Rx Only

cobas® CMV

Quantitative nucleic acid test for use on the cobas® 6800/8800 Systems

For in vitro diagnostic use

cobas® CMV	P/N: 07001029190
cobas® CMV Control Kit	P/N: 07001037190
cobas® NHP Negative Control Kit	P/N: 07002220190

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Intended 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.

Summary and explanation of the test

Background

Human cytomegalovirus (CMV) is a viral pathogen belonging to the herpes virus family found ubiquitously in communities worldwide.[1,2] In immunocompetent hosts, infections with CMV are often asymptomatic but primary lytic infection can present as an acute mononucleosis-like syndrome. Once acquired, CMV usually persists as a lifelong latent infection that may reactivate intermittently. Peripheral blood mononuclear cells of the myeloid lineage (but not lymphocytes) and endothelial cells appear to be the major sites of CMV infection.[3] CMV remains in a latent stage in monocytes/macrophages in humans.[2] Latently infected individuals may asymptomatically shed the virus in their body fluids (e.g., urine, saliva) and thus infect others. Immunocompromised individuals, including neonates, transplant recipients, and AIDS patients, are at high risk for developing severe primary CMV infections or reactivations of latent CMV that lead to a high rate of morbidity and mortality.[4] Severe manifestations of CMV disease include retinitis, polyradiculopathy, gastroenteritis, hepatitis, encephalitis, esophagitis, enterocolitis, pancreatitis, nephritis, donor organ rejection, pneumonitis, and CMV viral syndrome.[2,5,6]

Our current understanding of the relationship between CMV viremia and CMV disease in transplant patients comes from a variety of studies using different technologies, study populations, and end-points.[7-14] In general; higher viral loads are more closely associated with the risk of development of CMV disease. In patients with HIV/AIDS, CMV DNA levels have been correlated with the risk of CMV disease and overall mortality.[15-18] Current guidelines based on the precision of PCR tests suggest that the changes in serial viral load measurements should be at least 3-fold (0.5 $\log_{10}$) to represent biologically important changes.[10,11]

Historically, laboratory-developed methods of CMV DNA quantification have had a high degree of inter-laboratory and inter-assay variability.[19] The advent of an international standardization has improved comparability of assay results across laboratories, but discrepancies still exist due to commutability issues with the standard.

Rationale for NAT testing

Laboratory methods for diagnosing disseminated infection and active visceral disease for human CMV include isolation of virus by culture from peripheral blood leukocytes (PBL), histology on biopsies, serologic methods, measurement of pp65 antigenemia, and detection of CMV DNA by polymerase chain reaction (PCR).[20] Serology is only of value for determining whether a patient has been previously infected with CMV and is at risk of reactivation. Culture methods have poor predictive value, require greater than 48-hour turnaround time, and have limited use in immunocompromised patients. The pp65 antigenemia assay is labor intensive and requires that blood be processed within 6 hours of collection because of decrease in antigenemia upon storage.[21] The pp65 assay is also difficult to perform on neutropenic patients. Direct detection of CMV DNA by real-time PCR methods potentially offers a wide dynamic range, precision, and high sensitivity.

Explanation of the test

cobas® CMV is a quantitative test that is run on the **cobas®** 6800 System and **cobas®** 8800 System. **cobas®** CMV enables the detection and quantitation of CMV DNA in EDTA plasma of infected transplant patients. The viral load is quantified

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

Principles of the procedure

cobas® CMV is based on 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 software which assigns test results for all tests as either target not detected, CMV DNA detected < LLoQ (lower limit of quantitation), CMV DNA detected > ULoQ (upper limit of quantitation), or a value in the linear range LLoQ < x < ULoQ. Results can be reviewed directly on the system screen, exported, or printed as a report.

Nucleic acid from patient samples and added lambda DNA-QS molecules is simultaneously extracted. In summary, 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 protein, cellular debris and potential PCR inhibitors are removed with subsequent wash reagent steps and purified nucleic acid is eluted from the glass particles with elution buffer at elevated temperature.

Selective amplification of target nucleic acid from the sample is achieved by the use of target virus-specific forward and reverse primers which are selected from highly-conserved regions of the CMV DNA polymerase (UL54) gene. 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. A thermostable DNA polymerase enzyme is used for amplification. The target and DNA-QS sequences are amplified simultaneously utilizing a universal PCR amplification profile with predefined temperature steps and number of cycles. The master mix includes deoxyuridine triphosphate (dUTP), instead of deoxythimidine triphosphate (dTTP), which is incorporated into the newly synthesized DNA (amplicon).[22-24] Any contaminating amplicon from previous PCR runs is eliminated by the AmpErase enzyme, which is included in the PCR mix, when heated in the first thermal cycling step. However, newly formed amplicon are not eliminated since the AmpErase enzyme is inactivated once exposed to temperatures above 55°C.

The **cobas**® CMV master mix contains one detection probe specific for CMV target sequences and one for the DNA-QS. The probes are labeled with target-specific fluorescent reporter dyes allowing simultaneous detection of CMV target and DNA-QS in two different target channels.[25,26] The fluorescent signal of the intact probes is suppressed by the quencher dye. During the PCR amplification step, hybridization of the probe to the specific single-stranded DNA templates results in cleavage by the 5'-to-3' nuclease 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 probes are generated and the cumulative signal of the reporter dye is concomitantly increased. Real-time detection and discrimination of PCR products is accomplished by measuring the fluorescence of the released reporter dyes for the viral targets and DNA-QS.

Reagents and materials

The materials provided for **cobas**® CMV can be found in Table 1. Materials required, but not provided can be found in Table 2 through Table 4, Table 7 and Table 8.

Refer to the **Reagents and materials** section and **Precautions and handling requirements** section for the hazard information for the product.

cobas® CMV reagents and controls

All unopened reagents and controls shall be stored as recommended in Table 1 to Table 4.

Table 1 cobas® CMV

cobas® CMV
Store at 2-8°C
96 test cassette (P/N 07001029190)

Kit components	Reagent ingredients	Quantity per kit 96 tests
Proteinase Solution (PASE)	Tris buffer, < 0.05% EDTA, calcium chloride, calcium acetate, 8% (w/v) proteinase EUH210: Safety data sheet available on request. EUH208: Contains Subtilisin, 9014-01-1. May produce an allergic reaction.	13 mL
DNA Quantitation Standard (**DNA-QS**)	Tris buffer, < 0.05% EDTA, < 0.001% non-CMV DNA construct containing non-CMV primer binding and a unique probe region (non-infectious DNA), < 0.002% Poly rA RNA (synthetic), < 0.1% sodium azide	13 mL
Elution Buffer (EB)	Tris buffer, 0.2% methyl-4 hydroxybenzoate	13 mL
Master Mix Reagent 1 (MMX-R1)	Manganese acetate, potassium hydroxide, < 0.1% sodium azide	5.5 mL
CMV Master Mix Reagent 2 (CMV MMX-R2)	Tricine buffer, potassium acetate, 18% dimethyl sulfoxide, glycerol, < 0.1% Tween 20, EDTA, < 0.12% dATP, dCTP, dGTP, dUTPs, < 0.01% upstream and downstream CMV primers, < 0.01% Quantitation Standard forward and reverse primers, < 0.01% fluorescent-labeled oligonucleotide probes specific for CMV and the CMV Quantitation Standard,< 0.01% oligonucleotide aptamer, < 0.01% ZO5D DNA polymerase (microbial), < 0.1% AmpErase (uracil-N- glycosylase) enzyme (microbial), < 0.1% sodium azide	6 mL

Table 2 **cobas**® CMV Control Kit*

cobas® **CMV Control Kit (CMV CTL)** Store at 2–8°C (P/N 07001037190)			
Kit components	**Reagent ingredients**	**Quantity per kit**	**Safety symbol and warning****
CMV Low Positive Control (CMV L(+)C)	< 0.001% synthetic (plasmid) CMV DNA encapsulated in Lambda bacteriophage coat protein, normal human plasma, non-reactive by licensed tests for antibody to HCV, antibody to HIV-1/2, HBsAg, antibody to HBc; HIV-1 RNA, HIV-2 RNA, HCV RNA, HBV DNA, HEV RNA, WNV RNA and CMV DNA not detectable by PCR methods. 0.1% ProClin® 300 preservative***	4 mL (8 x 0.5 mL)	  **WARNING** H317: May cause an allergic skin reaction. P261: Avoid breathing dust/fume/gas/mist/vapours/spray. P272: Contaminated work clothing should not be allowed out of the workplace. P280: Wear protective gloves. P333 + P313: If skin irritation or rash occurs: Get medical advice/attention. P362 + P364: Take off contaminated clothing and wash it before reuse. P501: Dispose of contents/container to an approved waste disposal plant.
CMV High Positive Control (CMV H(+)C)	< 0.001% high titered synthetic (plasmid) CMV DNA encapsulated in Lambda bacteriophage coat protein, normal human plasma, non-reactive by licensed tests for antibody to HCV, antibody to HIV-1/2, HBsAg, antibody to HBc; HIV-1 RNA, HIV-2 RNA, HCV RNA, HBV DNA, HEV RNA, WNV RNA and CMV DNA not detectable by PCR methods. 0.1% ProClin® 300 preservative***	4 mL (8 x 0.5 mL)	  **WARNING** H317: May cause an allergic skin reaction. P261: Avoid breathing dust/fume/gas/mist/vapours/spray. P272: Contaminated work clothing should not be allowed out of the workplace. P280: Wear protective gloves. P333 + P313: If skin irritation or rash occurs: Get medical advice/attention. P362 + P364: Take off contaminated clothing and wash it before reuse. P501: Dispose of contents/container to an approved waste disposal plant.

* These reagents are not included in the **cobas**® CMV test kit, but are required to perform the test and available separately.

** Product safety labeling primarily follows EU GHS guidance

***Hazardous substance or mixture

Table 3 **cobas**® NHP Negative Control Kit*

cobas® NHP Negative Control Kit
Store at 2-8°C
(P/N 07002220190)

Kit components	Reagent ingredients	Quantity per kit	Safety symbol and warning**
Normal Human Plasma Negative Control (NHP-NC)	Normal human plasma, non-reactive by licensed tests for antibody to HCV, antibody to HIV-1/2, HBsAg, antibody to HBc; HIV-1 RNA, HIV-2 RNA, HCV RNA, HBV DNA, HEV RNA, WNV RNA, and CMV DNA not detectable by PCR methods. < 0.1% ProClin® 300 preservative***	16 mL (16 x 1 mL)	  **WARNING** H317: May cause an allergic skin reaction. P261: Avoid breathing dust/fume/gas/mist/vapours/spray. P272: Contaminated work clothing should not be allowed out of the workplace. P280: Wear protective gloves. P333 + P313: If skin irritation or rash occurs: Get medical advice/attention. P362 + P364: Take off contaminated clothing and wash it before reuse. P501: Dispose of contents/container to an approved waste disposal plant.

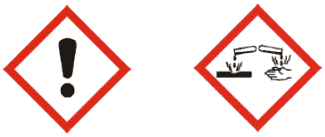
* These reagents are not included in the **cobas**® CMV test kit, but are required to perform the test and available separately.

** Product safety labeling primarily follows EU GHS guidance

***Hazardous substance or mixture

cobas omni reagents for sample preparation

Table 4 cobas omni reagents for sample preparation*

Reagents	Reagent ingredients	Quantity per kit	Safety symbol and warning**
cobas omni MGP Reagent (MGP) Store at 2–8°C (P/N 06997546190)	Magnetic glass particles, Tris buffer, 0.1% methyl-4 hydroxybenzoate, < 0.1% sodium azide	480 tests	Not applicable
cobas omni Specimen Diluent (SPEC DIL) Store at 2–8°C (P/N 06997511190)	Tris buffer, 0.1% methyl-4 hydroxybenzoate, < 0.1% sodium azide	4 x 875 mL	Not applicable
cobas omni Lysis Reagent (LYS) Store at 2–8°C (P/N 06997538190)	42.56% (w/w) guanidine thiocyanate***, 5% (w/v) polydocanol***, 2% (w/v) dithiothreitol, dihydro sodium citrate	4 x 875 mL	 **DANGER** H302 + H332: Harmful if swallowed or if inhaled. H318: Causes serious eye damage. H412: Harmful to aquatic life with long lasting effects. EUH032: Contact with acids liberates very toxic gas. P261: Avoid breathing dust/fume/gas/mist/vapours/spray. P273: Avoid release to the environment. P280: Wear eye protection/face protection. P304 + P340 + P312: IF INHALED: Remove person to fresh air and keep comfortable for breathing. Call a POISON CENTER/doctor if you feel unwell. P305+P351+P338 + P310: IF IN EYES Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Immediately call a POISON CENTER/doctor. P501: Dispose of contents/container to an approved waste disposal plant.
cobas omni Wash Reagent (WASH) Store at 15–30°C (P/N 06997503190)	Sodium citrate dihydrate, 0.1% methyl-4 hydroxybenzoate	4.2L	Not applicable

* These reagents are not included in the **cobas**® CMV test kit, but are required to perform the test and available separately.

** Product safety labeling primarily follows EU GHS guidance

***Hazardous substance or mixture

Reagent storage and handling requirements

Reagents shall be stored and will be handled as specified in Table 5 and Table 6.

When reagents are not loaded on the **cobas**® 6800/8800 Systems, store them at the corresponding temperature specified in Table 5.

Table 5 Reagent storage (when reagent is not on the system)

Reagent	Storage temperature
cobas® CMV – 96	2–8°C
cobas® CMV Control Kit	2–8°C
cobas® NHP Negative Control Kit	2–8°C
cobas omni Lysis Reagent	2–8°C
cobas omni MGP Reagent	2–8°C
cobas omni Specimen Diluent	2–8°C
cobas omni Wash Reagent	15–30°C

Reagents loaded onto the **cobas**® 6800/8800 Systems are stored at appropriate temperatures and their expiration is monitored by the system. The **cobas**® 6800/8800 Systems allow reagents to be used only if all of the conditions shown in Table 6 are met. The system automatically prevents use of expired reagents. Table 6 allows the user to understand the reagent handling conditions enforced by the **cobas**® 6800/8800 Systems.

Table 6 Reagent expiry conditions enforced by the **cobas**® 6800/8800 Systems

Reagent	Kit expiration date	Open-kit stability	Number of runs for which this kit can be used	On-board stability (cumulative time on board outside refrigerator)
cobas® CMV – 96	Date not passed**	30 days from first usage	Max 10 runs	Max 8 hours
cobas® CMV Control Kit	Date not passed**	Not applicable	Not applicable	Max 8 hours
cobas® NHP Negative Control Kit	Date not passed**	Not applicable	Not applicable	Max 10 hours
cobas omni Lysis Reagent	Date not passed**	30 days from loading*	Not applicable	Not applicable
cobas omni MGP Reagent	Date not passed**	30 days from loading*	Not applicable	Not applicable
cobas omni Specimen Diluent	Date not passed**	30 days from loading*	Not applicable	Not applicable
cobas omni Wash Reagent	Date not passed**	30 days from loading*	Not applicable	Not applicable

* Time is measured from the first time that reagent is loaded onto the **cobas**® 6800/8800 Systems.

**Reagents are not expired.

Additional materials required

Table 7 Materials and consumables for use on **cobas**® 6800/8800 Systems

Material	P/N
cobas omni Processing Plate	05534917001
cobas omni Amplification Plate	05534941001
cobas omni Pipette Tips	05534925001
cobas omni Liquid Waste Container	07094388001
Solid Waste Bag	07435967001
Solid Waste Container	07094361001

Instrumentation and software required

The **cobas**® 6800/8800 software and **cobas**® CMV analysis package shall be installed on the instrument(s). The Instrument Gateway (IG) server will be provided with the system.

Table 8 Instrumentation

Equipment	P/N
cobas® 6800 System (Option Moveable)	05524245001 and 06379672001
cobas® 6800 System (Fix)	05524245001 and 06379664001
cobas® 8800 System	05412722001
Sample Supply Module	06301037001

Refer to the **cobas**® 6800/8800 Systems Operator's Manual for additional information for primary and secondary sample tubes accepted on the instruments.

Note: Contact your local Roche representative for a detailed order list for sample racks, racks for clotted tips and rack trays accepted on the instruments.

Precautions and handling requirements

Warnings and precautions

As with any test procedure, good laboratory practice is essential to the proper performance of this assay. Due to the high sensitivity of this test, care should be taken to keep reagents and amplification mixtures free of contamination.

- For *in vitro* diagnostic use only.
- For prescription use only.
- All patient samples should be handled as if infectious, using good laboratory procedures as outlined in Biosafety in Microbiological and Biomedical Laboratories and in the CLSI Document M29-A4.[27,28] Only personnel proficient in handling infectious materials and the use of **cobas**® CMV and **cobas**® 6800/8800 Systems should perform this procedure.
- All human-sourced materials, such as specimens and controls, should be considered potentially infectious and should be handled with universal precautions. If spillage occurs, immediately disinfect with a freshly prepared solution of 0.5% sodium hypochlorite in distilled or deionized water (dilute household bleach 1:10) or follow appropriate site procedures.
- **cobas**® CMV Control Kit and **cobas**® NHP Negative Control Kit contain plasma derived from human blood. The source material has been tested by licensed antibody tests and found non-reactive for the presence of antibody to HCV, antibody to HIV-1/2, HBsAg, and antibody to HBc. Testing of normal human plasma by PCR methods also showed no detectable HIV-1 (Groups M and O) RNA, HIV-2 RNA, HCV RNA, HBV DNA, HEV RNA, WNV RNA, and CMV DNA. No known test method can offer complete assurance that products derived from human blood will not transmit infectious agents.
- **Do not freeze whole blood or any samples stored in primary tubes.**
- Use only supplied or specified required consumables to ensure optimal test performance.
- Safety Data Sheets (SDS) are available upon request from your local Roche representative.
- Closely follow procedures and guidelines provided to ensure that the test is performed correctly. Any deviation from the procedures and guidelines may affect optimal test performance.
- False positive results may occur if carryover of samples is not adequately controlled during sample handling and processing.

Reagent handling

- Handle all reagents, controls, and samples according to good laboratory practice in order to prevent carryover of samples or controls.
- Before use, visually inspect each reagent cassette, diluent, lysis reagent, and wash reagent to ensure that there are no signs of leakage. If there is any evidence of leakage, do not use that material for testing.
- **cobas omni** Lysis Reagent contains guanidine thiocyanate, a potentially hazardous chemical. Avoid contact of reagents with the skin, eyes, or mucous membranes. If contact does occur, immediately wash with generous amounts of water; otherwise, burns can occur.
  cobas® CMV test kits, **cobas omni** MGP Reagent, and **cobas omni** Specimen Diluent contain sodium azide as a preservative. Avoid contact of reagents with the skin, eyes, or mucous membranes. If contact does occur, immediately wash with generous amounts of water; otherwise, burns can occur. If these reagents are spilled, dilute with water before wiping dry. As sodium azide may react with lead and copper plumbing to form explosive metal azides, this reagent should be disposed of by flushing with copious amounts of water.
- Do not allow **cobas omni** Lysis Reagent, which contains guanidine thiocyanate, to contact sodium hypochlorite (bleach) solution or acids. This mixture can produce a highly toxic gas.
- Dispose of all materials that have come in contact with samples and reagents in accordance with country, state, and local regulations.

Good laboratory practice

- Do not pipette by mouth.
- Do not eat, drink, or smoke in designated work areas.
- Wear laboratory gloves, laboratory coats, and eye protection when handling samples and reagents. Gloves must be changed between handling samples and **cobas**® CMV kits and **cobas omni** reagents to prevent contamination. Avoid contaminating gloves when handling samples and controls.
- Wash hands thoroughly after handling samples and kit reagents, and after removing the gloves.
- Thoroughly clean and disinfect all laboratory work surfaces with a freshly prepared solution of 0.5% sodium hypochlorite in distilled or deionized water (dilute household bleach 1:10). Follow by wiping the surface with 70% ethanol.
- If spills occur on the **cobas**® 6800/8800 instrument, follow the instructions in the **cobas**® 6800/8800 Systems Operator's Manual to properly clean and decontaminate the surface of instrument(s).

Sample collection, transport, and storage

Note: Handle all samples and controls as if they are capable of transmitting infectious agents.

Store all samples at specified temperatures.

Sample stability is affected by elevated temperatures.

If using frozen samples in secondary tubes, place the samples at room temperature (15-30ºC) until completely thawed and then briefly mix (e.g. vortex for 3-5 seconds) and centrifuge to collect all sample volume at the bottom of the tube.

Samples

- Blood should be collected in BD Vacutainer® PPT™ Plasma PreparationTubes for Molecular Diagnostic Test Methods or in sterile tubes using EDTA as the anticoagulant. Follow the sample collection tube manufacturer instructions.
- Whole blood collected in BD Vacutainer® PPT™ Plasma PreparationTubes for Molecular Diagnostic Test Methods or in sterile tubes using EDTA as the anticoagulant may be stored and/or transported for up to 36 hours at 2-25°C prior to plasma preparation. Centrifugation should be performed according to manufacturer instructions.
- Plasma samples separated from whole blood within 24 hours of collection may be stored and/or transported for up to 6 days at 2-8°C or up to 12 weeks at -20°C ± 2°C. For long-term storage up to 6 months, temperatures at -75°C ± 15°C are recommended.
- Plasma samples are stable for up to four freeze/thaw cycles when frozen at -20°C ± 2°C.
- If samples are to be shipped, they should be packaged and labeled in compliance with applicable country and/or international regulations covering the transport of samples and etiologic agents.

Instructions for use

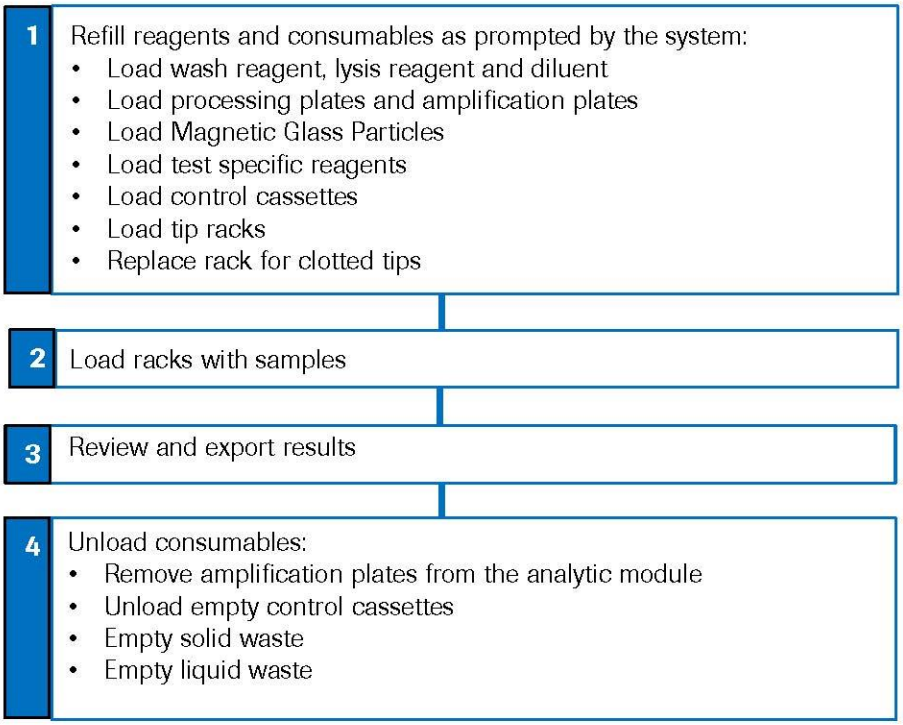
Procedural notes

- Do not use **cobas**® CMV test reagents, **cobas**® CMV Control Kit, **cobas**® NHP Negative Control Kit, or **cobas omni** reagents after their expiry dates.
- Do not reuse consumables. They are for one-time use only.
- Refer to the **cobas**® 6800/8800 Systems Operator's Manual for proper maintenance of instruments.

Running cobas® CMV

cobas® CMV can be run with a minimum sample volume of 500 µL of which 350 µL is processed. The test procedure as described in detail in the **cobas**® 6800/8800 Systems Operator's Manual must be followed. Figure 1 below summarizes the procedure.

Figure 1 **cobas**® CMV test procedure



Results

The **cobas**® 6800/8800 System automatically determines the CMV DNA concentration for the samples and controls. The CMV DNA concentration is expressed in International Units per milliliter (IU/mL).

Quality control and validity of results

- One negative control (-) C and two positive controls, a low positive control CMV L(+)C and a high positive control CMV H(+)C is processed with each batch.
- In the **cobas**® 6800/8800 software and/or report, check for flags and their associated results to ensure the batch validity.
- The batch is valid if no flags appear for all three controls, which includes one negative control and two positive controls: CMV L(+)C, CMV H(+)C. The negative control result is displayed as (-) C and the low and high positive controls are displayed as CMV L(+)C and CMV H(+)C.

Invalidation of results is performed automatically by the **cobas**® 6800/8800 software based on negative and positive control failures.

Control flags

Table 9 Control flags for negative and positive controls

Negative Control	Flag	Result	Interpretation
(-) C	Q02 (Control batch failed)	Invalid	An invalid result or the calculated titer result for the negative control is not negative.
Positive Control	**Flag**	**Result**	**Interpretation**
CMV L(+)C	Q02 (Control batch failed)	Invalid	An invalid result or the calculated titer result for the low positive control is not within the assigned range.
CMV H(+)C	Q02 (Control batch failed)	Invalid	An invalid result or the calculated titer result for the high positive control is not within the assigned range.

If the batch is invalid, repeat testing of the entire batch including samples and controls.

Interpretation of results

For a valid batch, check each individual sample for flags in the **cobas**® 6800/8800 software and/or report. The result interpretation should be as follows:

- A valid batch may include both valid and invalid sample results.

Table 10 Target results for individual target 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 concentrations 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.

Procedural limitations

- **cobas**® CMV has been evaluated only for use in combination with the **cobas**® CMV Control Kit, **cobas**® NHP Negative Control Kit, **cobas omni** MGP Reagent, **cobas omni** Lysis Reagent, **cobas omni** Specimen Diluent, and **cobas omni** Wash Reagent for use on the **cobas**® 6800/8800 Systems.
- **When adopting a new CMV assay for clinical use, laboratories should compare the performance of the new CMV assay to the previously used assay to assess any potentially clinical significant differences in the absolute value of CMV viral load reported.**
- Reliable results depend on proper sample collection, storage and handling procedures.
- This test has been validated only for use with EDTA plasma. Testing of other sample types with **cobas**® CMV may result in inaccurate results. Plasma viral load measurements are not directly comparable to those of other sample types.
- Quantitation of CMV DNA may be affected by sample collection methods, patient factors (i.e., age, presence of symptoms), and/or stage of infection.
- Results should be interpreted by qualified healthcare professionals in conjunction with clinical signs and symptoms and all other laboratory findings.
- Mutations within the highly-conserved regions of the CMV DNA polymerase (UL54) gene covered by **cobas**® CMV may affect primers and/or probe binding resulting in the under-quantitation of virus or failure to detect the presence of virus. The cobas CMV mitigates this risk through the use of redundant amplification primers.
- Negative test results do not preclude CMV infection or tissue-invasive CMV disease, and test results should therefore not be the sole basis for patient management decisions.
- Due to potential variability from measurements with different CMV assays, it is recommended that the same device (or assay) be used for the measurement of CMV viral load when managing CMV infection in individual patients.
- **cobas**® CMV is not intended for use as a screening test for the presence of CMV in blood or blood products and has not been evaluated as a diagnostic test to confirm the presence of CMV infection.
- Clinicians should take individual patient risk factors as well as current clinical guidelines into account when using CMV viral load results for the management of transplant patients.

Non-clinical performance evaluation

Key performance characteristics

Limit of detection (LoD)

The limit of detection (LoD) of **cobas**® CMV was determined by analysis of serial dilutions of the WHO International Standard (Merlin strain, glycoprotein B genotype 1) and verified for Glycoprotein B genotypes gB-2, gB-3 through gB-4 as well as for drug resistant CMV specimens. The overall concentration level with a hit rate of ≥ 95% is 34.5 IU/mL for EDTA plasma.

WHO International Standard

The limit of detection of **cobas**® CMV for the WHO International Standard was determined by analysis of serial dilutions of the 1st WHO International Standard for Human Cytomegalovirus DNA for Nucleic Acid Amplification Technology Assays (1st HCMV WHO International Standard) obtained from NIBSC, in CMV-negative human EDTA plasma. Panels of eight concentration levels plus a blank were tested over three lots of **cobas**® CMV test reagents, multiple runs, days, operators, and instruments.

The results for EDTA plasma are shown in Table 11 through Table 13. The study demonstrates that with the least sensitive lot, the concentration for which 95% hit rate is expected by PROBIT is 30.7 IU/mL with a 95% confidence range of 24.5-40.9 IU/mL in EDTA plasma. The lowest concentration level with a hit rate ≥ 95% is 34.5 IU/mL in EDTA plasma.

Table 11 CMV DNA WHO International Standard Limit of Detection in EDTA plasma, Lot 1

Input titer concentration (CMV DNA IU/mL)	Number of valid replicates (N)	Number of positives (n)	Hit rate (n/N) x100
92.0	63	63	100.0%
46.0	63	63	100.0%
34.5	62	62	100.0%
23.0	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%
0.0	63	0	0.0%
LoD by PROBIT at 95% hit rate	14.7 IU/mL 95% confidence range: 11.7 – 20.0 IU/mL		

Table 12 CMV DNA WHO International Standard limit of detection in EDTA plasma, Lot 2

Input titer concentration (CMV DNA IU/mL)	Number of valid replicates (N)	Number of positives (n)	Hit rate (n/N) x 100
92.0	63	63	100.0%
46.0	63	62	98.4%
34.5	63	62	98.4%
23.0	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%
0.0	63	0	0.0%
LoD by PROBIT at 95% hit rate	30.7 IU/mL 95% confidence range: 24.5 – 40.9 IU/mL		

Table 13 CMV DNA WHO International Standard limit of detection in EDTA plasma, Lot 3

Input titer concentration (CMV DNA IU/mL)	Number of valid replicates (N)	Number of positives (n)	Hit rate (n/N) x 100
92.0	63	63	100.0%
46.0	63	63	100.0%
34.5	63	63	100.0%
23.0	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%
0.0	63	0	0.0%
LoD by PROBIT at 95% hit rate	14.8 IU/mL 95% confidence range: 11.6 – 19.9 IU/mL		

Glycoprotein B genotypes gB-2, gB-3 and gB-4

CMV cell culture supernatants for three different Glycoprotein B genotypes (gB-2, gB-3 and gB-4) 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.

The combined results from three lots shown in Table 14 verify that – consistent with an LoD of 34.5 IU/mL – **cobas**® CMV detected CMV DNA for genotype gB-2 at a concentration of 17.25 IU/mL and for gB-3 and gB-4 at a

concentration of 34.5 IU/mL with a 95% hit rate. The achieved hit rates at 34.5 IU/mL verify the LoD for each of the three genotypes.

Table 14 CMV DNA genotypes gB-2 through gB-4 verification of limit of detection in EDTA plasma

Test concentration	17.25 IU/mL			34.5 IU/mL			51.75 IU/mL		
Genotype	Number of valid replicates (N)	Number of positives (n)	Hit rate (n/N)x100	Number of valid replicates (N)	Number of positives (n)	Hit rate (n/N)x100	Number of valid replicates (N)	Number of positives (n)	Hit rate (n/N)x100
gB-2	63	61	96.8%	63	63	100.0%	63	63	100.0%
gB-3	63	57	90.5%	63	63	100.0%	63	63	100.0%
gB-4	63	55	87.3%	63	63	100.0%	63	63	100.0%

Drug resistant CMV specimens (resistant against foscarnet or ganciclovir, valganciclovir and cidofovir)

Cell culture supernatants for two different drug resistant CMV specimens (one resistant against foscarnet and one resistant against ganciclovir, valganciclovir and cidofovir) 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.

The combined results from three lots shown in Table 15 verify that – consistent with an LoD of 34.5 IU/mL – **cobas**® CMV detected CMV DNA for two different drug resistant specimens at a concentration of 34.5 IU/mL with a 95% hit rate. The achieved hit rates at 34.5 IU/mL verify the LoD for both of the tested drug resistant CMV specimens.

Table 15 Drug resistant CMV specimens verification of limit of detection in EDTA plasma

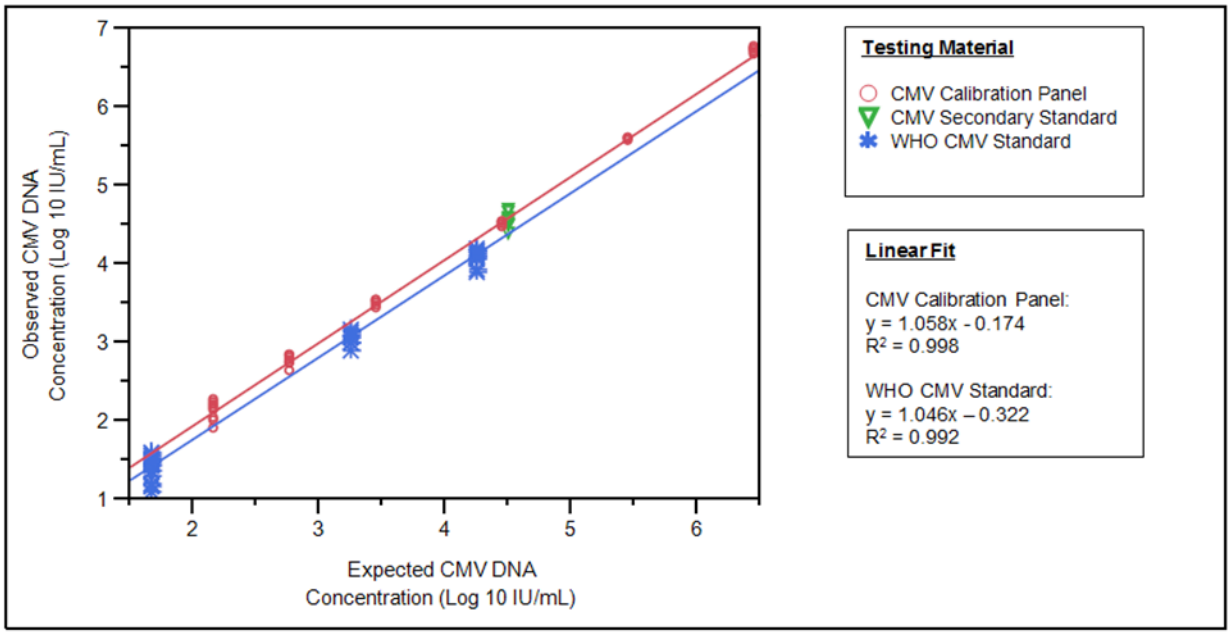
Test concentration		17.25 IU/mL			34.5 IU/mL			51.75 IU/mL		
Drug resistance	Mutation site in UL54	Number of valid replicates (N)	Number of positives (n)	Hit rate (n/N)x100	Number of valid replicates (N)	Number of positives (n)	Hit rate (n/N)x100	Number of valid replicates (N)	Number of positives (n)	Hit rate (n/N)x100
Foscarnet	E756Q	63	58	92.1%	63	63	100.0%	63	63	100.0%
Ganciclovir, Valganciclovir, Cidofovir	L545S	63	59	93.7%	63	63	100.0%	63	63	100.0%

Traceability to the 1st WHO International Standard for human Cytomegalovirus for Nucleic Acid Amplification Techniques (NAT)-based assays

Several standards and controls have been used during development of this test to provide traceability to the WHO standard [the 1st WHO International Standard for human Cytomegalovirus DNA for Nucleic Acid Amplification Techniques (NIBSC 09/162)[29]]. The standards used during development of the test include the HCMV WHO Standard, the RMS CMV Secondary Standard, and the RMS CMV Calibration Panel. The Standards and the Calibration Panel were tested. 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).

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 (Figure 2). The maximum deviation was obtained around the test LLoQ.

Figure 2 Traceability to WHO International Standard [bivariate fit of observed CMV DNA concentration (log_{10} IU/mL) by expected CMV DNA concentration (log_{10} IU/mL)] using **cobas**® CMV



Linear range

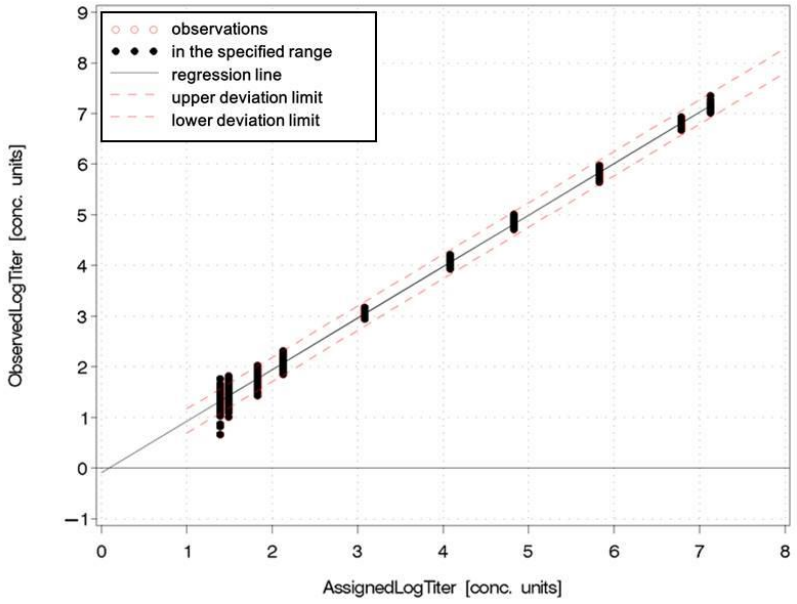
Linearity of the **cobas**® CMV was evaluated using a dilution series consisting of 10 panel members with CMV genotype gB-1 DNA concentrations spanning the assay linear range (2.45E+01 IU/mL to 1.34E+07 IU/mL). Each panel member was tested in 48 replicates across three lots of **cobas**® CMV test reagents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

cobas® CMV was demonstrated to be linear from 3.45E+01 IU/mL to 1.00E+07 IU/mL and shows an absolute deviation from the better fitting non-linear regression of less than ± 0.2 log_{10}. Across the linear range, the accuracy of the test was within ± 0.24 log_{10}.

The lower limit of quantitation (LLoQ) is 34.5 IU/mL, calculated based on a goal for acceptable total analytical error (TAE) of ≤ 1.0 log_{10}, where TAE = |bias| + 2 standard deviations in alignment with the CLSI EP-17A guideline, and TAE = SQUARE ROOT(2) x 2 standard deviations based on the "difference between 2 measurements" approach.

Based on the LLoQ and the determined linear range, as well as the medical value the linear measurement range of the test was set to 34.5-1.0E+07 IU/mL. The results of calculation and claimed LLoQ are shown in Table 18.

Figure 3 Linearity in EDTA plasma using CMV Merlin Virus as representative for Glycoprotein B (gB) Genotype 1



Linearity for Glycoprotein B genotypes gB-2, gB-3 and gB-4

The dilution series used in the verification of CMV Glycoprotein B genotypes linearity study of **cobas**® CMV consists of seven panel members spanning the intended linear range. Sixteen replicates were tested across two lots of **cobas**® CMV reagent for each level in EDTA plasma. The results of the study are presented in Table 16.

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 ± 0.2 $\log_{10}$.

Table 16 Linearity verification on gB-2, gB-3 and gB-4 Genotypes

CMV gB Genotype	Linear regression	Better fitting higher order model regression	Maximum difference between linear regression and the better fitting higher order model ($\log_{10}$ IU/mL)
2	y = 1.0225x - 0.0566	$y = -0.0091x^2 + 1.0931x - 0.1639$	-0.05
3	y = 1.0221x - 0.0705	N/A*	N/A*
4	y = 1.0361x - 0.1099	$y = -0.0196x^2 + 1.1877 - 0.3390$	-0.11

*The linear regression is the best fitting model.

Linearity for CMV drug resistant specimens (resistant against foscarnet or ganciclovir, valganciclovir and cidofovir)

The dilution series used in the linearity study for verification of CMV drug resistant specimens of **cobas®** CMV consists of seven panel members spanning the intended linear range. Sixteen replicates were tested across two lots of **cobas®** CMV reagent for each level in EDTA plasma. The results of the study are presented in Table 17.

The linearity within the linear range of **cobas®** CMV was verified for two CMV drug resistant specimens (resistant against foscarnet or ganciclovir, valganciclovir and cidofovir). The maximum deviation between the linear regression and the better fitting non-linear regression was equal to or less than ± 0.2 $\log_{10}$.

Table 17 Linearity verification on CMV drug resistant specimens

CMV drug resistant specimens	Linear regression	Better fitting higher order model regression	Maximum difference between linear regression and the better fitting higher order model ($\log_{10}$ IU/mL)
Foscarnet	y = 1.0285x - 0.1110	$y = -0.0165x^2 + 1.1570x - 0.3073$	-0.09
Ganciclovir, Valganciclovir, Cidofovir	y = 1.0182x - 0.0259	N/A*	N/A*

*The linear regression is the best fitting model.

Lower limit of quantitation

The analysis for LLOQ was performed with data obtained from the LoD study at concentration levels of 34.5 IU/mL, 46.0 IU/mL and 92.0 IU/mL. The LLoQ is the lowest titer within the linear range that is not lower than the LoD and meets the acceptance criterion for the Total Analytical Error (|Bias| + 2x SD) (TAE). The TAE criterion is ≤ 1$\log_{10}$.

The results of calculation and claimed LLoQ are shown in Table 18; the lower limit of quantitation (LLoQ) is 34.5 IU/mL.

Table 18 Lower Limit of Quantitation (LLoQ) of cobas® CMV using the WHO International Standard for Human Cytomegalovirus (HCMV) (NIBSC 09/162)

Lot	Nominal concentration (IU/mL)	$\log_{10}$ titer nominal	Mean $\log_{10}$ titer observed	SD ($\log_{10}$)	Absolute Bias	TAE (|Bias| + 2x SD)	Difference between Measurements in SD (= SQRT(2) x 2x SD)
1	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	0.20	0.53	0.46
2	34.5	1.54	1.42	0.19	0.11	0.50	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	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	1.80	0.18	0.16	0.52	0.51
3 lots combined	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	1.80	0.17	0.16	0.50	0.47

Precision – within laboratory

Precision of **cobas**® CMV was determined by analysis of serial dilutions of high titer cultured Virus (Merlin, 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 procedure on a fully automated **cobas**® 6800/8800 Systems. Therefore, the precision reported here represents all aspects of the test procedure. The results of the within-laboratory precision are shown in Table 19. The results of the variance component estimation are shown on the Table 20.

cobas® CMV showed high precision for three lots of reagents tested across a concentration range of 2.45E+01 IU/mL to 1.34+07 IU/mL.

Table 19 Within-laboratory precision of **cobas**® CMV*

Nominal concentration (IU/mL)	Assigned concentration (IU/mL)	Lot 1	Lot 2	Lot 3	All lots
		SD	SD	SD	Pooled SD
2.00E+07	1.34E+07	0.03	0.06	0.02	0.04
9.11E+06	6.11E+06	0.04	0.04	0.03	0.04
1.00E+06	6.71E+05	0.05	0.03	0.06	0.05
1.00E+05	6.71E+04	0.06	0.05	0.03	0.05
1.80E+04	1.21E+04	0.06	0.04	0.05	0.05
1.80E+03	1.21E+03	0.04	0.03	0.04	0.04
2.00E+02	1.34E+02	0.13	0.10	0.11	0.12
1.00E+02	6.71E+01	0.14	0.11	0.09	0.12
4.60E+01	3.09E+01	0.20	0.23	0.17	0.20
3.65E+01	2.45E+01	0.22	0.20	0.23	0.22

* Titer data are considered to be log-normally distributed and are analyzed following $\log_{10}$ transformation. Standard deviations (SD) columns present the total of the log-transformed titer for each of the three reagent lots.

Table 20 Lognormal Percent Coefficient of Variation (%CV) of **cobas**® CMV by positive panel and contributing components of variance

Nominal concentration (IU/mL)		Assigned concentration (IU/mL)			Instrument	Lot	Day	Run / Operator	Within Run	Total
Titer (IU/mL)	$\log_{10}$ titer (IU/mL)	Titer (IU/mL)	$\log_{10}$ titer (IU/mL)	N	%CV	%CV	%CV	%CV	%CV	%CV
2.00E+07	7.30	1.34E+07	7.13	48	0%	15%	4%	0%	8%	18%
9.11E+06	6.96	6.11E+06	6.79	48	0%	15%	7%	0%	7%	19%
1.00E+06	6.00	6.71E+05	5.83	48	0%	19%	0%	5%	9%	22%
1.00E+05	5.00	6.71E+04	4.83	48	0%	23%	2%	0%	11%	26%
1.80E+04	4.26	1.21E+04	4.08	48	8%	14%	5%	2%	8%	19%
1.80E+03	3.26	1.21E+03	3.08	48	0%	13%	0%	0%	9%	15%
2.00E+02	2.30	1.34E+02	2.13	48	0%	7%	0%	0%	29%	30%
1.00E+02	2.00	6.71E+01	1.83	48	0%	0%	21%	0%	28%	35%
4.60E+01	1.66	3.09E+01	1.49	48	0%	19%	23%	0%	52%	62%
3.65E+01	1.56	2.45E+01	1.39	47	10%	7%	28%	0%	63%	72%

* Titer data are considered to be log-normally distributed and the %CV values are analyzed as Lognormal CV(%) = sqrt(10^[SD^2 * ln(10)] - 1) * 100%

Analytical specificity

The analytical specificity of **cobas**® CMV was evaluated by testing a panel of microorganisms at a concentration of 1.00E+06 particles, copies, IU, genome equivalents or CFU/mL. Microorganisms were diluted into CMV DNA negative human EDTA plasma as well as human EDTA plasma containing (230 IU/mL) CMV DNA (Table 21). 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 $\log_{10}$ titer of each of the positive CMV samples

containing potentially cross-reacting organisms was within ± 0.5 $\log_{10}$ of the mean $\log_{10}$ titer of the respective positive spike control.

Table 21 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 pneumoniae*	
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 pyogenes*	
Human Papillomavirus	*Mycoplasma pneumoniae*	
JC virus	*Salmonella typhimurium*	
Parvovirus B19	*Streptococcus pneumoniae*	
Varicella-Zoster Virus		

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) in samples were tested in the presence (230 IU/mL) and absence of CMV DNA. The tested endogenous interferences were shown not to interfere with the test performance of **cobas**® CMV.

Moreover, the presence of autoimmune diseases such as systemic lupus erythematosus (SLE), rheumatoid arthritis (RA) and antinuclear antibody were tested.

In addition, drug compounds listed in Table 22 were tested at three times the C_{max} in presence (230 IU/mL) and absence of CMV DNA.

All potentially interfering substances have been 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 on all of the samples with CMV target. Furthermore, the mean $\log_{10}$ titer of each of the positive CMV samples containing potentially interfering substances was within ± 0.5 $\log_{10}$ of the mean $\log_{10}$ titer of the respective positive spike control.

Table 22 Drug compounds tested for interference with the quantitation of CMV DNA by **cobas**® CMV

Class of drug	Generic drug name	
Antimicrobial	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

Cross contamination

The cross-contamination rate for **cobas**® CMV 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 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%).

Clinical performance

Clinical reproducibility

The reproducibility of the **cobas**® CMV was evaluated in EDTA plasma on the **cobas**® 6800 System. Reproducibility and lot-to-lot variability testing was performed at 3 sites, 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 23 through Table 25 below.

Table 23 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 23 Attributable percentage of total variance, standard deviation, total precision standard deviation, and lognormal CV(%) of CMV DNA concentration (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%
CMV = cytomegalovirus; CV(%) = percent coefficient of variation; SD = standard deviation; sqrt = square root; CV(%) = percent coefficient of variation.

Table 24 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 24 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; CMV = cytomegalovirus; No. = number; sqrt = square root.

Table 25 below presents the reproducibility results for the negative panel member for the **cobas**® 6800 System.

Table 25 Reproducibility results for 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.

Clinical performance evaluation: solid organ transplant (SOT) population

This study was designed to evaluate the clinical concordance between **cobas**® CMV and the COBAS® AmpliPrep/COBAS® TaqMan® CMV Test in a solid organ transplant population. Residual frozen EDTA plasma samples prospectively collected from kidney transplant recipients participating in a phase 2a double-blinded randomized placebo-controlled trial of an anti-CMV prophylaxis regimen were tested. The assay target regions were sequenced for samples with an offset of > 0.5 log_{10} IU/mL between the two assays, as well as a representative set of samples without a measurement offset. Sequences associated with a mean offset > 0.9 log_{10} IU/mL were defined as "impactful." Only impactful sequences affecting the targets for the COBAS® AmpliPrep/COBAS® TaqMan® CMV Test were identified.

The demographic characteristics of the patient population are presented in Table 26.

Table 26 Demographics and baseline clinical characteristics of SOT subjects

Characteristics	Statistic
Total, N	107
Age (years)	
Mean ± SD	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%)

Note: Unknown category indicates subjects for whom the corresponding information is not available or not reported.

CMV = cytomegalovirus, SD = standard deviation.

Clinical concordance in the solid organ transplant (SOT) population

Agreement at baseline

Table 27 through Table 30 below show results of the concordance analysis, between **cobas**® CMV and TaqMan® CMV using thresholds: TND, < 1.37E+02 /≥ 1.37E+02 IU/mL , <5.00E+02 /≥ 5.00E+02 IU/mL and < 1.8E+03 /≥ 1.8E+03IU/mL, respectively from evaluable samples collected on the day of or immediately prior to treatment initiation.

Table 27 Concordance analysis of **cobas®** CMV and TaqMan® CMV Test results using threshold target not detected (paired samples at baseline anti-CMV therapy initiation) in the SOT population

Baseline	**TaqMan® CMV Test**			
cobas® CMV	**Target Not Detected**	**Detected**	**Total**	**Row Agreement (95% Exact CI)[a]**
Target Not Detected	9	0	9	100.0% (66.4%, 100.0%)
Detected	2	60	62	96.8% (88.8%, 99.6%)
Total	11	60	71	
Column Agreement (95% Exact CI)[a]	81.8% (48.2%, 97.7%)	100.0% (94.0%, 100.0%)		
Overall Percent Agreement (95% Exact CI)[a]	97.2% (90.2%, 99.7%)			
p-value[b]	0.5000			

Note: Only paired samples evaluable for clinical concordance analysis at Baseline were included in this table.
a Assumed independence between all samples.
b Calculated using McNemar's Test.
1 IU/mL = 1.1 copy/mL.

Table 28 Concordance analysis of **cobas®** CMV and TaqMan® CMV Test results using threshold 1.37E+02 IU/mL (paired samples at baseline anti-CMV therapy initiation) in the SOT population

Baseline	**TaqMan® CMV Test**			
cobas® CMV	**< 1.37E+02 IU/mL (< 2.137 $\log_{10}$ IU/mL)**	**≥ 1.37E+02 IU/mL (≥ 2.137 $\log_{10}$ IU/mL)**	**Total**	**Row Agreement (95% Exact CI)[a]**
< 1.37E+02 IU/mL (< 2.137 $\log_{10}$ IU/mL)	24	1	25	96.0% (79.6%, 99.9%)
≥1.37E+02 IU/mL (≥ 2.137 $\log_{10}$ IU/mL)	5*	41	46	89.1% (76.4%, 96.4%)
Total	29	42	71	
Column Agreement (95% Exact CI)[a]	82.8% (64.2%, 94.2%)	97.6% (87.4%, 99.9%)		
Overall Percent Agreement (95% Exact CI)[a]	91.5% (82.5%, 96.8%)			
p-value[b]	0.2188			

Note: Only paired samples evaluable for clinical concordance analysis at Baseline were included in this table.
Sample with a "Target Not Detected" or a detectable viral load below 1.37E+02 IU/mL result was categorized as "< 1.37E+02 IU/mL (< 2.137 $\log_{10}$ IU/mL)".
*Among the 5 subjects with discordant samples, 2 subjects were found to have impactful sequence mismatch.
a Assumed independence between all samples.
b Calculated using McNemar's Test.
1 IU/mL = 1.1 copy/mL.

Table 29 Concordance analysis of **cobas®** CMV and TaqMan® CMV test results using threshold 5.00E+02 IU/mL (paired samples at baseline anti-CMV therapy initiation) in the SOT population

Baseline	**TaqMan® CMV Test**			
cobas® CMV	**< 5.00E+02 IU/mL (< 2.699 log_{10} IU/mL)**	**≥ 5.00E+02 IU/mL (≥ 2.699 log_{10} IU/mL)**	**Total**	**Row Agreement (95% Exact CI)[a]**
< 5.00E+02 IU/mL (< 2.699 log_{10} IU/mL)	33	2	35	94.3% (80.8%, 99.3%)
≥ 5.00E+02 IU/mL (≥ 2.699 log_{10} IU/mL)	7*	29	36	80.6% (64.0%, 91.8%)
Total	40	31	71	
Column Agreement (95% Exact CI)[a]	82.5% (67.2%, 92.7%)	93.5% (78.6%, 99.2%)		
Overall Percent Agreement (95% Exact CI)[a]	87.3% (77.3%, 94.0%)			
p-value[b]	0.1797			

Note: Only paired samples evaluable for clinical concordance analysis at Baseline were included in this table. Sample with a "Target Not Detected" or a detectable viral load below 5.00E+02 IU/mL result was categorized as "< 5.00E+02 IU/mL (< 2.699 log_{10} IU/mL)".

*Among the 7 subjects with discordant samples, 3 subjects were found to have impactful sequence mismatch.

a Assumed independence between all samples.

b Calculated using McNemar's Test.

1 IU/mL = 1.1 copy/mL.

Table 30 Concordance analysis of **cobas®** CMV and TaqMan® CMV test results using threshold 1.8E+03 IU/mL (paired samples at baseline anti-CMV therapy initiation) in the SOT population

Baseline	**TaqMan® CMV Test**			
cobas® CMV	**< 1.8E+03 IU/mL (< 3.255 log_{10} IU/mL)**	**≥ 1.8E+03 IU/mL (≥ 3.255 log_{10} IU/mL)**	**Total**	**Row Agreement (95% Exact CI)[a]**
< 1.8E+03 IU/mL (< 3.255 log_{10} IU/mL)	48	0	48	100.0% (92.6%, 100.0%)
≥ 1.8E+03 IU/mL (≥ 3.255 log_{10} IU/mL)	4*	19	23	82.6% (61.2%, 95.0%)
Total	52	19	71	
Column Agreement (95% Exact CI)[a]	92.3% (81.5%, 97.9%)	100.0% (82.4%, 100.0%)		
Overall Percent Agreement (95% Exact CI)[a]	94.4% (86.2%, 98.4%)			
p-value[b]	0.1250			

Note: Only paired samples evaluable for clinical concordance analysis at Baseline were included in this table. Sample with a "Target Not Detected" or a detectable viral load below 1.8E+03 IU/mL result was categorized as "< 1.8E+03 IU/mL (< 3.255 log_{10} IU/mL)".

* Among the 4 subjects with discordant samples, 1 subject was found to have impactful sequence mismatch.

a Assumed independence between all samples.

b Calculated using McNemar's Test.

1 IU/mL = 1.1 copy/mL.

Resolution analysis per day

Table 31 presents a concordance analysis of CMV episode resolution for SOT subjects at Day 14, Day 21, Day 28, Day 35, and Day 49 post anti-CMV therapy initiation.

Table 31 Concordance analysis of CMV episode resolution for subjects who initiated anti-CMV therapy in the SOT population

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

Note: Among the subjects included in Day 14 table, 2 subjects were found to have impactful sequence mismatch

Time point Post Anti-CMV Therapy Initiation		TaqMan® CMV Test			
	cobas® CMV	**Resolution of CMV Episode[a]**	**No Resolution of CMV Episode**	**Total**	**Row Agreement (95% Exact CI)**
Day 21	Resolution of CMV Episode[a]	0	0	0	NC
	No Resolution of CMV Episode	1	50	51	98.0% (89.6%, 100.0%)
	Total	1	50	51	
	Column Agreement (95% Exact CI)	0.0% (0.0%, 97.5%)	100.0% (92.9%, 100.0%)		
	Overall Percent Agreement (95% Exact CI)	98.0% (89.6%, 100.0%)			
	p-value[b]	NC			

Note: Among the subjects included in Day 21 table 2 subjects were found to have impactful sequence mismatch.

Time point Post Anti-CMV Therapy Initiation		TaqMan® CMV Test			
	cobas® CMV	**Resolution of CMV Episode[a]**	**No Resolution of CMV Episode**	**Total**	**Row Agreement (95% Exact CI)**
Day 28	Resolution of CMV Episode[a]	6	0	6	100.0% (54.1%, 100.0%)
	No Resolution of CMV Episode	4	46	50	92.0% (80.8%, 97.8%)
	Total	10	46	56	
	Column Agreement (95% Exact CI)	60.0% (26.2%, 87.8%)	100.0% (92.3%, 100.0%)		
	Overall Percent Agreement (95% Exact CI)	92.9% (82.7%, 98.0%)			
	p-value[b]	0.1250			

Note: Among the subjects included in Day 28 table, 3 subjects were found to have impactful sequence mismatch.

Time point Post Anti-CMV Therapy Initiation		TaqMan® CMV Test			
	cobas® CMV	**Resolution of CMV Episode[a]**	**No Resolution of CMV Episode**	**Total**	**Row Agreement (95% Exact CI)**
Day 35	Resolution of CMV Episode[a]	16	1	17	94.1% (71.3%, 99.9%)
	No Resolution of CMV Episode	8	31	39	79.5% (63.5%, 90.7%)
	Total	24	32	56	
	Column Agreement (95% Exact CI)	66.7% (44.7%, 84.4%)	96.9% (83.8%, 99.9%)		
	Overall Percent Agreement (95% Exact CI)	83.9% (71.7%, 92.4%)			
	p-value[b]	0.0391			

Note: Among the subjects included in Day 35 table, 3 subjects were found to have impactful sequence mismatch.

Time point Post Anti-CMV Therapy Initiation		TaqMan® CMV Test			
	cobas® CMV	**Resolution of CMV Episode[a]**	**No Resolution of CMV Episode**	**Total**	**Row Agreement (95% Exact CI)**
Day 49	Resolution of CMV Episode[a]	38	0	38	100.0% (90.7%, 100.0%)
	No Resolution of CMV Episode	7	12	19	63.2% (38.4%, 83.7%)
	Total	45	12	57	
	Column Agreement (95% Exact CI)	84.4% (70.5%, 93.5%)	100.0% (73.5%, 100.0%)		
	Overall Percent Agreement (95% Exact CI)	87.7% (76.3%, 94.9%)			
	p-value[b]	0.0156			

Note: Among the subjects included in this table, 4 subjects were found to have impactful sequence mismatch.
[a] Resolution of CMV episode was defined by 2 consecutive samples (preferably sampled one week apart) that were tested below the LLoQ of TaqMan® CMV Test (137 IU/mL), which is consistent with what is recommended in current guidelines; ie, 2 consecutive "negative" samples have been recommended as a viral load endpoint for treatment of acute CMV episodes.
[b] Calculated using McNemar's Test.
CI = confidence interval; NC = not calculable; SOT = solid organ transplant

When used to aid in determining resolution of viremic episodes at Day 14, Day 21, Day 28, Day 35, and Day 49 (post anti-CMV therapy initiation), the OPA between **cobas®** CMV and TaqMan® CMV Test ranged from 83.9% to 100% (Table 32).

Table 32 Overall percentage agreement by resolution status (not resolved/resolved) resolution for subjects who initiated anti-CMV therapy in the SOT population

Time Point	Agreement Not Resolved	Agreement Resolved	Overall Percent Agreement	95% Exact CI Overall Percent Agreement
Day 14	100.0% (40/40)	NC	100.0% (40/40)	(91.2%, 100.0%)
Day 21	100.0% (50/50)	0.0% (0/1)	98.0% (50/51)	(89.6%, 100.0%)
Day 28	100.0% (46/46)	60.0% (6/10)	92.9% (52/56)	(82.7%, 98.0%)
Day 35	96.9% (31/32)	66.7% (16/24)	83.9% (47/56)	(71.7%, 92.4%)
Day 49	100.0% (12/12)	84.4% (38/45)	87.7% (50/57)	(76.3%, 94.9%)

Note: Resolution of CMV episode was defined by 2 consecutive samples (preferably sampled one week apart) that were tested below the LLoQ of TaqMan® CMV Test (137 IU/mL), which is consistent with what is recommended in current guidelines; i.e., 2 consecutive "negative" samples have been recommended as a viral load endpoint for treatment of acute CMV episodes.

2 out of the total 40 samples at Day 14 were from subjects found to have impactful sequence mismatch.

2 out of the total 51 samples at Day 21 were from subjects found to have impactful sequence mismatch.

3 out of the total 56 samples at Day 28 were from subjects found to have impactful sequence mismatch.

3 out of the total 56 samples at Day 35 were from subjects found to have impactful sequence mismatch.

4 out of the total 57 samples at Day 49 were from subjects found to have impactful sequence mismatch.

CMV = cytomegalovirus; LLoQ = lower limit of quantitation; NC = not calculable; SOT = solid organ transplant

Overall agreements among different viral load levels

Table 33 below shows the concordance of viral load results of **cobas®** CMV and the TaqMan® CMV Test for all 1898 paired samples evaluable in the SOT population of the clinical concordance study.

Table 33 Summary of concordance analyses (all paired samples) in the SOT population

All Paired Samples	TaqMan® CMV Test (log_{10} IU/mL)						
cobas® CMV (log_{10} IU/mL)	**Target Not Detected**	**< 2.137**	**2.137 to < 2.699**	**2.699 to < 3.255**	**3.255 to < 3.899**	**≥ 3.899**	**Total**
Target Not Detected	1,022	8	0	0	0	0	1,030
< 2.137	168	193	6	0	0	0	367
2.137 to < 2.699	3[a]	76	61	8	0	0	148
2.699 to < 3.255	0	12[c]	73	63	1	0	149
3.255 to < 3.899	1[b]	5[d]	8[e]	44	58	0	116
≥ 3.899	0	0	3[f]	1[b]	45	39	88
Total	1,194	294	151	116	104	39	1,898

Note: All 1898 paired samples evaluable for clinical concordance analysis were included in this table. The lower limit of quantitation (LLoQ) is 3.45E+01 IU/mL for **cobas®** CMV and 1.37E+02 IU/mL for TaqMan® CMV Test. log_{10} (1.37E+02) = 2.137; log_{10} (5.0E+02) = 2.699; log_{10} (1.8E+03) = 3.255; log_{10} (7.943E+03) = 3.899.

a These discrepant samples were sequenced and 2 out of 3 were found to contain a significant impact mutation.

b This discrepant sample was sequenced and was found to contain a significant impact mutation.

c 8 of the 12 discrepant samples derived from 5 subjects and all 8 samples were sequenced and found to a contain significant impact mutation.

d These 5 discrepant samples derived from 3 subjects; they were sequenced and all 5 were found to contain a significant impact mutation.

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

f These 3 discrepant samples derived from 2 subjects; they were sequenced and all 3 were found to contain a significant impact mutation.

Table 34 below shows the summary of concordance of viral load results by different thresholds (Target Not Detected, 137 IU/mL, 500 IU/mL, and 1800 IU/mL) for all paired samples in the SOT population.

Table 34 Summary of concordance of viral load results by different thresholds for all paired samples in the SOT population

	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% (1022/1194)	**98.9%** 97.8%, 99.5% (696/704)	**90.5%** 89.1%, 91.8% (1718/1898)
137 IU/mL (2.1 log_{10} IU/mL*)	**93.5%** 92.1%, 94.7% (1391/1488)	**98.5%** 96.8%, 99.5% (404/410)	**94.6%** 93.5%, 95.5% (1795/1898)
500 IU/mL (2.7 log_{10} IU/mL)**	**93.8%** 92.5%, 94.9% (1537/1639)	**96.9%** 94.0%, 98.7% (251/259)	**94.2%** 93.1%, 95.2% (1788/1898)
1800 IU/mL (3.3 log_{10} IU/mL*)**	**96.5%** 95.5%, 97.3% (1693/1755)	**99.3%** 96.2%, 100.0% (142/143)	**96.7%** 95.8%, 97.4% (1835/1898)

Note: Only paired samples 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 2.7log_{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.

Table 35 below shows the concordance of viral load results of **cobas**® CMV and the TaqMan® CMV Test for all 272 paired samples evaluable at Day14, Day 21, Day 28, Day 35, or Day 49 post anti-CMV therapy initiation in the SOT population.

Table 35 Summary of concordance analyses (paired samples at timepoints of interest post anti-CMV therapy initiation) in the SOT population

All time points of interest	**TaqMan® CMV Test (log_{10} IU/mL)**						
cobas® CMV (log_{10} IU/mL)	**Target Not Detected**	**< 2.137**	**2.137 to < 2.699**	**2.699 to < 3.255**	**3.255 to < 3.899**	**≥ 3.899**	**Total**
Target Not Detected	24	3	0	0	0	0	27
< 2.137	36	42	1	0	0	0	79
2.137 to < 2.699	0	27	18	0	0	0	45
2.699 to < 3.255	0	4[a]	25	16	0	0	45
3.255 to < 3.899	0	2[b]	1[c]	21	12	0	36
≥ 3.899	0	0	2[b]	0	26	12	40
Total	60	78	47	37	38	12	272

Note: Only paired samples evaluable for clinical concordance analysis at time points of interest (Day 14, Day 21, Day 28, Day 35 or Day 49 post anti-CMV therapy initiation) were included in this table. The lower limit of quantitation (LLoQ) is 3.45E+01 IU/mL for **cobas**® CMV and 1.37E+02 IU/mL for TaqMan® CMV Test. log_{10} (1.37E+02) = 2.137; log_{10} (5.0E+02) = 2.699; log_{10} (1.8E+03) = 3.255; log_{10} (7.943E+03) = 3.899.

[a] These 4 samples were sequenced and two of the 4 discrepant samples were found to contain a significant impact mutation.

[b] These 2 discrepant samples were sequenced and both were found to contain a significant impact mutation.

[c] The discrepant sample was sequenced and found to contain a significant impact mutation.

Table 36 below shows the summary of concordance of viral load results by different thresholds (Target Not Detected, 137 IU/mL, 500 IU/mL, and 1800 IU/mL) for all paired samples evaluable at Day14, Day 21, Day 28, Day 35, or Day 49 post anti-CMV therapy initiation in the SOT population.

Table 36 Summary of concordance of viral load results by different thresholds for paired samples at Day 14, Day 21, Day 28, Day 35 or Day 49 post anti-CMV therapy initiation in the SOT population

	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%** 68.1%, 82.9% (105/138)	**99.3%** 95.9%, 100.0% (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)	**100.0%** 95.8%, 100.0% (87/87)	**87.5%** 83.0%, 91.2% (238/272)
1800 IU/mL (3.3 log_{10} IU/mL*)**	**88.3%** 83.3%, 92.2% (196/222)	**100.0%** 92.9%, 100.0% (50/50)	**90.4%** 86.3%, 93.7% (246/272)

Note: Only paired samples evaluable for clinical concordance analysis at Day 14, Day 21, Day 28, Day 35 and Day 49 post anti-CMV therapy initiation 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 2.7 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.

Method comparison in the solid organ transplant population

A method comparison study was conducted to evaluate the performance of **cobas**® CMV as compared to another FDA approved CMV viral load test, the TaqMan® CMV Test. The study used 543 paired samples including 381 CMV positive samples from the phase 2a double-blinded randomized placebo-controlled trial of an anti-CMV prophylaxis regimen referenced above, supplemented by 64 leftover specimens from transplant patients and 98 contrived samples made by spiking cultured CMV (Merlin strain) into CMV negative EDTA plasma.

Table 37 along with Figure 4 through Figure 6 present the Deming regression of the viral load (log_{10} IU/mL) results from **cobas**® CMV and the TaqMan® CMV Test for all sites combined for the solid organ transplant population.

Table 37 Parameter estimates of Deming regression between viral loads (log_{10} IU/mL) in the SOT population (**cobas**® CMV Versus TaqMan® CMV Test)

Samples	Number of Paired Samples	Parameter	Parameter Estimate	Standard Error	95% CI[a] 95% CI[b]	r
Clinical and Spiked	543	Intercept	0.348 0.407*	0.033	(0.283, 0.413) (0.356, 0.462)	0.98
		Slope	0.961 0.945*	0.009	(0.944, 0.979) (0.933, 0.957)	
Clinical	445	Intercept	0.193 0.229*	0.037	(0.120, 0.266) (0.160, 0.301)	0.97
		Slope	1.023 1.010*	0.010	(1.002, 1.044) (0.992, 1.030)	
Spiked	98	Intercept	0.012 N/A	0.063	(-0.114, 0.138) N/A	0.99
		Slope	0.985 N/A	0.013	(0.960, 1.010) N/A	

Note: Twenty-six samples from nine subjects were excluded from method comparison analyses due to impactful sequence mismatch. 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.

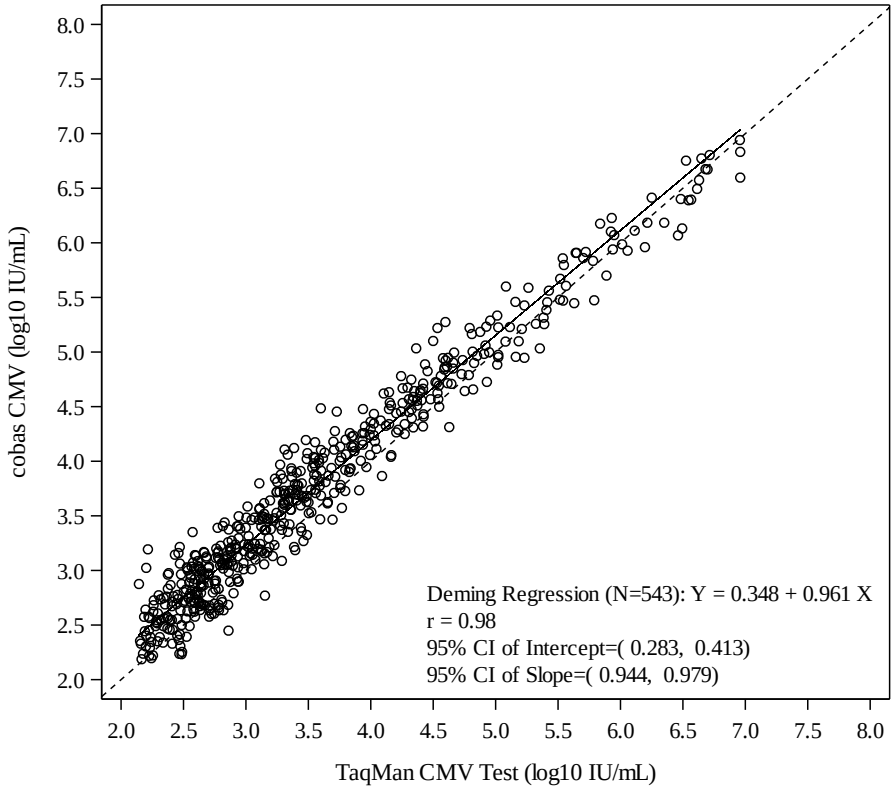
a Assumed independence between all samples.

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

* Denotes the 50th percentile of the bootstrapped distribution of parameter estimates.

CI = confidence interval; N/A = not applicable; r = correlation coefficient.

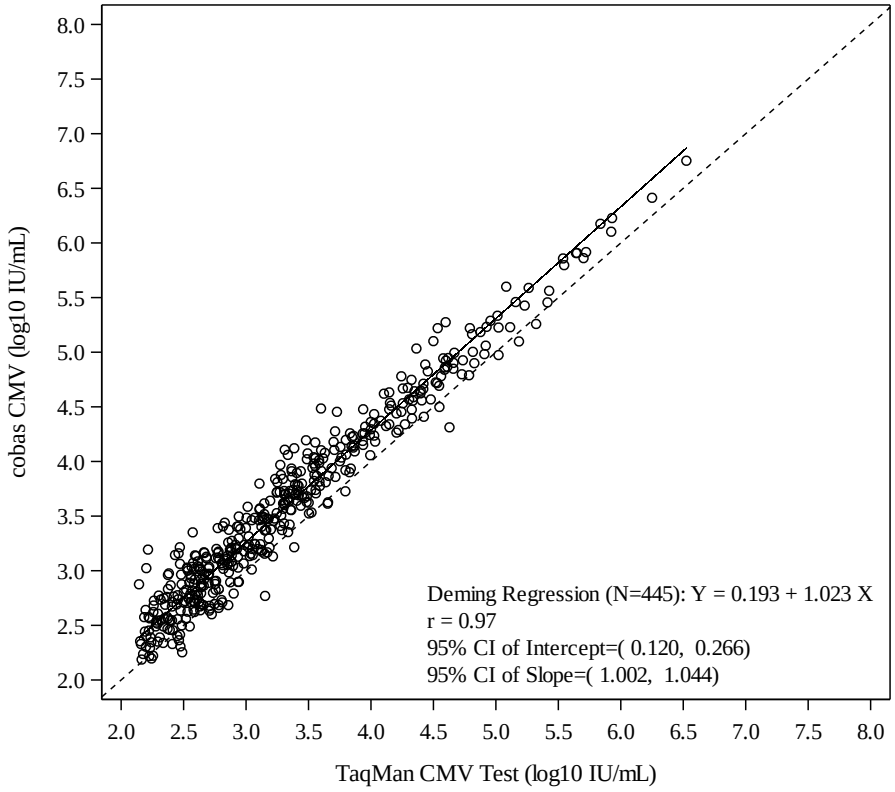
Figure 4 Deming linear regression plot of viral loads (log_{10} IU/mL) in the SOT population (**cobas**® CMV Versus TaqMan® CMV Test; clinical and spiked samples)



Note: Twenty-six samples from nine subjects were excluded from method comparison analyses due to impactful sequence mismatch.
The figure 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.

CI = confidence interval;
r = correlation coefficient.

Figure 5 Deming linear regression plot of viral loads (log_{10} IU/mL) in the SOT population (**cobas**® CMV Versus TaqMan® CMV Test; clinical samples)

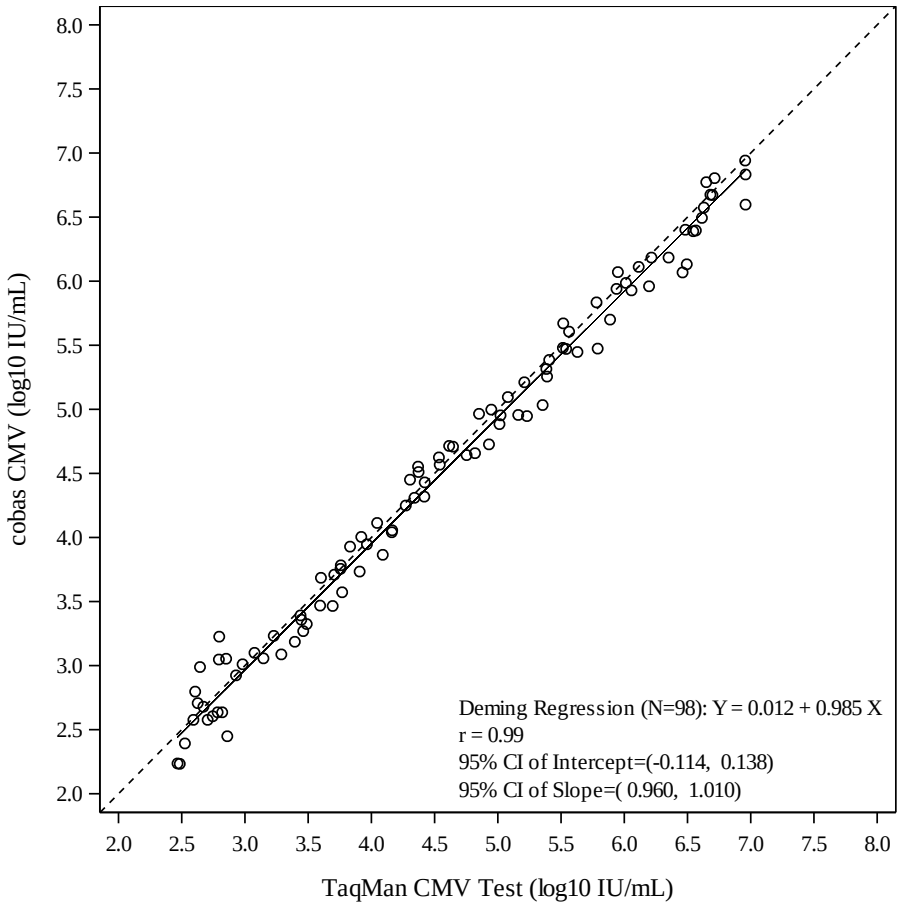


Note: Twenty-six samples from nine subjects were excluded from method comparison analyses due to impactful sequence mismatch.

The figure 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.

CI = confidence interval;
r = correlation coefficient.

Figure 6 Deming linear regression plot of viral loads (log_{10} IU/mL) in the SOT population (**cobas**® CMV Versus TaqMan® CMV Test; spiked samples)



Note: CI = confidence interval;
r = correlation coefficient.

Bias at selected viral load levels

Table 38 below presents the bias between **cobas**® CMV and the TaqMan® CMV Test at five selected viral load levels from 2.14 $\log_{10}$ IU/mL to 7.00 $\log_{10}$ IU/mL with associated non-transformed equivalents.

Table 38 Bias between **cobas**® CMV and TaqMan® CMV Test ($\log_{10}$ IU/mL) at five selected viral load levels (clinical and spiked samples)

Samples	Viral load level (Per TaqMan® CMV Test)	Systematic Difference
Clinical and Spiked	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 (1.19E+03 IU/mL)
	4.000 $\log_{10}$ IU/ml (1.00E+04 IU/ml)	0.192 $\log_{10}$ IU/ml (5.56E+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)
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 (1.53E+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)
Spiked	2.137 $\log_{10}$ IU/ml (1.37E+02 IU/ml)	-0.020 $\log_{10}$ IU/ml (-6.19E+00 IU/mL)
	2.699 $\log_{10}$ IU/ml (5.00E+02 IU/ml)	-0.028 $\log_{10}$ IU/ml (-3.17E+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+06 IU/mL)

a Difference in IU/mL calculated as $10^{(\text{cobas® CMV estimate log10 IU/mL})} - 10^{(\text{TaqMan® CMV Test Viral Load Level log10 IU/mL})}$.

Mean paired difference

Table 39 below present 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.[30]

Table 39 Mean of paired viral load differences of **cobas**® CMV minus TaqMan® CMV Test (log_{10} IU/mL) at representative decision intervals (IU/mL) in the SOT population

Samples	Representative Decision Intervals[a] (IU/mL)	N	Mean of Paired Difference (log_{10} IU/mL)	SE for Mean of Paired Difference (log_{10} IU/mL)	95% CI (log_{10} IU/mL)
Clinical and Spiked	1.37E+02 to < 2.0E+03	275	0.234	0.013	(0.208, 0.260)
	2.0E+03 to < 2.0E+04	143	0.260	0.019	(0.223, 0.296)
	2.0E+04 to < 1.0E+05	62	0.195	0.025	(0.145, 0.245)
	≥ 1.0E+05	63	0.012	0.025	(-0.039, 0.062)
	Overall	543	0.211	0.010	(0.191, 0.230)
Clinical	1.37E+02 to < 2.0E+03	253	0.256	0.013	(0.230, 0.282)
	2.0E+03 to < 2.0E+04	122	0.317	0.016	(0.285, 0.350)
	2.0E+04 to < 1.0E+05	47	0.251	0.027	(0.196, 0.305)
	≥ 1.0E+05	23	0.201	0.030	(0.139, 0.262)
	Overall	445	0.269	0.009	(0.251, 0.288)
Spiked	1.37E+02 to < 2.0E+03	22	-0.017	0.044	(-0.108, 0.074)
	2.0E+03 to < 2.0E+04	21	-0.074	0.024	(-0.125, -0.024)
	2.0E+04 to < 1.0E+05	15	0.021	0.031	(-0.045, 0.086)
	≥ 1.0E+05	40	-0.097	0.022	(-0.141, -0.053)
	Overall	98	-0.056	0.015	(-0.087, -0.025)

Note: Twenty-six samples from nine subjects were excluded from method comparison analyses due to impactful sequence mismatch. 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 within the linear range on both assays were categorized into representative decision intervals based on the TaqMan® CMV Test result (IU/mL).

a Equivalent representative decision intervals (IU/mL) for 1.37E+02 to < 2.0E+03 (IU/mL) = 2.137 to < 3.301 (log_{10}IU/mL), 2.0E+03 to < 2.0E+04 (IU/mL) = 3.301 to < 4.301 (log_{10}IU/mL), 2.0E+04 to < 1.0E+05 (IU/mL) = 4.301 to < 5.000 (log_{10}IU/mL) and ≥ 1.0E+05 (IU/mL) =≥ 5.000 (log_{10}IU/mL).

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

Allowable total difference (ATD)

Table 40 along with Figure 7 through Figure 9 below, present the ATD results using the individual paired differences between **cobas**® CMV and the TaqMan® CMV Test versus their average at representative thresholds and calculates the percentage of paired results in the ATD zone.

Table 40 Percentage of samples in the SOT population falling in Allowable Total Difference (ATD) zone intervals (IU/mL) (**cobas**® CMV Versus TaqMan® CMV Test)

Samples	Interval Category	Interval Range[a] (IU/mL)	Percentage of Paired Samples within ATD Zone % (n/N)
Clinical and Spiked	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		94.3% (512/543)
Clinical	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		93.0% (414/445)
Spiked	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		100.0% (98/98)

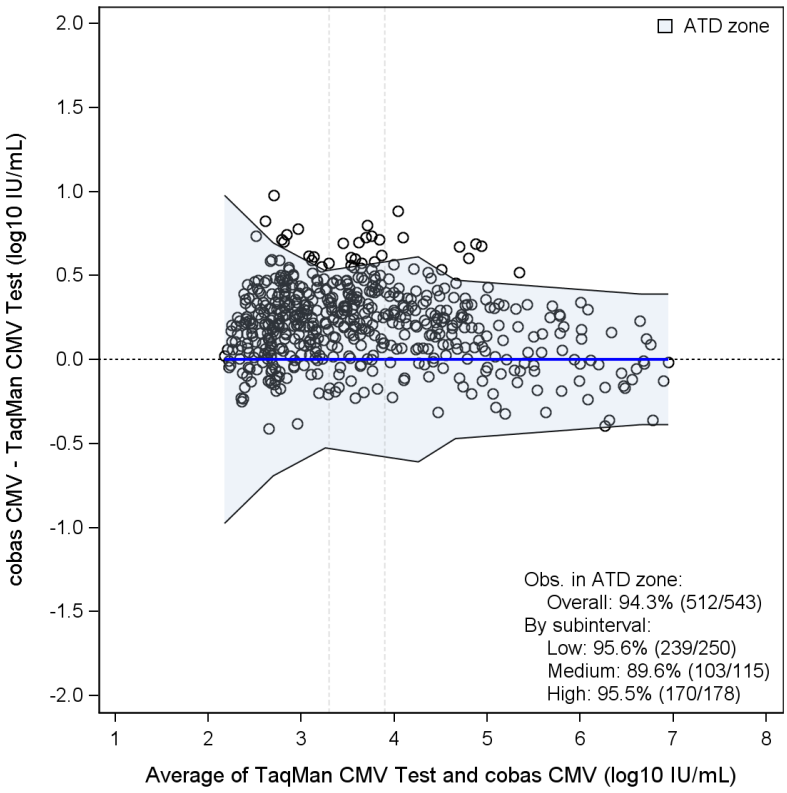
Note: Twenty-six samples from nine subjects were excluded from method comparison analyses due to impactful sequence mismatch. The table only includes paired samples with paired results that were each within 1.37E+02 IU/mL to 9.1E+06 IU/mL, the overlapping linear range of both assays. Paired results were categorized into viral load intervals based on the TaqMan® CMV Test result (IU/mL). ATD Zone = Allowable Total Difference Zone.

a Equivalent medically relevant intervals (IU/mL) for 1.37E+02 to < 2.0E+03, 2.0E+03 to < 8.0E+03 and 8.0E+03 to 9.1E0 in log10 IU/mL are, respectively, 2.137 to < 3.301, 3.301 to < 3.903 and 3.903 to 6.959.

N = total number of paired samples within the appropriate interval.

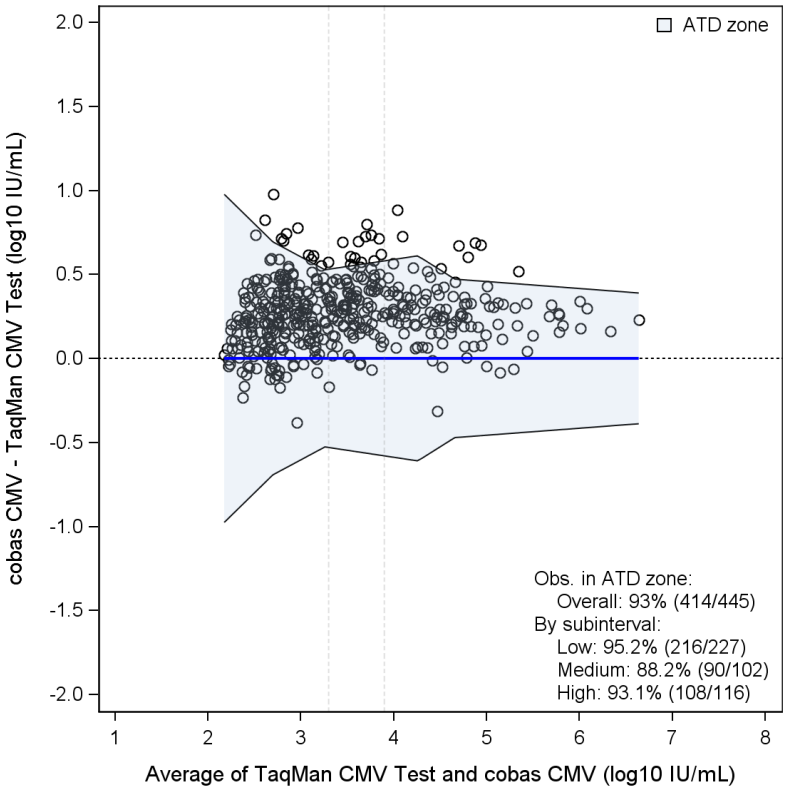
n = number of paired samples included in the ATD Zone within the appropriate interval.

Figure 7 Allowable Total Difference (ATD) plot of individual viral load differences versus their average (log_{10} IU/mL) in the SOT population (**cobas**® CMV versus TaqMan® CMV Test; clinical and spiked samples)



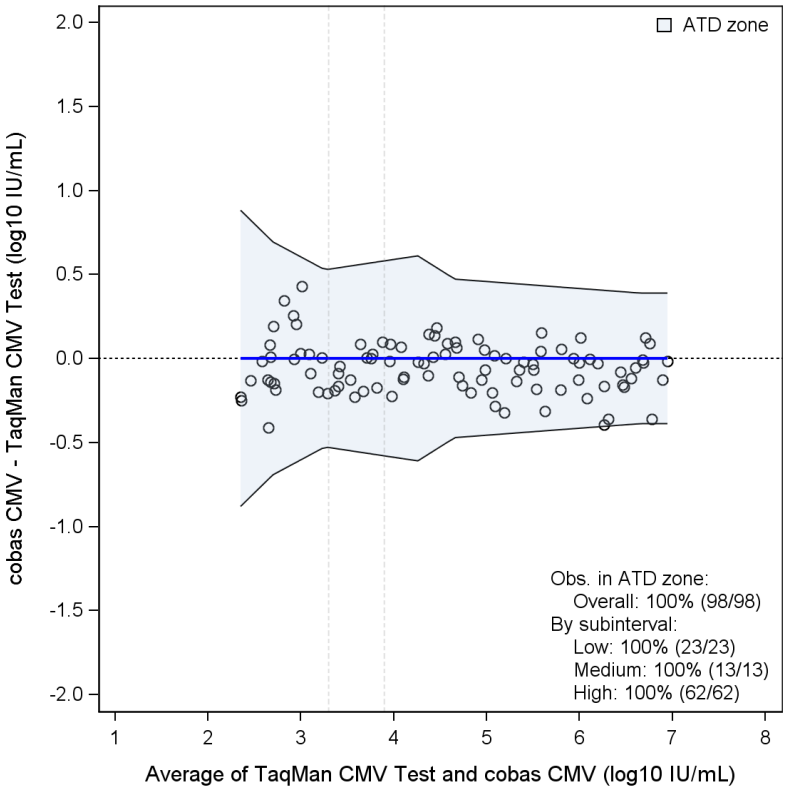
Note: Twenty-six samples from nine subjects were excluded from method comparison analyses due to impactful sequence mismatch. The figure only includes paired samples with paired results that were each within 1.37E+02 IU/mL to 9.1E+06 IU/mL, the overlapping linear range of both assays. Paired results were categorized into viral load intervals based on the TaqMan® CMV Test result (IU/mL).

Figure 8 Allowable Total Difference (ATD) plot of individual viral load differences versus their average (log_{10} IU/mL) in the SOT population (**cobas**® CMV versus TaqMan® CMV Test; clinical samples)



Note: Twenty-six samples from nine subjects were excluded from method comparison analyses due to impactful sequence mismatch. The figure 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).

Figure 9 Allowable Total Difference (ATD) plot of individual viral load differences versus their average (log_{10} IU/mL) in the SOT population (**cobas**® CMV versus TaqMan® CMV Test; spiked samples)



Agreement with negative samples

Thirty CMV IgG negative samples were tested on each assay and results are presented in Table 41.

Table 41 Results of CMV IgG-negative specimens (**cobas**® CMV versus TaqMan® CMV Test)

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

CMV = cytomegalovirus; IgG = immunoglobulin G.

Clinical performance evaluation: hematopoietic stem cell transplant (HSCT) population

The study was designed to evaluate the concordance between **cobas**® CMV and the COBAS® AmpliPrep/COBAS® TaqMan® CMV Test in a hematopoietic stem cell transplant (HSCT) population. Residual samples from a Phase 2, randomized, double blind, placebo-controlled dose-ranging multicenter clinical trial of brincidofovir for CMV prophylaxis[2] were tested.

All evaluable samples tested were collected over time from a total of 258 subjects. The assay target regions were sequenced samples with an offset of > 0.5 $\log_{10}$ IU/mL between the two assays, as well as a representative set of samples without a measurement offset. Sequences associated with a mean offset > 0.9 $\log_{10}$ IU/mL were defined as "impactful." Only impactful sequences affecting the targets for the COBAS® AmpliPrep/COBAS® TaqMan® CMV Test were identified.

Table 42 below summarizes the demographics and baseline clinical characteristics of the 258 subjects.

Table 42 Demographics and baseline clinical characteristics of HSCT subjects

Characteristics	Statistic
Total, N	258
Age (years)	
Mean ± SD	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%)

Note: A subject whose information was not available or not reported was categorized as "Unknown" for the corresponding characteristic. The following cohorts are included in the Anti-CMV prophylaxis regimen category for Study Arm: CMX001 Treatment Cohort 1, CMX001 Treatment Cohort 2, CMX001 Treatment Cohort 3 and CMX001 Treatment Cohort 4.
CMV = cytomegalovirus; SD = standard deviation.

Clinical concordance in the HSCT population

Agreement at baseline based on viral load thresholds

Table 43 shows the agreement between **cobas**® CMV and TaqMan® CMV Test using a Target Not Detected threshold at Baseline for subjects that initiated anti-CMV therapy.

Table 43 Concordance analysis of **cobas**® and TaqMan® CMV Test results using a threshold of target not detected in the HSCT population

Baseline	**TaqMan® CMV Test**			
cobas® CMV	**Target Not Detected**	**Detected**	**Total**	**Row Agreement (95% Exact CI)**
Target Not Detected	11	0	11	100.0% (71.5%, 100.0%)
Detected	8*	48	56	85.7% (73.8%, 93.6%)
Total	19	48	67	
Column Agreement (95% Exact CI)	57.9% (33.5%, 79.7%)	100.0% (92.6%, 100.0%)		
Overall Percent Agreement (95% Exact CI)	88.1% (77.8%, 94.7%)			
p-value[a]	0.0078			

Note: Only paired samples evaluable for clinical concordance analysis at Baseline for subjects that initiated anti-CMV therapy were included in this table.

* 1 of the 8 discrepant samples was from impactful sequence mismatch subjects.

a Calculated using McNemar's Test.

CI = confidence interval.

Table 44 shows the agreement between **cobas**® CMV and TaqMan® CMV Test using a 1.37E+02 IU/mL threshold at Baseline for subjects that initiated anti-CMV therapy.

Table 44 Concordance analysis of **cobas**® and TaqMan® CMV Test results using threshold 1.37E+02 IU/mL in the HSCT population

Baseline	**TaqMan® CMV Test**			
cobas® CMV	**< 1.37E+02 IU/mL (< 2.137 $\log_{10}$ IU/mL)**	**≥ 1.37E+02 IU/mL (≥ 2.137 $\log_{10}$ IU/mL)**	**Total**	**Row Agreement (95% Exact CI)**
< 1.37E+02 IU/mL (< 2.137 $\log_{10}$ IU/mL)	36	1	37	97.3% (85.8%, 99.9%)
≥ 1.37E+02 IU/mL (≥ 2.137 $\log_{10}$ IU/mL)	1	29	30	96.7% (82.8%, 99.9%)
Total	37	30	67	
Column Agreement (95% Exact CI)	97.3% (85.8%, 99.9%)	96.7% (82.8%, 99.9%)		
Overall Percent Agreement (95% Exact CI)	97.0% (89.6%, 99.6%)			
p-value[a]	1.0000			

Note: Only paired samples evaluable for clinical concordance analysis at Baseline for subjects that initiated anti-CMV therapy were included in this table. Sample with a "Target Not Detected" or a detectable viral load below 1.37E+02 IU/mL result was categorized as "< 1.37E+02 IU/mL (< 2.137 $\log_{10}$ IU/mL)".

0 of the 2 discrepant samples were from impactful sequence mismatch subjects.

a Calculated using McNemar's Test.

1.0E+00 IU/mL = 1.1 copy/mL.

CI = confidence interval.

Table 45 shows the agreement between **cobas**® CMV and TaqMan® CMV Test using a 5.0E+02 IU/mL threshold at Baseline for subjects that initiated anti-CMV therapy.

Table 45 Concordance analysis of **cobas**® and TaqMan® CMV Test results using threshold 5.0E+02 IU/mL in the HSCT population

Baseline	TaqMan® CMV Test			
cobas® CMV	**< 5.0E+02 IU/mL (< 2.699 $\log_{10}$ IU/mL)**	**≥ 5.0E+02 IU/mL (≥ 2.699 $\log_{10}$ IU/mL)**	**Total**	**Row Agreement (95% Exact CI)**
< 5.0E+02 IU/mL (< 2.699 $\log_{10}$ IU/mL)	43	1	44	97.7% (88.0%, 99.9%)
≥ 5.0E+02 IU/mL (≥ 2.699 $\log_{10}$ IU/mL)	0	23	23	100.0% (85.2%, 100.0%)
Total	43	24	67	
Column Agreement (95% Exact CI)	100.0% (91.8%, 100.0%)	95.8% (78.9%, 99.9%)		
Overall Percent Agreement (95% Exact CI)	98.5% (92.0%, 100.0%)			
p-value [a]	1.0000			

Note: Only paired samples evaluable for clinical concordance analysis at Baseline for subjects that initiated anti-CMV therapy were included in this table. Sample with a "Target Not Detected" or a detectable viral load below 5.0E+02 IU/mL result was categorized as "< 5.0E+02 IU/mL (< 2.699 $\log_{10}$ IU/mL)".

0 of the 1 discrepant sample were from impactful sequence mismatch subjects.

a Calculated using McNemar's Test.

1.0E+00 IU/mL = 1.1 copy/mL.

CI = confidence interval.

Table 46 shows the agreement between **cobas**® CMV and TaqMan® CMV Test using a 1.8E+03 IU/mL threshold at Baseline for subjects that initiated anti-CMV therapy.

Table 46 Concordance analysis of **cobas**® and TaqMan® CMV Test results using threshold 1.8 E+03 IU/mL in the HSCT population

Baseline	**TaqMan® CMV Test**			
cobas® CMV	**< 1.8E+03 IU/mL (< 3.255 $\log_{10}$ IU/mL)**	**≥ 1.8E+03 IU/mL (≥ 3.255 $\log_{10}$ IU/mL)**	**Total**	**Row Agreement (95% Exact CI)**
< 1.8E+03 IU/mL (< 3.255 $\log_{10}$ IU/mL)	48	0	48	100.0% (92.6%, 100.0%)
≥ 1.8E+03 IU/mL (≥ 3.255 $\log_{10}$ IU/mL)	2	17	19	89.5% (66.9%, 98.7%)
Total	50	17	67	
Column Agreement (95% Exact CI)	96.0% (86.3%, 99.5%)	100.0% (80.5%, 100.0%)		
Overall Percent Agreement (95% Exact CI)	97.0% (89.6%, 99.6%)			
p-value[a]	0.5000			

Note: Only paired samples evaluable for clinical concordance analysis at Baseline for subjects that initiated anti-CMV therapy were included in this table. Sample with a "Target Not Detected" or a detectable viral load below 1.8E+03 IU/mL result was categorized as "< 1.8E+03 IU/mL (< 3.255 $\log_{10}$ IU/mL)".

0 of the 2 discrepant samples were from impactful sequence mismatch subjects.

a Calculated using McNemar's Test.

1.0E+00 IU/mL = 1.1 copy/mL; 1.8E+03 IU/mL = 2000 copies/mL.

CI = confidence interval.

Resolution of CMV episode analysis

Table 47 below shows the concordance analyses of CMV episode resolution by time point for viremic subjects who initiated anti-CMV therapy.

Table 47 Concordance analysis of CMV episode resolution by time point for viremic HSCT subjects who initiated anti-CMV therapy

Time Point					
		TaqMan® CMV Test			
	cobas® CMV	**Resolution of CMV Episode[a]**	**No Resolution of CMV Episode**	**Total**	**Row Agreement (95% Exact CI)**
Day 14	Resolution of CMV Episode[a]	0	0	0	NC
	No Resolution of CMV Episode	0	14	14	100.0% (76.8%, 100.0%)
	Total	0	14	14	
	Column Agreement (95% Exact CI)	NC	100.0% (76.8%, 100.0%)		
	Overall Percent Agreement (95% Exact CI)	100.0% (76.8%, 100.0%)			

		TaqMan® CMV Test			
	cobas® CMV	**Resolution of CMV Episode[a]**	**No Resolution of CMV Episode**	**Total**	**Row Agreement (95% Exact CI)**
Day 21	Resolution of CMV Episode[a]	1	0	1	100.0% (2.5%, 100.0%)
	No Resolution of CMV Episode	0	12	12	100.0% (73.5%, 100.0%)
	Total	1	12	13	
	Column Agreement (95% Exact CI)	100.0% (2.5%, 100.0%)	100.0% (73.5%, 100.0%)		
	Overall Percent Agreement (95% Exact CI)	100.0% (75.3%, 100.0%)			

		TaqMan® CMV Test			
	cobas® CMV	**Resolution of CMV Episode[a]**	**No Resolution of CMV Episode**	**Total**	**Row Agreement (95% Exact CI)**
Day 28	Resolution of CMV Episode[a]	2	0	2	100.0% (15.8%, 100.0%)
	No Resolution of CMV Episode	0	7	7	100.0% (59.0%, 100.0%)
	Total	2	7	9	
	Column Agreement	100.0% (15.8%, 100.0%)	100.0% (59.0%, 100.0%)		
	Overall Percent Agreement	100.0% (66.4%, 100.0%)			

Time Point					
		TaqMan® CMV Test			
	cobas® CMV	**Resolution of CMV Episode[a]**	**No Resolution of CMV Episode**	**Total**	**Row Agreement (95% Exact CI)**
Day 49	Resolution of CMV Episode [a]	3	0	3	100.0% (29.2%, 100.0%)
	No Resolution of CMV Episode	0	1	1	100.0% (2.5%, 100.0%)
	Total	3	1	4	
	Column Agreement	100.0% (29.2%, 100.0%)	100.0% (2.5%, 100.0%)		
	Overall Percent Agreement	100.0% (39.8%, 100.0%)			

Note: Only subjects with paired results evaluable for clinical concordance analysis at either Day 14, 21, 28 or 49 post anti-CMV therapy initiation and with a resolution status available for each respective assay were included in this table. Two subjects had resolution of CMV episode on both assays at Day 28 and their resolution statuses were carried forward to Day 49. None of the subjects included in this analysis showed impactful sequence mismatch.

a Resolution of CMV episode was defined by 2 consecutive samples (preferably sampled one week apart) that were tested below the LLoQ of TaqMan® CMV Test (137 IU/mL), which is consistent with what is recommended in current guidelines; i.e., 2 consecutive "negative" samples have been recommended as a viral load endpoint for treatment of acute CMV episodes.

CMV = cytomegalovirus.

Table 48 below shows the overall percent agreements from the concordance analysis of CMV episode resolution between **cobas**® CMV and TaqMan® CMV Test for viremic subjects at Day 14, Day 21, Day 28, and Day 49. The OPA was estimated as 100% for all time points of interest. Hence, the acceptance criterion for OPA was met.

Table 48 Overall percent agreement from concordance analysis of CMV episode resolution for viremic HSCT subjects who initiated anti-CMV therapy

Time Point	Overall Percent Agreement Not Resolved	Overall Percent Agreement Resolved	Overall Percent Agreement	95% Exact CI Overall Percent Agreement
Day 14	100.0% (14/14)	NC	100.0% (14/14)	(76.8%, 100.0%)
Day 21	100.0% (12/12)	100.0% (1/1)	100.0% (13/13)	(75.3%, 100.0%)
Day 28	100.0% (7/7)	100.0% (2/2)	100.0% (9/9)	(66.4%, 100.0%)
Day 49	100.0% (1/1)	100.0% (3/3)	100.0% (4/4)	(39.8%, 100.0%)

Note: Two subjects had resolution of CMV episode on both assays at Day 28 and their resolution statuses were carried forward to Day 49. None of the subjects included in this analysis showed impactful sequence mismatch. Resolution of CMV episode was defined by 2 consecutive samples (preferably sampled one week apart) that were tested below the LLoQ of TaqMan® CMV Test (1.37E+02 IU/mL), which is consistent with what is recommended in current guidelines; i.e., 2 consecutive "negative" samples have been recommended as a viral load endpoint for treatment of acute CMV episodes.

CMV = cytomegalovirus; LLoQ = lower limit of quantitation; NC = not calculable.

Overall agreement at viral load levels

Table 49 below shows the overall agreement of viral load results of cobas® CMV and the TaqMan® CMV Test for all 1367 paired samples in the clinical concordance study.

Table 49 Overall agreement between viral load results of **cobas®** CMV and TaqMan® CMV in the HSCT population

All Paired Samples	**TaqMan® CMV Test (log_{10} IU/mL)**						
cobas® CMV (log_{10} IU/mL)	**Target Not Detected**	**< 2.137**	**2.137 to < 2.699**	**2.699 to < 3.255**	**3.255 to < 3.899**	**≥ 3.899**	**Total**
Target Not Detected	918	23	0	0	1	1	943
< 2.137	154	138	9	0	0	0	301
2.137 to < 2.699	0	13	24	5	0	0	42
2.699 to < 3.255	1*	1	17	17	0	0	36
3.255 to 3.899	0	0	0	8	16	1	25
> 3.899	0	0	0	0	10	10	20
Total	1,073	175	50	30	27	12	1,367

Note: All paired samples evaluable for clinical concordance analysis were included in this table. The lower limit of quantitation (LLoQ) is 3.45E+01 IU/mL for **cobas®** CMV and 1.37E+02 IU/mL for TaqMan® CMV Test. Results were categorized into one of the five viral load ranges based on the IU/mL result of each respective assay.
Seven samples from three subjects with impactful sequence mismatch are included in this table.

* The sample is from a subject with impactful sequence mismatch.

log10 (1.37E+02) = 2.137; log10 (5.0E+02) = 2.699; log10 (1.8E+03) = 3.255; log10 (7.943E+03) = 3.899.

Table 50 below shows the summary concordance of viral load results for all paired samples from HSCT patients using different thresholds (Target Not Detected, 137 IU/mL, 500 IU/mL and 1800 IU/mL).

Table 50 Summary concordance of viral load results for HSCT patients using different thresholds (all paired samples)

Threshold	Percent Agreement < Threshold 95% Exact CI (n/N)	Percent Agreement ≥ Threshold 95% Exact CI (n/N)	Overall Percent Agreement 95% Exact CI (n/N)
Target Not Detected	**85.6%** (83.3%, 87.6%) (918/1073)	**91.5%** (87.7%, 94.4%) (269/294)	**86.8%** (84.9%, 88.6%) (1187/1367)
1.37E+02 IU/mL (2.137 log10 IU/mL)	**98.8%** (98.0%, 99.3%) (1233/1248)	**90.8%** (84.1%, 95.3%) (108/119)	**98.1%** (97.2%, 98.8%) (1341/1367)
5.0E+02 IU/mL (2.699 log10 IU/mL)	**98.5%** (97.7%, 99.1%) (1279/1298)	**89.9%** (80.2%, 95.8%) (62/69)	**98.1%** (97.2%, 98.8%) (1341/1367)
1.8E+03 IU/mL (3.255 log10 IU/mL)	**99.4%** (98.8%, 99.7%) (1320/1328)	**94.9%** (82.7%, 99.4%) (37/39)	**99.3%** (98.7%, 99.6%) (1357/1367)

Note: All paired samples evaluable for clinical concordance analysis were included in this table. The LOD of the **cobas®** CMV test is 3.45E+01 IU/mL. The LOD of the TaqMan® CMV test is 1.37E+02 IU/mL.

95% confidence intervals (CI) were calculated by the exact method assuming independence between all samples.
1 IU/mL = 1.1 copy/mL.

Table 51 below shows the overall agreement of viral load results of **cobas®** CMV and the TaqMan® CMV Test for samples taken from those patients that initiated anti-CMV therapy and taken at protocol defined time points of interest post anti-CMV therapy initiation.

Table 51 Overall agreement between viral of **cobas®** CMV and TaqMan® CMV from samples at time points of interest post anti-CMV therapy initiation in the HSCT population

All Time Points	TaqMan® CMV Test (log_{10} IU/mL)						
cobas® CMV (log_{10} IU/mL)	**Target Not Detected**	**< 2.137**	**2.137 to < 2.699**	**2.699 to < 3.255**	**3.255 to < 3.899**	**≥ 3.899**	**Total**
Target Not Detected	17	1	0	0	0	0	18
< 2.137	10	8	0	0	0	0	18
2.137 to < 2.699	0	0	0	0	0	0	0
2.699 to < 3.255	1*	0	2	2	0	0	5
3.255 to 3.899	0	0	0	2	0	0	2
> 3.899	0	0	0	0	1	1	2
Total	28	9	2	4	1	1	45

Note: Only paired samples evaluable for clinical concordance analysis at time points (Day 14, Day 21, Day 28 or Day 49) were included in this table. The lower limit of quantitation (LLoQ) is 3.45E+01 IU/mL for **cobas®** CMV and 1.37E+02 IU/mL for TaqMan® CMV Test. Results were categorized into one of the five viral load ranges based on the IU/mL result of each respective assay.
* The sample is from a subject with impactful sequence mismatch.
log10 (1.37E+02) = 2.137; log10 (5.0E+02) = 2.699; log10 (1.8E+03) = 3.255; log10 (7.943E+03) = 3.899.

Table 52 below shows the summary concordance of viral load results for paired samples at time points of interest post anti-CMV therapy initation from HSCT patients using different thresholds (Target Not Detected, 137 IU/mL, 500 IU/mL and 1800 IU/mL).

Table 52 Summary concordance of viral load results for HSCT Patients Using Different Thresholds (Samples at time points of interest post anti-CMV therapy initiation)

Threshold	Percent Agreement < Threshold 95% Exact CI (n/N)	Percent Agreement ≥ Threshold 95% Exact CI (n/N)	Overall Percent Agreement 95% Exact 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)
1.37E+02 IU/mL (2.137 log10 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)
5.0E+02 IU/mL (2.699 log10 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)
1.8E+03 IU/mL (3.255 log10 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: All paired samples evaluable for clinical concordance analysis were included in this table. The LOD of the **cobas**® CMV test is 3.45E+01 IU/mL. The LOD of the TaqMan® CMV test is 1.37E+02 IU/mL.

95% confidence intervals (CI) were calculated by the exact method assuming independence between all samples.
1 IU/mL = 1.1 copy/mL.

Method comparison in the hematopoietic stem cell transplant population

A method comparison study was conducted to evaluate the performance of **cobas**® CMV as compared to another FDA approved CMV viral load test, the TaqMan® CMV Test for the Hematopoietic Stem Cell Transplant population. The study used 204 paired samples including 107 CMV positive samples from the phase 2 CMV prophylaxis trial referenced above, supplemented by 97 spiked samples made by spiking negative plasma from HSCT recipients with cultured CMV virus (Merlin strain).

Table 53 presents the parameter estimates of Deming regression of the viral load ($\log_{10}$ IU/mL) results of **cobas**® CMV and TaqMan® CMV Test by sample type.

Table 53 Parameter estimates of Deming regression between viral loads (log_{10} IU/mL) between **cobas®** CMV and TaqMan® CMV Test in the HSCT population by sample type

Sample Type	Number of Paired Samples	Parameter	Parameter Estimate	Standard Error	95% CI[a] 95% Bootstrap CI[b]	r
Clinical and Spiked	204	Intercept	0.145 0.172*	0.041	(0.064, 0.227) (0.132, 0.219)	0.99
		Slope	0.990 0.982*	0.009	(0.972, 1.008) (0.972, 0.990)	
Clinical	107	Intercept	-0.146 -0.188*	0.106	(-0.356, 0.064) (-0.462, -0.008)	0.96
		Slope	1.110 1.125*	0.034	(1.041, 1.178) (1.066, 1.217)	
Spiked	97	Intercept	-0.097 N/A	0.063	(-0.223, 0.028) N/A	0.99
		Slope	1.025 N/A	0.012	(1.000, 1.049) N/A	

Note: Seven samples from three subjects were excluded from method comparison analyses due to impactful sequence mismatch. The table only included paired clinical and spiked samples with results each within 1.37E+02 to 9.1E+06 IU/mL, the common linear range of both assays.

a Assumed independence between all samples.

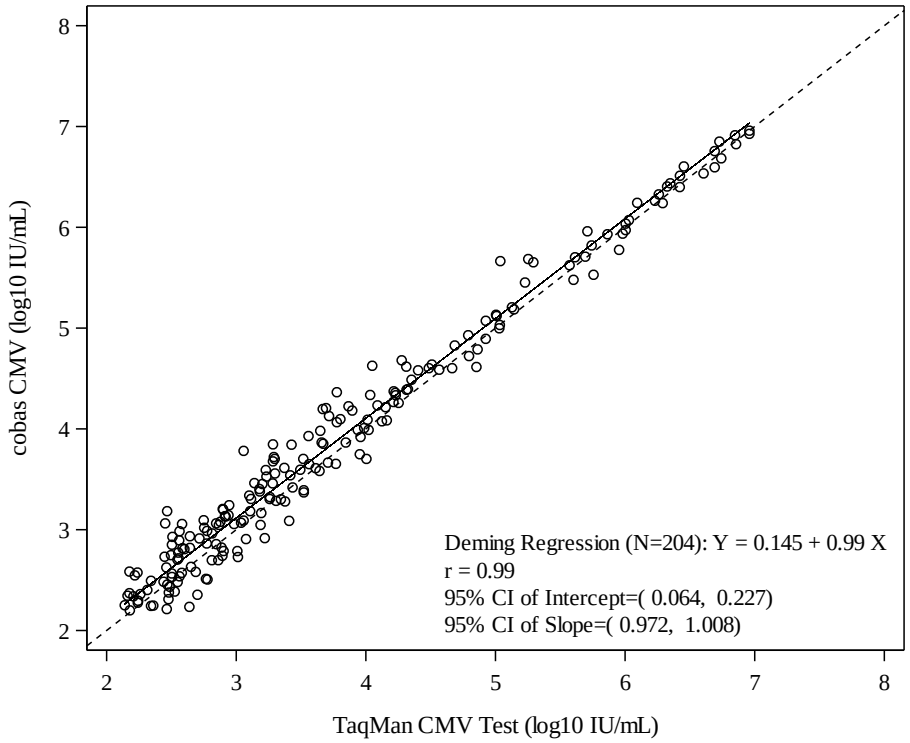
b Adjusted correlation between samples from the same subject by the bootstrap method with 500 iterations.

* Denotes the 50th percentile of the bootstrapped distribution of parameter estimates.

CI = confidence interval; **cobas®** CMV = **cobas®** CMV for use on the **cobas®** 6800/8800 Systems; N/A = not applicable; r = correlation coefficient

Figure 10 below presents the plot for the Deming regression of the viral load (log_{10} IU/mL) results of **cobas®** CMV and the TaqMan® CMV Test from clinical and spiked samples combined.

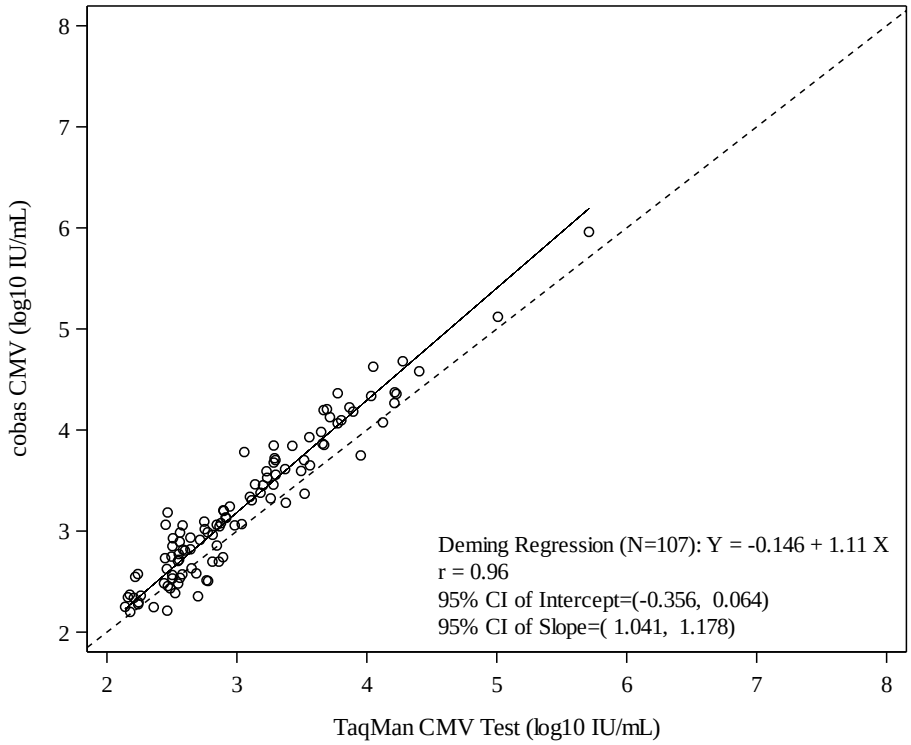
Figure 10 Deming linear regression plot of viral loads (log_{10} IU/mL) in the HSCT population (clinical and spiked samples)



Note: Seven samples from three subjects were excluded from method comparison analyses due to impactful sequence mismatch.
CI = confidence interval; r = correlation coefficient.

Figure 11 below presents the plot for the Deming regression of the viral load (log_{10} IU/mL) results of **cobas®** CMV and the TaqMan® CMV Test from clinical samples.

Figure 11 Deming linear regression plot of viral loads (log_{10} IU/mL) in the HSCT population (clinical samples)

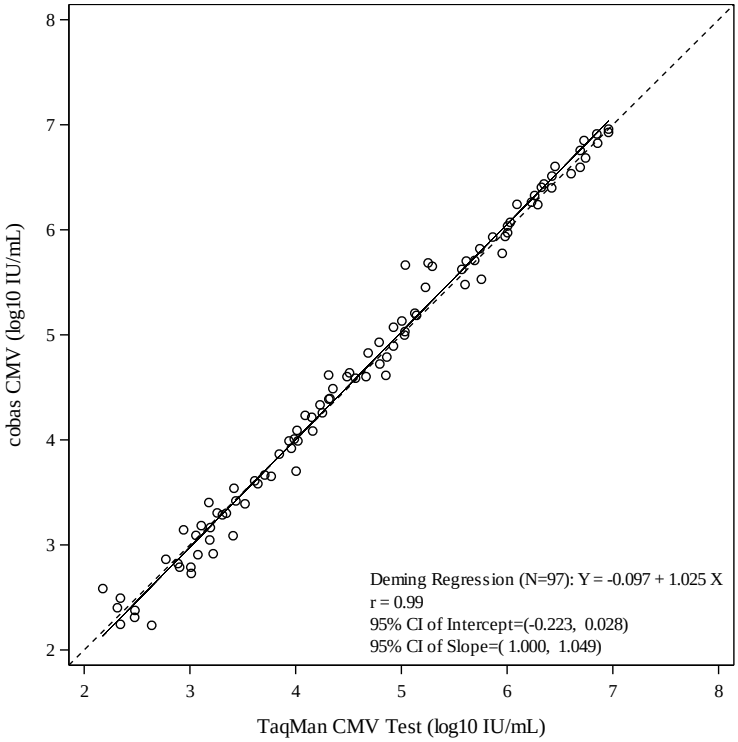


Note: Seven samples from three subjects were excluded from method comparison analyses due to impactful sequence mismatch.

CI = confidence interval; r = correlation coefficient.

Figure 12 below presents the plot for the Deming regression of the viral load (log_{10} IU/mL) results of **cobas**® CMV and the TaqMan® CMV Test from spiked samples.

Figure 12 Deming linear regression plot of viral loads (log_{10} IU/mL) in the HSCT population (spiked samples)



CI = confidence interval; r = correlation coefficient.

Bias at selected viral levels

Table 54 below presents the bias between **cobas**® CMV and TaqMan® CMV Test at five selected viral load levels from 2.14 log_{10} IU/mL to 7.00 log_{10} IU/mL with associated non-transformed equivalents.

Table 54 Bias between **cobas**® CMV and TaqMan® CMV Test (log10 IU/mL) at five selected viral load levels in the HSCT population (clinical and spiked samples)

Sample Type	Viral load level (per TaqMan® CMV Test)	Systematic Difference between the cobas® CMV and the TaqMan® CMV Test
Clinical and Spiked	2.137 log10 IU/ml (1.37E+02 IU/ml)	0.124 log10 IU/ml (4.51E+01 IU/mL)
	2.699 log10 IU/ml (5.00E+02 IU/ml)	0.118 log10 IU/ml (1.56E+02 IU/mL)
	3.255 log10 IU/ml (1.80E+03 IU/ml)	0.112 log10 IU/ml (5.32E+02 IU/mL)
	4.000 log10 IU/ml (1.00E+04 IU/ml)	0.105 log10 IU/ml (2.74E+03 IU/mL)
	7.000 log10 IU/ml (1.00E+07 IU/ml)	0.075 log10 IU/ml (1.89E+06 IU/mL)
Clinical	2.137 log10 IU/ml (1.37E+02 IU/ml)	0.089 log10 IU/ml (3.12E+01 IU/mL)
	2.699 log10 IU/ml (5.00E+02 IU/ml)	0.151 log10 IU/ml (2.08E+02 IU/mL)
	3.255 log10 IU/ml (1.80E+03 IU/ml)	0.212 log10 IU/ml (1.13E+03 IU/mL)
	4.000 log10 IU/ml (1.00E+04 IU/ml)	0.294 log10 IU/ml (9.68E+03 IU/mL)
	7.000 log10 IU/ml (1.00E+07 IU/ml)	0.624 log10 IU/ml (3.21E+07 IU/mL)
Spiked	2.137 log10 IU/ml (1.37E+02 IU/ml)	-0.044 log10 IU/ml (-1.31E+01 IU/mL)
	2.699 log10 IU/ml (5.00E+02 IU/ml)	-0.030 log10 IU/ml (-3.29E+01 IU/mL)
	3.255 log10 IU/ml (1.80E+03 IU/ml)	-0.016 log10 IU/ml (-6.36E+01 IU/mL)
	4.000 log10 IU/ml (1.00E+04 IU/ml)	0.003 log10 IU/ml (6.93E+01 IU/mL)
	7.000 log10 IU/ml (1.00E+07 IU/ml)	0.078 log10 IU/ml (1.97E+06 IU/mL)

Mean paired difference

Table 55 below shows the bias estimate as the observed mean of paired viral load difference by sample type. The overall systematic bias was estimated as 0.107 $\log_{10}$ IU/mL on average throughout the common linear range for combined clinical and spiked samples. The table also shows the bias estimate stratified by representative decision intervals.

Table 55 Mean of paired viral load difference (log_{10} IU/mL) between **cobas**® CMV and TaqMan® CMV Test at representative decision intervals (IU/mL) in HSCT population by sample type

Sample Type	Representative decision Intervals (IU/mL)[a]	N	Mean of Paired Difference (log_{10} IU/mL)	SE for Mean of Paired Difference (log_{10} IU/mL)	95% CI (log_{10} IU/mL)
Clinical and Spiked	1.37E+02 to < 2.0E+03	98	0.126	0.023	(0.080, 0.171)
	2.0E+03 to < 2.0E+04	49	0.121	0.032	(0.058, 0.184)
	2.0E+04 to < 1.0E+05	16	0.061	0.033	(-0.009, 0.131)
	1.0E+05 to 9.1E+06	41	0.062	0.024	(0.013, 0.110)
	Overall	204	0.107	0.014	(0.078, 0.135)
Clinical	1.37E+02 to < 2.0E+03	77	0.170	0.024	(0.122, 0.219)
	2.0E+03 to < 2.0E+04	27	0.241	0.041	(0.157, 0.326)
	2.0E+04 to < 1.0E+05	1	0.178	-	-
	1.0E+05 to 9.1E+06	2	0.181	0.070	(-0.705, 1.068)
	Overall	107	0.188	0.021	(0.148, 0.229)
Spiked	1.37E+02 to < 2.0E+03	21	-0.037	0.043	(-0.127, 0.053)
	2.0E+03 to < 2.0E+04	22	-0.027	0.025	(-0.079, 0.025)
	2.0E+04 to < 1.0E+05	15	0.053	0.034	(-0.020, 0.126)
	1.0E+05 to 9.1E+06	39	0.056	0.025	(0.006, 0.106)
	Overall	97	0.017	0.016	(-0.015, 0.048)

Note: Seven samples from three subjects were excluded from method comparison analyses due to impactful sequence mismatch. The table only included paired combined clinical and spiked samples with results each within 1.37E+02 to 9.1E+06 IU/mL, the common linear range of both assays. Paired results were categorized into medically relevant intervals based on the TaqMan® CMV Test result (IU/mL).CI = confidence interval; N = number of paired samples; SE = standard error.

[a] Equivalent representative decision intervals (IU/mL) for 1.37E+02 to < 2.0E+03 (IU/mL) = 2.137 to < 3.301 (log_{10}IU/mL), 2.0E+03 to < 2.0E+04 (IU/mL) = 3.301 to < 4.301 (log_{10}IU/mL), 2.0E+04 to < 1.0E+05 (IU/mL) = 4.301 to < 5.000 (log_{10}IU/mL) and ≥ 1.0E+05 (IU/mL) = ≥ 5.000 (log_{10}IU/mL).

Allowable total difference

Table 56 below shows the percentage of results within low, medium and high intervals of the Allowable Total Difference zone by sample type.

Table 56 Percentage of samples at low, medium and high intervals within the Allowable Total Difference zone in the HSCT population by sample type

Sample Type	Interval Category	Interval Range (IU/mL)[a]	Percentage of Samples within ATD Zone
Clinical and Spiked	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 9.1E+06	98.8% (81/82)
	Overall		98.0% (200/204)
Clinical	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 9.1E+06	100.0% (18/18)
	Overall		97.2% (104/107)
Spiked	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 9.1E+06	98.4% (63/64)
	Overall		99.0% (96/97)

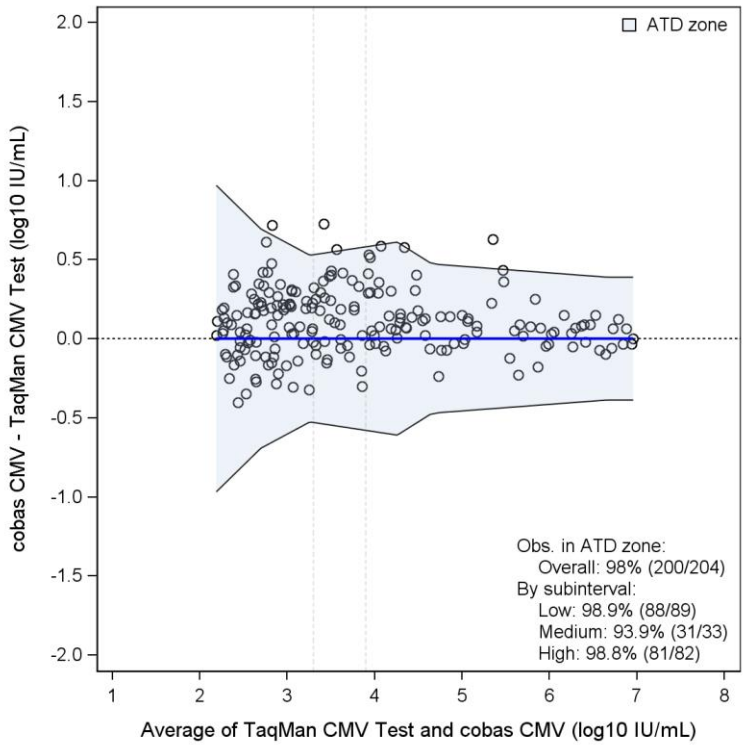
Note: Seven samples from three subjects were excluded from method comparison analyses due to impactful sequence mismatch. The table only included paired samples with results each within 1.37E+02 to 9.1E+06 IU/mL, the common linear range of both assays. Paired results were categorized into the intervals based on the TaqMan® CMV Test result (IU/mL).

ATD = allowable total difference.

a Equivalent medically relevant intervals (IU/mL) for 1.37E+02 to < 2.0E+03, 2.0E+03 to < 8.0E+03 and 8.0E+03 to 9.1E+06 in log10 IU/mL are, respectively, 2.137 to < 3.301, 3.301 to < 3.903 and 3.903 to 6.959.

Figure 13 below presents the Allowable Total Difference plot of the viral load (log_{10} IU/mL) results of **cobas®** CMV and the TaqMan® CMV Test from clinical and spiked samples combined.

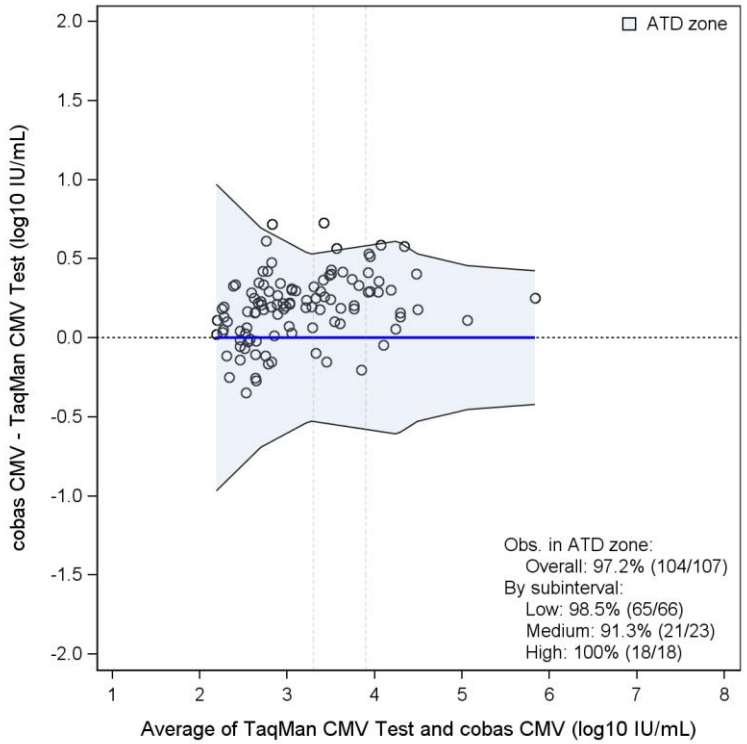
Figure 13 Allowable Total Difference (ATD) plot of viral load difference (log_{10} IU/mL) in the HSCT population (clinical and spiked samples)



Note: Seven samples from three subjects were excluded
from method comparison analyses due to impactful sequence mismatch.
ATD = allowable total difference; Obs. = observed paired results.

Figure 14 below presents the Allowable Total Difference plot of the viral load (log_{10} IU/mL) results of **cobas**® CMV and the TaqMan® CMV Test from clinical samples.

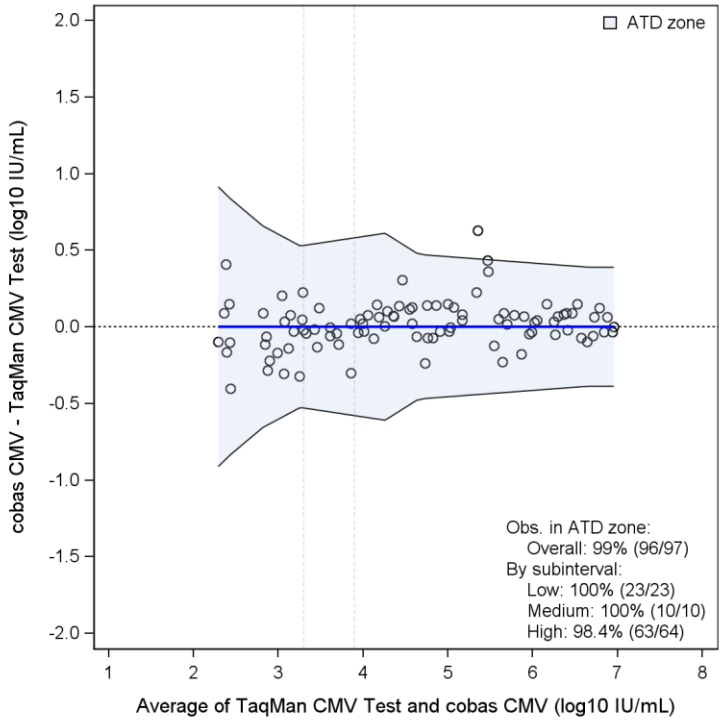
Figure 14 Allowable Total Difference (ATD) plot of viral load difference (log_{10} IU/mL) in the HSCT population (clinical samples)



Note: Seven samples from three subjects were excluded
from method comparison analyses due to impactful sequence mismatch.
ATD = allowable total difference; Obs. = observed paired results.

Figure 15 below presents the Allowable Total Difference plot of the viral load (log_{10} IU/mL) results of **cobas**® CMV and the TaqMan® CMV Test from spiked samples.

Figure 15 Allowable Total Difference (ATD) plot of viral load difference (log_{10} IU/mL) in the HSCT population (spiked samples)



ATD = allowable total difference; Obs. = observed paired results.

Agreement with negative samples

Thirty CMV IgG negative samples from HSCT patients were tested on each assay and their results are presented in Table 57 below.

Table 57 Results of CMV IgG-Negative Specimens Tested on **cobas**® CMV and TaqMan® CMV Test

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

Note: The lower limit of quantitation is 34.5 IU/mL for **cobas**® CMV and 1.37E+02 IU/mL for TaqMan® CMV Test.

IgG = immunoglobulin G.

Conclusion

cobas® CMV quantitates the level of CMV DNA in EDTA plasma with good agreement to the FDA-approved TaqMan® CMV Test. The results of these studies demonstrate the clinical concordance of **cobas**® CMV with TaqMan® CMV Test when used for treatment monitoring in solid organ transplant patients and hematopoietic stem cell transplant patients.

Additional information

Key test features

Sample type	EDTA plasma
Minimum amount of sample required	500 µL
Sample processing volume	350 µL
Analytical sensitivity	34.5 IU/mL
Linear range	34.5 IU/mL to 1E+07 IU/mL
Specificity	100% (one-sided 95% confidence interval: 99.5%)
Genotypes detected	CMV Glycoprotein B Genotype 1-4
Drug resistant CMV specimens detected	CMV specimens resistant against ganciclovir, valganciclovir, cidofovir and foscarnet

Symbols

The following symbols are used in labeling for Roche PCR diagnostic products.

Table 58 Symbols used in labeling for Roche PCR diagnostics products

	Ancillary Software		*In Vitro* Diagnostic Medical Device
	Authorized Representative in the European community		Lower Limit of Assigned Range
	Barcode Data Sheet		Manufacturer
	Batch code		Store in the dark
	Biological Risks		Contains sufficient for <*n*> tests
	Catalogue number		Temperature Limit
	Consult instructions for use		Test Definition File
	Contents of kit		Upper Limit of Assigned Range
	Distributed by		Use-by date
	For IVD Performance Evaluation Only		Global Trade Item Number
	This product fulfills the requirements of the European Directive 98/79 EC for *in vitro* diagnostic medical devices.		

US Customer Technical Support 1-800-526-1247

Manufacturer and distributors

Table 59 Manufacturer and distributors

Manufactured in United States

Roche Diagnostics GmbH
Sandhofer Strasse 116
68305 Mannheim, Germany
www.roche.com

Roche Diagnostics
9115 Hague Road
Indianapolis, IN 46250-0457 USA
(For Technical Assistance call the
Roche Response Center
toll-free: 1-800-526-1247)

Trademarks and patents

See http://www.roche-diagnostics.us/patents

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Document revision

Document Revision Information	
Doc Rev. 1.0 05/2017	First Publishing.
Doc Rev. 2.0 08/2017	Revised footnote corresponding to * in **Table 28** and **Table 29**. Corrected $\log_{10}$ value from 2.2 to 2.7 in **Table 34** and **Table 36**. Corrected header format for **Table 31** and **Table 47**. Revised Note of **Table 47** and **Table 48** to remove specific subject information. Corrected format errors throughout document. Please contact your local Roche Representative if you have any questions.