.48
	92	1.96	1.76	0.16	0.2	0.53	0.46
2*	34.5	1.54	1.42	0.19	0.11	0.5	0.55
	46	1.66	1.63	0.22	0.03	0.47	0.61
	92	1.96	1.84	0.16	0.12	0.44	0.46
3	23	1.36	1.15	0.25	0.21	0.72	0.72
	34.5	1.54	1.32	0.25	0.22	0.71	0.70
	46	1.66	1.48	0.24	0.18	0.66	0.67
	92	1.96	1.8	0.18	0.16	0.52	0.51
Across lots	23	1.36	1.17	0.27	0.2	0.74	0.77
	34.5	1.54	1.34	0.25	0.19	0.69	0.70
	46	1.66	1.51	0.21	0.15	0.57	0.59
	92	1.96	1.8	0.17	0.16	0.5	0.47

* Concentration 23 IU/mL for Lot 2 did not meet 95% hit rate in the LOD study above and was therefore not included in this analysis.

The LLOQ was determined to be 23 IU/mL for lots 1 and 3 and 34.5 IU/mL for lot 2, calculated based on the calculation of the Total Analytical Error (TAE) and the difference between two measurements. The claimed LLOQ for the **cobas** CMV test is 34.5 IU/mL.

4. Linear Range

a. Linear Range for Glycoprotein B Genotype gB-1

Linearity of the **cobas** CMV test was evaluated using a dilution series consisting of 10 panel members with CMV genotype gB-1 DNA concentrations spanning the range of 2.45E+01 IU/mL to 1.34E+07 IU/mL. Each panel member was tested in 48 replicates across three lots of **cobas** CMV test reagents and the results of the study are presented in Table 6 and Figure 3 below.

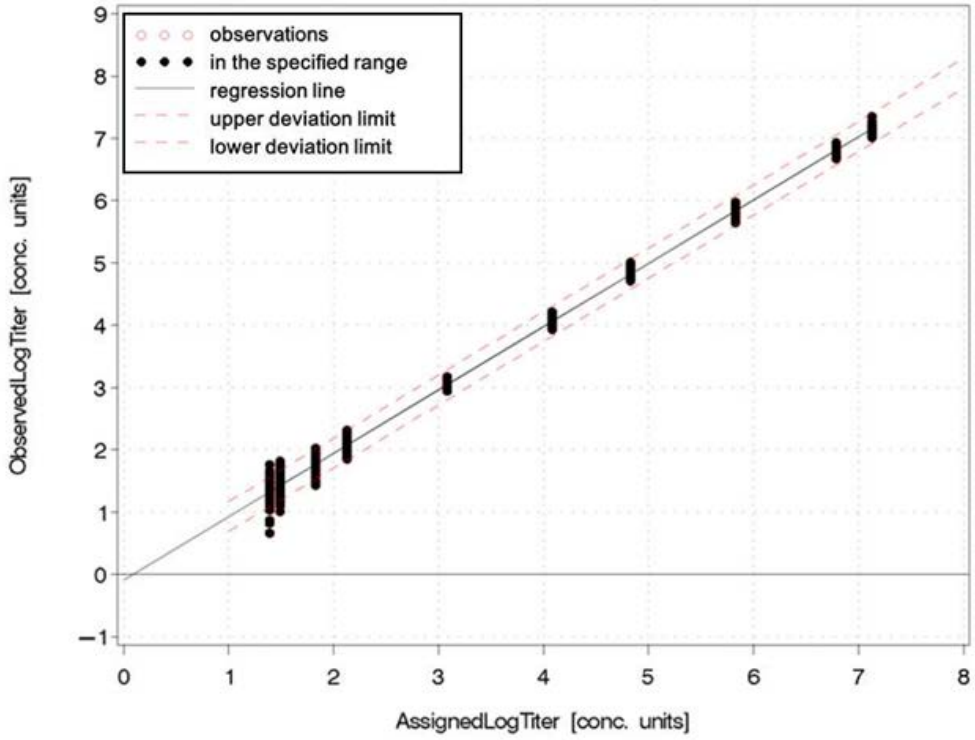
The **cobas** CMV test was demonstrated to be linear from 2.45E+01 IU/mL to 1.34E+07 IU/mL and shows an absolute deviation from the better fitting non-linear regression of less than ± 0.2 log_{10} IU/mL. Across the linear range, the accuracy of the test was within ± 0.1 log_{10} IU/mL.

Based on the LLOQ (34.5 IU/mL) and the determined linear range, the claimed linear measurement range of the **cobas** CMV test is 34.5 – 1.0E+07 IU/mL.

Table 6: cobas CMV Linearity with CMV Genotype gB-1

GT	Linear Equation HCV Genotype Linearity Study (Intercept and Slope in log_{10} IU/mL)	Maximum Difference Between 1st Order Model and Higher Order Model (log_{10} IU/mL)
gB-1	y= 1.0169x + -0.089	n.a.
Note: The linear model (1st order) was observed to be the best fit model after analysis of the combined three lot data as no higher order model is significant. Hence there is no deviation to be shown between 1st order model and any higher order model.		

Figure 3: Linear Range Determination in EDTA Plasma with Genotype gB-1*



*Units on Axis in log_{10} IU/mL

b. Linear Range for Glycoprotein B Genotypes gB-2, gB-3, and gB-4

The dilution series used in the verification of linearity for CMV genotypes gB-2, gB-3, and gB-4 consisted of seven panel members for each genotype spanning the intended linear range of 34.5 – 1.0E+07 IU/mL. Panel members were prepared in EDTA plasma. Sixteen replicates were tested across two lots of **cobas** CMV reagents for each level.

All relevant genotypes (gB-2, gB-3 and gB-4) were detected within the linear range for EDTA plasma established for the predominant genotype gB-1. The linearity within the linear range of **cobas** CMV was verified for all three CMV Glycoprotein B genotypes

(gB-2, gB-3 and gB-4. The maximum deviation between the linear regression and the better fitting non-linear regression was equal to or less than ± 0.1 log_{10}.

Table 7: cobas CMV Linearity Using CMV Genotype gB-2, 3 and 4

GT	Linear Equation HCV Genotype Linearity Study (Intercept and Slope in log_{10} IU/mL)	Maximum Difference Between 1st Order Model and Higher Order Model (log_{10} IU/mL)
gB-2	y= 1.0225 x + -0.0566	n.a.*
gB-3	y= 1.0221 x + -0.0705	n.a.*
gB-4	y= 1.0361 x + -0.1099	-0.11

* n.a.: The linear model (1st order) was observed to be the best fit model after analysis of the combined three lot data as no higher order model is significant. Hence there is no deviation to be shown between 1st order model and any higher order model.

c. Linear Range for CMV Drug Resistant Isolates

The dilution series used in the verification of linearity for CMV drug resistant isolates for the **cobas** CMV test consisted of seven panel members spanning the intended linear range of 34.5 – 1.0E+07 IU/mL. Sixteen replicates were tested across two lots of **cobas** CMV reagent for each level in EDTA plasma.

The linearity within the linear range of **cobas** CMV was verified for two CMV drug resistant isolates (one isolate resistant against foscarnet and one isolate resistant against ganciclovir, valganciclovir and cidofovir). Table 8 shows that the maximum deviation between the linear regression and the better fitting non-linear regression was equal to or less than ± 0.1 log_{10} IU/mL.

Table 8: cobas CMV Linearity Using Drug Resistant CMV

Resistant Phenotype	Linear Equation HCV Genotype Linearity Study (Intercept and Slope in log_{10} IU/mL)	Maximum Difference Between 1st Order Model and Higher Order Model (log_{10} IU/mL)
Ganciclovir /Valganciclovir /Cidofovir	y= 1.0182 x + -0.0259	n.a.*
Foscarnet	y= 1.0285 x + -0.111	-0.09

* The linear model (1st order) was observed to be the best fit model after analysis of the combined two lot data as no higher order model is significant. Hence there is no deviation to be shown between 1st order model and any higher order model.

The claimed linear range of the **cobas** CMV assay, based on the linearity results, was determined to be between 34.5 and 1 x 10^7 IU/mL, or 1.54 log_{10} IU/mL to 7.00 log_{10} IU/mL, for CMV in EDTA plasma, with maximum deviation from linearity of less than or equal to 0.1 log_{10} IU/mL.

5. Precision – Within Laboratory

The precision of the **cobas** CMV test was determined by analysis of serial dilutions of high titer cultured virus (Merlin strain, gB-1 genotype) in CMV negative EDTA plasma. Ten dilution levels were tested in 48 replicates for each level across three lots of **cobas** CMV test reagents using three instruments and three operators over 12 days. Each sample was carried through the entire **cobas** CMV test procedure. Therefore, the reported precision represents all aspects of the test procedure.

The precision data were subjected to multivariate analysis accounting for: reagent lots, operators/instruments, days, runs and within-run replicates. No outliers were removed for analysis of results. The results are shown in Table 9.

Table 9: Within-Laboratory Precision of the cobas CMV test – Lot Specific Standard Deviation (SD*)

Nominal Concentration		Assigned Concentration		Lot 1	Lot 2	Lot 3	All Lots
(IU/mL)	Log_{10} (IU/mL)	(IU/mL)	Log_{10} (IU/mL)	SD	SD	SD	Pooled SD
2.00E+07	7.30	1.34E+07	7.13	0.03	0.06	0.02	0.04
9.11E+06	6.96	6.11E+06	6.79	0.04	0.04	0.03	0.04
1.00E+06	6.00	6.71E+05	5.83	0.05	0.03	0.06	0.05
1.00E+05	5.00	6.71E+04	4.83	0.06	0.05	0.03	0.05
1.80E+04	4.26	1.21E+04	4.08	0.06	0.04	0.05	0.05
1.80E+03	3.26	1.21E+03	3.08	0.04	0.03	0.04	0.04
2.00E+02	2.30	1.34E+02	2.13	0.13	0.10	0.11	0.12
1.00E+02	2.00	6.71E+01	1.83	0.14	0.11	0.09	0.12
4.60E+01	1.66	3.09E+01	1.49	0.20	0.23	0.17	0.20
3.65E+01	1.56	2.45E+01	1.39	0.22	0.20	0.23	0.22

* Standard deviations (SD) was calculated based on log_{10}-transformed results from the **cobas** CMV test.

6. Reproducibility

The reproducibility of the **cobas** CMV test was evaluated with the Merlin strain (gB-1) diluted into EDTA plasma on the **cobas** 6800 System. Reproducibility and lot-to-lot variability testing was performed at 3 sites (2 external clinical sites and one internal site), using 3 reagent lots. Two operators at each site tested each reagent lot for 6 days (3 days for Operator 1 and 3 days for Operator 2). Two runs were performed each day; 3 replicates of each panel member were performed for each run. Data were analyzed using a mixed model to estimate total variance. The evaluation results are summarized in Table 10 through Table 14 below.

Table 10 below shows the clinical reproducibility of the assay at points across the linear range. The relative contributions of different factors to the observed variance are shown.

Table 10: Attributable Percentage of Total Variance, Total Precision Standard Deviation, and Lognormal CV(%) of CMV DNA Concentrations (log_{10} IU/mL) by Positive Panel Member

CMV DNA Concentration (log_{10} IU/mL)			Percent Contribution to Total Variance (Lognormal CV(%)) Standard Deviation[c]					Total Precision	
Expected	Observed Mean[a]	Number of Tests[b]	Lot	Site	Operator /Day	Run	Within -Run	SD[d]	Log-normal CV(%)[e]
2.01	2.07	324	1% (2.97) 0.0129	6% (6.49) 0.0282	0% (0.00) 0.0000	3% (4.47) 0.0194	90% (25.15) 0.1076	0.114	26.61
3.26	3.27	322	10% (4.29) 0.0186	13% (4.85) 0.0210	3% (2.50) 0.0109	0% (0.00) 0.0000	74% (11.71) 0.0507	0.059	13.64
3.86	3.90	324	23% (7.26) 0.0315	0% (0.00) 0.0000	0% (0.22) 0.0010	0% (0.00) 0.0000	77% (13.50) 0.0584	0.066	15.36
6.70	6.74	324	15% (5.16) 0.0224	3% (2.31) 0.0100	1% (1.52) 0.0066	0% (0.00) 0.0000	81% (11.98) 0.0518	0.058	13.35

Note: The table only includes results with detectable viral load..
[a] Calculated using SAS MIXED procedure.
[b] Number of valid tests with detectable viral load.
[c] Calculated using the variance component from the SAS MIXED procedure.
[d] Calculated using the total variability from the SAS MIXED procedure.
[e] Lognormal CV(%) = sqrt(10^[SD^2 * ln(10)] - 1) * 100.

Table 11 below shows the estimated detectable viral load difference for each positive panel member. The detectable fold difference can be used to assess statistically significant changes in a patient's viral load when measured serially.

Table 11: Detectable Viral Load Difference by Positive Panel Member

CMV DNA Concentration (log_{10} IU/mL)						
Expected	**Observed Mean**	**No. of Tests[a]**	**Total Precision Standard Deviation (log_{10} IU/mL)**	**Standard Deviation of Difference Between Two Measurements[b]**	**95% Confidence Limit[c] (± log_{10} IU/mL)**	**Detectable Fold Difference[d]**
2.01	2.07	324	0.11	0.16	0.31	2.06
3.26	3.27	322	0.06	0.08	0.16	1.46
3.86	3.90	324	0.07	0.09	0.18	1.53
6.70	6.74	324	0.06	0.08	0.16	1.45

Note: The table only includes results with detectable viral load. The lower limit of quantitation (LLOQ) for the assay is 3.45E+01 IU/mL, and the upper limit of quantitation (ULoQ) is 1.0E+07 IU/mL.
[a] Number of valid tests with detectable viral load.
[b] Standard deviation of difference between two measurements = sqrt[2 * (total precision standard deviation)^2].
[c] 95% CL = 1.96 * standard deviation of difference between two measurements.
[d] Detectable Fold Difference = 10^(1.96 * sqrt(2 * (total standard deviation)^2)).
CL = confidence limit.

Table 12 below presents the reproducibility results for the **cobas** 6800 System using negative panel member. 100% of test results were negative.

Table 12: Reproducibility Results With the Negative Panel Member

Expected CMV DNA Concentration	Number of Valid Tests	Positive Results	Negative Results	Negative Percent Agreement[a]	95% Exact CI[b]
Negative	323	0	323	100.00	(98.86, 100.00)

[a] Negative Percent Agreement = (number of negative results / total valid tests in negative panel member)*100%.
[b] Calculated using the Clopper-Pearson exact binomial confidence interval method.
CI = confidence interval; CMV = cytomegalovirus.

7. Analytical Specificity - Cross-Reactivity

The analytical specificity of **cobas** CMV was evaluated by diluting a panel of microorganisms to a concentration of 1.00E+06 particles, copies, IU, genome equivalents or CFU/mL. Organisms were spiked into negative human EDTA plasma and into human EDTA plasma containing 230 IU/mL CMV DNA (Table 13). Each sample was tested in replicates of three. None of the non-CMV pathogens interfered with test performance. Negative results were obtained with **cobas** CMV for all microorganism samples without CMV target and positive results were obtained for all of the microorganism samples with CMV target. Furthermore, the mean log_{10} IU/mL of each of the positive CMV samples containing potentially cross-reacting organisms was within ± 0.5 log_{10} IU/mL of the mean log_{10} IU/mL of the respective positive spike control.

Table 13: Microorganisms Tested for Cross-Reactivity

Viruses	Bacteria	Yeast and Fungi
Adenovirus type 5	Propionibacterium acnes	Aspergillus niger
BK Polyomavirus	Staphylococcus aureus	Candida albicans
Epstein-Barr Virus	Chlamydia trachomatis	Cryptococcus neoformans
Hepatitis B Virus	Clostridium perfringens	
Hepatitis C Virus	Enterococcus faecalis	
Herpes Simplex Virus type1	Escherichia coli	
Herpes Simplex Virus type 2	Klebsiella pneumonia	
Human Herpes Virus type-6	Listeria monocytogenes	
Human Herpes Virus type-7	Mycobacterium avium	
Human Herpes Virus type-8	Neisseria gonorrhoeae	
Human Immunodeficiency Virus-1	Staphylococcus epidermidis	
Human Immunodeficiency Virus-2	Streptococcus pyrogenes	
Human Papillomavirus	Mycoplasma pneumonia	
JC virus	Salmonella typhimurium	
Parvovirus B19	Streptococcus pneumonia	
Varicella-Zoster Virus		

8. Analytical Specificity – Interfering Substances

Elevated levels of triglycerides (34.5 g/L), conjugated bilirubin (0.25 g/L), unconjugated bilirubin (0.25 g/L), albumin (58.7 g/L), hemoglobin (2.9 g/L) and human DNA (2 mg/L) were tested in samples in the presence and absence of CMV DNA (230 IU/mL). The tested endogenous interfering substances were shown not to interfere with the performance of the **cobas** CMV test.

Specimens from patients with autoimmune diseases such as systemic lupus erythematosus (SLE, n=6 specimens), rheumatoid arthritis (RA; n=7 specimens) and antinuclear antibody (ANA; n=7 specimens) were tested with CMV DNA spiked to 230 IU/mL (3 replicates per specimen) and without spiked CMV DNA (one replicate per specimen).

In addition, drug compounds listed in Table 14 were tested at three times the C_{max} in the presence (230 IU/mL) and absence of CMV DNA.

All potentially interfering substances tested were shown to not interfere with the test performance. Negative results were obtained with **cobas** CMV for all samples without CMV target and positive results were obtained with all of the samples with CMV target. Furthermore, the mean $\log_{10}$ IU/mL of each of the positive CMV samples containing potentially interfering substances was within ± 0.5 $\log_{10}$ IU/mL of the mean $\log_{10}$ IU/mL of the respective positive spike control.

Table 14: Drug Compounds Tested for Interference with the Quantitation of CMV DNA by cobas CMV

Class of Drug	Generic Drug Name	
Antibimicrobial	Cefotetan Clavulanate potassium Fluconazole Piperacillin Tazobactam sodium	Sulfamethoxazole Ticarcillin disodium Trimethoprim Vancomycin
Compounds for Treatment of Herpes Viruses	Ganciclovir Valganciclovir	Cidofovir Foscarnet
Immune Suppressant	Azathioprine Cyclosporine Everolimus Mycophenolate mofetil Mycophenolic acid	Prednisone Sirolimus Tacrolimus

9. Cross-Contamination

The cross-contamination rate for the **cobas** CMV test was determined by testing 240 replicates of a normal, CMV DNA negative human EDTA-plasma sample and 225 replicates of a high titer CMV sample at 1.00E+06 IU/mL. In total, five runs were performed with positive and negative samples arranged in a checkerboard configuration. All 240 replicates of the negative sample were negative, resulting in a cross-contamination rate of 0% (95% confidence interval 0%-1.5%).

B. Animal Studies

Not applicable

C. Additional Studies

None

X. SUMMARY OF PRIMARY CLINICAL STUDIES

To establish a reasonable assurance of safety and effectiveness of viral load testing with the **cobas** CMV test for use as an aid in the management of CMV in solid organ transplant (SOT) recipients and hematopoietic stem cell transplant (HSCT) recipients, clinical performance evaluation of the **cobas** CMV test carried out with EDTA plasma specimens left over from clinical studies that tested investigational prophylactic anti-CMV treatment regimens. The use of left-over specimens in the clinical performance evaluation of the **cobas** CMV test did not require an IDE for either study. Data from these clinical performance studies were the basis for the PMA approval decision.

The objective for the studies listed below was to evaluate the **cobas** CMV test with respect to clinical decision making (i.e., Clinical Concordance study) and numeric viral

load results (i.e., Method Comparison study) by comparing the **cobas** CMV test results to the test results obtained with the previously approved COBAS AmpliPrep/COBAS TaqMan CMV Test. EDTA plasma specimens from SOT and Hematopoetic Stem Cell Transplant (HSCT) patient populations were used in all studies. A summary of the clinical studies is presented in the sections below.

A. Clinical Concordance in SOT Patients
B. Method Comparison in SOT Patients
C. Clinical Concordance in HSCT Patients
D. Method Comparison in HSCT Patients

A. Clinical Concordance in SOT Patients

1. Study Design

EDTA plasma samples were obtained from kidney transplant recipients at high risk for CMV viremia (CMV D+/R-) and who participated in a phase 2a international, multicenter, randomized, placebo-controlled (two-armed), double-blind clinical trial of a prophylactic treatment. Samples were prospectively collected for the drug trial and stored frozen; leftover. De-identified samples were then used for performance validation of the **cobas** CMV test.

Subjects in the drug trial were provided with either the prophylactic treatment (treatment arm) or a placebo (control arm). Patients were managed with serial plasma CMV viral load testing as per the institutions standard of care.

Blood was drawn and tested from the study subjects at least weekly for the first 12 weeks after transplant and then biweekly for a subsequent 12 weeks. Participants who developed CMV viremia had anti-CMV treatment initiated and underwent more frequent blood draws for viral load testing (at least once per week until 2 sequential negative test results were obtained). The key timepoints as defined by the **cobas** CMV testing protocol were Baseline, Day 7, 14, 21, 28, 35, 42 and 49.

SOT patient samples in the parent drug study were collected between December 2012 and October 2014. The database for this PMA included n=107 SOT patients.

EDTA plasma samples were tested with the **cobas** CMV test and with an FDA approved comparator test (**cobas** AmpliPrep/**cobas** TaqMan CMV Test abbreviated as TaqMan CMV Test for this document) and concordance of CMV viral load measurements was assessed (see below under "Analysis"). For the **cobas** CMV device study testing was performed at 3 external clinical sites and one manufacturer's site. The sponsor internal site performed comparator testing only. The external clinical sites performed **cobas** testing of the specimens. For further discussion of the analysis methods, see Section 4, Analysis, below.

Inclusion and Exclusion Criteria

Enrollment in the parent drug study was limited to patients who met the following clinical inclusion criteria:

- ≥ 18 years of age
- Signed Informed Consent Form
- Seronegative for CMV (based on CMV immunoglobulin G) before transplantation
- Recipient of primary or secondary renal allograft from a living or cadaveric CMVseropositive donor

Enrollment in the **cobas** CMV testing study was limited to patients who met the following inclusion criteria:

- Patients in the parent study with more than 5 samples with sufficient volume to permit testing with the **cobas** CMV test and the TaqMan CMV Test

Patients were not permitted to enroll in the studies if the inclusion criteria were not met.

Follow-up schedule and endpoints

See Study Design (above).

2. Accountability of SOT Subjects

A total of 107 subjects participated in the parent drug study and provided a total of 1,913 specimens. All specimens were assessed for entry into the clinical performance evaluation of the **cobas** CMV test.

For additional accountability with respect to the different analysis performed on the study cohort, please refer to the analysis sections below (section 4).

3. SOT Study Population Demographics and Baseline Parameters

The demographics of the study population are representative for a study performed in the US that assesses CMV in SOT transplant patients.

Table 15: Demographics and Baseline Clinical Characteristics of SOT Subjects

Characteristics	Statistic
Total, N	107
Age (years)	
Mean ± Standard Deviation	49 ± 13.6
Median	50
Range	18 - 76
Gender, n(%)	
Male	74 (69.2%)
Female	33 (30.8%)
Ethnicity, n(%)	
Hispanic / Latino	10 (9.3%)
Not Hispanic / Not Latino	91 (85.0%)
Unknown	6 (5.6%)
Race, n(%)	
Asian	1 (0.9%)
Black / African-American	16 (15.0%)
White	88 (82.2%)
Other	2 (1.9%)
Immunosuppression Induction, n(%)	
Yes	26 (24.3%)
No	81 (75.7%)
Study Arm, n(%)	
Anti-CMV Prophylaxis Regimen	53 (49.5%)
Placebo	54 (50.5%)
CMV Serology Status, n(%)	
Donor Positive, Recipient Negative	107 (100.0%)

4. Analysis

The concordance of CMV measurements between the **cobas** CMV test and the TaqMan CMV Test with specimens from SOT patients was assessed with respect to the following clinical assessments:

a. Agreement of SOT Patient Viral Load Results Using Different Threshold Values

Viral load guided initiation of anti-CMV medication in the management of transplant patients depends on multiple patient risk factors as well as the experience of the treating physicians and the institution's standard of care. Hence, no universally applicable treatment threshold can be validated. Consequently, result concordance between the

cobas CMV test and the TaqMan CMV Test was analyzed using four different arbitrary thresholds: Target Not Detected [LOD], 137 IU/mL [2.137 $\log_{10}$ IU/mL], 500 IU/mL[2.699 $\log_{10}$ IU/mL] and 1,800 IU/mL [3.255 $\log_{10}$ IU/mL]. The analysis included all evaluable samples of the clinical study independent of treatment and independent of the specific time elapsed post treatment initiation.

In addition, results from the **cobas** CMV and the TaqMan CMV tests were analyzed in a 6x6 table that takes into account that the SD for the reproducibility of the TaqMan CMV Test was less than ± 0.3 $\log_{10}$ IU/mL. The categories in the table derived from the threshold value (in $\log_{10}$ IU/mL) ± 0.6 $\log_{10}$ IU/mL (2x the SD of the reproducibility) with the log-transformed nominal threshold values in $\log_{10}$ rounded to one significant figure.The analysis is based on the assumption that samples with values above a threshold of 2.7 $\log_{10}$ IU/mL IU/mL are considered unlikely to be below the threshold of 2.1 $\log_{10}$ IU/mL because of random measurement error. Similarly, samples with values above a threshold of 3.3 $\log_{10}$ IU/mL IU/mL are considered unlikely to be below the threshold of 2.7 $\log_{10}$ IU/mL and samples with values above a threshold of 3.9 $\log_{10}$ IU/mL IU/mL are considered unlikely to be below the threshold of 3.3 $\log_{10}$ IU/mL because of random measurement error. Therefore samples were considered discordant only if they were discrepant across more than the immediately adjacent categories.

Analysis Specific Accountability:
A total of 1,898 samples from the 107 subjects had paired results (i.e., valid results with both, the **cobas** CMV test and the TaqMan CMV Test) and were included in the analysis.

b. Agreement of SOT Patient Viral Load Results at Baseline in Order to Assess Initiation of Anti-CMV Therapy

The **cobas** CMV test and TaqMan CMV test results were analyzed as described under 4.a. (above) but analysis was performed only for paired evaluable samples collected at baseline. For the pupose of this analysis measurements prior to initiation of anti-CMV therapy were considered in the baseline analysis as well because they were used to guide the decision to initiate anti-CMV treatment.

Analysis Specific Accountability:
Of the 107 subjects in the clinical study, 71 subjects initiated therapy; 125 of their samples with paired results were evaluable and collected at baseline or prior to anti-CMV treatment initiation and were included in the analysis.

c. Agreement of SOT Patient Viral Load Results in Order to Assess Response to Anti-CMV Therapy

The **cobas** CMV and TaqMan CMV test results were analyzed as described under 4.a. (above), but analysis was performed for all paired evaluable samples collected at the protocol defined timepoints post anti-CMV therapy initiation (Day 14, Day 21, Day 28, Day 35, and Day 49).

Analysis Specific Accountability:
Of the 107 total subjects in the clinical study, 71 subjects initiated therapy; 272 of their samples with paired results were evaluable and collected at protocol defined time points post anti-CMV therapy initiation and were included in the analysis.

d. Agreement of SOT Patient Viral Load Results in Order to Determine When to Stop Anti-CMV Therapy

An analysis was carried out to determine the concordance between the **cobas** CMV test and the TaqMan CMV Test when used to aid in determining whether or not to stop anti-CMV treatment at visit times of Day 14, Day 21, Day 28, Day 35, and Day 49 (post anti-CMV therapy initiation). The analysis considered all viremic patients who started anti-CMV medication (n=71).

The following Definitions were used:

- **Baseline:** Baseline was defined as the date the anti-CMV medication was started.

- **Days of Interest:** Testing days of interest were Day 14, Day 21, Day 28, Day 35, and Day 49 and were defined relative to the Baseline with a window of ±3 days.

- **Viremic Episode:** A viral load of ≥137 IU/mL (or ≥ 2.137 $\log_{10}$ IU/mL) for both tests was used to define a viremic episode.

- **Resolution of Viremia:** Consistent with current guideline recommendations resolution of the viremic episode is defined by two consecutive measurements below the LLOQ of the comparator test (i.e., <137 IU/mL or < 2.137 $\log_{10}$ IU/mL) and/or Target Not Detected (TND) with the samples taken one week apart.

For subjects in the cohort who had multiple episodes of viremia, once the subject met the criterion for resolution of their initial CMV episode at a given timepoint, that resolution status was carried forward for the corresponding assay and recurrent episodes of viremia were not analyzed anymore.

Not all subjects have all samples available at each of the time points. Missing data points were imputed through linear regression if surrounding datapoints with viral loads > 137 IU/mL were available. Imputation was performed across a maximum of two missing data points (2 weeks).

A missing data point can be considered as “not resolved” for the purpose of resolution analysis if the previous data point was viremic because two consecutive viral load measurements of < 137 IU/mL (or undetectable CMV DNA) are required to define a resolution of the viremic episode.

Analysis Specific Acountability:
Of the 107 subjects that were included in the Clinical Concordance analysis, the following number of subjects were excluded for the purpose of the resolution analysis:

- Thirty-nine (39) subjects with 609 samples were excluded for the resolution analysis because they did not inititate therapy during the course of the study or they did not have viral load measurements at any of the required time points so that neither the occurrence of a viremic episode nor the resolution of a viremic episode could be determined.
- Six (6) subjects who initiated therapy but were not viremic at any of the collected time points were excluded because neither a viremic episode nor its resolution could be determined.
- One (1) subject was excluded because the subject became viremic only at Day 42 so that resolution of viremia could not be analyzed.

A total of 61 subjects started anti-CMV therapy during the course of the parent drug study; 1,184 samples from these subjects provided paired viral load measurements from the **cobas** CMV and the TaqMan CMV tests at any of the following time points and were included in the resolution analysis: Baseline, Day 7, 14, 21, 28, 35, 42 and 49.

5. Safety and Effectiveness Results

The key safety and effectiveness (performance) outcomes for the Clinical Concordance study of Solid Organs Transplant (SOT) patients are presented below in Tables 16 to 29.

Performance evaluation was based on 107 patients evaluable for Clinical Concordance. Adverse effect reporting is not applicable to this study as it is a retrospective study with leftover specimens and no patient management was based on the results of this device during the study.

a. Agreement of SOT Patient Viral Load Results Using Different Threshold Values

Table 16 below considers all 1,898 specimens for which a result was available for both the **cobas** CMV and TaqMan CMV tests (so called “paired results”). These specimens were obtained from 107 subjects (viremic and non-viremic). Concordance for the viral load results of the specimens is provided in Table 17.

Table 16: Concordance Analysis of Samples From SOT Patients Using Different Thresholds (All Paired Samples)

		TaqMan CMV		Total
		Target Not Detected	**Detected**	
cobas CMV	**Target Not Detected**	1,022	8	1,030
	Detected	172[1]	696	868
Total		1,194	704	1,898

		< 137 IU/mL (< 2.1 log_{10} IU/mL*)	**≥ 137 IU/mL (≥ 2.1 log_{10} IU/mL*)**	**Total**
cobas CMV	**< 137 IU/mL (< 2.1 log_{10} IU/mL*)**	1,391	6	1397
	≥ 137 IU/mL (≥ 2.1 log_{10} IU/mL*)	97[2]	404	501
Total		1,488	410	1,898

		< 500 IU/mL (< 2.7 log_{10} IU/mL)**	**≥ 500 IU/mL (≥ 2.7 log_{10} IU/mL**)**	**Total**
cobas CMV	**< 500 IU/mL (< 2.7 log_{10} IU/mL**)**	1,537	8	1,545
	≥ 500 IU/mL (≥ 2.7 log_{10} IU/mL)**	102[3]	251	353
Total		1,639	259	1,898

		< 1,800 IU/mL (< 3.3 log_{10} IU/mL*)**	**≥ 1,800 IU/mL (≥ 3.3 log_{10} IU/mL***)**	**Total**
cobas CMV	**< 1,800 IU/mL (< 3.3 log_{10} IU/mL***)**	1,693	1	1,694
	≥ 1,800 IU/mL (≥ 3.3 log_{10} IU/mL*)**	62[4]	142	204
Total		1,755	143	1,898

All paired samples evaluable for Clinical Concordance analysis were included in this table. Note: All paired samples evaluable for Clinical Concordance analysis were included in this table. Samples with a "Target Not Detected" results were categorized as "< Threshold in IU/mL".

[1] 28 of the 172 discordant samples were sequenced and showed impactful sequenced mismatch

[2] 25 of the 97 discordant samples were sequenced and showed impactful sequenced mismatch

[3] 27 of the 102 discordant samples were sequenced and showed impactful sequenced mismatch

[4] 19 of the 62 discordant samples were sequenced and showed impactful sequenced mismatch

* Threshold Log_{10} of 2.137 abbreviated as 2.1 log_{10} IU/mL

** Threshold Log_{10} of 2.699 abbreviated as 2.2log_{10} IU/mL

*** Threshold Log_{10} of 3.255 abbreviated as 3.3 log_{10} IU/mL

Table 17: Summary Concordance of Viral Load Results from SOT Patients Using Different Thresholds

	Percent Agreement < Threshold 95% CI (n/N)	**Percent Agreement ≥ Threshold (n/N)** 95% CI (n/N)	**Overall Percent Agreement** 95% CI (n/N)
Target Not Detected	**85.6** 83.5%, 87.5% (1,022/1,194)	**98.9** 97.8%, 99.5% (696/704)	**90.5** 89.1%, 91.8% (1,718/1,898)
137 IU/mL (2.1 log_{10} IU/mL*)	**93.5** 92.1%, 94.7 (1,391/1,488)	**98.5** 96.8%, 99.5% (404/410)	**94.6** 93.5%, 95.5% (1,795/1,898)
500 IU/mL (2.7 log_{10} IU/mL)**	**93.8** 92.5%, 94.9% (1,537/1,639)	**96.9** 94.0%, 98.7% (251/259)	**94.2** 93.1%, 95.2% (1,788/1,898)
1,800 IU/mL (3.3 log_{10} IU/mL*)**	**96.5** 95.5%, 97.3% (1,693/1,755)	**99.3** 96.2%, 100% (142/143)	**96.7** 95.8%, 97.4% (1,835/1,898)

Note: All paired samples evaluable for Clinical Concordance analysis were included in this table. Samples with a "Target Not Detected" results were categorized as "< Threshold in IU/mL".
* Log_{10} of 2.137 abbreviated as 2.1 log_{10} IU/mL
** Log_{10} of 2.699 abbreviated as 2.2log_{10} IU/mL
*** Log_{10} of 3.255 abbreviated as 3.3 log_{10} IU/mL.
95% confidence interval (CI) calculated by exact method assuming independence between all samples.
1 IU/mL = 1.1 copy/mL.

The concordance analysis of viral load results as determined by the **cobas** CMV and TaqMan CMV tests was further analyzed in the 6x6 Table (Table 18) below as described in section A.4.a. (above). Samples were considered discordant if they were discrepant across more than the immediately adjacent categories; this analysis considers the reproducibility of the **cobas** CMV test.

Most samples were not discrepant by more than 1 category from the diagonal. The 4 samples with wider discrepancies (see footnotes a to d in Table 18) were sequenced and a total of 12 out of 13 were found to contain a CMV variant with a significant mismatch mutation in one of the TaqMan CMV Test primer binding sites.

Agreements were found to be as follows:

Agreement for TND:	99.7% (1,190/1,194)
Agreement for Detected < 2.1 log_{10} IU/mL:	94.2% (277/294)
Agreement for 2.1 to < 2.7 log_{10} IU/mL:	92.75% (140/151)
Agreement for 2.7 to < 3.3 log_{10} IU/mL:	99.1% (115/116)
Agreement for 3.3 to < 3.9 log_{10} IU/mL:	100% (104/104)
Agreement for ≥ 3.9 log_{10} IU/mL:	100% (39/39)

Table 18: Concordance Analysis for Samples From SOT Patients (All Paired Samples)

cobas CMV (log_{10} IU/mL)	Target Not Detected	TaqMan CMV Test (log_{10} IU/mL) < 2.1	2.1 to < 2.7	2.7 to < 3.3	3.3 to < 3.9	≥ 3.9	Total
Target Not Detected	1,022	8	0	0	0	0	1,030
< 2.1*	168	193	6	0	0	0	367
2.1 to < 2.7	3[a]	76	61	8	0	0	148
2.7 to < 3.3	0	12[c]	73	63	1	0	149
3.3 to < 3.9	1[b]	5[d]	8[e]	44	58	0	116
≥ 3.9	0	0	3[f]	1[g]	45	39	88
Total	1,194	294	151	116	104	39	1,898

Note: All 1898 paired samples evaluable for Clinical Concordance analysis from all 71 subjects were included in this table.

*The LLOQ is 34.5 IU/mL for the **cobas** CMV and 137 IU/mL for TaqMan CMV Test. Therefore for the **cobas** CMV test the category of <137 IU/mL includes non-quantificable results < the LLOQ and quantifiable results ≥ 34.5 IU/mL but < 137 IU/mL.

log_{10} (137) = 2.137 (abbreviated above as 2.1); log_{10} (1,800) = 3.255 (abbreviated above as 3.3); log_{10} (7,943) = 3.900 (abbreviated above as 3.9).

[a] These samples were sequenced and 2 out of 3 were found to contain a significant impact mutation in the forward primer binding site of the Taqman CMV test.

[b] This sample was sequenced and was found to contain a significant impact mutation in the forward primer binding site of the Taqman CMV test.

[c] 8 of the 12 discordant samples derived from 5 subjects and all 8 samples were sequenced and found to have significant impact mutations.

[d] These 5 samples derived from 3 subjects; they were sequenced and all 5 were found to contain a significant impact mutation in the forward primer binding site of the Taqman CMV test.

[e] 7 of the 8 discordant samples derived from 3 subjects and all 7 samples were sequenced and found to have a significant impact mutation.

[f] These 3 samples derived from 2 subjects; they were sequenced and all 3 were found to contain a significant impact mutation in the forward primer binding site of the Taqman CMV test.

[g] The one discrepant sample was found to have significant impact mutation

b. Agreement of SOT Patient Viral Load Results at Baseline Using Different Threshold Values

Table 19 below considers all 71 subjects that had paired baseline viral load results. If the baseline sample was missing the sample immediately prior to treatment initiation was used instead (if available). Concordance for the viral load result of these 71 samples is provided in Table 19.

Table 19: Concordance Analysis for Baseline Samples From SOT Patients Using Different Thresholds

		TaqMan CMV		Total
		Target Not Detected	Detected	
cobas CMV	Target Not Detected	9	0	9
	Detected	2[1]	60	62
Total		11	60	71

		< 137 IU/mL (< 2.1 log_{10} IU/mL*)	≥ 137 IU/mL (≥ 2.1 log_{10} IU/mL*)	Total
cobas CMV	< 137 IU/mL (< 2.1 log_{10} IU/mL*)	24	1	25
	≥ 137 IU/mL (≥ 2.1 log_{10} IU/mL*)	5[2]	41	46
Total		29	42	71

		< 500 IU/mL (< 2.7 log_{10} IU/mL**)	≥ 500 IU/mL (≥ 2.7 log_{10} IU/mL**)	Total
cobas CMV	< 500 IU/mL (< 2.7 log_{10} IU/mL**)	33	2	35
	≥ 500 IU/mL (≥ 2.7 log_{10} IU/mL**)	7[3]	29	36
Total		40	31	71

		< 1,800 IU/mL (< 3.3 log_{10} IU/mL***)	≥ 1,800 IU/mL (≥ 3.3 log_{10} IU/mL***)	Total
cobas CMV	< 1,800 IU/mL (< 3.3 log_{10} IU/mL***)	48	0	48
	≥ 1,800 IU/mL (≥ 3.3 log_{10} IU/mL***)	4[4]	19	23
Total		52	19	71

Only paired samples at or before baseline and evaluable for Clinical Concordance analysis were included in this table. Samples with a "Target Not Detected" results were categorized as "< threshold value in IU/mL".

[1] 0 of the 2 discordant samples derived from subjects with an impactful sequence mismatch; discrepancy of results was <0.5 log_{10} IU/mL for both discrepant samples.

[2] 2 of the 5 discordant samples derived from subjects with an impactful sequence mismatch and for three samples the discrepancy of results was >0.5 log_{10} IU/mL including the two samples with impactfull sequence mismatch.

[3] 3 of the 7 discordant samples derived from subjects with an impactful sequence mismatch and all three had discrepancies >0.5log_{10} IU/mL. For the remaining 4 discrepant samples the discrepancy was <0.5 log IU/mL.

[4] 1 of the 4 discordant samples derived from subject with impactful sequence mismatch. Two of the discordant samples had discrepancies of >0.5 log_{10} IU/mL (including the sample with the sequence mismatch); the other two samples had discrepancies <0.5 log_{10} IU/mL.
* Threshold Log_{10} of 2.137 abbreviated as 2.1 log_{10} IU/mL
** Threshold Log_{10} of 2.699 abbreviated as 2.2log_{10} IU/mL
*** Threshold Log_{10} of 3.255 abbreviated as 3.3 log_{10} IU/mL.

Table 20: Summary Concordance of Viral Load Results for Baseline Samples From SOT Using Different Thresholds

	Percent Agreement < Threshold 95% CI (n/N)	**Percent Agreement ≥ Threshold (n/N)** 95% CI (n/N)	**Overall Percent Agreement** 95% CI (n/N)
Target Not Detected	**81.8%** 48.2%, 97.7% (9/11)	**100.0%** 94.0%, 100.0% (60/60)	**97.2%** 90.2%, 99.7% (69/71)
137 IU/mL (2.1 log_{10} IU/mL*)	**82.8%** 64.2%, 94.2% (24/29)	**97.6%** 87.4%, 99.9% (41/42)	**91.5%** 82.5%, 96.8% (65/71)
500 IU/mL (2.7 log_{10} IU/mL)**	**82.5%** 67.2%, 92.7% (33/40)	**93.5%** 78.6%, 99.2% (29/31)	**87.3%** 77.3%, 94.0% (62/71)
1,800 IU/mL (3.3 log_{10} IU/mL*)**	**92.3%** 81.5%, 97.9% (48/52)	**100.0%** 82.4%, 100.0% (19/19)	**94.4%** 86.2%, 98.4% (67/71)

Note: Only paired samples at or before baseline and evaluable for Clinical Concordance analysis were included in this table. Samples with a "Target Not Detected" results were categorized as "< threshold value in IU/mL".
* Log_{10} of 2.137 abbreviated as 2.1 log_{10} IU/mL
** Log_{10} of 2.699 abbreviated as 2.2log_{10} IU/mL
*** Log_{10} of 3.255 abbreviated as 3.3 log_{10} IU/mL.
CI: 2-sided 95% confidence interval calculated by exact method assuming independence between all samples.

The concordance analysis of viral load results as determined by the **cobas** CMV and TaqMan CMV tests was further analyzed in the 6x6 Table (Table 21) below as described in section A.4.a. (above). Samples were considered discordant if they were discrepant across more than the immediately adjacent categories; this analysis considers the reproducibility of the **cobas** CMV test. Most samples were not discrepant by more than 1 category from the diagonal. The two samples with wider discrepancy (see footnote a in Table 21) were sequenced and found to contain a CMV variant with a significant mismatch mutation in one of the TaqMan CMV Test primer binding sites.

Agreements were found to be as follows:

Agreement for TND:	100% (11/11)
Agreement for < 2.1 log_{10} IU/mL:	88.9% (16/18)
Agreement for 2.1 to < 2.7 log_{10} IU/mL:	100% (11/11)

Agreement for 2.7 to < 3.3 log_{10} IU/mL: 100% (12/12)
Agreement for 3.3 to < 3.9 log_{10} IU/mL: 100% (15/15)
Agreement for ≥ 3.9 log_{10} IU/mL: 100% (4/4)

Table 21: Concordance Analysis for Baseline Samples From SOT Patients

		TaqMan CMV Test (log_{10} IU/mL)					
cobas CMV (log_{10} IU/mL)	**Target Not Detected**	**< 2.1**	**2.1 to < 2.7**	**2.7 to < 3.3**	**3.3 to <3.9**	**≥ 3.9**	**Total**
Target Not Detected	9	0	0	0	0	0	9
< 2.1*	2	13	1	0	0	0	16
2.1 to < 2.7	0	3	5	2	0	0	10
2.7 to < 3.3	0	1[a]	5	7	0	0	13
3.3 to <3.9	0	1[a]	0	3	15	0	19
≥ 3.9	0	0	0	0	0	4	4
Total	11	18	11	12	15	4	71

Note: Only paired samples at or before baseline and evaluable for Clinical Concordance analysis were included in this table. Samples with a "Target Not Detected" results were categorized as "< threshold value in IU/mL".
*The LLOQ is 34.5 IU/mL for the **cobas** CMV and 137 IU/mL for TaqMan CMV Test. Therefore for the **cobas** CMV test the category of <137 IU/mL includes non-quantificable results < the LLOQ and quantifiable results ≥ 34.5 IU/mL but < 137 IU/mL.
Log_{10} of 2.137 abbreviated as 2.1 log_{10} IU/mL
Log_{10} of 2.699 abbreviated as 2.2log_{10} IU/mL
Log_{10} of 3.255 abbreviated as 3.3 log_{10} IU/mL
Log_{10} of 7,943 abbreviated as 3.9 log_{10} IU/mL
[a] This sample was sequenced and found to contain a significant impact mutation in the forward primer binding site of the Taqman CMV test.

c. Agreement of SOT Patients Viral Load Results in Order to Assess Response to Anti-CMV Therapy

Tables 22 and 23 provide an analysis of concordance with respect to viral load results for the 272 paired results from the 71 subjects who started anti-CMV therapy and had samples collected at any of the protocol defined days post therapy initiation (i.e., Days 7, 14, 21, 28, 35, and 49 post treatment).

The concordance analysis of viral load results as determined by the **cobas** CMV and TaqMan CMV tests was further analyzed in the 6x6 Table (Table 24) below as described in section 4. a. (above). Samples were considered discordant if they were discrepant across more than the immediately adjacent categories; this analysis considers the reproducibility of the **cobas** CMV test.

Except for the categories < 2.1 log_{10} IU/mL and 2.1 to < 2.7 log_{10} IU/mL all categories had 100% agreement between the **cobas** CMV and the TaqMan CMV Test. Agreements for the categories < 2.1 log_{10} IU/mL and 2.1 to < 2.7 log_{10} IU/mL was 92.3% (72/78) and 96.1% (74/77), respectively. However, 7 out of 9 discrepant samples were samples that

contained a significant impact mutation in the primer binding site of the TaqMan CMV test.

Table 22: Concordance Analysis of cobas CMV and TaqMan CMV Test Results for Samples from SOT Patients Collected at Protocol Defined Days Post Treatment Initiation Using Different Thresholds (n=272 Samples)

		TaqMan CMV		Total
		Target Not Detected	**Detected**	
cobas CMV	**Target Not Detected**	24	3	27
	Detected	36[1]	209	245
Total		60	212	272

		< 137 IU/mL (< 2.1 log_{10} IU/mL*)	**≥ 137 IU/mL (≥ 2.1 log_{10} IU/mL*)**	**Total**
cobas CMV	**< 137 IU/mL (< 2.1 log_{10} IU/mL*)**	105	1	106
	≥ 137 IU/mL (≥ 2.1 log_{10} IU/mL*)	33[2]	133	166
Total		138	134	272

		< 500 IU/mL (< 2.7 log_{10} IU/mL)**	**≥ 500 IU/mL (≥ 2.7 log_{10} IU/mL**)**	**Total**
cobas CMV	**< 500 IU/mL (< 2.7 log_{10} IU/mL**)**	151	0	151
	≥ 500 IU/mL (≥ 2.7 log_{10} IU/mL)**	34[3]	87	121
Total		185	87	272

		< 1,800 IU/mL (< 3.3 log_{10} IU/mL*)**	**≥ 1,800 IU/mL (≥ 3.3 log_{10} IU/mL***)**	**Total**
cobas CMV	**< 1,800 IU/mL (< 3.3 log_{10} IU/mL***)**	196	0	196
	≥ 1,800 IU/mL (≥ 3.3 log_{10} IU/mL*)**	26[4]	50	76
Total		222	50	272

Note: Samples with a "Target Not Detected" or a detectable viral load below the threshold result were categorized as "< Threshold IU/mL. Note: LLOD **cobas** CMV = 34.5 IU/mL; LLOQ TaqMan CMV = 137 IU/mL. 1 IU/mL = 1.1 copy/mL**.**

[1] 4 of the 36 discordant samples were sequenced and showed impactful sequenced mismatch

[2] 8 of the 33 discordant samples were sequenced and showed impactful sequenced mismatch
[3] 8 of the 34 discordant samples were sequenced and showed impactful sequenced mismatch
[4] 6 of the 26 discordant samples were sequenced and showed impactful sequenced mismatch
* Threshold $\log_{10}$ of 2.137 abbreviated as 2.1 $\log_{10}$ IU/mL
** Threshold $\log_{10}$ of 2.699 abbreviated as 2.2 $\log_{10}$ IU/mL
*** Threshold $\log_{10}$ of 3.255 abbreviated as 3.3 $\log_{10}$ IU/mL.

Table 23: Summary of Concordance of Viral Load Results by Different Baseline Threshold

	Percent Agreement < Threshold 95% CI (n/N)	**Percent Agreement ≥ Threshold (n/N)** 95% CI (n/N)	**Overall Percent Agreement** 95% CI (n/N)
Target Not Detected	**40.0** 27.6%, 53.5% (24/60)	**98.6** 95.9%, 99.7% (209/212)	**85.7** 80.9%, 89.6% (233/272)
137 IU/mL (2.1 $\log_{10}$ IU/mL*)	**76.1** 68.2%, 82.9 (105/138)	**99.3** 95.9%, 100% (133/134)	**87.5** 83.0%, 91.2% (238/272)
500 IU/mL (2.7 $\log_{10}$ IU/mL)**	**81.6** 75.3%, 86.9% (151/185)	**100** 95.8%, 100% (87/87)	**87.5** 83.0%, 91.2% (238/272)
1,800 IU/mL (3.3 $\log_{10}$ IU/mL*)**	**88.3** 83.3%, 92.2% (196/222)	**100** 92.9%, 100% (50/50)	**90.4** 86.3%, 93.7% (246/272)

Note: Samples with a "Target Not Detected" or a detectable viral load below the threshold result were categorized as "< Threshold IU/mL**.**
* Log_{10} of 2.137 abbreviated as 2.1 $\log_{10}$ IU/mL
** Log_{10} of 2.699 abbreviated as 2.2$\log_{10}$ IU/mL
*** Log_{10} of 3.255 abbreviated as 3.3 $\log_{10}$ IU/mL.
CI: 2-sided 95% confidence interval calculated by exact method assuming independence between all samples.

Table 24: Concordance Analysis for Samples From SOT Patients Collected at Protocol Defined Time Points Post Treatment Initiation

	TaqMan CMV Test (log_{10} IU/mL)						
cobas CMV (log_{10} IU/mL)	**Target Not Detected**	**< 2.1**	**2.1 to < 2.7**	**2.7 to < 3.3**	**3.3 to < 3.9**	**≥ 3.9**	**Total**
Target Not Detected	24	3	0	0	0	0	27
< 2.1*	36	42	1	0	0	0	79
2.1 to < 2.7	0	27	18	0	0	0	45
2.7 to < 3.3	0	4[a]	25	16	0	0	45
3.3 to < 3.9	0	2[b]	1[c]	21	12	0	36
≥ 3.9	0	0	2[b]	0	26	12	40
Total	60	78	47	37	38	12	272

Note: A total of 272 paired samples evaluable for Clinical Concordance analysis from 68 viremic subjects at protocol defined time points (Day 14, Day 21, Day 28, Day 35 or Day 49 post anti-CMV therapy initiation) were included in this table.

*The LLOQ is 34.5 IU/mL for the **cobas** CMV and 137 IU/mL for TaqMan CMV Test. Therefore for the **cobas** CMV test the category of <137 IU/mL includes non-quantifiable results < the LLOQ and quantifiable results ≥ 34.5 IU/mL but < 137 IU/mL.

log_{10} (137) = 2.137 (abbreviated above as 2.1); log_{10} (1,800) = 3.255 (abbreviated above as 3.3); log_{10} (7,943) = 3.900 (abbreviated above as 3.9).

[a] two of the 4 discrepant samples were sequenced and found to contain a significant impact mutation in the forward primer binding site of the Taqman CMV test.

[b] These 2 samples were sequenced and both were found to contain a significant impact mutation in the forward primer binding site of the Taqman CMV test.

[c] The discrepant sample was sequenced and found to contain a significant impact mutation in the forward primer binding site of the Taqman CMV test.

d. Agreement of SOT Patients Viral Load Results in Order to Determine When to Stop Anti-CMV Therapy

The concordance analysis between the **cobas** CMV test and the TaqMan CMV Test when used to aid in determining whether or not to stop anti-CMV treatment at visit times of Day 14, Day 21, Day 28, Day 35, and Day 49 (post anti-CMV therapy initiation) considered all viremic patients who started anti-CMV medication (n=71).

From the total of n=71 SOT recipients who initiated therapy n=4 subjects were excluded because they did not have any viral load measurements at any of the protocol define timepoints so that a viremic episode could not be defined and a resolution thereof could not be determined. From the remaining n=67 subjects who started therapy n=7 only had negative viral load results up to ≥ Day 42 and hence resolution of viremia could therefore not be assessed within the protocol defined time points (up to Day 49). Therefore the resolution analysis below contains samples from n=60 subjects.

Viremia was defined as a viral load of ≥137 IU/mL with both assays, the **cobas** CMV and the FDA approved comparator test. Resolution of viremia is defined as two consecutive samples with a "Target Not Detected" result or a detectable viral load below 137 IU/mL.

Table 25: Concordance Analysis of cobas CMV and TaqMan CMV Test Results When Used to Determining Resolution of Viremia for SOT Patients

Day 14 Post Anti-CMV Therapy Initiation			
	TaqMan CMV		
cobas CMV	**Resolution of CMV Episode**	**No Resolution of CMV Episode**	**Total**
Resolution of CMV Episode	0	0	0
No Resolution of CMV Episode	0	40	40
Total	0	40	40
Column Agreement (95% Exact CI)[a]	**NC**	**100.0% (91.2%, 100.0%)**	
Overall Percent Agreement (95% CI)[a]	**100.0% (91.2%, 100.0%)**		

Day 21 Post Anti-CMV Therapy Initiation			
	TaqMan CMV		
cobas CMV	**Resolution of CMV Episode**	**No Resolution of CMV Episode**	**Total**
Resolution of CMV Episode	0	0	0
No Resolution of CMV Episode	1	50	51
Total	1	50	51
Column Agreement (95% CI)[a]	0.0% (0.0%, 95%)	100.0% (92.9%, 100.0%)	
Overall Percent Agreement (95% CI)[a]	98.0% (89.6%, 100%)		

Day 28 Post Anti-CMV Therapy Initiation			
	TaqMan CMV		
cobas CMV	**Resolution of CMV Episode**	**No Resolution of CMV Episode**	**Total**
Resolution of CMV Episode	6	0	6
No Resolution of CMV Episode	4	46	50
Total	10	46	56
Column Agreement (95% CI)[a]	**60.0% (26.2%, 87.8%)**	**100.0% (92.3%, 100.0%)**	
Overall Percent Agreement (95% CI)[a]	**92.9% (82.7%, 98.0%)**		

Day 35 Post Anti-CMV Therapy Initiation			
	TaqMan CMV		
cobas CMV	**Resolution of CMV Episode**	**No Resolution of CMV Episode**	**Total**
Resolution of CMV Episode	16	1	17
No Resolution of CMV Episode	8	31	39
Total	24	32	56
Column Agreement (95% CI)[a]	66.7% (44.7%, 84.4%)	96.9% (83.8%, 99.9%)	
Overall Percent Agreement (95% CI)[a]	83.9% (71.7%, 92.4%)		

Day 49 Post Anti-CMV Therapy Initiation			
	TaqMan CMV		
cobas CMV	**Resolution of CMV Episode**	**No Resolution of CMV Episode**	**Total**
Resolution of CMV Episode	38	0	38
No Resolution of CMV Episode	7	12	19
Total	45	12	57
Column Agreement (95% CI)[a]	**84.4% (70.5%, 93.5%)**	**100.0% (73.5%, 100.0%)**	
Overall Percent Agreement (95% CI)[a]	**87.7% (76.3%, 94.9%)**		

Only paired samples evaluable for Clinical Concordance analysis from Non-ViremicSubjects at each of the indicated Days Post Anti-CMV Therapy Initiation were included in this table. Sample with a "Target Not Detected" or a detectable viral load below 137 IU/mL result was categorized as "< 2.1 $\log_{10}$ IU/mL (< 137 IU/mL)."

[a] CI calculated with exact method

NC = Not calculable

6. Subgroup Analysis

No Subgroup analysis was performed

7. Pediatric Extrapolation

In this premarket application, existing clinical data was not leveraged to support approval of a pediatric patient population.

B. Method Comparison in SOT Patients

1. Study Design

The objective of this study was to compare the results of clinical specimens tested with the **cobas** CMV for use on the **cobas** 6800/8800 Systems (**cobas** CMV) to the results obtained with and FDA approved comparator (**cobas** AmpliPrep/ **cobas** TaqMan CMV Test (TaqMan CMV Test) at clinically relevant viral load levels. The method comparison study was conducted using a subset of samples from the Clinical Concordance study (see section A above) some additional high positive clinical samples (not part of the Clinical Concordance study) as well as contrived samples in order to cover the measuring range of the assay. Spiking for the preparation of contrived samples was performed by spiking negative SOT plasma with cultured CMV virus (Merlin Strain).

2. Accountability

There was a total of 612 valid samples that had paired results from the **cobas** CMV and the TaqMan CMV test. The 612 samples consisted of 472 clinical (including 68 cross sectional and 404 longitudinal samples), 110 spiked, and 30 negative samples with at least one valid result on both assays that were included in the method comparison study. Longitudinal samples were derived from the Clinical Concordance study that is described in Section A above. Twenty-seven (27) clinical samples and 12 contrived samples were excluded. Thus, there was a total of 543 paired samples (445 clinical and 98 contrived combined) that had valid results with both the **cobas** CMV and TaqMan CMV tests and had results within the common linear range of both assays.

Table 26: Accountability for SOT Method Comparison Study

	Total Samples	Invalid	Excluded Samples	Valid	Valid but Non-Evaluable**	Evaluable
Clinical	472	0	26*	446	1	445
Contrived	110	0	0	110	12	98
Total	582	0	26	**556**	13	543

*Samples from 8 subjects of the Clinical Concordance study and 3 cross sectional clinical samples were sequenced and were shown to have a CMV variant that contained a mutation in the primer binding site of the TaqMan CMV test.
Samples had either the **cobas CMV or the TaqMan CMV test result or both out of linear range

In addition 30 CMV immunoglobulin G (IgG)-negative, CMV DNA negative clinical samples from SOT donors with valid Target Not Detected (TND) results on both assays were tested to evaluate clinical specificity of the assay but were excluded from the regression analysis of viral load.

3. Study Population Demographics and Baseline Parameters

Please refer to Section A for the Demographics and Baseline parameters of the clinical study cohort.

4. Analysis

The method comparison study included the analyses listed below. All analyses were provided separately for clinical and contrived samples as well as pooled for both types of sample:

a. Deming Regression Analysis
b. Analysis of the Mean Paired Difference
c. Bias
d. Analysis of the Allowable Total Difference
e. Agreement with Negative Samples

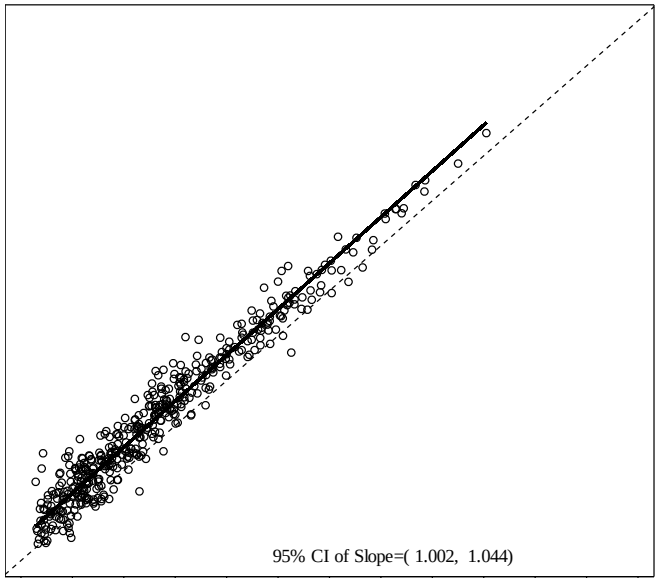
5. Safety and Effectiveness Results

a. Deming Regession Analysis

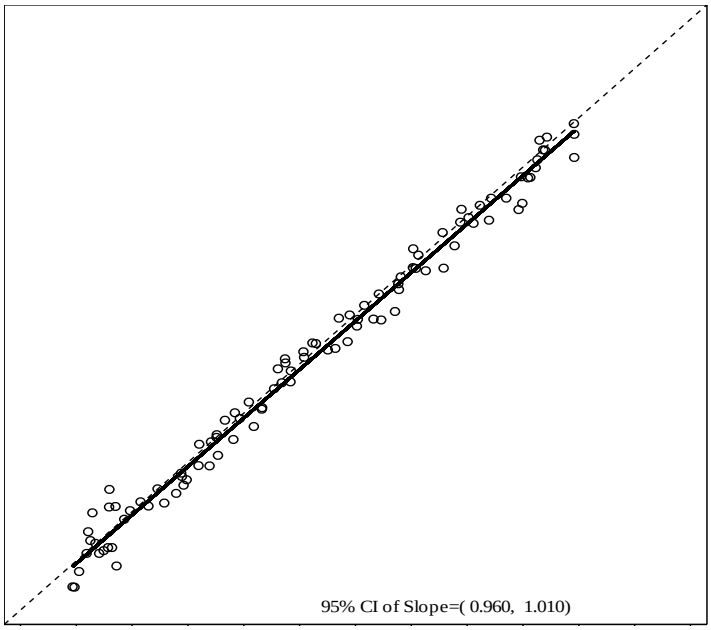
Figure 4 present the Deming regression of the viral load (log_{10} IU/mL) results from the **cobas** CMV and TaqMan CMV tests for all sites combined for the SOT population. Regression is analyzed in Table 27 below.

Figure 4: Deming Linear Regression Plot of Viral Loads (log10 IU/mL) for SOT patients (cobas CMV Versus TaqMan CMV Test – All Clinical Sites)

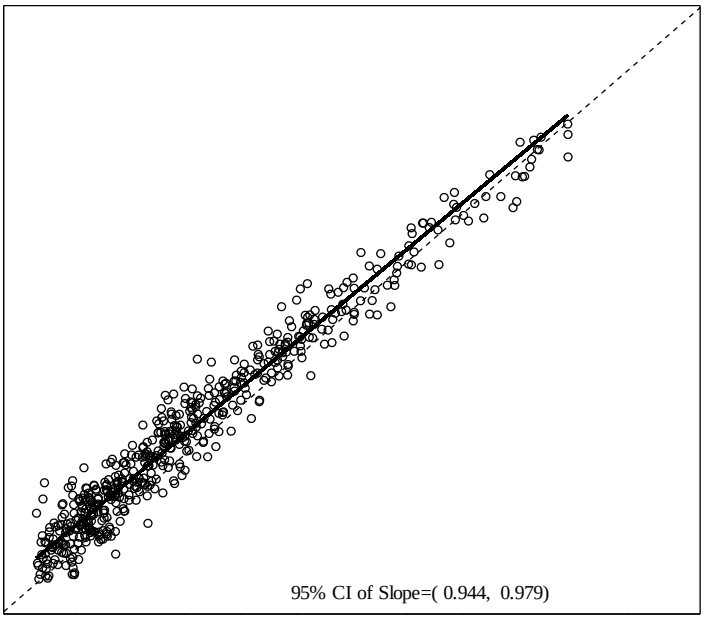
Clinical Samples



Contrived Samples



Clinical + Contrived Samples



CI = confidence interval; r = correlation coefficient

Table 27: Parameter Estimates of Deming Regression Between Viral Loads (log_{10} IU/mL) for SOT Patients (All Clinical Sites)

	Number of Paired Samples	Parameter	Parameter Estimate*	Standard Error [log_{10}]	95% CI [log_{10}]	r	Parameter Estimate (95% CI[a]) Non-Log Transformed Data
Clinical Samples	**445**	Intercept	0.193	0.037	(0.120, 0.266)[a] (0.160,0.301)[b]	0.97	1783 (-547, 4112)
		Slope	1.023	0.010	(1.002, 1.044)[a] (0.992, 1.030)[b]		1.675 (1.570, 1.781)
Contrived Samples	**98**	Intercept	0.012	0.063	(0.114, 0.138)[a]	0.99	-95 (-138, -34)
		Slope	0.985	0.013	(0.960, 1.010)[a]		1.675 (1.881, 2.049)
Clinical + Contrived Samples	**543**	Intercept	0.348	0.033	(0.283, 0.413)[a] (0.356,0.462)[b]	0.98	14568 (-1064, 30200)
		Slope	0.961	0.009	(0.944, 0.979)[a] (0.993, 0.957)[b]		0.877 (0.683, 1.070)

* Intercept in log_{10} IU/mL

[a] Assumed independence between all samples

[b] Adjusted correlation between samples from same subjects by the bootstrap method with 500 iterations

b. Analysis of the Mean Paired Difference

Table 28 below presents the mean paired difference for SOT patient samples between the **cobas** CMV test and the TaqMan CMV test at representative thresholds and associated 95% CIs calculated using the paired t-test.

Table 28: Mean of Paired Viral Load Differences Between the cobas CMV and the TaqMan CMV Test (log_{10} IU/mL) for SOT Patients at Representative Decision Intervals (IU/mL)

	Representative Decision Intervals (log_{10} IU/mL)	N	Mean of Paired Differences (log_{10} IU/mL)	SE for Mean of Paired Differences (log_{10} IU/mL)	95% CI (log_{10} IU/mL)
Clinical Samples	≥ 2.14 to < 3.00	253	0.256	0.013	(0.230, 0.282)
	≥ 3.00 to < 4.00	122	0.317	0.016	(0.285, 0.350)
	≥ 4.00 to < 5.00	47	0.251	0.027	(0.196, 0.305)
	≥ 5.00	23	0.201	0.030	(0.139, 0.262)
	Overall	445	0.269	0.009	(0.251, 0.288)
Contrived Samples	≥ 2.14 to < 3.00	22	-0.017	0.044	(-0.108, 0.074)
	≥ 3.00 to < 4.00	21	-0.074	0.024	(-0.125, -0.024)
	≥ 4.00 to < 5.00	15	0.021	0.031	(-0.045, 0.086)
	≥ 5.00	40	-0.097	0.022	(-0.141, -0.053)
	Overall	98	-0.056	0.015	(-0.087, -0.025)
Clinical + Contrived Samples	≥ 2.14 to < 3.00	275	0.234	0.013	(0.208, 0.260)
	≥ 3.00 to < 4.00	143	0.260	0.019	(0.223, 0.296)
	≥ 4.00 to < 5.00	62	0.195	0.025	(0.145, 0.245)
	≥ 5.00	63	0.012	0.025	(-0.039, 0.062)
	Overall	543	0.211	0.010	(0.191, 0.230)

Note: The table only included paired samples with paired results that were each within 137 IU/mL to 9.1E+06 IU/mL, the overlapping linear range of both assays. Paired results within the linear range on both assays were categorized into representative decision intervals based on the TaqMan CMV Test result (IU/mL).
Decision levels :

≥ 2.14 to < 3.00 log_{10} IU/mL = ≥ 1.37E+02 to < 2.0E+03 IU/mL
≥ 3.00 to < 4.00 log_{10} IU/mL = ≥ 2.0E+03 to < 2.0E+04 IU/mL
≥ 4.00 to < 5.00 log_{10} IU/mL = ≥ 2.0E+04 to < 1.0E+05 IU/mL
≥ 5.00 log_{10} IU/mL = ≥ 1.0E+05 IU/mL

CI = confidence interval; N = number of paired samples; SE = standard error.

c. Analysis of Bias

Table 29 below presents the mean paired difference between the **cobas** CMV test and the TaqMan CMV test at representative thresholds and associated 95% CIs calculated using the paired t-test.

Table 29: Bias/Systematic Difference Between the cobas CMV Test and the TaqMan CMV Test for SOT Patients at Selected Viral Load Levels

	Viral Load Level (Per Comparator)	Systematic Difference
Clinical	2.137 $\log_{10}$ IU/ml (1.37E+02 IU/ml)	0.242 $\log_{10}$ IU/ml (1.02E+02 IU/mL)
	2.699 $\log_{10}$ IU/ml (5.00E+02 IU/ml)	0.255 $\log_{10}$ IU/ml (4.00E+02 IU/mL)
	3.255 $\log_{10}$ IU/ml (1.80E+03 IU/ml)	0.268 $\log_{10}$ IU/ml (1.54E+03 IU/mL)
	4.000 $\log_{10}$ IU/ml (1.00E+04 IU/ml)	0.285 $\log_{10}$ IU/ml (9.28E+03 IU/mL)
	7.000 $\log_{10}$ IU/ml (1.00E+07 IU/ml)	0.354 $\log_{10}$ IU/ml (1.26E+07 IU/mL)
Contrived	2.137 $\log_{10}$ IU/ml (1.37E+02 IU/ml)	-0.020 $\log_{10}$ IU/ml (-6.10E+00 IU/mL)
	2.699 $\log_{10}$ IU/ml (5.00E+02 IU/ml)	-0.029 $\log_{10}$ IU/ml (-3.20E+01 IU/mL)
	3.255 $\log_{10}$ IU/ml (1.80E+03 IU/ml)	-0.037 $\log_{10}$ IU/ml (-1.46E+02 IU/mL)
	4.000 $\log_{10}$ IU/ml (1.00E+04 IU/ml)	-0.048 $\log_{10}$ IU/ml (-1.05E+03 IU/mL)
	7.000 $\log_{10}$ IU/ml (1.00E+07 IU/ml)	-0.093 $\log_{10}$ IU/ml (-1.93E+07 IU/mL)
Clinical + Contrived	2.137 $\log_{10}$ IU/ml (1.37E+02 IU/ml)	0.265 $\log_{10}$ IU/ml (1.15E+02 IU/mL)
	2.699 $\log_{10}$ IU/ml (5.00E+02 IU/ml)	0.243 $\log_{10}$ IU/ml (3.74E+02 IU/mL)
	3.255 $\log_{10}$ IU/ml (1.80E+03 IU/ml)	0.221 $\log_{10}$ IU/ml (1.195E+03 IU/mL)
	4.000 $\log_{10}$ IU/ml (1.00E+04 IU/ml)	0.192 $\log_{10}$ IU/ml (5.560E+03 IU/mL)
	7.000 $\log_{10}$ IU/ml (1.00E+07 IU/ml)	0.075 $\log_{10}$ IU/ml (1.89E+07 IU/mL)

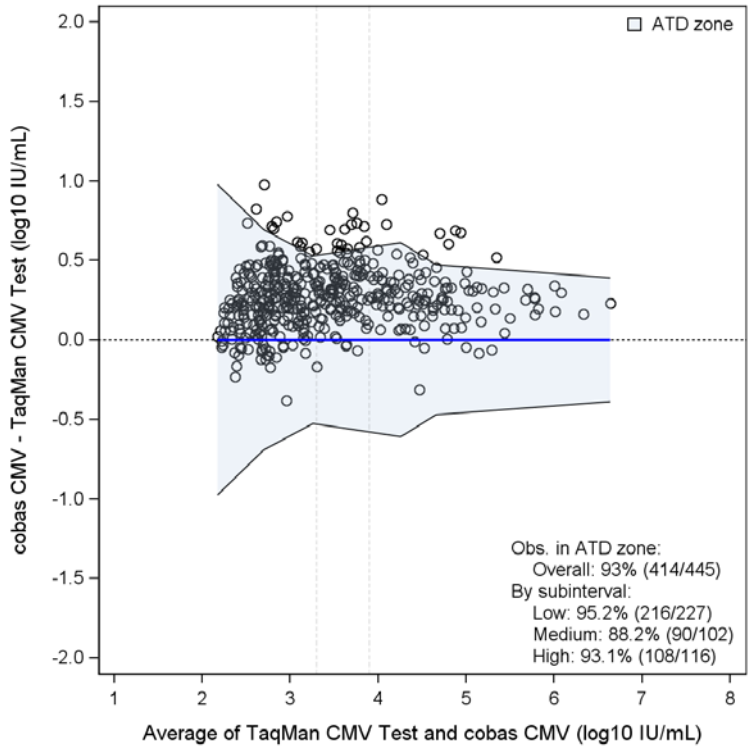
d. Analysis of the Allowable Total Difference (ATD)

The ATD zone was constructed for two measurements of the TaqMan CMV Test based on the reproducibility of the TaqMan CMV Test. In addition, the percentages of the samples at low, medium, and high sub-intervals that fall within the ATD zone were calculated. Viral load sub-intervals were determined using the log10 IU/mL based on the TaqMan CMV Test for each paired sample and are defined as outlined in Table 30 below.

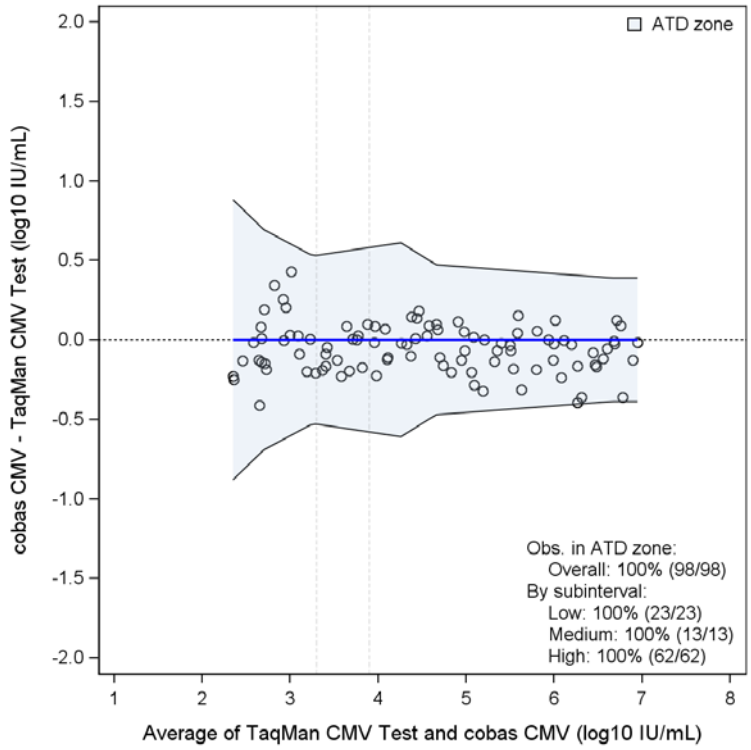
The resulting graphs are shown in Figure 5 below for clinical, contrived and combined (clinical+contrived) data sets. Table 30 demonstrates that 93% (414/445) of paired observations fell within the ATD zone for clinical samples.
For contrived samples 100% of the results fell within the ATD zone and within each of the sub intervals.

Figure 5: Allowable Total Difference (ATD) of Viral Load Difference (log_{10} IU/mL) for SOT Patients (All Clinical Sites Combined)

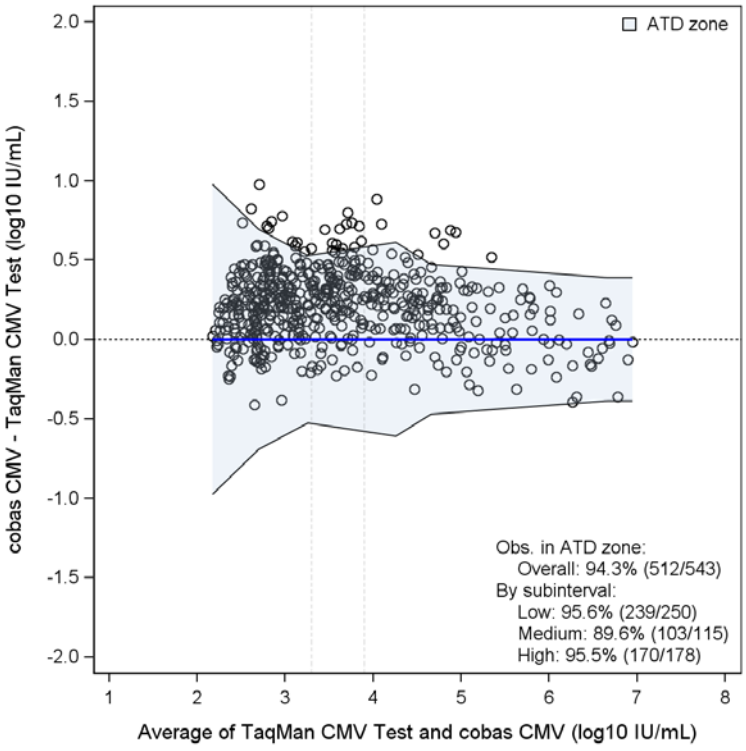
Clinical Samples



Contrived Samples



Clinical + Contrived Samples



CI = confidence interval; r = correlation coefficient

Table 30: Percentage of Samples in the SOT Population that Fall in Allowable Total Difference (ATD) Zone Intervals (IU/mL)

	Interval Category	Interval Range (IU/mL)	Percentage of Paired Samples Within ATD Zone % (n/N)
Clinical[1]	Low	1.37E+02 to < 2.0E+03	95.2% (216/227)
	Medium	2.0E+03 to < 8.0E+03	88.2% (90/102)
	High	8.0E+03 to 9.10E+06	93.1% (108/116)
	Overall	N/A	93.0% (414/445)
Contrived	Low	1.37E+02 to < 2.0E+03	100.0% (23/23)
	Medium	2.0E+03 to < 8.0E+03	100.0% (13/13)
	High	8.0E+03 to 9.10E+06	100.0% (62/62)
	Overall	N/A	100.0% (98/98)
Clinical[1] + Contrived	Low	1.37E+02 to < 2.0E+03	95.6% (239/250)
	Medium	2.0E+03 to < 8.0E+03	89.6% (103/115)
	High	8.0E+03 to 9.10E+06	95.5% (170/178)
	Overall	N/A	94.3% (512/543)

Note: The table only includes paired samples with paired results that were each within 1.37E+02 IU/mL to 9.1E+06 IU/mL, the overlapping linear range of both assays. Paired results were categorized into viral load intervals based on the TaqMan CMV Test result (IU/mL).

[1] Twenty-six samples from nine subjects were excluded from method comparison analyses due to impactful sequence mismatch.

N = total number of paired samples within the appropriate interval; n = number of paired samples included in the ATD Zone within the appropriate interval.

e. Agreement with Negative Samples

Thirty CMV immunoglobulin G (IgG)-negative clinical samples from SOT patients were tested on each assay (the **cobas** CMV test and the TaqMan CMV Test) to evaluate clinical specificity of the assay. All samples resulted in "Target Not Detected" results for both tests and resulted in 100% specificity (Table 31).

Table 31: Results of CMV IgG-Negative SOT Patient Specimens

		TaqMan CMV Test (IU/mL)			
		Target Not Detected	**< 1.37E+02**	**≥ 1.37E+02**	**Total**
cobas CMV (IU/mL)	**Target Not Detected**	30	0	0	30
	< 1.37E+02	0	0	0	0
	≥ 1.37E+02	0	0	0	0
	Total	30	0	0	30

Note: The lower limit of quantitation (LLOQ) is 137 IU/mL for TaqMan CMV Test.
IgG = immunoglobulin G

C. Clinical Concordance in HSCT Patients

1. Study Design

The overall objective of this study was to assess whether CMV viral loads measured with the **cobas** CMV test are concordant with measurements of the same samples with an FDA approved comparator test (TaqMan CMV Test) and are therefore informative in aiding in the management of Hematopoetic Stem Cell Transplant (HSCT) recipients.

Longitudinal samples were obtained from HSCT recipients who participated in a Phase 2, randomized, double blind, placebo-controlled dose-ranging multicenter clinical trial for a new drug candidate for CMV prophylaxis. HSCT patients were followed for a period of up to 19 weeks, with EDTA plasma samples drawn weekly for the first 11 weeks, followed by monthly sampling for the remaining time. The study conformed to the standard of care, which was to initiate pre-emptive treatment in patients with significant CMV viremia.

Residual, frozen EDTA plasma samples were from HSCT patients who: (1) participated in the CMV prophylaxis drug trial and (2) fulfilled the inclusion criteria. EDTA plasma samples were tested with the **cobas** CMV test and the TaqMan CMV Test. The concordance of CMV measurements with the **cobas** CMV test and the TaqMan CMV Test CMV test in HSCT patients was assessed with respect to significant clinical decisions in the same manner as described above for SOT recipients (see under "Analysis").

HSCT patient samples in the parent drug study were collected between December 2009 and November 2011. Samples from 258 HSCT patients were used for the **cobas** CMV Clinical Concordance study and were tested with the **cobas** CMV test at three investigational sites; TaqMan testing was performed at the internal site only.

The key timepoints as defined by the **cobas** CMV testing protocol were Baseline, Days 7, 14, 21, 28, 35, and 49.

Inclusion and Exclusion Criteria

Enrollment in the parent drug trial was limited to patients who met the following clinical inclusion criteria:

- ≥ 18 years of age
- Recipient of an allogeneic HSCT within 28 days of enrollment
- Seropositive for CMV (based on CMV IgG) before transplantation
- No detectable CMV viremia or CMV disease between the transplant and the first day dose of the experimental prophylactic treatment
- Suitable consent for diagnostic study

Enrollment in the **cobas** CMV testing study was limited to patients who met the following inclusion criteria:

- Patients in the parent drug study with 5 or more samples, each with sufficient volume to permit testing with the **cobas** CMV test and the TaqMan CMV Test
- The Clinical Concordance analysis was limited to a subset of the drug study subjects who had sufficient data points for the data to be informative. The subjects included in this analysis had a viremic episode (according to the CMV viral load test used in the parent drug study) and still had a minimum of 4 consecutive weekly samples collected during this episode available for testing with both, the **cobas** CMV and the TaqMan CMV tests in the diagnostic study.

Patients were not permitted to enroll in the studies if inclusion criteria were not met.

Follow-up schedule and endpoints

See Study Design (above)

2. Accountability of PMA Cohort

A total of 305 total subjects participated in the parent drug study; however only 258 subjects had documented informed consent forms. The 258 subjects contributed 1,392 samples that provided at least one valid result on each assay. Samples that did not generate a valid result on both, the **cobas** CMV and the TaqMan CMV tests were excluded (so called "paired" samples). A total of 257 subjects with 1,367 paired samples were included in the Clinical Concordance study.

For additional accountability with respect to the different analysis a. to b. (see below) please refer to the analysis sections below.

3. HSCT Study Population Demographics and Baseline Parameters

The demographics of the study population are representative for a study performed in the U.S. that assesses CMV in HSCT transplant patients.

Table 32: Demographics and Baseline Clinical Characteristics of HSCT Subjects

Characteristics	Statistic
Total, N	258
Age (years)	
Mean ± Standard Deviation	51 ± 12.3
Median	51
Range	21 - 71
Gender, n(%)	
Male	144 (55.8%)
Female	114 (44.2%)
Ethnicity, n(%)	
Hispanic / Latino	24 (9.3%)
Not Hispanic / Not Latino	230 (89.1%)
Unknown	4 (1.6%)
Race, n(%)	
Asian	15 (5.8%)
Black / African-American	10 (3.9%)
White	228 (88.4%)
Other	5 (1.9%)
Study Arm, n(%)	
Anti-CMV Prophylaxis Regimen	164 (63.6%)
Placebo	61 (23.6%)
Screen Failure	33 (12.8%)

4. Analysis

The concordance of CMV measurements between the **cobas** CMV test and the TaqMan CMV Test in HSCT patients was assessed with respect to the following clinical asessments:

a. Agreement of HSCT Patient Viral Load Results Using Different Threshold Values

Viral load guided initiation of anti-CMV medication in the management of transplant patients depends on multiple patient risk factors as well as the experience of the treating physicians and the institution's standard of care. Hence, no universally applicable treatment threshold can be validated. Consequently, result concordance between the **cobas** CMV test and the TaqMan CMV Test was analyzed using four different arbitrary thresholds: Target Not Detected [LOD], 137 IU/mL [2.137 log10 IU/mL], 500 IU/mL [2.699 log10 IU/mL] and 1,800 IU/mL [3.255 log10 IU/mL]. The analysis included all

evaluable samples of the clinical study independent of treatment and independent of the specific time elapsed post treatment initiation.

In addition, results from the **cobas** CMV and the TaqMan CMV tests were analyzed in a 6x6 table that takes into account that the SD for the reproducibility of the TaqMan CMV Test was less than ± 0.3 $\log_{10}$ IU/mL. The categories in the table derived from the threshold value (in $\log_{10}$ IU/mL) ± 0.6 $\log_{10}$ IU/mL (2x the SD of the reproducibility) with the log-transformed nominal threshold values in $\log_{10}$ rounded to one significant figure.The analysis is based on the assumption that samples with values above a threshold of 2.7 $\log_{10}$ IU/mL IU/mL are considered unlikely to be below the threshold of 2.1 $\log_{10}$ IU/mL because of random measurement error. Similarly, samples with values above a threshold of 3.3 $\log_{10}$ IU/mL IU/mL are considered unlikely to be below the threshold of 2.7 $\log_{10}$ IU/mL and samples with values above a threshold of 3.9 $\log_{10}$ IU/mL IU/mL are considered unlikely to be below the threshold of 3.3 $\log_{10}$ IU/mL because of random measurement error. Therefore samples were considered discordant only if they were discrepant across more than the immediately adjacent categories

Analysis Specific Accountability:

Of the 257 subjects n=1,367 samples had paired results (i.e., valid results with both, the **cobas** CMV and the FDA approved comparator test) and were included in the Clinical Concordance analysis.

b. Agreement of HSCT Patient Viral Load Results at Baseline in Order to Assess Initiation of Anti-CMV Therapy

The **cobas** CMV and TaqMan CMV test results were analyzed as described under 4.a. (above) but analysis was performed only for paired evaluable samples collected at baseline. For the pupose of this analysis, measurements prior to initiation of anti-CMV therapy were considered in the baseline analysis as well because they were used to inform the decision to initiate anti-CMV treatment.

Analysis Specific Accountability:
Of the 257 total HSCT subjects in the clinical study, 71 subjects initiated anti-CMV therapy. One subject who initiated anti-CMV therapy was excluded because the subject's sample had an invalid **cobas** CMV result. Three subjects did not have results collected at or prior to baseline. Thus, the analysis included the remaining 67 subjects who provided 91 samples with paired results that were evaluable and collected at baseline or prior to anti-CMV treatment initiation.

c. Agreement of HSCT Patient Viral Load Results in Order to Assess Response to Anti-CMV Therapy

The **cobas** CMV and TaqMan CMV test results were analyzed as described under 4.a. (above) but analysis was performed for all paired evaluable samples collected at the protocol defined timepoints post anti-CMV therapy initiation (Day 14, Day 21, Day 28, Day 35, and Day 49).

Analysis Specific Accountability:
Of the 257 total HSCT subjects in the Clinical Concordance study for the **cobas** CMV test, 71 subjects initiated anti-CMV therapy. However, only 17 of them had samples (total of 45 samples) with paired results that were evaluable and collected at protocol defined time points post anti-CMV therapy initiation and were included in the analysis.

d. Agreement of HSCT Patients Viral Load Results in Order to Determine When to Stop Anti-CMV Therapy.

An analysis was carried out to determine the concordance between the **cobas** CMV test and the TaqMan CMV Test when used to aid in determining whether or not to stop anti-CMV treatment at visit times of Day 14, Day 21, Day 28, Day 35, and Day 49 (post anti-CMV therapy initiation). The analysis considered all viremic patients who started anti-CMV medication (n=71).

The following Definitions were used:

- **Baseline:** Baseline was defined as the date the anti-CMV medication was started.

- **Days of Interest:** Testing days of interest were Day 14, Day 21, Day 28, Day 35, and Day 49 and were defined relative to the Baseline with a window of ±3 days.

- **Viremic Episode:** A viral load of ≥137 IU/mL (or ≥ 2.137 $\log_{10}$ IU/mL) for both tests was used to define a viremic episode.

- **Resolution of Viremia:** Consistent with current guideline recommendations resolution of the viremic episode is defined by two consecutive measurements below the LLOQ of the comparator test (i.e., <137 IU/mL or < 2.137 $\log_{10}$ IU/mL) and/or Target Not Detected (TND) with the samples taken one week apart.

For subjects in the cohort who had multiple episodes of viremia, once the subject met the criterion for resolution of their initial CMV episode at a given timepoint, that resolution status was carried forward for the corresponding assay and recurrent episodes of viremia were not analyzed anymore.

Note that not all subjects have all samples available at each of the time points. Missing data points were imputed through linear regression if surrounding datapoints with viral loads > 137 IU/mL were available. Imputation was performed across a maximum of two missing data points (2 weeks).

A missing data point can be considered as “not resolved” for the purpose of resolution analysis if the previous data point was viremic because two consecutive viral load measurements of < 137 IU/mL (or undetectable CMV DNA) are required to define a resolution of the viremic episode.

Analysis Specific Accountability:
Of the 257 subjects (1367 samples) that were included in the Clinical Concordance analysis the following number of subjects were excluded for the purpose of resolution analysis:

- One hundred and eighty-six (186) subjects with 1,054 samples were excluded for the resolution analysis because they did not have documented anti-CMV medication administered during the course of the study.
- Fifty-four (54) subjects were excluded because a viremic episode could not be defined and/or the resolution of the viremic episode could not be determined.

A total of 17 study subjects who received therapy had a viremic episode and had paired viral load measurements that allowed the resolution analysis of the viremic episode (i.e., a positive viral load measurement ≥137 IU/mL) at least at one of the protocol defined time points post anti-CMV therapy initiation (i.e., Day 14, Day 21, Day 28, Day 35, and Day 49) and were included in the analysis.

Note: The concordance of the **cobas** CMV and the TaqMan CMV tests was not assessed for Day 35 because the number of subjects that could be analyzed (n=2) was not meaningful since both subjects had already resolved viremia and the resolution status carried forward from days 21 and 28.

5. Safety and Effectiveness Results

The key safety and effectiveness (performance) outcomes for the Clinical Concordance study of Hematopoetics Stem Cell Transplant (HSCT) patients are presented below in Tables 33 to 48.

Performance evaluation was based on 257 patients evaluable for Clinical Concordance. Adverse effect reporting is not applicable to this study as it is a retrospective study with leftover specimens and no patient management was based on the results of the investigational device.

a. Agreement of HSCT Patient Viral Load Results Using Different Threshold Values

Tables 33 and 34 below consider all 1,367 specimens for which a result was available for both the **cobas** CMV and TaqMan CMV tests. These specimens were obtained from 257 subjects (viremic and non-viremic). Analysis was performed at four different thresholds: Target Not detected (TND), 137 IU/mL, 500 IU/mL and 1,800 IU/mL.

Table 33: Concordance Analysis of Viral Load Results for HSCT Patients Using Different Threshold (All Paired Samples)

		TaqMan CMV		Total
		Target Not Detected	Detected	
cobas CMV	Target Not Detected	918	25	943
	Detected	155	269	424
Total		1,073	294	1,367[1]

		< 137 IU/mL (< 2.1 log_{10} IU/mL*)	≥ 137 IU/mL (≥ 2.1 log_{10} IU/mL*)	Total
cobas CMV	< 137 IU/mL (< 2.1 log_{10} IU/mL*)	1,233	11	1,244
	≥ 137 IU/mL (≥ 2.1 log_{10} IU/mL*)	15	108	123
Total		1,248	119	1,367[2]

		< 500 IU/mL (< 2.7 log_{10} IU/mL**)	≥ 500 IU/mL (≥ 2.7 log_{10} IU/mL**)	Total
cobas CMV	< 500 IU/mL (< 2.7 log_{10} IU/mL**)	1,279	7	1,286
	≥ 500 IU/mL (≥ 2.7 log_{10} IU/mL**)	19	62	81
Total		1,298	69	1,367[3]

		< 1,800 IU/mL (< 3.3 log_{10} IU/mL***)	≥ 1,800 IU/mL (≥ 3.3 log_{10} IU/mL***)	Total
cobas CMV	< 1,800 IU/mL (< 3.3 log_{10} IU/mL***)	1,320	2	1,322
	≥ 1,800 IU/mL (≥ 3.3 log_{10} IU/mL***)	8	27	45
Total		1,328	39	1,367[4]

All paired samples evaluable for Clinical Concordance analysis were included in this table. Samples with a "Target Not Detected" or a detectable viral load below 1,800 IU/mL result were categorized as "< 1,800 IU/mL (< 3.255 log_{10} IU/mL)**.**

[1] 3 of the 180 discordant samples were sequenced and showed impactful sequenced mismatch

[2] 2 of the 26 discordant samples were sequenced and showed impactful sequenced mismatch

[3] 3 of the 26 discordant samples were sequenced and showed impactful sequenced mismatch

[4] None of the samples with impactful sequenced mismatches was discrepant using this threshold

* Threshold Log_{10} of 2.137 abbreviated as 2.1 log_{10} IU/mL

** Threshold Log_{10} of 2.699 abbreviated as 2.2log_{10} IU/mL

*** Threshold Log_{10} of 3.255 abbreviated as 3.3 log_{10} IU/mL.

Table 34: Summary Concordance of Viral Load Results for HSCT Patients Using Different Thresholds (All Paired Samples)

	Percent Agreement < Threshold 95% CI (n/N)	**Percent Agreement ≥ Threshold (n/N)** 95% CI (n/N)	**Overall Percent Agreement** 95% CI (n/N)
Target Not Detected	**85.6** 95% CI: 83.3%, 87.6% (918/1,073)	**91.5** (95% CI: 87.7%, 94.4%) (269/294)	**86.8** 95% CI: 84.9%, 88.6% (1,187/1,367)
137 IU/mL (2.1 log_{10} IU/mL*)	**98.85** 95% CI: 98.0%, 99.3 (1,233/1,248)	**90.8** 95% CI: 84.1%, 93.5% (108/119)	**98.1** 95% CI: 97.2%, 98.8% (1,341/1,367)
500 IU/mL (2.7 log_{10} IU/mL)**	**98.5** 95% CI: 97.7%, 99.1% (1,279/1,298)	**89.9** 95% CI: 80.2%, 95.8% (62/69)	**98.1** 95% CI: 97.2%, 98.8% (1,341/1,367)
1,800 IU/mL (3.3 log_{10} IU/mL*)**	**99.4** 95% CI: 98.8%, 99.7% (1,320/1,328)	**94.9** 95% CI: 82.7%, 99.4% (37/39)	**99.3** 95% CI: 98.7%, 99.6% (1,357/1,367)

Note: All paired samples evaluable for Clinical Concordance analysis were included in this table. Samples with a "Target Not Detected" or a detectable viral load below 1,800 IU/mL result were categorized as "< 1,800 IU/mL (< 3.255 log_{10} IU/mL). The LOD of the **cobas** CMV is 34.5 IU/mL, the LOD of the TaqMan CMV test is 137 IU/mL.
* Log_{10} of 2.137 abbreviated as 2.1 log_{10} IU/mL
** Log_{10} of 2.699 abbreviated as 2.2log_{10} IU/mL
*** Log_{10} of 3.255 abbreviated as 3.3 log_{10} IU/mL.
95% confidence interval (CI) calculated by exact method assuming independence between all samples.
1 IU/mL = 1.1 copy/mL; 1,800 IU/mL = 2,000 copies/mL.
HSCT = Hematopoetic Stem Cell Transplant.

The concordance analysis of viral load results as determined by the **cobas** CMV and TaqMan CMV tests was further analyzed in the 6x6 Table (Table 35) below as described in section C.4.a. (above). Samples were considered discordant if they were discrepant across more than the immediately adjacent categories; this analysis considers the reproducibility of the **cobas** CMV test.

Most samples were not discrepant by more than 1 category. Of the 3 samples with wider discrepancies (see footnotes a and b in Table 35), one was found to contain a CMV variant with a significant mismatch mutation in one of the TaqMan CMV Test primer binding sites.

Agreements were found to be as follows:

Agreement for TND:	99.9%	(1072/1,073)
Agreement for Detected < 2.1 log_{10} IU/mL:	98.1%	(152/175)
Agreement for 2.1 to < 2.7 log_{10} IU/mL:	100%	(50/50)

Agreement for 2.7 to < 3.3 log_{10} IU/mL: 100% (30/30)
Agreement for 3.3 to < 3.9 log_{10} IU/mL: 96.3% (26/27)
Agreement for ≥ 3.9 log_{10} IU/mL: 91.2% (11/12)

Table 35: Concordance Analysis for HSCT Patients (All Paired Samples)

		TaqMan CMV Test (log_{10} IU/mL)					
cobas CMV (log_{10} IU/mL)	**Target Not Detected**	**< 2.1**	**2.1 to < 2.7**	**2.7 to < 3.3**	**3.3 to < 3.9**	**≥ 3.9**	**Total**
Target Not Detected	918	23	0	0	1[b]	1[b]	943
< 2.1*	154	138	9	0	0	0	301
2.1 to < 2.7	0	13	24	5	0	0	42
2.7 to < 3.3	1[a]	1	17	17	0	0	36
3.3 to < 3.9	0	0	0	8	16	1	25
≥ 3.9	0	0	0	0	10	10	20
Total	1,073	175	50	30	27	12	1,367

Note: All 1367 paired samples evaluable for Clinical Concordance analysis from all 257 subjects were included in this table.
*The LLOQ is 34.5 IU/mL for the **cobas** CMV and 137 IU/mL for TaqMan CMV Test. Therefore for the **cobas** CMV test the category of <137 IU/mL includes non-quantifiable results < the LLOQ and quantifiable results ≥ 34.5 IU/mL but < 137 IU/mL
log_{10} (137) = 2.137 (abbreviated above as 2.1); log_{10} (1,800) = 3.255 (abbreviated above as 3.3); log_{10} (7,943) = 3.900 (abbreviated above as 3.9).
[a] This samples was sequenced and was found to contain a significant impact mutation in the forward primer binding site of the Taqman CMV test.
[b] These samples failed sequencing but the initial viral load result of these samples in the prophylactic drug trial was negative and hence concordant with the **cobas** CMV test. Retesting of a 1:10 dilution of these samples with the TaqMan CMV test resulted in TND results for both samples.

b. Agreement of HSCT Patient Viral Load Results at Baseline Using Different Threshold Values

Table 36 below considers all 67 subjects who had paired viral load results at baseline. For subjects with a missing baseline sample the sample immediately prior to therapy initiation was used. Concordance for the viral load result of these 67 samples is provided in Table 37.

Table 36: Concordance Analysis for Baseline Samples from HSCT Patients Using Different Thresholds

		TaqMan CMV		Total
		Target Not Detected	**Detected**	
cobas CMV	**Target Not Detected**	11	0	11
	Detected	8[1]	48	56
Total		19	48	67

		< 137 IU/mL (< 2.1 log_{10} IU/mL*)	**≥ 137 IU/mL (≥ 2.1 log_{10} IU/mL*)**	**Total**
cobas CMV	**< 137 IU/mL (< 2.1 log_{10} IU/mL*)**	36	1	37
	≥ 137 IU/mL (≥ 2.1 log_{10} IU/mL*)	1[1]	29	30
Total		49	42	67

		< 500 IU/mL (< 2.7 log_{10} IU/mL)**	**≥ 500 IU/mL (≥ 2.7 log_{10} IU/mL**)**	**Total**
cobas CMV	**< 500 IU/mL (< 2.7 log_{10} IU/mL**)**	43	1[3]	44
	≥ 500 IU/mL (≥ 2.7 log_{10} IU/mL)**	0	23	23
Total		43	24	67

		< 1,800 IU/mL (< 3.3 log_{10} IU/mL*)**	**≥ 1,800 IU/mL (≥ 3.3 log_{10} IU/mL***)**	**Total**
cobas CMV	**< 1,800 IU/mL (< 3.3 log_{10} IU/mL***)**	48	0	48
	≥ 1,800 IU/mL (≥ 3.3 log_{10} IU/mL*)**	2[1]	17	19
Total		50	17	67

Only paired samples at or before baseline and evaluable for Clinical Concordance analysis were included in this table. Samples with a "Target Not Detected" results were categorized as "< Threshold value in IU/mL".

[1] The discrepant samples did not have an impactful sequence mismatch. Discordance was <0.5 log.

* Threshold Log_{10} of 2.137 abbreviated as 2.1 log_{10} IU/mL

** Threshold Log_{10} of 2.699 abbreviated as 2.2log_{10} IU/mL

*** Threshold Log_{10} of 3.255 abbreviated as 3.3 log_{10} IU/mL.

Table 37: Summary Concordance of Viral Load Results for Baseline Samples from HSCT Patients Using Different Thresholds

	Percent Agreement < Threshold 95% CI (n/N)	**Percent Agreement ≥ Threshold (n/N)** 95% CI (n/N)	**Overall Percent Agreement** 95% CI (n/N)
Target Not Detected	**57.9%** (33.5%, 79.7%) 11/19	**100.0%** (92.6%, 100.0%) 48/48	**88.1%** (77.8%, 94.7%) 59/59
137 IU/mL (2.1 log_{10} IU/mL*)	**97.3%** (85.8%, 99.9%) 36/37	**96.7%** (82.8%, 99.9%) 29/30	**97.0%** (89.6%, 99.6%) 65/67
500 IU/mL (2.7 log_{10} IU/mL)**	**100.0%** (91.8%, 100.0%) 43/43	**95.8%** (78.9%, 99.9%) 23/24	**98.5%** (92.0%, 100.0%) 66/67
1,800 IU/mL (3.3 log_{10} IU/mL*)**	**96.0%** (86.3%, 99.5%) 48/50	**100.0%** (80.5%, 100.0%) 17/17	**97.0%** (89.6%, 99.6%) 65/67

Note: Only paired samples at or before baseline and evaluable for Clinical Concordance analysis were included in this table. Samples with a "Target Not Detected" results were categorized as "< threshold value in IU/mL".
* Log_{10} of 2.137 abbreviated as 2.1 log_{10} IU/mL
** Log_{10} of 2.699 abbreviated as 2.2log_{10} IU/mL
*** Log_{10} of 3.255 abbreviated as 3.3 log_{10} IU/mL.
95% confidence interval (CI) calculated by exact method assuming independence between all samples.
1 IU/mL = 1.1 copy/mL.
HSCT = Hematopoetic stem cell transplant

The concordance of viral load results as determined by the **cobas** CMV and TaqMan CMV tests was further analyzed in the 6x6 Table (Table 38) below as described in section C.4.a. (above)1,8007,943Samples were considered discordant if they were discrepant across more than the immediately adjacent categories; this analysis considers the reproducibility of the **cobas** CMV test.

None of the samples were discrepant by more than 1 category. Therefore the agreements were found to be 100% for all categories.

Table 38: Concordance Analysis for Baseline Samples from HSCT Patients

cobas CMV (log_{10} IU/mL)	Target Not Detected	TaqMan CMV Test (log_{10} IU/mL) < 2.1	2.1 to < 2.7	2.7 to < 3.3	3.3 to 3.9	≥ 3.9	Total
Target Not Detected	11	0	0	0	0	0	11
< 2.1*	8	17	1	0	0	0	26
2.1 to < 2.7	0	1	5	1	0	0	12
2.7 to < 3.3	0	0	0	4	0	0	
3.3 to 3.9	0	0	0	2	7	1	9
≥ 3.9	0	0	0	0	3	6	9
Total	19	18	5	7	10	7	67

Note: Only paired samples at or before baseline and evaluable for Clinical Concordance analysis were included in this table. Samples with a "Target Not Detected" results were categorized as "< threshold value in IU/mL".
*The LLOQ is 34.5 IU/mL for the **cobas** CMV and 137 IU/mL for TaqMan CMV Test. Therefore for the **cobas** CMV test the category of <137 IU/mL includes non-quantifiable results < the LLOQ and quantifiable results ≥ 34.5 IU/mL but < 137 IU/mL.
Log_{10} of 2.137 abbreviated as 2.1 log_{10} IU/mL
Log_{10} of 2.699 abbreviated as 2.2log_{10} IU/mL
Log_{10} of 3.255 abbreviated as 3.3 log_{10} IU/mL
Log_{10} of 7,943 abbreviated as 3.9 log_{10} IU/mL

c. Agreement of HSCT Patients Viral Load Results in Order to Assess Response to Anti-CMV Therapy

Tables 39 and 40 provide an analysis of concordance of viral load results when used to assess response to therapy and included the 45 paired results from 17 subjects who initiated anti-CMV therapy and for whom samples were collected at any of the protocol defined days post therapy initiation (i.e., Days 7, 14, 21, 28, and 49 post treatment).

Table 39: Concordance Analysis for Samples From HSCT Patients Collected at Protocol Defined Days Post Treatment Initiation Using Different Thresholds (n=45 Samples)

		TaqMan CMV		Total
		Target Not Detected	**Detected**	
cobas CMV	**Target Not Detected**	17	1	18
	Detected	11[1]	16	27
Total		28	17	45

		< 137 IU/mL (< 2.1 log_{10} IU/mL*)	**≥ 137 IU/mL (≥ 2.1 log_{10} IU/mL*)**	**Total**
cobas CMV	**< 137 IU/mL (< 2.1 log_{10} IU/mL*)**	36	0	36
	≥ 137 IU/mL (≥ 2.1 log_{10} IU/mL*)	1[2]	8	9
Total		37	8	45

		< 500 IU/mL (< 2.7 log_{10} IU/mL)**	**≥ 500 IU/mL (≥ 2.7 log_{10} IU/mL**)**	**Total**
cobas CMV	**< 500 IU/mL (< 2.7 log_{10} IU/mL**)**	36	0	36
	≥ 500 IU/mL (≥ 2.7 log_{10} IU/mL)**	3[3]	6	9
Total		39	6	45

		< 1,800 IU/mL (< 3.3 log_{10} IU/mL*)**	**≥ 1,800 IU/mL (≥ 3.3 log_{10} IU/mL***)**	**Total**
cobas CMV	**< 1,800 IU/mL (< 3.3 log_{10} IU/mL***)**	41	0	41
	≥ 1,800 IU/mL (≥ 3.3 log_{10} IU/mL*)**	2[4]	2	4
Total		43	2	45

Samples with a "Target Not Detected" or a detectable viral load below 1,800 IU/mL result were categorized as "< 1,800 IU/mL (< 3.255 log_{10} IU/mL)**.**

[1] 1 of the 11 discordant samples were sequenced and showed impactful sequenced mismatch

[2] The 1 discordant sample was sequenced and showed impactful sequenced mismatch

[3] 1 of the 3 discordant samples was sequenced and showed impactful sequenced mismatch

[4] none of the 2 discrepant samples were from subjects with impactful sequenced mismatch

* Threshold Log_{10} of 2.137 abbreviated as 2.1 log_{10} IU/mL

** Threshold Log_{10} of 2.699 abbreviated as 2.2log_{10} IU/mL

*** Threshold Log_{10} of 3.255 abbreviated as 3.3 log_{10} IU/mL.

Table 40: Summary Concordance for Samples From HSCT Patients Collected at Protocol Defined Days Post Treatment Initiation Using Different Thresholds (n=45 Samples)

	Percent Agreement < Threshold 95% CI (n/N)	**Percent Agreement ≥ Threshold (n/N)** 95% CI (n/N)	**Overall Percent Agreement** 95% CI (n/N)
Target Not Detected	**60.7%** 40.6%, 78.5% (17/28)	**94.1%** 71.3%, 99.9% (16/17)	**73.3%** 58.1%, 85.4% (33/45)
137 IU/mL (2.1 $\log_{10}$ IU/mL*)	**97.3%** 85.8%, 99.9% (36/37)	**100.0%** 63.1%, 100.0% (8/8)	**97.8%** 88.2%, 99.9% (44/45)
500 IU/mL (2.7 $\log_{10}$ IU/mL)**	**92.3%** 79.1%, 98.4% (36/39)	**100.0%** 54.1%, 100.0% (6/6)	**93.3%** 81.7%, 98.6% (42/45)
1,800 IU/mL (3.3 $\log_{10}$ IU/mL*)**	**95.3%** 84.2%, 99.4% (41/43)	**100.0%** 15.8%, 100.0% (2/2)	**95.6%** 84.9%, 99.5% (43/45)

Note: Samples with a "Target Not Detected" or a detectable viral load below 1,800 IU/mL result were categorized as "< 1,800 IU/mL (< 3.255 $\log_{10}$ IU/mL). The LOD of the **cobas** CMV is 34.5 IU/mL, the LOD of the TaqMan CMV test is 137 IU/mL.
* Log_{10} of 2.137 abbreviated as 2.1 $\log_{10}$ IU/mL
** Log_{10} of 2.699 abbreviated as 2.2$\log_{10}$ IU/mL
*** Log_{10} of 3.255 abbreviated as 3.3 $\log_{10}$ IU/mL.
95% confidence interval (CI) calculated by exact method assuming independence between all samples.
1 IU/mL = 1.1 copy/mL; 1,800 IU/mL = 2,000 copies/mL.
HSCT = Hematopoetic Stem Cell Transplant.

The concordance of viral load results as determined by the **cobas** CMV and TaqMan CMV tests was further analyzed in the 6x6 Table (Table 41) below as described in section C.4.a. (above). Samples were considered discordant if they were discrepant across more than the immediately adjacent categories; this analysis considers the reproducibility of the **cobas** CMV test.

Most samples were not discrepant by more than 1 category. The sample with wider discrepancy (see footnote a in Table 41) was considered discordant and was found to contain a significant impact mutation in the forward primer binding site of the TaqMan CMV test.

The agreements between the **cobas** CMV and TaqMan CMV test results for all, except the "Target Not Detected" category were 100%. Agreement for the TND category is 96.4% (27/28); the only discrepant sample contained a significant impact mutation in the forward primer binding site of the Taqman CMV test.

Table 41: Concordance Analysis for Samples From HSCT Patients Collected at Protocol Defined Time Points Post Treatment Initiation

	TaqMan CMV Test (log_{10} IU/mL)						
cobas CMV (log_{10} IU/mL)	**Target Not Detected**	**< 2.1**	**2.1 to < 2.7**	**2.7 to < 3.3**	**3.3 to 3.9**	**≥ 3.9**	**Total**
Target Not Detected	17	1	0	0	0	0	18
< 2.1*	10	8	0	0	0	0	18
2.1 to < 2.7	0	0	0	0	0	0	0
2.7 to < 3.3	1[a]	0	2	2	0	0	5
3.3 to 3.9	0	0	0	2	0	0	2
≥ 3.9	0	0	0	0	1	1	2
Total	28	9	2	4	1	1	45

Note: A total of 45 paired samples evaluable for Clinical Concordance analysis from 17 viremic subjects at protocol defined time points (Day 14, Day 21, Day 28, Day 35 or Day 49 post anti-CMV therapy initiation) were included in this table.

*The LLOQ is 34.5 IU/mL for the **cobas** CMV and 137 IU/mL for TaqMan CMV Test. Therefore for the **cobas** CMV test the category of <137 IU/mL includes non-quantificable results < the LLOQ and quantifiable results ≥ 34.5 IU/mL but < 137 IU/mL.

log_{10} (137) = 2.137 (abbreviated above as 2.1); log_{10} (1,800) = 3.255 (abbreviated above as 3.3); log_{10} (7,943) = 3.900 (abbreviated above as 3.9).

[a] This samples was sequenced and was found to contain a significant impact mutation in the forward primer binding site of the Taqman CMV test.

d. Agreement of HSCT Patients Viral Load Results in Order to Determine When to Stop Anti-CMV Therapy

The concordance analysis between the **cobas** CMV test and TaqMan CMV tests when used to aid in determining whether or not to stop anti-CMV treatment at visit times of Day 14, Day 21, Day 28, Day 35, and Day 49 (post anti-CMV therapy initiation) considered all 17 viremic HSCT patients who started anti-CMV medication and for whom a viremic episode and the resolution status thereof could be determined according to the definition and methods outlined in the analysis section above. Results are summarized in Table 42 below.

Viremia was defined as a viral load of ≥ 137 IU/mL with both the **cobas** CMV and the TaqMan CMV tests. Resolution of viremia was defined as two consecutive samples with a "Target Not Detected" result or a detectable viral load below 137 IU/mL.

Table 42:
Concordance Analysis of cobas CMV and TaqMan CMV Test Results When Used to Determining Resolution of Viremia for HSCT Patients

Day 14 Post Anti-CMV Therapy Initiation			
	TaqMan CMV		
cobas CMV	**Resolution of CMV Episode**	**No Resolution of CMV Episode**	**Total**
Resolution of CMV Episode	0	0	0
No Resolution of CMV Episode	0	14	14
Total	0	14	14
Column Agreement (95% CI)[a]	**NC**	**100.0% (76.8%, 100.0%)**	
Overall Percent Agreement (95% CI)[a]	**100.0% (80.7%, 100.0%)**		
Day 21 Post Anti-CMV Therapy Initiation			
	TaqMan CMV		
cobas CMV	**Resolution of CMV Episode**	**No Resolution of CMV Episode**	**Total**
Resolution of CMV Episode	1	0	1
No Resolution of CMV Episode	0	12	12
Total	1	12	13
Column Agreement (95% CI)[a]	100.0% (5%, 100.0%)	**100.0% (73.5%, 100.0%)**	
Overall Percent Agreement (95% CI)[a]	100.0% (79.4%, 100.0%)		
Day 28 Post Anti-CMV Therapy Initiation			
	TaqMan CMV		
cobas CMV	**Resolution of CMV Episode**	**No Resolution of CMV Episode**	**Total**
Resolution of CMV Episode	2	0	2
No Resolution of CMV Episode	0	7	7
Total	2	7	9
Column Agreement (95% CI)[a]	**100.0% (15.8%, 100.0%)**	**100.0% (59.0.2%, 100.0%)**	
Overall Percent Agreement (95% CI)[a]	**100.0% (66.4%, 100.0%)**		

Day 49 Post Anti-CMV Therapy Initiation			
	TaqMan CMV		
cobas CMV	**Resolution of CMV Episode**	**No Resolution of CMV Episode**	**Total**
Resolution of CMV Episode	3	0	3
No Resolution of CMV Episode	0	1	1
Total	3	1	4
Column Agreement (95% CI)[a]	**100.0% (29.2%, 100.0%)**	100.0% (2.5%, 100.0%)	
Overall Percent Agreement (95% CI)[a]	**100.0% (39.8%, 100.0%)**		

Only paired samples evaluable for Clinical Concordance analysis from Non-ViremicSubjects at each of the indicated Days post anti-CMV therapy initiation were included in this table. Samples with a "Target Not Detected" or a detectable viral load below 137 IU/mL result were categorized as "< 2.1 $\log_{10}$ IU/mL (< 137 IU/mL)."

[a] CI calculated by exact method and assuming independence between all samples

NC = Not calculable

6. Subgroup Analysis

No Subgroup analysis was performed

7. Pediatric Extrapolation

In this premarket application, existing clinical data was not leveraged to support approval of a pediatric patient population.

D. Method Comparison in HSCT Patients

1. Study Design

The objective of this study was to compare the results of clinical specimens from HSCT recipients tested with the **cobas** CMV and the (TaqMan CMV tests at clinically relevant viral load levels. The method comparison study was conducted using a subset of samples from the Clinical Concordance study (see section A above) as well as contrived samples in order to cover the measuring range of the assay. Spiking for the preparation of contrived samples was performed by spiking negative HSCT plasma with cultured CMV virus (Merlin Strain).

2. Accountability

There was a total of 1,506 valid paired samples that consisted of 1,367 clinical longitudinal samples from the Clinical Concordance study (Section C, Clinical Concordance in HSCT Patients; above), 109 spiked, and 30 negative samples with at least one valid result on both assays that were included in the method comparison study. From this sample cohort 1,253 clinical samples and 12 contrived samples were valid but

excluded. The 30 CMV immunoglobulin G (IgG)-negative samples were tested to evaluate clinical specificity of the assay but were excluded from the regression analysis of viral load.

Hence, there was a total of 204 paired samples (107 clinical and 97 contrived) that had valid results with both the **cobas** CMV and TaqMan CMV tests (so called "paired results") and had results within the common linear range of both assays.

Table 43: Accountability for HSCT Method Comparison Study

	Total samples	Invalid	Excluded Samples	Valid	Valid but Non-Evaluable**	Evaluable
Clinical	1,392	25	7*	1,360	1,253	107
Contrived	110	1	0	109	12	97
Total	1,502	26	7	1,499	1,295	204

*Samples from 3 subjects of the Clinical Concordance study were sequenced during and were shown to have a CMV variant that contained a mutation in the primer binding site of the TaqMan CMV Test.
Samples had either the **cobas CMV or the TaqMan CMV test result or both out of linear range.

3. Study Population Demographics and Baseline Parameters

Please refer to Section C for the Demographics and Baseline parameters of the clinical HSCT study cohort.

4. Analysis

The Method comparison included the analyses listed below that were provided in the submission. All analysis was provided separately for clinical and contrived samples as well as for both samples pooled:

a. Regression Analysis by Deming
b. Analysis of the Mean Paired Difference
c. Bias
d. Analysis of the Allowable Total Difference
e. Agreement with negative Samples

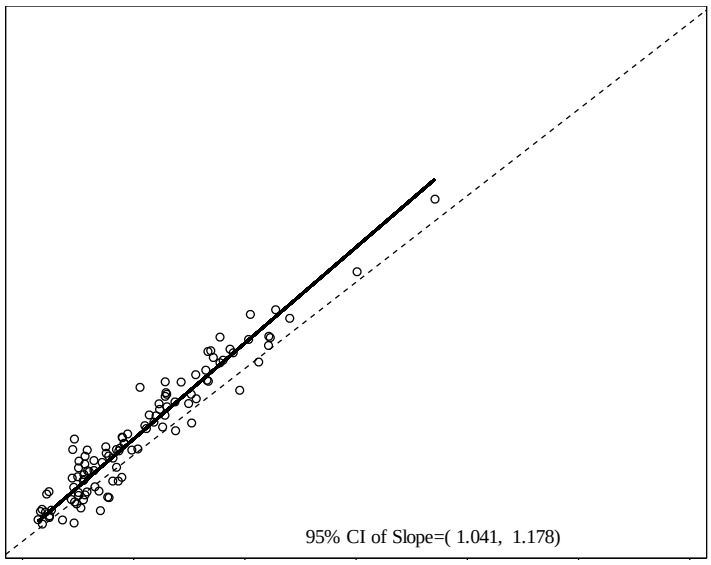
5. Safety and Effectiveness Results

a. Deming Regession Analysis

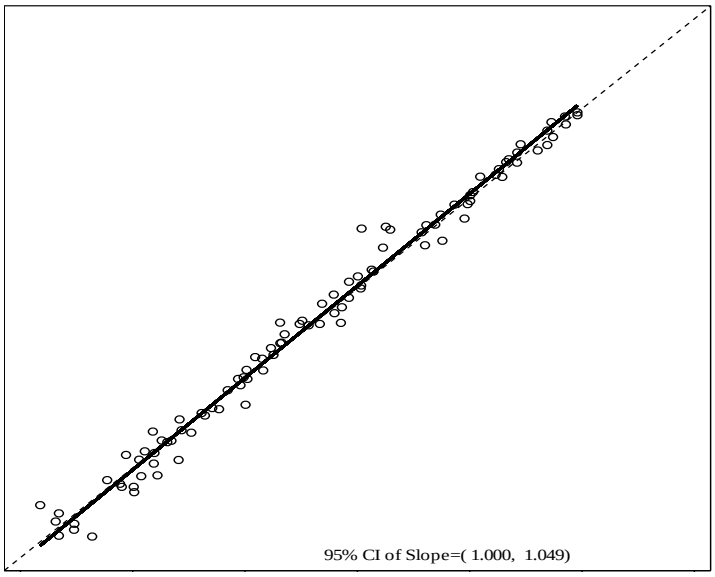
Figure 5 present the Deming regression of the viral load (log_{10} IU/mL) results from the **cobas** CMV and TaqMan CMV tests for all sites combined for the SOT population. Regression is analyzed in Table 44 below.

Figure 5: Deming Linear Regression Plot of Viral Loads (log10 IU/mL) for HSCT Patients (All Clinical Sites)

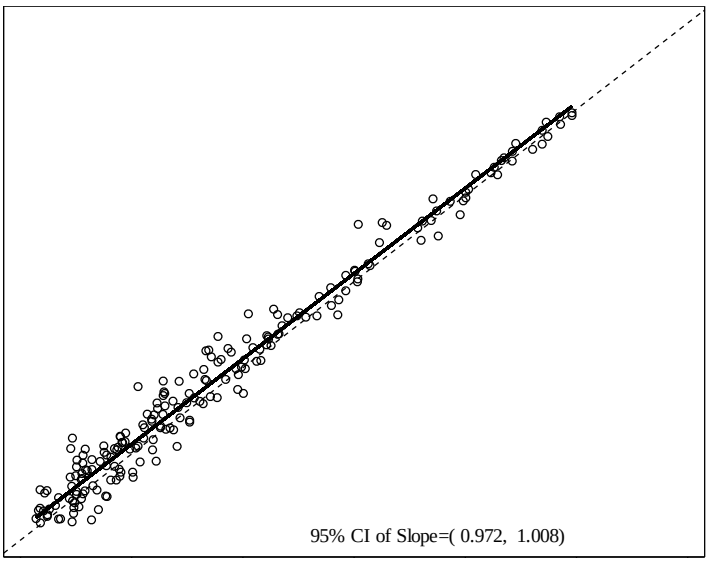
Clinical Samples



Contrived Samples



Clinical + Contrived Samples



CI = confidence interval; r = correlation coefficient

Table 44: Parameter Estimates of Deming Regression Between Viral Loads (log_{10} IU/mL) for HSCT Patients (All Clinical Sites)

	Number of Paired Samples	Parameter	Parameter Estimate [log_{10}]	Standard Error [log_{10}]	95% CI [log_{10}]	r	Parameter Estimate (95% CI[a]) Non-Log Transformed Data
Clinical Samples	**107**	Intercept	-0.146	0.106	(-0.356, 0.064)[a] (-0.462, -0.008)[b]	0.96	-169.93 (-2629, 2288.9)
		Slope	1.110	0.034	(1.041, 1.178)[a] (1.066, 1.217)[b]		1.771 (1.111, 2.430)
Contrived Samples	**97**	Intercept	-0.097	0.063	(-0.223, 0.028)[a]	0.99	23121.611 (-13619, 59862)
		Slope	1.025	0.012	(1.000, 1.049)[a]		1.026 (0.932, 1.119)
Clinical + Contrived Samples	**204**	Intercept	0.145	0.041	(0.064, 0.227)[a] (0.132,0.219)[b]	0.99	13021.279 (-3193, 29236)
		Slope	0.990	0.009	(0.972, 1.008)[a] (0.972, 0.990)[b]		1.028 (0.937, 1.119)

[a] Assumed independence between all samples
[b] Adjusted correlation between samples from same subjects by the bootstrap method with 500 iterations

b. Analysis of the Mean Paired Difference

Table 45 below presents the mean paired difference for HSCT patient samples between **cobas** CMV and the TaqMan CMV Test at representative thresholds and associated 95% CIs calculated using the paired t-test.

Table 45: Mean of Paired Viral Load Differences Between the cobas CMV and the TaqMan CMV Test (log_{10} IU/mL) for HSCT Patients at Representative Decision Intervals (IU/mL)

	Representative Decision Intervals (IU/mL)	N	Mean of Paired Differences (log_{10} IU/mL)	SE for Mean of Paired Differences (log_{10} IU/mL)	95% CI (log_{10} IU/mL)
Clinical Samples	≥ 2.14 to < 3.00	77	0.017	0.024	(0.122, 0.219)
	≥ 3.00 to < 4.00	27	0.241	0.041	(0.157, 0.326)
	≥ 4.00 to < 5.00	1	0.178	-	-
	≥ 5.00	2	0.181	0.070	(-0.705, 1.068)
	Overall	107	0.188	0.021	(0.148, 0.229)
Contrived Samples	≥ 2.14 to < 3.00	21	-0.037	0.043	(-0.127, 0.053)
	≥ 3.00 to < 4.00	22	-0.027	0.025	(-0.079, 0.025)
	≥ 4.00 to < 5.00	15	0.053	0.034	(-0.020, 0.126)
	≥ 5.00	39	0.056	0.025	(0.006, 0.106)
	Overall	97	0.017	0.016	(-0.015, 0.048)
Clinical + Contrived Samples	≥ 2.14 to < 3.00	98	0.126	0.023	(0.080, 0.171)
	≥ 3.00 to < 4.00	49	0.121	0.032	(0.058, 0.184)
	≥ 4.00 to < 5.00	16	0.061	0.033	(-0.009, 0.131)
	≥ 5.00	41	0.062	0.024	(0.013, 0.110)
	Overall	204	0.107	0.014	(0.078, 0.135)

Note: The table only included paired samples with paired results that were each within 137 IU/mL to 9.1E+06 IU/mL, the overlapping linear range of both assays. Paired results within the linear range on both assays were categorized into representative decision intervals based on the TaqMan CMV Test result (IU/mL).
Decision levels :
≥ 2.14 to < 3.00 log_{10} IU/mL = ≥ 1.37E+02 to < 2.0E+03 IU/mL
≥ 3.00 to < 4.00 log_{10} IU/mL = ≥ 2.0E+03 to < 2.0E+04 IU/mL
≥ 4.00 to < 5.00 log_{10} IU/mL = ≥ 2.0E+04 to < 1.0E+05 IU/mL
≥ 5.00 log_{10} IU/mL = ≥ 1.0E+05 IU/mL
CI = confidence interval; N = number of paired samples; SE = standard error

c. Analysis of Bias

Table 46 below presents the mean paired difference between **cobas** CMV and the TaqMan CMV Test at representative thresholds and associated 95% CIs calculated using the paired t-test.

Table 46: Bias/Systematic Difference Between the cobas CMV and the TaqMan CMV Test for HSCT Patients at Selected Viral Load Levels

	Viral Load Level (Per Comparator)	Systematic Difference
Clinical	2.137 $\log_{10}$ IU/ml (1.37E+02 IU/ml)	0.089 $\log_{10}$ IU/ml (3.12E+01 IU/mL)
	2.699 $\log_{10}$ IU/ml (5.00E+02 IU/ml)	0.151 $\log_{10}$ IU/ml (2.08E+02 IU/mL)
	3.255 $\log_{10}$ IU/ml (1.80E+03 IU/ml)	0.212 $\log_{10}$ IU/ml (1.13E+03 IU/mL)
	4.000 $\log_{10}$ IU/ml (1.00E+04 IU/ml)	0.294 $\log_{10}$ IU/ml (9.68E+03 IU/mL)
	7.000 $\log_{10}$ IU/ml (1.00E+07 IU/ml)	0.624 $\log_{10}$ IU/ml (3.21E+07 IU/mL)
Contrived	2.137 $\log_{10}$ IU/ml (1.37E+02 IU/ml)	-0.044 $\log_{10}$ IU/ml (-1.31E+01 IU/mL)
	2.699 $\log_{10}$ IU/ml (5.00E+02 IU/ml)	-0.030 $\log_{10}$ IU/ml (- 3.29E+01 IU/mL)
	3.255 $\log_{10}$ IU/ml (1.80E+03 IU/ml)	-0.016 $\log_{10}$ IU/ml (-6.36E+01 IU/mL)
	4.000 $\log_{10}$ IU/ml (1.00E+04 IU/ml)	0.003 $\log_{10}$ IU/ml (6.93E+01 IU/mL)
	7.000 $\log_{10}$ IU/ml (1.00E+07 IU/ml)	0.078 $\log_{10}$ IU/ml (1.97E+06 IU/mL)
Clinical + Contrived	2.137 $\log_{10}$ IU/ml (1.37E+02 IU/ml)	0.124 $\log_{10}$ IU/ml (4.51E+01 IU/mL)
	2.699 $\log_{10}$ IU/ml (5.00E+02 IU/ml)	0.118 $\log_{10}$ IU/ml (1.56E+02 IU/mL)
	3.255 $\log_{10}$ IU/ml (1.80E+03 IU/ml)	0.113 $\log_{10}$ IU/ml (5.32E+02 IU/mL)
	4.000 $\log_{10}$ IU/ml (1.00E+04 IU/ml)	0.105 $\log_{10}$ IU/ml (2.74E+03 IU/mL)
	7.000 $\log_{10}$ IU/ml (1.00E+07 IU/ml)	0.075 $\log_{10}$ IU/ml (1.89E+06 IU/mL)

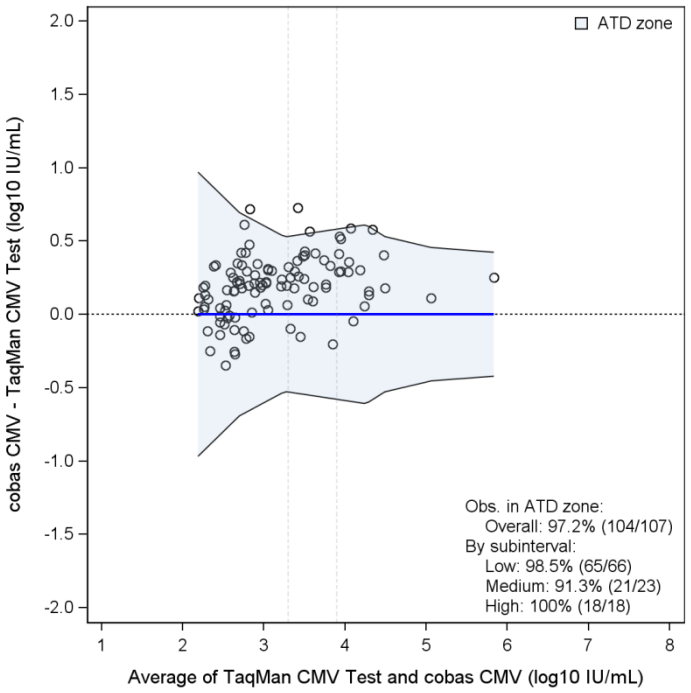
d. Analysis of the Allowable Total Difference (ATD)

The ATD zone was constructed for two measurements of the TaqMan CMV Test based on the reproducibility of the TaqMan CMV Test. In addition, the percentages of the samples at low, medium, and high sub-intervals that fall within the ATD zone were calculated. Viral load sub-intervals were determined using the log10 IU/mL based on the TaqMan CMV Test for each paired sample and are defined as outlined in Table 47 below.

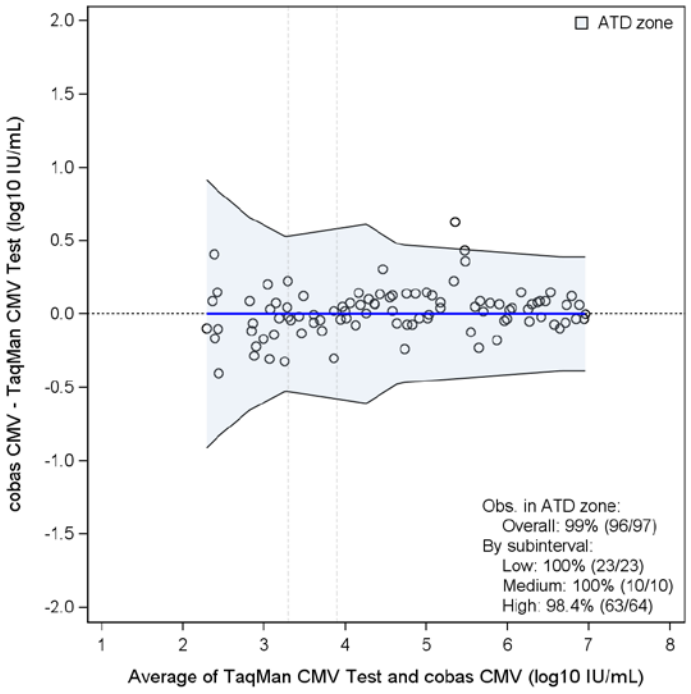
The resulting graphs are shown in Figure 6 below for clinical, contrived and combined (clinical + contrived) data sets. Table 47 demonstrates that 97.2% (104/107) of paired observations fell within the ATD zone for clinical samples.
For contrived samples 99% of the results fall within the ATD zone (96/97).

Figure 6: Allowable Total Difference (ATD) of Viral Load Difference (log_{10} IU/mL) for HSCT Patients (All Clinical Sites Combined)

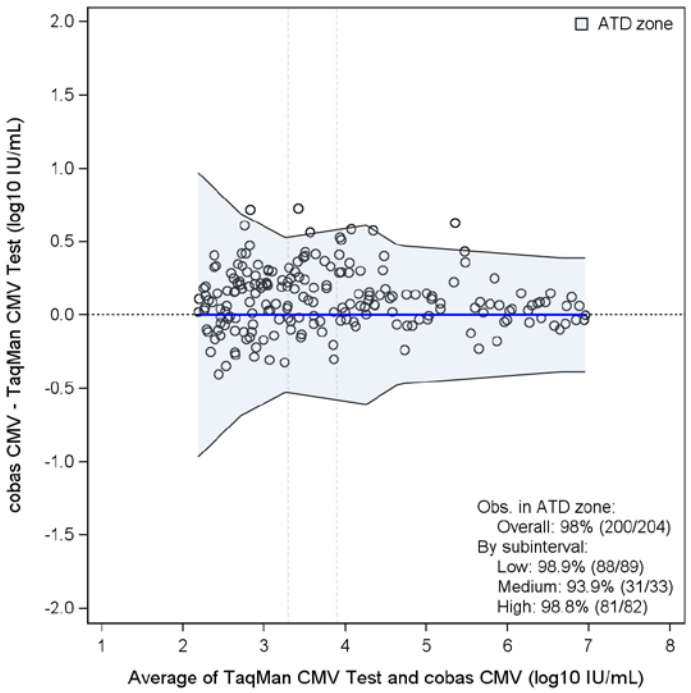
Clinical Samples



Contrived Samples



Clinical + Contrived Samples



CI = confidence interval; r = correlation coefficient

Table 47: Percentage of Samples in the HSCT Population that Fall in Allowable Total Difference (ATD) Zone Intervals (IU/mL)

	Interval Category	Interval Range (IU/mL)	Percentage of Paired Samples Within ATD Zone % (n/N)
Clinical[1]	Low	1.37E+02 to < 2.0E+03	98.5% (65/66)
	Medium	2.0E+03 to < 8.0E+03	91.3% (21/23)
	High	8.0E+03 to 9.10E+06	100.0% (18/18)
	Overall	N/A	97.2% (104/107)
Contrived	Low	1.37E+02 to < 2.0E+03	100.0% (23/23)
	Medium	2.0E+03 to < 8.0E+03	100.0% (10/10)
	High	8.0E+03 to 9.10E+06	98.4% (63/64)
	Overall	N/A	99.0% (96/97)
Clinical[1] + Contrived	Low	1.37E+02 to < 2.0E+03	98.9% (88/89)
	Medium	2.0E+03 to < 8.0E+03	93.9% (31/33)
	High	8.0E+03 to 9.10E+06	98.8% (81/82)
	Overall	N/A	98.0% (200/204)

Note: The table only includes paired samples with paired results that were each within 1.37E+02 IU/mL to 9.1E+06 IU/mL, the overlapping linear range of both assays. Paired results were categorized into viral load intervals based on the TaqMan CMV Test result (IU/mL).
[1] Seven samples from three subjects were excluded from method comparison analyses due to impactful sequence mismatch.
N = total number of paired samples within the appropriate interval; n = number of paired samples included in the ATD Zone within the appropriate interval.

e. Agreement with Negative Samples

Thirty CMV immunoglobulin G (IgG)-negative samples from HSCT donors were tested on both assays to evaluate clinical specificity of the **cobas** CMV test and results are presented in Table 48.

Table 48: Results of CMV IgG-Negative HSCT Patient Specimens

		TaqMan CMV Test (IU/mL)			
		Target Not Detected	**< 1.37E+02**	**≥ 1.37E+02**	**Total**
cobas CMV (IU/mL)	**Target Not Detected**	30	0	0	30
	< 1.37E+02	0	0	0	0
	≥ 1.37E+02	0	0	0	0
	Total	30	0	0	30

Note: The lower limit of quantitation (LLOQ) is 137 IU/mL for TaqMan CMV Test.
IgG = immunoglobulin G

E. Financial Disclosure

The Financial Disclosure by Clinical Investigators regulation (21 CFR 54) requires applicants who submit a marketing application to include certain information concerning the compensation to, and financial interests and arrangement of, any clinical investigator conducting clinical studies covered by the regulation. The pivotal clinical study included 4 investigators. None of the clinical investigators had disclosable financial interests/arrangements as defined in sections 54.2(a), (b), (c), and (f). The information provided does not raise any questions about the reliability of the data.

XI. PANEL MEETING RECOMMENDATION AND FDA'S POST-PANEL ACTION

In accordance with the provisions of section 515(c)(3) of the act as amended by the Safe Medical Devices Act of 1990, this PMA was not referred to the Microbiology Panel, an FDA advisory committee, for review and recommendation because the information in the PMA substantially duplicates information previously reviewed by this panel.

XII. CONCLUSIONS DRAWN FROM PRECLINICAL AND CLINICAL STUDIES

A. Effectiveness Conclusions

The effectiveness of the **cobas** 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 **cobas** CMV test for aiding in the management of solid-organ transplant patients and hematopoetic stem cell 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

The risks of the device are based on nonclinical laboratory studies as well as data collected in clinical studies conducted to support PMA approval as described above. Based on the results of the analytical and clinical laboratory studies, the **cobas** CMV, 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 Determination

The probable benefits of the device are also based on data collected in clinical studies conducted to support PMA approval as described above. When used for the proposed intended use, benefits to both the clinician and patient include confirmation that CMV viral load is responding to treatment as anticipated and an approximation of the time that therapy can be discontinued if appropriate. The device can also be used effectively in aiding the decision to initiate antiviral therapy post transplantation in

the context of all other clinical aspects of the patient and other laboratory measurements.

The pivotal clinical studies were clinical concordance and method comparison studies in SOT patients and similar clinical concordance and method comparison studies in HSCT patients. The quality and the study were both robust as multiple samples were obtained per patient (for SOT this was > 5 samples/patient), and samples at clinically significant decision points were obtained.

Although for both patient populations the majority of patients were of white race and male, there was significant representation of other races and females, and results are generalizable across all race, ethnic, and gender subpopulations.

Additional factors to be considered in determining probable risks and benefits for the cobas CMV device included:

The risk from an inaccurately high result is the misinterpretation that a patient is not responding to treatment. This is mitigated by the known likelihood that most patients respond to treatment, understanding of the time course of response, by serial and/or repeat measurement of CMV viral load and clinical evaluation which would likely show symptomatic improvement.

The consequences of a false-negative measurement may be relatively more substantial. The current 'standard of care' per accepted guidelines is that patient treated with antiviral medication for CMV and monitored via CMV viral load measurements should continue therapy until two serial measurements are undetectable. Hence, measurement of viral load in the setting of very low levels of viremia, i.e., near the LoQ of the assay, may lead either to prolonged therapy (i.e., patient has not 'fully responded' by serial undetectable measurements) or to treatment discontinued early due to an undetectable level. This may be mitigated by local practice; as experience with these assays evolves at local institutions, it is likely that 'low level' positive results in the context of otherwise negative results will be recognized and that therapy would be unlikely to be unnecessarily prolonged.

It is important to recognize that the current standard of care has evolved in the absence of a recognized diagnostic standard for CMV viral load, i.e., either a measurement reference standard or a standardized definition. Overall substantial differences in treatment duration due to the use of different assays are unlikely given the strong analytical validation, and differences in clinical outcome are even less likely. There is no evidence from the studies performed by the sponsor that risks are greater than for any of the previously approved assays.

1. Patient Perspectives

   This submission did not include specific information on patient perspectives for this device.

In conclusion, given the available information above, the data support that for management of SOT and HSCT patients who are undergoing anti-CMV therapy, 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 **cobas** CMV 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 studies and the statistical analysis of clinical data in this application have shown that serial CMV DNA levels measured with the **cobas** CMV are informative for assessing the virological response to treatment in solid organ transplant patients and hematopoetic stem cell 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.

XIII. CDRH DECISION

CDRH issued an approval order on June 1, 2017.

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

XIV. APPROVAL SPECIFICATIONS

Directions for use: See device labeling.

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

Post-approval Requirements and Restrictions: See approval order.