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: 09040897190
cobas® CMV Control Kit	P/N: 09040919190
cobas® NHP Negative Control Kit	P/N: 09051554190

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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 diagnosis and management of CMV in solid organ transplant patients and in hematopoietic stem cell transplant patients. The test can be used in these populations to assess the need to initiate antiviral treatment. 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.

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.[5-7]

Our current understanding of clinically-relevant thresholds for the development of CMV disease comes from a variety of studies using different technologies, study populations, and end-points.[8-14] In general; higher viral loads are more closely associated with the risk of development of CMV disease. The relationship between viremia and disease is sigmoidal; ie, the risk of CMV disease increases significantly after CMV viral load reaches a "critical threshold." For example, when using a laboratory-developed whole blood CMV DNA assay to test liver transplant recipients, the critical threshold was ≥ 5 $\log_{10}$ copies/mL of CMV DNA.[12] In patients with HIV/AIDS, CMV DNA levels have been correlated with the risk of CMV disease and overall mortality.[15-18]

However, current laboratory-developed methods of CMV DNA quantification are limited by a lack of standardized results, which can lead to a high degree of inter-laboratory and inter-assay variability.[19] Validating the reproducibility of CMV DNA viral load is critical to ensuring consistency of results for the management of patients with CMV disease. 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. Since variability is greatest at low concentrations, viral load changes may need to be more than 5-fold (0.7 $\log_{10}$) when the titer values are near the assay's lower limit of quantification, to be considered significant.[11]

While the exact threshold is still a subject of debate due to assay-to-assay variability, the critical threshold concept appears valid and has been reported in natural history studies showing that higher viral load values correlate with increased risk for the

development of CMV disease.[8-13] One study using the COBAS® AMPLICOR CMV MONITOR Test established a cutoff for predicting disease between 2,000 and 5,000 copies/mL in CMV seropositive liver transplant recipients.[10]

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 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. The high positive and low positive external controls are manufactured by dilution from stock material with a titer traceable to CMV WHO International Standard. Each Amplification/Detection kit lot is calibrated traceable to CMV WHO International Standard.

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

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 192 test cassette (P/N 09040897190)		
Kit components	**Reagent ingredients**	**Quantity per kit** **192 tests**
Proteinase Solution (PASE)	Tris buffer, < 0.05% EDTA, calcium chloride, calcium acetate, 8% proteinase EUH210: Safety data sheets available on request. EUH208: Contains subtilisin. May produce an allergic reaction.	22.3 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	21.2 mL
Elution Buffer (EB)	Tris buffer, 0.2% methyl-4 hydroxybenzoate	21.2 mL
Master Mix Reagent 1 (MMX-R1)	Manganese acetate, potassium hydroxide, < 0.1% sodium azide	7.5 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, < 0.1% ZO5D DNA polymerase, < 0.10% AmpErase (uracil-N- glycosylase) enzyme (microbial), < 0.1% sodium azide	9.7 mL

Table 2 **cobas**® CMV Control Kit

cobas® CMV Control Kit Store at 2–8°C (P/N 09040919190)			
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, CMV DNA not detectable by PCR methods. 0.1% ProClin® 300 preservative**	4 mL (8 x 0.5mL)	  **WARNING** H317: May cause an allergic skin reaction. H412: Harmful to aquatic life with long lasting effects. P261: Avoid breathing dust/fume/gas/ mist/vapours/spray. P273: Avoid release to the environment. 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. 55965-84-9 Reaction mass of: 5-chloro-2-methyl-4-isothiazolin-3-one [EC no. 247-500-7]and 2-methyl-2H -isothiazol-3- one [EC no. 220-239-6] (3:1)
CMV High Positive Control **(CMV H(+)C)**	< 0.001% synthetic (plasmid) CMV DNA encapsulated in Lambda bacteriophage coat protein, normal human plasma, CMV DNA not detectable by PCR methods. 0.1% ProClin® 300 preservative**	4 mL (8 x 0.5mL)	  **WARNING** H317: May cause an allergic skin reaction. H412: Harmful to aquatic life with long lasting effects. P261: Avoid breathing dust/fume/gas/ mist/vapours/spray. P273: Avoid release to the environment. 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. 55965-84-9 Reaction mass of: 5-chloro-2-methyl-4-isothiazolin-3-one [EC no. 247-500-7]and 2-methyl-2H -isothiazol-3- one [EC no. 220-239-6] (3:1)

* Product safety labeling primarily follows EU GHS guidance

**Hazardous substance

Table 3 cobas® NHP Negative Control Kit

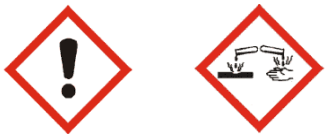
cobas® NHP Negative Control Kit Store at 2-8°C (P/N 09051554190)			
Kit components	**Reagent ingredients**	**Quantity per kit**	**Safety symbol and warning***
Normal Human Plasma Negative Control **(NHP-NC)**	Normal human plasma, CMV DNA not detectable by PCR methods. < 0.1% ProClin® 300 preservative**	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. 55965-84-9 Reaction mass of: 5-chloro-2-methyl-4-isothiazolin-3-one [EC no. 247-500-7]and 2-methyl-2H -isothiazol-3- one [EC no. 220-239-6] (3:1)

* Product safety labeling primarily follows EU GHS guidance

**Hazardous substance

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)	43% (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. H314: Causes severe skin burns and 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 protective gloves/ protective clothing/ eye protection/ face protection. P303 + P361 + P353 IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water. P304 + P340 + P310: IF INHALED: Remove person to fresh air and keep comfortable for breathing. Call a POISON CENTER/doctor. 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. 593-84-0 Guanidinium thiocyanate 9002-92-0 Polidocanol 3483-12-3 (R*,R*)-1,4-dimercaptobutane-2,3-diol
cobas omni Wash Reagent (WASH) Store at 15–30°C (P/N 06997503190)	Sodium citrate dihydrate, 0.1% methyl-4 hydroxybenzoate	4.2 L	Not applicable

* These reagents are not included in the **cobas**® CMV test kit. See listing of additional materials required (Table 7).

** Product safety labeling primarily follows EU GHS guidance

***Hazardous substance

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 – 192	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 – 192	Date not passed	90 days from first usage	Max 40 runs	Max 40 hours
cobas® CMV Control Kit	Date not passed	Not applicable[a]	Not applicable	Max 8 hours
cobas® NHP Negative Control Kit	Date not passed	Not applicable[a]	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

[a] Single use reagent

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

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
cobas omni Lysis Reagent	06997538190
cobas omni MGP Reagent	06997546190
cobas omni Specimen Diluent	06997511190
cobas omni Wash Reagent	06997503190
Solid Waste Bag and Solid Waste Container or Solid Waste Bag With Insert and Kit Drawer	07435967001 and 07094361001 or 08030073001 and 08387281001

Instrumentation and software required

The **cobas**® 6800/8800 software and **cobas**® CMV analysis package must 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 User Assistance and/or User Guide 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.
- **cobas**® CMV has not been evaluated for use as a screening test for the presence of CMV in blood or blood products.
- 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 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 PCR methods and showed no detectable 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 on 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.
- Inform your local competent authority about any serious incidents which may occur when using this assay.

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.
- Do not allow **cobas omni** Lysis Reagent, which contains guanidine thiocyanate, to contact sodium hypochlorite (bleach) solution. 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 User Assistance and/or User Guide to properly clean and decontaminate the surface of instrument(s).

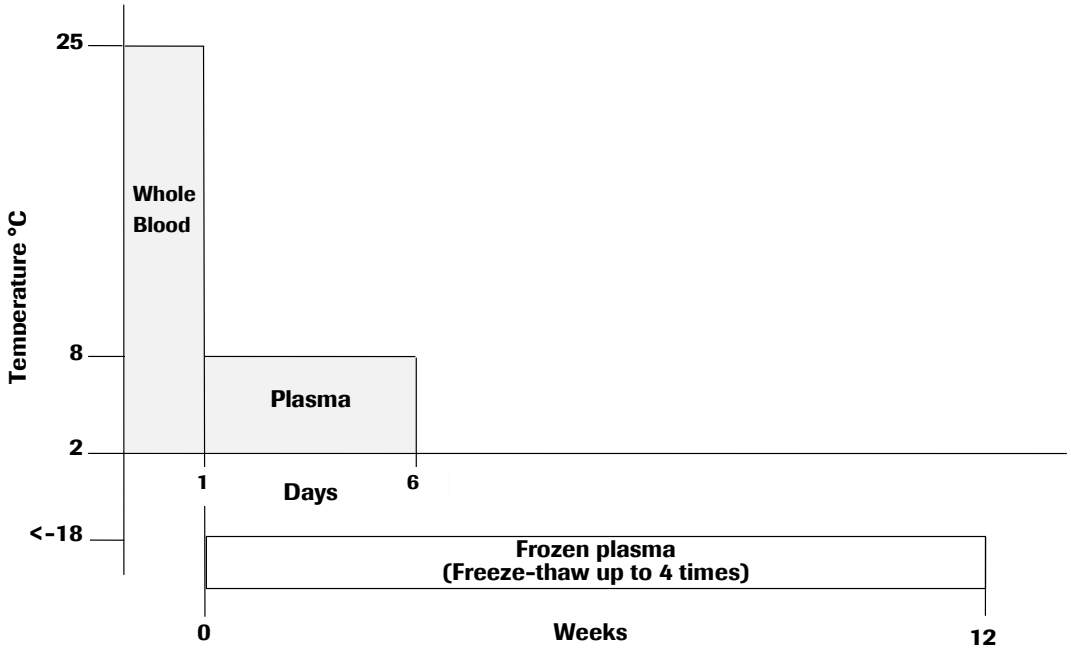
Sample collection, transport, and storage

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. Refer to Figure 1.
- 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 24 hours at 2-25°C prior to plasma preparation. Centrifugation should be performed according to manufacturer instructions.
- Upon separation plasma samples may be stored in secondary tubes for up to 6 days at 2-8°C or up to 12 weeks at ≤ -18°C.
- Plasma samples are stable for up to four freeze/thaw cycles when frozen at ≤ -18°C.

Figure 1 Sample storage conditions



- 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.

Note: Alternatively, 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, but then separated plasma cannot be stored for longer and needs to be analyzed directly.

Instructions for use

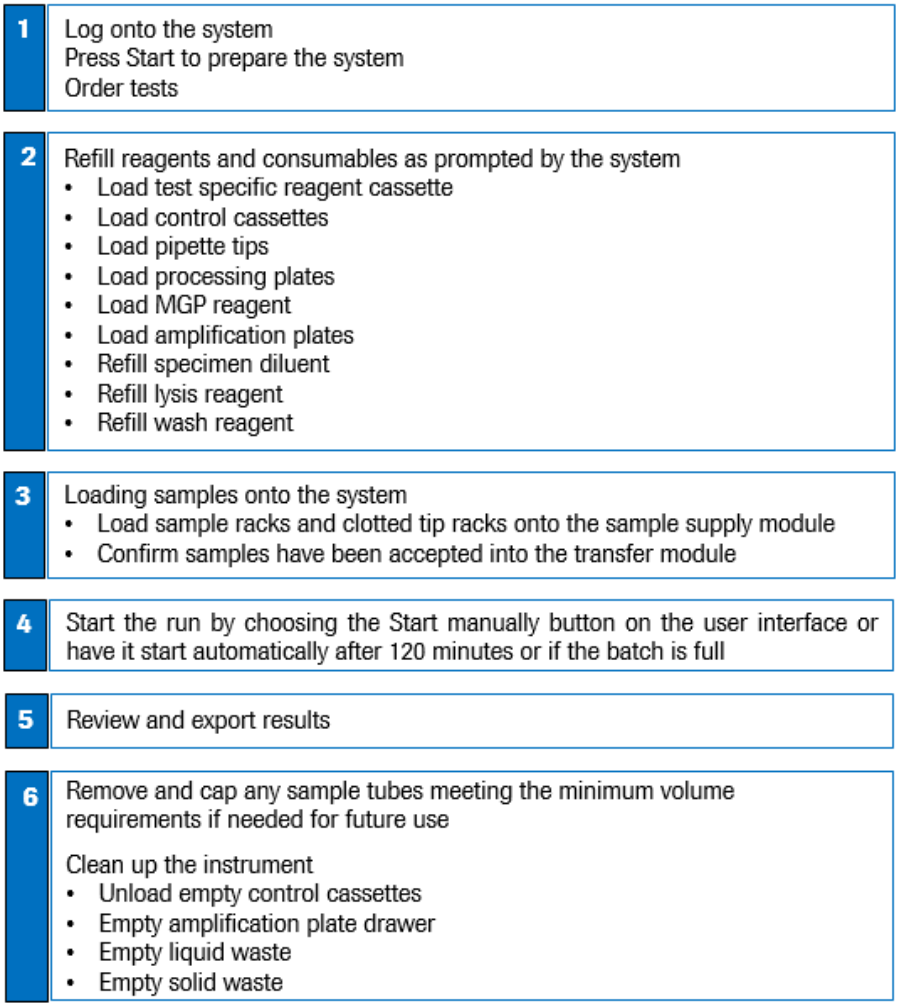
Procedural notes

- 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 User Assistance and/or User Guide for proper maintenance of instruments.

Running cobas® CMV

cobas® CMV can be run with one required sample volume of minimally 500 µL. The test procedure is described in detail in the **cobas**® 6800/8800 User Assistance and/or User Guide. Figure 2 below summarizes the procedure.

Figure 2 cobas® CMV procedure



Results

The **cobas**® 6800/8800 Systems automatically determine 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 titers above the upper limit of quantitation (ULoQ). If a quantitative result is desired, the original sample should be diluted with CMV-negative human EDTA plasma and the test should be repeated. Multiply the reported result by the dilution factor.

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.
- 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.
- 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. **cobas**® CMV mitigates this risk, through the use of redundant amplification primers.
- Due to inherent differences between technologies, it is recommended that, prior to switching from one technology to the next, users perform method correlation studies in their laboratory to qualify technology differences. Users should follow their own specific policies/procedures.
- **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.

Non-clinical performance evaluation

Key performance characteristics

Limit of Detection (LoD)

WHO International Standard

The limit of detection of **cobas**® CMV 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. The study demonstrates that **cobas**® CMV detected CMV DNA at a concentration of 23 IU/mL or greater with a hit rate of ≥ 95%.

Table 11 Limit of detection in EDTA plasma

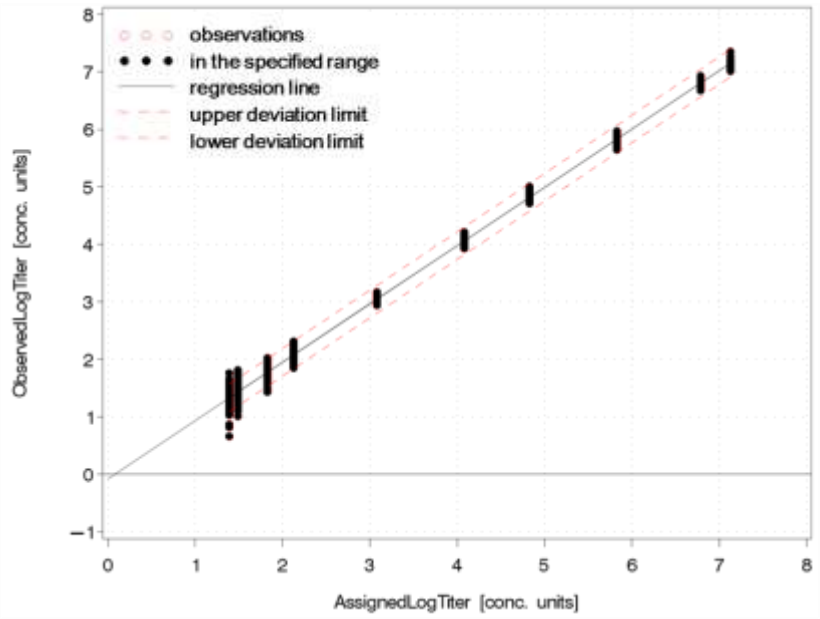
Input titer concentration (CMV DNA IU/mL)	Number of valid replicates	Number of positives	Hit rate in %
92.0	189	189	100.00
46.0	189	188	99.47
34.5	188	187	99.47
23.0	189	181	95.77
11.5	189	158	83.60
5.8	189	117	61.60
2.9	189	66	34.92
1.4	189	28	14.81
0.0	189	0	0.00
LoD by PROBIT at 95% hit rate	20.6 IU/mL 95% confidence range: 17.9 – 24.3 IU/mL		

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 2.45E+01 IU/mL to 1.34E+07 IU/mL.

Figure 3 Linear range determination in EDTA plasma



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 insturments 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 are shown in Table 12.

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 12 Within-laboratory precision of **cobas**® CMV

Nominal concentration (IU/mL)	Assigned concentration (IU/mL)	EDTA plasma			
		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

Genotype verification

The performance of **cobas**® CMV on CMV Glycoprotein B genotypes was evaluated by:

- Verification of the limit of detection for Glycoprotein B genotypes 2 through 4
- Verification of the linear range for genotypes 2 through 4

Verification of limit of detection for the 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 results are shown in Table 13. These results verify that **cobas**® CMV detected CMV DNA for three different genotypes at concentrations of 34.5 IU/mL with a hit rate of ≥ 95%.

Table 13 CMV DNA genotype verification of limit of detection

	17.25 IU/mL			34.5 IU/mL			51.75 IU/mL		
Genotype	**Number of valid replicates**	**Number of positives**	**Hit rate in % (95%CI*)**	**Number of valid replicates**	**Number of positives**	**Hit rate in % (95%CI*)**	**Number of valid replicates**	**Number of positives**	**Hit rate in % (95%CI*)**
gB-2	63	61	96.8 (99.6 %)	63	63	100.0 (100.0)	63	63	100.0 (100.0)
gB-3	63	57	90.5 (96.4%)	63	63	100.0 (100.0)	63	63	100.0 (100.0)
gB-4	63	55	87.3 (94.4%)	63	63	100.0 (100.0)	63	63	100.0 (100.0)

* Upper one-sided 95% confidence interval

Verification of linear range for genotypes gB-2, gB-3 and gB-4

The dilution series used in the verification of genotypes linearity study of **cobas**® CMV consisted of seven panel members spanning the assay linear range. Testing was conducted with two lots of **cobas**® CMV reagent, 16 replicates per level were tested in EDTA plasma.

The linear range of **cobas**® CMV was verified for all three genotypes (gB-2, gB-3 and gB-4).

Drug resistant CMV specimens verification

The performance of **cobas**® CMV on CMV drug resistant specimens was evaluated by:

- Verification of the limit of detection for drug resistant CMV specimens (resistant against Ganciclovir, Valganciclovir, Cidofovir or Foscarnet)
- Verification of the linear range for drug resistant CMV specimens (resistant against Ganciclovir, Valganciclovir, Cidofovir or Foscarnet)

Verification of limit of detection for the drug resistant CMV specimens (resistant against Foscarnet or Ganciclovir, Valganciclovir and Cidofovir)

Cell culture supernatants for two different drug resistant CMV specimens (resistant against Foscarnet or 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 results are shown in Table 14. These results verify that **cobas**® CMV detected CMV DNA for two different specimens resistant against Foscarnet or Ganciclovir, Valganciclovir and Cidofovir at concentrations of 34.5 IU/mL with a hit rate of ≥ 95%.

Table 14 Drug resistant CMV specimens verification of limit of detection

Drug resistance	Mutation site in UL54	17.25 IU/mL			34.5 IU/mL			51.75 IU/mL		
		Number of valid replicates	Number of positives	Hit rate in % (95%CI*)	Number of valid replicates	Number of positives	Hit rate in % (95%CI*)	Number of valid replicates	Number of positives	Hit rate in % (95%CI*)
Forscarnet	E756Q	63	58	92.1 (97.4 %)	63	63	100.0 (100.0)	63	63	100.0 (100.0)
Ganciclovir, Valganciclovir, Cidofovir	L545S	63	59	93.7 (98.2%)	63	63	100.0 (100.0)	63	63	100.0 (100.0)

* Upper one-sided 95% confidence interval

Verification of linear range for CMV drug resistant specimens (resistant against Foscarnet or Ganciclovir, Valganciclovir and Cidofovir)

The dilution series used in the verification of CMV drug resistant specimens linearity study of **cobas**® CMV consisted of seven panel members spanning the assay linear range. Testing was conducted with two lots of **cobas**® CMV reagent, 16 replicates per level were tested in EDTA plasma.

The linear range of **cobas**® CMV was verified for all two CMV drug resistant specimens (resistant against Foscarnet or Ganciclovir, Valganciclovir and Cidofovir).

Specificity

The specificity of **cobas**® CMV was determined by analyzing CMV negative EDTA plasma samples from individual donors. Six hundred and eight individual EDTA plasma samples were tested with two lots of **cobas**® CMV reagents. All samples tested negative for CMV DNA. In the test panel the specificity of **cobas**® CMV was 100% (lower one-sided 95% confidence limit: 99.5%).

Analytical specificity

The analytical specificity of **cobas**® CMV was evaluated by diluting a panel of microorganisms to a concentration of 1.00E+06 particles, copies, IU, genome equivalents or CFU/mL with CMV DNA positive and CMV DNA negative EDTA plasma. The specific organisms tested are listed in Table 15. Each panel member was evaluated with **cobas**® CMV. None of the non-CMV pathogens were shown to interfere with test performance.

Table 15 Microorganisms tested for cross-reactivity

Viruses	Bacteria	Yeast
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		

Analytical specificity – interfering substances

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

The impact of the presence of autoimmune diseases such as systemic lupus erythematosus (SLE), rheumatoid arthritis (RA) and antinuclear antibody was also evaluated in the presence and absence of CMV DNA. In addition, drug compounds listed in Table 16 were tested at three times the Cmax in presence and absence of CMV DNA.

All potentially interfering substances have been shown to not interfere with the test performance.

Table 16 Drug compounds tested for interference with the quantitation of CMV DNA by **cobas**® CMV

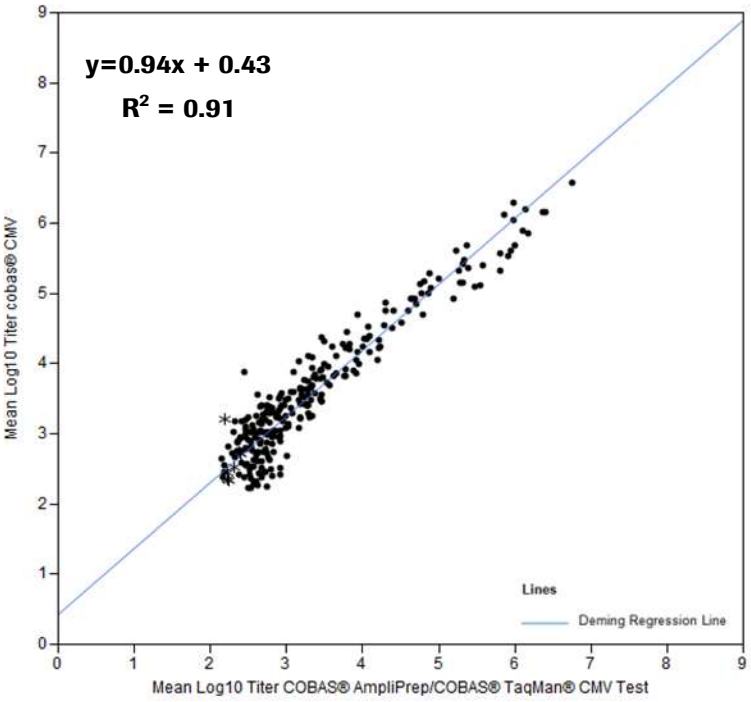
Class of drug	Generic drug name	
Antibimicrobial	Cefotetan	Sulfamethoxazole
	Clavulanate potassium	Ticarcillin disodium
	Fluconazole	Trimethoprim
	Piperacillin	Vancomycin
	Tazobactam sodium	
Compounds for Treatment of Herpes Viruses	Ganciclovir	Cidofovir
	Valganciclovir	Foscarnet
Immune suppressant	Azathioprine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

Performance compared to COBAS® AmpliPrep/COBAS® TaqMan® CMV Test

The performance of the **cobas**® CMV test and the COBAS® AmpliPrep/COBAS® TaqMan® CMV Test were compared by analysis of EDTA plasma specimens from CMV-infected patients. A total of 275 EDTA plasma specimens tested in duplicate and representing all CMV genotypes were valid and within the quantitation range of both tests. Deming regression analysis was performed.

The Deming regression results are shown in Figure 4.

Figure 4 Regression analysis of **cobas**® CMV vs CAP/CTM CMV Quantitative Test



Whole system failure

The whole system failure rate for **cobas**® CMV was determined by testing 100 replicates of EDTA plasma spiked with a CMV positive clinical specimen. These samples were tested at a concentration of approximately 3 x LoD.

The results of this study determined that all replicates were valid and positive for the CMV target, resulting in a whole system failure rate of 0% (95% confidence interval 0%-3.6%).

Cross contamination

The cross-contamination rate for **cobas**® CMV was determined by testing 240 replicates of a normal, CMV 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 17 through Table 19 below.

Table 17 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 17 Attributable percentage of total variance (%TV), total precision standard deviation (SD), and lognormal CV(%) of CMV DNA concentration (log_{10} IU/mL) by positive panel member

Expected CMV DNA Conc. (log_{10} IU/mL)	Observed Mean[a] CMV DNA Conc. (log_{10} IU/mL)	No. of Tests[b]	Lot %TV[c] (CV%)[e] SD[d]	Site %TV[c] (CV%)[e] SD[d]	Operator /Day %TV[c] (CV%)[e] SD[d]	Run %TV[c] (CV%)[e] SD[d]	Within -Run %TV[c] (CV%)[e] SD[d]	Total Precision SD[f]	Total Precision (CV%)[g]
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] %TV = Percent contribution to Total Variance

[d] Calculated using variance component from the SAS MIXED procedure.

[e] CV% = Lognormal percent coefficient of variation = sqrt(10^[SD^2 * ln(10)] - 1) * 100

[f] Calculated using total variability from the SAS MIXED procedure.

[g] Calculated using total variability from the SAS MIXED procedure.

DNA = deoxyribonucleic acid; CMV = cytomegalovirus; Conc. = concentration; SD = standard deviation; sqrt = square root; No. = number

Table 18 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 18 Detectable viral load difference by positive panel member

Expected CMV DNA Conc. (log_{10} IU/mL)	Observed Mean CMV DNA Conc. (log_{10} IU/mL)	No. of Tests[a]	Total Precision Standard Deviation (log_{10} IU/mL)	Standard Deviation of Difference Between Two Measurements[b]	95% CL[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 = Confidence Limit = 1.96 * standard deviation of difference between two measurements.

[d] Detectable Fold Difference = 10^(1.96 * sqrt(2 * (total standard deviation)^2)).

DNA = deoxyribonucleic acid; CMV = cytomegalovirus; No. = number; sqrt = square root.

Table 19 below presents the reproducibility results for the negative panel member for the **cobas**® 6800 System.

Table 19 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.

DNA = deoxyribonucleic acid; CMV = cytomegalovirus; CI = confidence interval.

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 (referred to as TaqMan® CMV) 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 20.

Table 20 Demographics and baseline clinical characteristics of SOT subjects

Characteristics	Groups	Statistics
Number of Subjects	Total, N	107
Age (years)	Mean ± SD	49 ± 13.6
Age (years)	Median	50
Age (years)	Range	18 - 76
Gender, n(%)	Male	74 (69.2%)
Gender, n(%)	Female	33 (30.8%)
Ethnicity, n(%)	Hispanic / Latino	10 (9.3%)
Ethnicity, n(%)	Not Hispanic / Not Latino	91 (85.0%)
Ethnicity, n(%)	Unknown	6 (5.6%)
Race, n(%)	Asian	1 (0.9%)
Race, n(%)	Black / African-American	16 (15.0%)
Race, n(%)	White	88 (82.2%)
Race, n(%)	Other	2 (1.9%)
Immunosuppression Induction, n(%)	Yes	26 (24.3%)
Immunosuppression Induction, n(%)	No	81 (75.7%)
Study Arm, n(%)	Anti-CMV Prophylaxis Regimen	53 (49.5%)
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 21 through Table 24 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+03 IU/mL, respectively from evaluable samples collected on the day of or immediately prior to treatment initiation.

Table 21 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 cobas® CMV	TaqMan® CMV Test Target Not Detected	TaqMan® CMV Test 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 22 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 cobas® CMV	TaqMan® CMV Test < 1.37E+02 IU/mL (< 2.137 $\log_{10}$ IU/mL)	TaqMan® CMV Test ≥ 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 23 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 cobas® CMV	TaqMan® CMV Test < 5.00E+02 IU/mL (< 2.699 $\log_{10}$ IU/mL)	TaqMan® CMV Test ≥ 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 24 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 cobas® CMV	**TaqMan® CMV Test < 1.8E+03 IU/mL (< 3.255 $\log_{10}$ IU/mL)**	**TaqMan® CMV Test ≥ 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 25 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 25 Concordance analysis of CMV episode resolution for subjects who initiated anti-CMV therapy in the SOT population

Time point Post Anti-CMV Therapy Initiation	cobas® CMV	TaqMan® CMV Test Resolution of CMV Episode[a]	TaqMan® CMV Test No Resolution of CMV Episode	Total	Row Agreement (95% Exact CI)
Day 14	Resolution of CMV Episode[a]	0	0	0	NC
Day 14	No Resolution of CMV Episode	0	40	40	100.0% (91.2%, 100.0%)
Day 14	Total	0	40	40	
Day 14	Column Agreement (95% Exact CI)	NC	100.0% (91.2%, 100.0%)	-	-
Day 14	Overall Percent Agreement (95% Exact CI)	100.0% (91.2%, 100.0%)	-	-	-
Day 14	p-value[b]	NC	-	-	-
Day 21	Resolution of CMV Episode[a]	0	0	0	NC
Day 21	No Resolution of CMV Episode	1	50	51	98.0% (89.6%, 100.0%)
Day 21	Total	1	50	51	
Day 21	Column Agreement (95% Exact CI)	0.0% (0.0%, 97.5%)	100.0% (92.9%, 100.0%)	-	-
Day 21	Overall Percent Agreement (95% Exact CI)	98.0% (89.6%, 100.0%)	-	-	-
Day 21	p-value[b]	NC	-	-	-
Day 28	Resolution of CMV Episode[a]	6	0	6	100.0% (54.1%, 100.0%)
Day 28	No Resolution of CMV Episode	4	46	50	92.0% (80.8%, 97.8%)
Day 28	Total	10	46	56	-
Day 28	Column Agreement (95% Exact CI)	60.0% (26.2%, 87.8%)	100.0% (92.3%, 100.0%)	-	-
Day 28	Overall Percent Agreement (95% Exact CI)	92.9% (82.7%, 98.0%)	-	-	-

Time point Post Anti-CMV Therapy Initiation	cobas® CMV	TaqMan® CMV Test Resolution of CMV Episode[a]	TaqMan® CMV Test No Resolution of CMV Episode	Total	Row Agreement (95% Exact CI)
Day 28	p-value[b]	0.1250	-	-	-
Day 35	Resolution of CMV Episode[a]	16	1	17	94.1% (71.3%, 99.9%)
Day 35	No Resolution of CMV Episode	8	31	39	79.5% (63.5%, 90.7%)
Day 35	Total	24	32	56	-
Day 35	Column Agreement (95% Exact CI)	66.7% (44.7%, 84.4%)	96.9% (83.8%, 99.9%)	-	-
Day 35	Overall Percent Agreement (95% Exact CI)	83.9% (71.7%, 92.4%)	-	-	-
Day 35	p-value[b]	0.0391	-	-	-
Day 49	Resolution of CMV Episode[a]	38	0	38	100.0% (90.7%, 100.0%)
Day 49	No Resolution of CMV Episode	7	12	19	63.2% (38.4%, 83.7%)
Day 49	Total	45	12	57	-
Day 49	Column Agreement (95% Exact CI)	84.4% (70.5%, 93.5%)	100.0% (73.5%, 100.0%)	-	-
Day 49	Overall Percent Agreement (95% Exact CI)	87.7% (76.3%, 94.9%)	-	-	-
Day 49	p-value[b]	0.0156	-	-	-

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

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

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

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

Among the subjects included in Day49 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 26).

Table 26 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 27 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 27 Summary of concordance analyses (all paired samples) in the SOT population

All Paired Samples cobas® CMV (log_{10} IU/mL)	TaqMan® CMV Test (log_{10} IU/mL) Target Not Detected	TaqMan® CMV Test (log_{10} IU/mL) < 2.137	TaqMan® CMV Test (log_{10} IU/mL) 2.137 to < 2.699	TaqMan® CMV Test (log_{10} IU/mL) 2.699 to < 3.255	TaqMan® CMV Test (log_{10} IU/mL) 3.255 to < 3.899	TaqMan® CMV Test (log_{10} IU/mL) ≥ 3.899	TaqMan® CMV Test (log_{10} IU/mL) 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 28 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 28 Summary of concordance of viral load results by different thresholds for all paired samples in the SOT population

All Paired Samples cobas® CMV	**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 29 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 29 Summary of concordance analyses (paired samples at timepoints of interest post anti-CMV therapy initiation) in the SOT population

All time points of interest cobas® CMV (log_{10} IU/mL)	**TaqMan® CMV Test (log_{10} IU/mL) Target Not Detected**	**TaqMan® CMV Test (log_{10} IU/mL) < 2.137**	**TaqMan® CMV Test (log_{10} IU/mL) 2.137 to < 2.699**	**TaqMan® CMV Test (log_{10} IU/mL) 2.699 to < 3.255**	**TaqMan® CMV Test (log_{10} IU/mL) 3.255 to < 3.899**	**TaqMan® CMV Test (log_{10} IU/mL) ≥ 3.899**	**TaqMan® CMV Test (log_{10} IU/mL) 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 30 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 30 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

All time points of interest cobas® CMV	**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 31along with Figure 5 through Figure 7 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 31 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
Clinical and Spiked	543	Slope	0.961 0.945*	0.009	(0.944, 0.979) (0.933, 0.957)	0.98
Clinical	445	Intercept	0.193 0.229*	0.037	(0.120, 0.266) (0.160, 0.301)	0.97
Clinical	445	Slope	1.023 1.010*	0.010	(1.002, 1.044) (0.992, 1.030)	0.97
Spiked	98	Intercept	0.012 N/A	0.063	(-0.114, 0.138) N/A	0.99
Spiked	98	Slope	0.985 N/A	0.013	(0.960, 1.010) N/A	0.99

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.

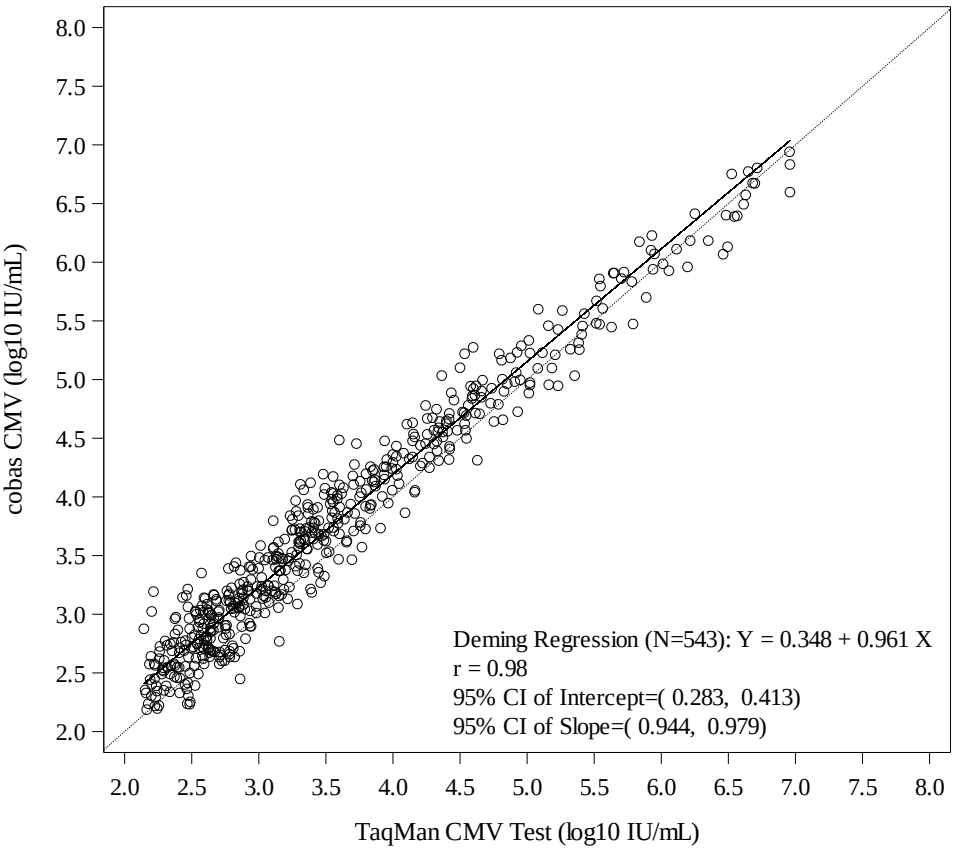
[a] Assumed independence between all samples.

[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.

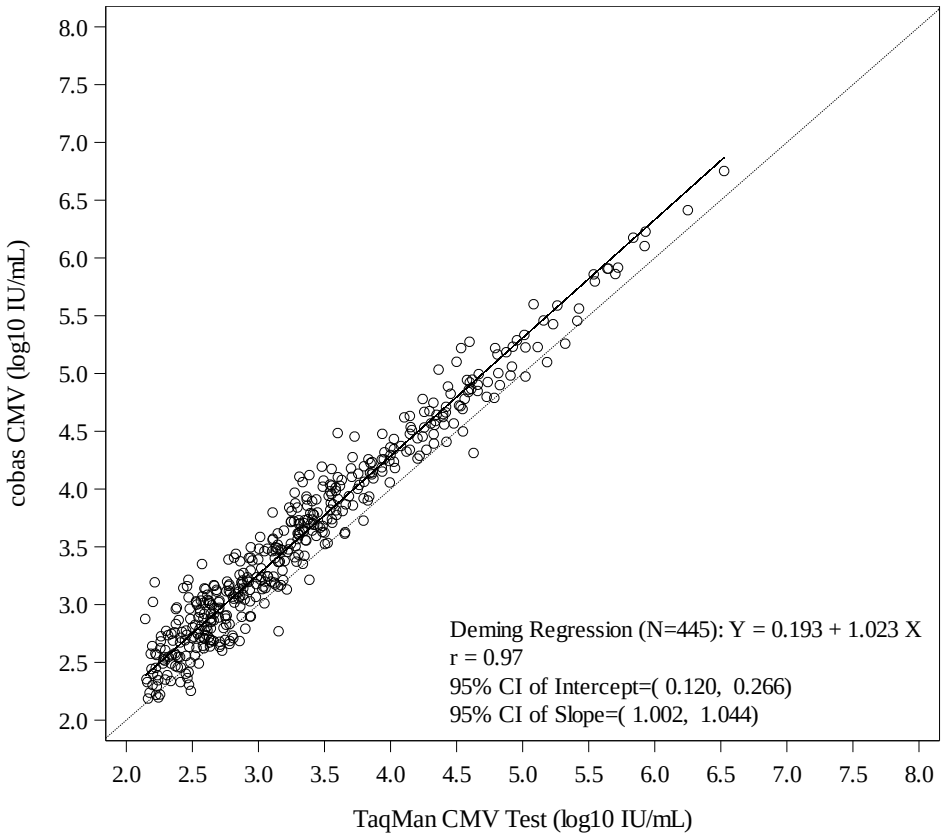
Figure 5 Deming linear regression plot of viral loads (log_{10} IU/mL) in the SOT population (**cobas**® CMV Versus TaqMan® CMV Test; clinical and spiked samples)



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.

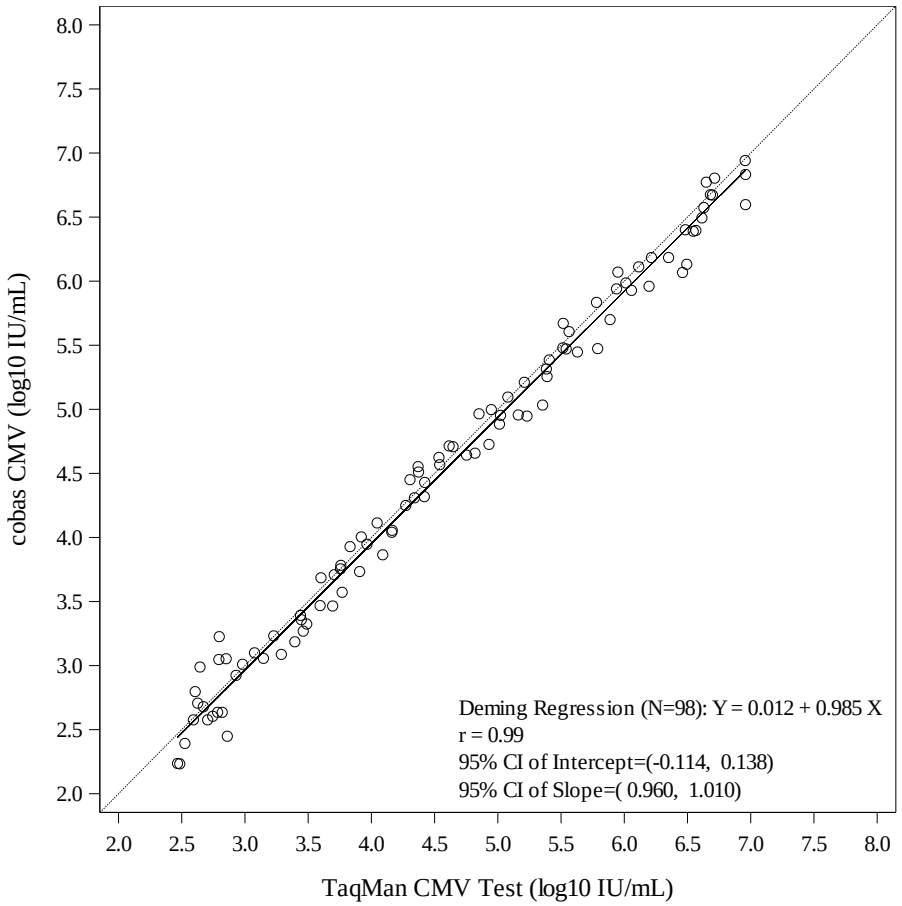
Figure 6 Deming linear regression plot of viral loads (log_{10} IU/mL) in the SOT population (**cobas**® CMV Versus TaqMan® CMV Test; clinical samples)



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 7 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 32below 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 32 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[a]
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)
Clinical and Spiked	2.699 $\log_{10}$ IU/ml (5.00E+02 IU/ml)	0.243 $\log_{10}$ IU/ml (3.74E+02 IU/mL)
Clinical and Spiked	3.255 $\log_{10}$ IU/ml (1.80E+03 IU/ml)	0.221 $\log_{10}$ IU/ml (1.19E+03 IU/mL)
Clinical and Spiked	4.000 $\log_{10}$ IU/ml (1.00E+04 IU/ml)	0.192 $\log_{10}$ IU/ml (5.56E+03 IU/mL)
Clinical and Spiked	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)
Clinical	2.699 $\log_{10}$ IU/ml (5.00E+02 IU/ml)	0.255 $\log_{10}$ IU/ml (4.00E+02 IU/mL)
Clinical	3.255 $\log_{10}$ IU/ml (1.80E+03 IU/ml)	0.268 $\log_{10}$ IU/ml (1.53E+03 IU/mL)
Clinical	4.000 $\log_{10}$ IU/ml (1.00E+04 IU/ml)	0.285 $\log_{10}$ IU/ml (9.28E+03 IU/mL)
Clinical	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)
Spiked	2.699 $\log_{10}$ IU/ml (5.00E+02 IU/ml)	-0.028 $\log_{10}$ IU/ml (-3.17E+01 IU/mL)
Spiked	3.255 $\log_{10}$ IU/ml (1.80E+03 IU/ml)	-0.037 $\log_{10}$ IU/ml (-1.46E+02 IU/mL)
Spiked	4.000 $\log_{10}$ IU/ml (1.00E+04 IU/ml)	-0.048 $\log_{10}$ IU/ml (-1.05E+03 IU/mL)
Spiked	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 $10^{(\textbf{cobas}^{\text{®}}\text{ CMV estimate log10 IU/mL})} - 10^{(\text{TaqMan}^{\text{®}}\text{ CMV Test Viral Load Level log10 IU/mL})}$.

Mean paired difference

Table 33 below presents the mean paired difference between **cobas**® CMV and the TaqMan® CMV Test at representative thresholds and associated 95% CIs calculated using the paired t-test.[29]

Table 33 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)
Clinical and Spiked	2.0E+03 to < 2.0E+04	143	0.260	0.019	(0.223, 0.296)
Clinical and Spiked	2.0E+04 to < 1.0E+05	62	0.195	0.025	(0.145, 0.245)
Clinical and Spiked	≥ 1.0E+05	63	0.012	0.025	(-0.039, 0.062)
Clinical and Spiked	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)
Clinical	2.0E+03 to < 2.0E+04	122	0.317	0.016	(0.285, 0.350)
Clinical	2.0E+04 to < 1.0E+05	47	0.251	0.027	(0.196, 0.305)
Clinical	≥ 1.0E+05	23	0.201	0.030	(0.139, 0.262)
Clinical	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)
Spiked	2.0E+03 to < 2.0E+04	21	-0.074	0.024	(-0.125, -0.024)
Spiked	2.0E+04 to < 1.0E+05	15	0.021	0.031	(-0.045, 0.086)
Spiked	≥ 1.0E+05	40	-0.097	0.022	(-0.141, -0.053)
Spiked	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 34 along with Figure 8 through Figure 10 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 34 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)
Clinical and Spiked	Medium	2.0E+03 to < 8.0E+03	89.6% (103/115)
Clinical and Spiked	High	8.0E+03 to 9.10E+06	95.5% (170/178)
Clinical and Spiked	Overall	-	94.3% (512/543)
Clinical	Low	1.37E+02 to < 2.0E+03	95.2% (216/227)
Clinical	Medium	2.0E+03 to < 8.0E+03	88.2% (90/102)
Clinical	High	8.0E+03 to 9.10E+06	93.1% (108/116)
Clinical	Overall	-	93.0% (414/445)
Spiked	Low	1.37E+02 to < 2.0E+03	100.0% (23/23)
Spiked	Medium	2.0E+03 to < 8.0E+03	100.0% (13/13)
Spiked	High	8.0E+03 to 9.10E+06	100.0% (62/62)
Spiked	Overall	-	100.0% (98/98)

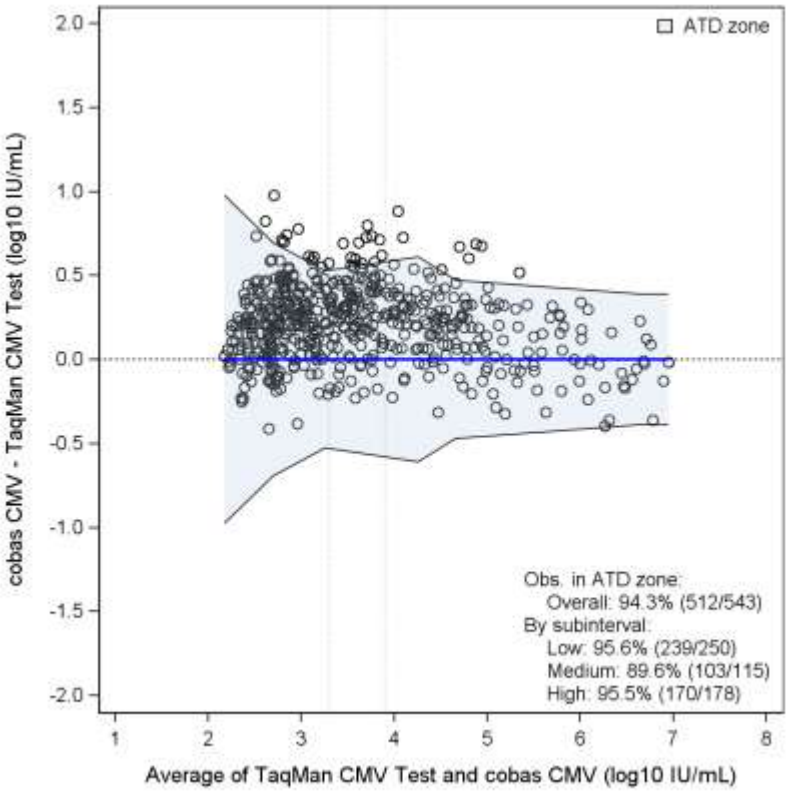
Note: Twenty-six samples from nine subjects were excluded from method comparison analyses due to impactful sequence mismatch. The table only includes paired samples with paired results that were each within 1.37E+02 IU/mL to 9.1E+06 IU/mL, the overlapping linear range of both assays. Paired results were 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 $\log_{10}$ 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.

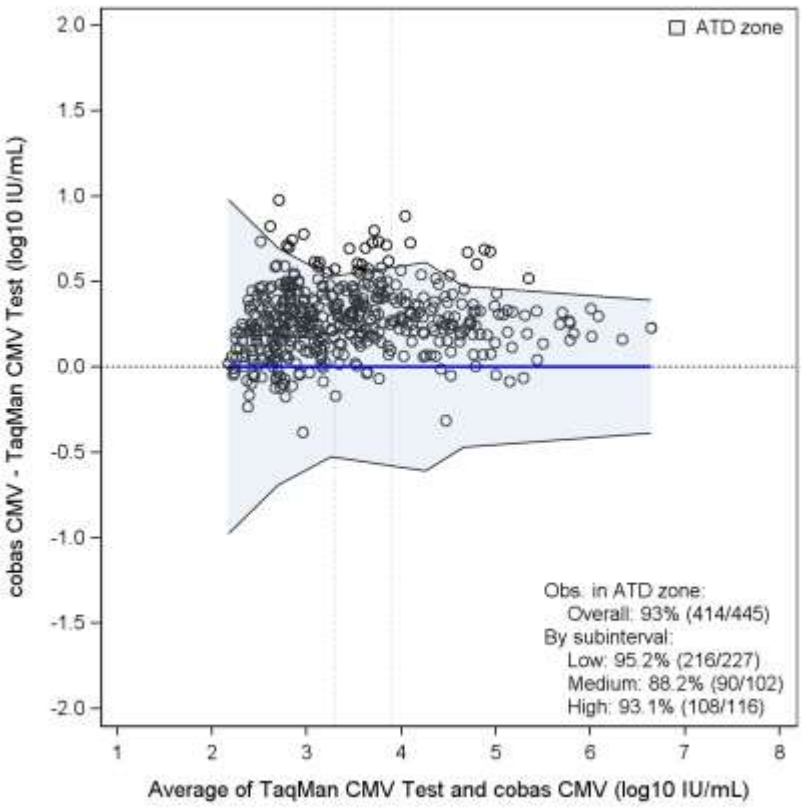
Figure 8 Allowable Total Difference (ATD) plot of individual viral load differences versus their average (log_{10} IU/mL) in the SOT population (**cobas**® CMV versus TaqMan® CMV Test; clinical and spiked samples)



ATD = allowable total difference; Obs. = observations.

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).

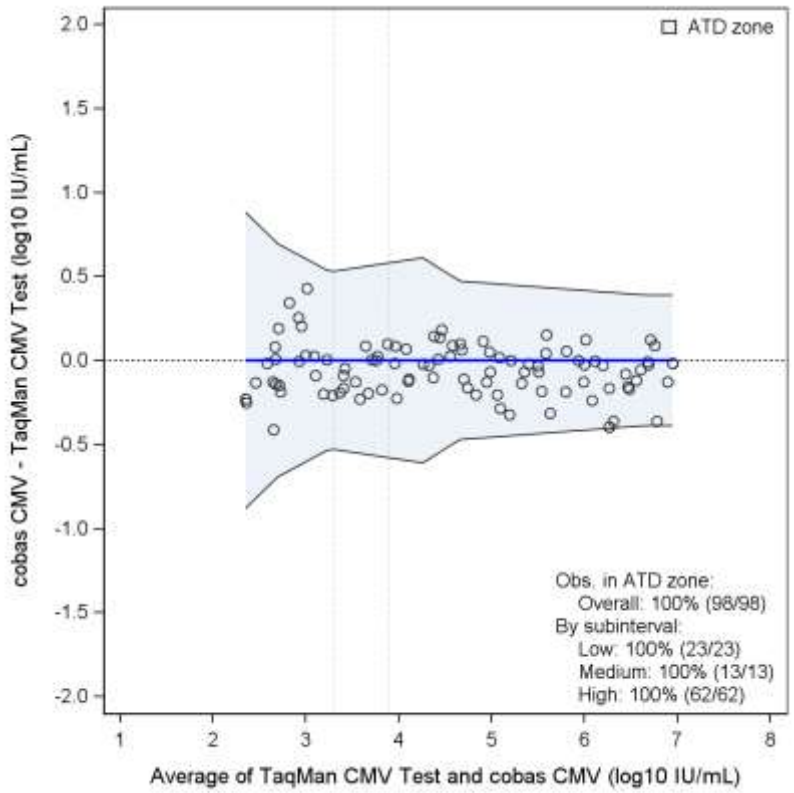
Figure 9 Allowable Total Difference (ATD) plot of individual viral load differences versus their average (log_{10} IU/mL) in the SOT population (**cobas**® CMV versus TaqMan® CMV Test; clinical samples)



ATD = allowable total difference; Obs. = observations.

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 10 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)



ATD = allowable total difference; Obs. = observations.

Agreement with negative samples

Thirty CMV IgG negative samples were tested on each assay and results are presented in Table 35.

Table 35 Results of CMV IgG-negative specimens (**cobas**® CMV versus TaqMan® CMV Test)

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

Note: The lower limit of quantitation (LLoQ) is 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[21] 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 36 below summarizes the demographics and baseline clinical characteristics of the 258 subjects.

Table 36 Demographics and baseline clinical characteristics of HSCT subjects

Characteristic	Groups	Statistics
Total number of Subjects	Total, N	258
Age (years)	Mean ± SD	51 ± 12.3
Age (years)	Median	51
Age (years)	Range	21 - 71
Gender, n(%)	Male	144 (55.8%)
Gender, n(%)	Female	114 (44.2%)
Ethnicity, n(%)	Hispanic / Latino	24 (9.3%)
Ethnicity, n(%)	Not Hispanic / Not Latino	230 (89.1%)
Ethnicity, n(%)	Unknown	4 (1.6%)
Race, n(%)	Asian	15 (5.8%)
Race, n(%)	Black / African-American	10 (3.9%)
Race, n(%)	White	228 (88.4%)
Race, n(%)	Other	5 (1.9%)
Study Arm, n(%)	Anti-CMV Prophylaxis Regimen	164 (63.6%)
Study Arm, n(%)	Placebo	61 (23.6%)
Study Arm, n(%)	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 37 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 37 Concordance analysis of **cobas**® and TaqMan® CMV Test results using a threshold of target not detected in the HSCT population

Baseline cobas® CMV	TaqMan® CMV Test Target Not Detected	TaqMan® CMV Test 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 38 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 38 Concordance analysis of **cobas**® and TaqMan® CMV Test results using threshold 1.37E+02 IU/mL in the HSCT population

Baseline cobas® CMV	TaqMan® CMV Test < 1.37E+02 IU/mL (< 2.137 $\log_{10}$ IU/mL)	TaqMan® CMV Test ≥ 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 39 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 39 Concordance analysis of **cobas**® and TaqMan® CMV Test results using threshold 5.0E+02 IU/mL in the HSCT population

Baseline cobas® CMV	TaqMan® CMV Test < 5.0E+02 IU/mL (< 2.699 log_{10} IU/mL)	TaqMan® CMV Test ≥ 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 40 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 40 Concordance analysis of **cobas**® and TaqMan® CMV Test results using threshold 1.8 E+03 IU/mL in the HSCT population

Baseline cobas® CMV	TaqMan® CMV Test < 1.8E+03 IU/mL (< 3.255 log_{10} IU/mL)	TaqMan® CMV Test ≥ 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 41 below shows the concordance analyses of CMV episode resolution by time point for viremic subjects who initiated anti-CMV therapy.

Table 41 Concordance analysis of CMV episode resolution by time point for viremic HSCT subjects who initiated anti-CMV therapy

Time Point	cobas® CMV	TaqMan® CMV Test Resolution of CMV Episode[a]	TaqMan® CMV Test No Resolution of CMV Episode	Total	Row Agreement (95% Exact CI)
Day 14	Resolution of CMV Episode[a]	0	0	0	NC
Day 14	No Resolution of CMV Episode	0	14	14	100.0% (76.8%, 100.0%)
Day 14	Total	0	14	14	-
Day 14	Column Agreement (95% Exact CI)	NC	100.0% (76.8%, 100.0%)	-	-
Day 14	Overall Percent Agreement (95% Exact CI)	100.0% (76.8%, 100.0%)	-	-	-
Day 21	Resolution of CMV Episode[a]	1	0	1	100.0% (2.5%, 100.0%)
Day 21	No Resolution of CMV Episode	0	12	12	100.0% (73.5%, 100.0%)
Day 21	Total	1	12	13	-
Day 21	Column Agreement (95% Exact CI)	100.0% (2.5%, 100.0%)	100.0% (73.5%, 100.0%)	-	-
Day 21	Overall Percent Agreement (95% Exact CI)	100.0% (75.3%, 100.0%)	-	-	-
Day 28	Resolution of CMV Episode[a]	2	0	2	100.0% (15.8%, 100.0%)
Day 28	No Resolution of CMV Episode	0	7	7	100.0% (59.0%, 100.0%)
Day 28	Total	2	7	9	-
Day 28	Column Agreement	100.0% (15.8%, 100.0%)	100.0% (59.0%, 100.0%)		-
Day 28	Overall Percent Agreement	100.0% (66.4%, 100.0%)	-	-	-
Day 49	Resolution of CMV Episode [a]	3	0	3	100.0% (29.2%, 100.0%)
Day 49	No Resolution of CMV Episode	0	1	1	100.0% (2.5%, 100.0%)

Time Point	cobas® CMV	TaqMan® CMV Test Resolution of CMV Episode[a]	TaqMan® CMV Test No Resolution of CMV Episode	Total	Row Agreement (95% Exact CI)
Day 49	Total	3	1	4	-
Day 49	Column Agreement	100.0% (29.2%, 100.0%)	100.0% (2.5%, 100.0%)	-	-
Day 49	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 42 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 42 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 43 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 43 Overall agreement between viral load results of **cobas**® CMV and TaqMan® CMV in the HSCT population

All Paired Samples cobas® CMV (log_{10} IU/mL)	**TaqMan® CMV Test (log_{10} IU/mL) Target Not Detected**	**TaqMan® CMV Test (log_{10} IU/mL) < 2.137**	**TaqMan® CMV Test (log_{10} IU/mL) 2.137 to < 2.699**	**TaqMan® CMV Test (log_{10} IU/mL) 2.699 to < 3.255**	**TaqMan® CMV Test (log_{10} IU/mL) 3.255 to < 3.899**	**TaqMan® CMV Test (log_{10} IU/mL) ≥ 3.899**	**TaqMan® CMV Test (log_{10} IU/mL) 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.

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.

Table 44 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 44 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 log_{10} 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 log_{10} 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 log_{10} 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; LOD = limit of detection.

Table 45 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 45 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 cobas® CMV (log_{10} IU/mL)	TaqMan® CMV Test (log_{10} IU/mL)Target Not Detected	TaqMan® CMV Test (log_{10} IU/mL) < 2.137	TaqMan® CMV Test (log_{10} IU/mL) 2.137 to < 2.699	TaqMan® CMV Test (log_{10} IU/mL) 2.699 to < 3.255	TaqMan® CMV Test (log_{10} IU/mL) 3.255 to < 3.899	TaqMan® CMV Test (log_{10} IU/mL) ≥ 3.899	TaqMan® CMV Test (log_{10} IU/mL) 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.
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.

Table 46 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 46 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 ($2.137 \log_{10}$ IU/mL)	**97.3%** (85.8%, 99.9%) (36/37)	**100.0%** (63.1%, 100.0%) (8/8)	**97.8%** (88.2%, 99.9%) (44/45)
5.0E+02 IU/mL ($2.699 \log_{10}$ IU/mL)	**92.3%** (79.1%, 98.4%) (36/39)	**100.0%** (54.1%, 100.0%) (6/6)	**93.3%** (81.7%, 98.6%) (42/45)
1.8E+03 IU/mL ($3.255 \log_{10}$ IU/mL)	**95.3%** (84.2%, 99.4%) (41/43)	**100.0%** (15.8%, 100.0%) (2/2)	**95.6%** (84.9%, 99.5%) (43/45)

Note: 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; LOD = limit of detection.

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 47 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 47 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
Clinical and Spiked	204	Slope	0.990 0.982*	0.009	(0.972, 1.008) (0.972, 0.990)	0.99
Clinical	107	Intercept	-0.146 -0.188*	0.106	(-0.356, 0.064) (-0.462, -0.008)	0.96
Clinical	107	Slope	1.110 1.125*	0.034	(1.041, 1.178) (1.066, 1.217)	0.96
Spiked	97	Intercept	-0.097 N/A	0.063	(-0.223, 0.028) N/A	0.99
Spiked	97	Slope	1.025 N/A	0.012	(1.000, 1.049) N/A	0.99

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.

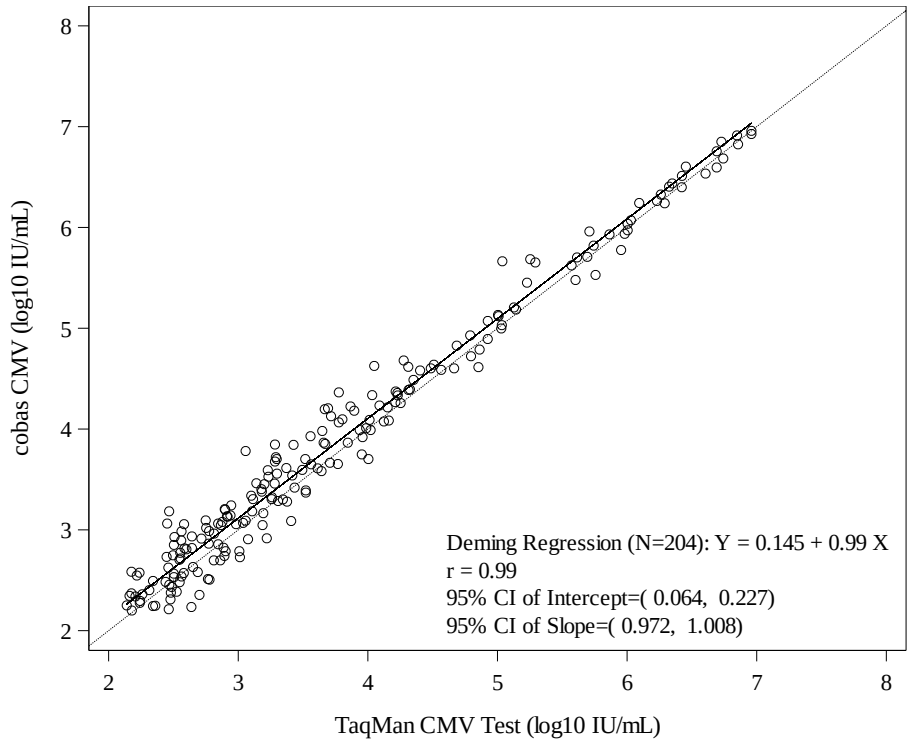
[b] Adjusted correlation between samples from the same subject by the bootstrap method with 500 iterations.

* 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 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 and spiked samples combined.

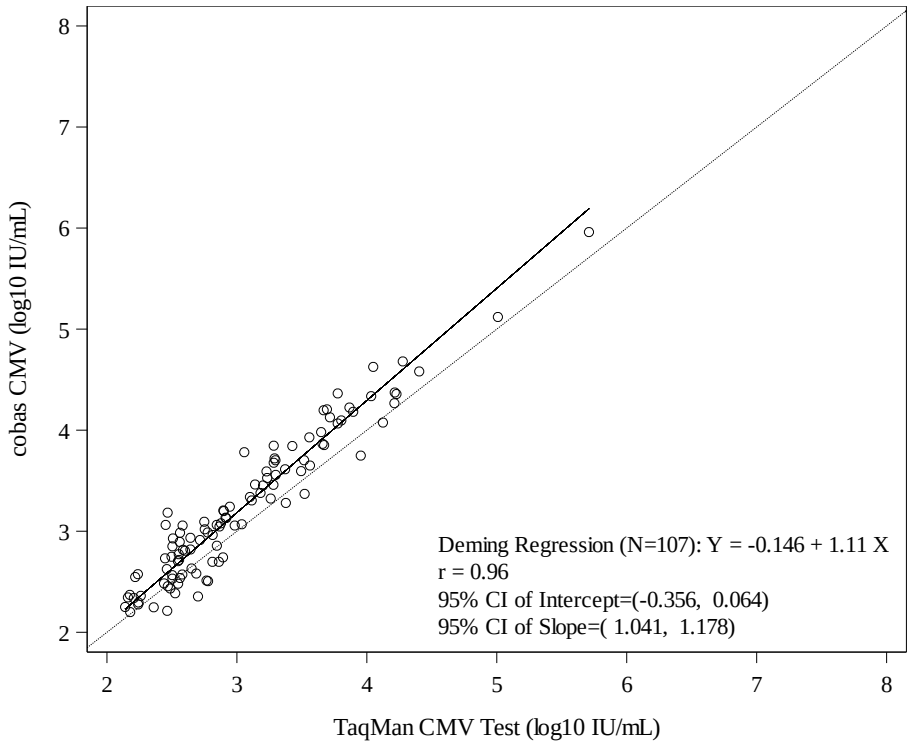
Figure 11 Deming linear regression plot of viral loads (log_{10} IU/mL) in the HSCT population (clinical and spiked samples)



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

Figure 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 clinical samples.

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

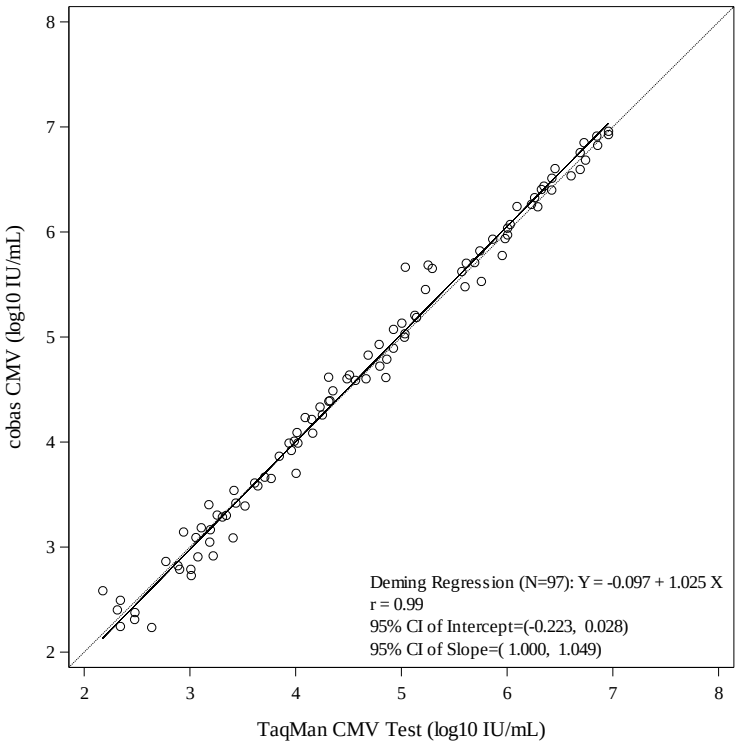


Note: Seven samples from three subjects were excluded from method comparison analyses due to impactful sequence mismatch.

CI = confidence interval; r = correlation coefficient.

Figure 13 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 13 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 48 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 48 Bias between **cobas**® CMV and TaqMan® CMV Test (log_{10} 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 log_{10} IU/ml (1.37E+02 IU/ml)	0.124 log_{10} IU/ml (4.51E+01 IU/mL)
Clinical and Spiked	2.699 log_{10} IU/ml (5.00E+02 IU/ml)	0.118 log_{10} IU/ml (1.56E+02 IU/mL)
Clinical and Spiked	3.255 log_{10} IU/ml (1.80E+03 IU/ml)	0.112 log_{10} IU/ml (5.32E+02 IU/mL)
Clinical and Spiked	4.000 log_{10} IU/ml (1.00E+04 IU/ml)	0.105 log_{10} IU/ml (2.74E+03 IU/mL)
Clinical and Spiked	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)	0.089 log_{10} IU/ml (3.12E+01 IU/mL)
Clinical	2.699 log_{10} IU/ml (5.00E+02 IU/ml)	0.151 log_{10} IU/ml (2.08E+02 IU/mL)
Clinical	3.255 log_{10} IU/ml (1.80E+03 IU/ml)	0.212 log_{10} IU/ml (1.13E+03 IU/mL)
Clinical	4.000 log_{10} IU/ml (1.00E+04 IU/ml)	0.294 log_{10} IU/ml (9.68E+03 IU/mL)
Clinical	7.000 log_{10} IU/ml (1.00E+07 IU/ml)	0.624 log_{10} IU/ml (3.21E+07 IU/mL)
Spiked	2.137 log_{10} IU/ml (1.37E+02 IU/ml)	-0.044 log_{10} IU/ml (-1.31E+01 IU/mL)
Spiked	2.699 log_{10} IU/ml (5.00E+02 IU/ml)	-0.030 log_{10} IU/ml (-3.29E+01 IU/mL)
Spiked	3.255 log_{10} IU/ml (1.80E+03 IU/ml)	-0.016 log_{10} IU/ml (-6.36E+01 IU/mL)
Spiked	4.000 log_{10} IU/ml (1.00E+04 IU/ml)	0.003 log_{10} IU/ml (6.93E+01 IU/mL)
Spiked	7.000 log_{10} IU/ml (1.00E+07 IU/ml)	0.078 log_{10} IU/ml (1.97E+06 IU/mL)

Mean paired difference

Table 49 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 49 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)
Clinical and Spiked	2.0E+03 to < 2.0E+04	49	0.121	0.032	(0.058, 0.184)
Clinical and Spiked	2.0E+04 to < 1.0E+05	16	0.061	0.033	(-0.009, 0.131)
Clinical and Spiked	1.0E+05 to 9.1E+06	41	0.062	0.024	(0.013, 0.110)
Clinical and Spiked	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)
Clinical	2.0E+03 to < 2.0E+04	27	0.241	0.041	(0.157, 0.326)
Clinical	2.0E+04 to < 1.0E+05	1	0.178	-	-
Clinical	1.0E+05 to 9.1E+06	2	0.181	0.070	(-0.705, 1.068)
Clinical	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)
Spiked	2.0E+03 to < 2.0E+04	22	-0.027	0.025	(-0.079, 0.025)
Spiked	2.0E+04 to < 1.0E+05	15	0.053	0.034	(-0.020, 0.126)
Spiked	1.0E+05 to 9.1E+06	39	0.056	0.025	(0.006, 0.106)
Spiked	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 50 below shows the percentage of results within low, medium and high intervals of the Allowable Total Difference zone by sample type.

Table 50 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)
Clinical and Spiked	Medium	2.0E+03 to < 8.0E+03	93.9% (31/33)
Clinical and Spiked	High	8.0E+03 to 9.1E+06	98.8% (81/82)
Clinical and Spiked	Overall	-	98.0% (200/204)
Clinical	Low	1.37E+02 to < 2.0E+03	98.5% (65/66)
Clinical	Medium	2.0E+03 to < 8.0E+03	91.3% (21/23)
Clinical	High	8.0E+03 to 9.1E+06	100.0% (18/18)
Clinical	Overall	-	97.2% (104/107)
Spiked	Low	1.37E+02 to < 2.0E+03	100.0% (23/23)
Spiked	Medium	2.0E+03 to < 8.0E+03	100.0% (10/10)
Spiked	High	8.0E+03 to 9.1E+06	98.4% (63/64)
Spiked	Overall	-	99.0% (96/97)

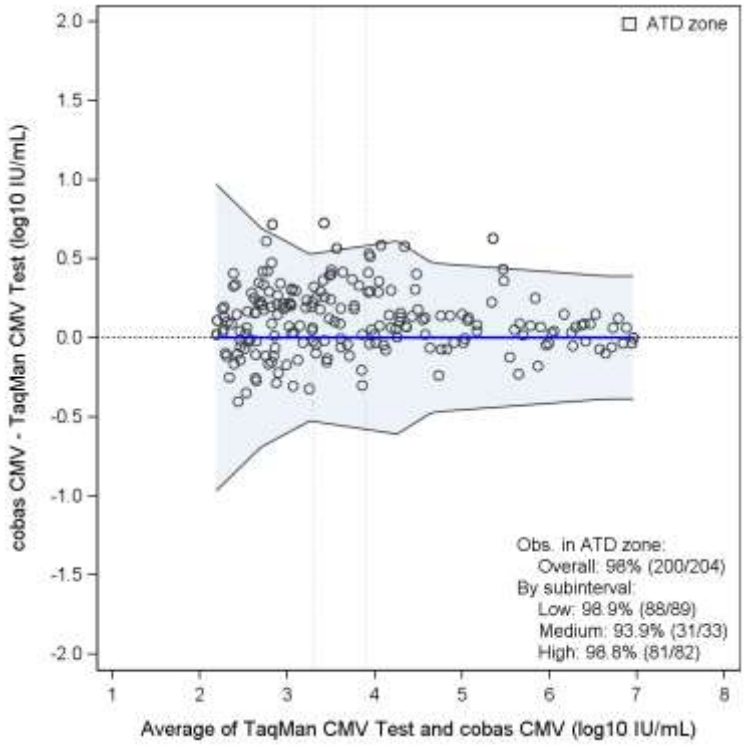
Note: Seven samples from three subjects were excluded from method comparison analyses due to impactful sequence mismatch. The table only included paired samples with results each within 1.37E+02 to 9.1E+06 IU/mL, the common linear range of both assays. Paired results were categorized into the intervals 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 $\log_{10}$ IU/mL are, respectively, 2.137 to < 3.301, 3.301 to < 3.903 and 3.903 to 6.959.

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 and spiked samples combined.

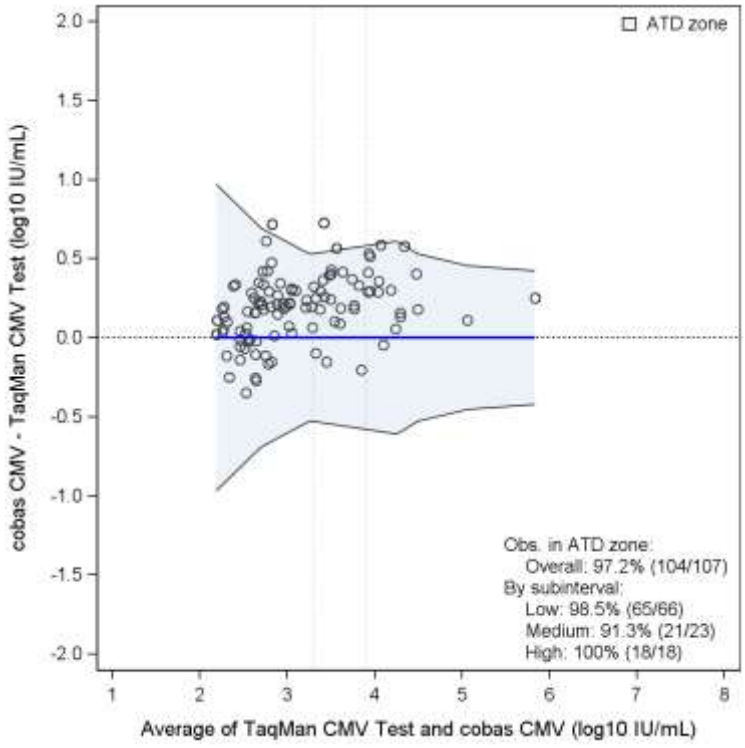
Figure 14 Allowable Total Difference (ATD) plot of viral load difference (log_{10} IU/mL) in the HSCT population (clinical and spiked samples)



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

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 clinical samples.

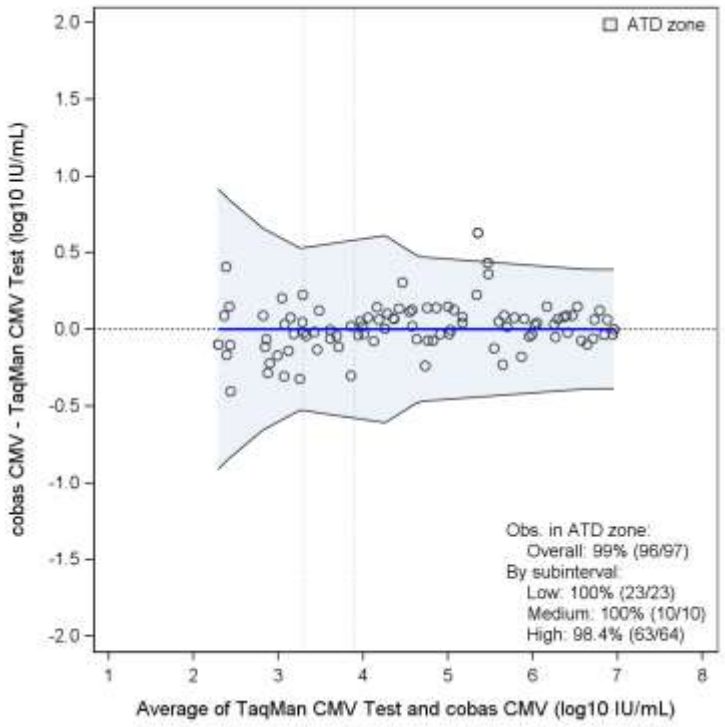
Figure 15 Allowable Total Difference (ATD) plot of viral load difference (log_{10} IU/mL) in the HSCT population (clinical samples)



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

Figure 16 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 16 Allowable Total Difference (ATD) plot of viral load difference (log_{10} IU/mL) in the HSCT population (spiked samples)



ATD = allowable total difference; Obs. = observations.

Agreement with negative samples

Thirty CMV IgG negative samples from HSCT patients were tested on each assay and their results are presented in Table 51 below.

Table 51 Results of CMV IgG-Negative Specimens Tested on **cobas**® CMV and TaqMan® CMV Test

cobas® CMV	**TaqMan® CMV Test Target Not Detected**	**TaqMan® CMV Test < 1.37E+02 IU/mL**	**TaqMan® CMV Test ≥ 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%
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 52 Symbols used in labeling for Roche PCR diagnostics products

Age/DOB	Age or Date of Birth
SW	Ancillary Software
Assigned Range [copies/mL]	Assigned Range (copies/mL)
Assigned Range [IU/mL]	Assigned Range (IU/mL)
EC REP	Authorized representative in the European Community
BARCODE	Barcode Data Sheet
LOT	Batch code
	Biological risks
REF	Catalogue number
Collect Date	Collect date
	Consult instructions for use
Σ	Contains sufficient for <*n*> tests
CONTENT	Contents of kit
CONTROL	Control
	Date of manufacture
D	Distributed by
	Do not re-use
	Female
	For IVD performance evaluation only
GTIN	Global Trade Item Number
IVD	*In vitro* diagnostic medical device
LLR	Lower Limit of Assigned Range
	Male
	Manufacturer
CONTROL −	Negative control
NON STERILE	Non sterile
#	Patient number
?	Patient Name


Peel here

CONTROL + Positive control

QS copies/PCR QS copies per PCR reaction, use the QS copies per PCR reaction in calculation of the results.

QS IU/PCR QS IU per PCR reaction, use the QS International Units (IU) per PCR reaction in calculation of the results.

SN Serial number

Site Site

Procedure Standard Standard Procedure

STERILE EO Sterilized using ethylene oxide


Store in the dark


Temperature limit

Test Definition File

CE CE marking of conformity; this device is in conformity with the applicable requirements for CE marking of an *in vitro* diagnostic medical device


This way up

UDI Unique Device Identification

Procedure UltraSensitive Ultrasensitive Procedure

ULR Upper Limit of Assigned Range

Urine Fill Line Urine Fill Line

Rx Only US Only: Federal law restricts this device to sale by or on the order of a physician.


Use-by date


Device for near-patient testing


Device Not for Near Patient Testing


Device for self-testing


Device not for self-testing

Technical Support

For technical support (assistance) please reach out to your local affiliate:
https://www.roche.com/about/business/roche_worldwide.htm

Manufacturer and distributors

Table 53 Manufacturer and distributors

Roche Molecular Systems, Inc.
1080 US Highway 202 South
Branchburg, NJ 08876 USA
www.roche.com

Made in USA

Roche Diagnostics GmbH
Sandhofer Strasse 116
68305 Mannheim, Germany

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

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