Rx Only

cobas® CMV

Quantitative nucleic acid test
for use on the cobas® 5800/6800/8800 Systems

For in vitro diagnostic use

cobas® CMV	P/N: 09040897190
For use on the cobas® 5800 System:	
cobas® CMV Control Kit	P/N: 09040919190
cobas® NHP Negative Control Kit	P/N: 09051554190
For use on the cobas® 6800/8800 Systems:	
cobas® CMV Control Kit	P/N: 07001037190 or P/N: 09040919190
cobas® NHP Negative Control Kit	P/N: 07002220190 or 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 management of CMV in solid organ transplant patients and in hematopoietic stem cell transplant patients. In patients receiving anti-CMV therapy, serial DNA measurements can be used to assess viral response to treatment.

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

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

Summary and explanation of the test

Background

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

Our current understanding of the relationship between CMV viremia and CMV disease in transplant patients comes from a variety of studies using different technologies, study populations, and end-points.[7-13] In general, higher viral loads are more closely associated with the risk of development of CMV disease. In patients with HIV/AIDS, CMV DNA levels have been correlated with the risk of CMV disease and overall mortality.[14-17] Current guidelines based on the precision of PCR tests suggest that the changes in serial viral load measurements should be at least 3-fold (0.5 $\log_{10}$) to represent biologically important changes.[10]

Historically, laboratory-developed methods of CMV DNA quantification have had a high degree of inter-laboratory and inter-assay variability.[18] The advent of an international standardization has improved comparability of assay results across laboratories, but discrepancies still exist due to commutability issues with the standard.

Rationale for NAT testing

Laboratory methods for diagnosing disseminated infection and active visceral disease for human CMV include isolation of virus by culture from peripheral blood leukocytes (PBL), histology on biopsies, serologic methods, measurement of pp65 antigenemia, and detection of CMV DNA by polymerase chain reaction (PCR).[19] 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.[20] 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**® 5800 System, **cobas**® 6800 System and **cobas**® 8800 System. **cobas**® CMV enables the detection and quantitation of CMV DNA in EDTA plasma of infected transplant patients. The viral load is quantified against a non-CMV DNA quantitation standard (DNA-QS), which is introduced into each specimen during sample processing. The DNA-QS also functions to monitor for the entire sample preparation and PCR amplification process. In addition, the test utilizes three external controls: a high titer positive, a low titer positive, and a negative control.

Principles of the procedure

cobas® CMV is based on fully automated sample preparation (nucleic acid extraction and purification) followed by PCR amplification and detection. The **cobas**® 5800 System is designed as one integrated instrument. 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**® 5800 or **cobas**® 6800/8800 System 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).[21-23] 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.[24,25] 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

The materials provided for **cobas**® CMV can be found in Table 1. Materials required, but not provided can be found in Table 2 through Table 4, Table 8 and Table 9.

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

Table 1 cobas® CMV

(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% (w/v) proteinase, glycerol EUH210: Safety data sheet 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, < 0.01% Z05D DNA polymerase (microbial), < 0.1% AmpErase (uracil-N- glycosylase) enzyme (microbial), < 0.1% sodium azide	9.7 mL

Table 2 cobas® CMV Control Kit

(CMV CTL)

Store at 2–8°C
For use on the **cobas**® 5800 System (P/N 09040919190)

For use on the **cobas**® 6800/8800 Systems (P/N 07001037190 or 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 0.5 mL)	 **WARNING** H317: May cause an allergic skin reaction. H412: Harmful to aquatic life with long lasting effects. P261: Avoid breathing mist or vapours. 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-2H-isothiazol-3-one and 2- methyl-2H-isothiazol-3-one (3:1)
CMV High Positive Control (CMV H(+)C)	< 0.001% high titered 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 0.5 mL)	 **WARNING** H317: May cause an allergic skin reaction. H412 Harmful to aquatic life with long lasting effects. P261: Avoid breathing mist or vapours. 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-2H-isothiazol-3-one and 2- methyl-2H-isothiazol-3-one (3:1)

* Product safety labeling primarily follows EU GHS guidance
** Hazardous substance or mixture

Table 3 cobas® NHP Negative Control Kit

(NHP-NC)

Store at 2-8°C
For use on the **cobas**® 5800 System (P/N 09051554190)

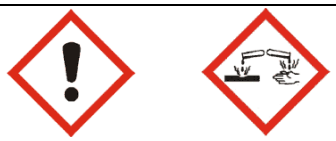
For use on the **cobas**® 6800/8800 Systems (P/N 07002220190 or P/N 09051554190)

Kit components	Reagent ingredients	Quantity per kit	Safety symbol and warning*
Normal Human Plasma Negative Control (NHP-NC)	Normal human plasma, non-reactive by licensed tests for antibody to HCV, antibody to HIV-1/2, HBsAg, antibody to HBc; HIV-1 RNA, HIV-2 RNA, HCV RNA, and HBV 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 or mixture

cobas® omni reagents for sample preparation

Table 4 cobas® omni reagents for sample preparation

Reagents	Reagent ingredients	Quantity per kit	Safety symbol and warning*
cobas® omni MGP Reagent (MGP) Store at 2–8°C (P/N 06997546190)	Magnetic glass particles, Tris buffer, 0.1% methyl-4 hydroxybenzoate, < 0.1% sodium azide	480 tests	Not applicable
cobas® omni Specimen Diluent (SPEC DIL) Store at 2–8°C (P/N 06997511190)	Tris buffer, 0.1% methyl-4 hydroxybenzoate, < 0.1% sodium azide	4 x 875 mL	Not applicable
cobas® omni Lysis Reagent (LYS) Store at 2–8°C (P/N 06997538190)	42.56% (w/w) guanidine thiocyanate**, 5% (w/v) polydocanol**, 2% (w/v) dithiothreitol**, dihydro sodium citrate	4 x 875 mL	 **DANGER** H302 + H332: Harmful if swallowed or if inhaled. H314: Causes severe skin burns and serious eye damage. H412: Harmful to aquatic life with long lasting effects. EUH032: Contact with acids liberates very toxic gas. P261: Avoid breathing dust/fume/gas/ mist/vapours/spray. P273: Avoid release to the environment. P280: Wear 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. Immediately 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

* Product safety labeling primarily follows EU GHS guidance

**Hazardous substance or mixture

Reagent storage and handling requirements

Reagents must be stored and handled as specified in Table 5, Table 6 and Table 7.

When reagents are not loaded on the **cobas**® 5800 or **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	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

Reagent handling requirements for the cobas® 5800 System

Reagents loaded onto the **cobas**® 5800 System are stored at appropriate temperatures and their expiration is monitored by the system. The system allows 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**® 5800 System.

Table 6 Reagent expiry conditions enforced by the **cobas**® 5800 System

Reagent	Kit expiration date	Open-kit stability	Number of runs for which this kit can be used	On-board stability
cobas® CMV	Date not passed	90 days from first usage	Max 40 runs	Max 36 days**
cobas® CMV Control Kit	Date not passed	Not applicable*	Not applicable	Max 36 days**
cobas® NHP Negative Control Kit	Date not passed	Not applicable*	Not applicable	Max 36 days**
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

* Single use reagents

** Time is measured from the first time that reagent is loaded onto the **cobas**® 5800 System.

Reagent handling requirements for the cobas® 6800/8800 Systems

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 7 are met. The system automatically prevents use of expired reagents. Table 7 allows the user to understand the reagent handling conditions enforced by the **cobas**® 6800/8800 Systems.

Table 7 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	Date not passed	90 days from first usage	Max 40 runs	Max 40 hours
cobas® CMV Control Kit	Date not passed	Not applicable*	Not applicable	Max 8 hours
cobas® NHP Negative Control Kit	Date not passed	Not applicable*	Not applicable	Max 10 hours
cobas® **omni** Lysis Reagent	Date not passed	30 days from loading**	Not applicable	Not applicable
cobas® **omni** MGP Reagent	Date not passed	30 days from loading**	Not applicable	Not applicable
cobas® **omni** Specimen Diluent	Date not passed	30 days from loading**	Not applicable	Not applicable
cobas® **omni** Wash Reagent	Date not passed	30 days from loading**	Not applicable	Not applicable

*Single use reagents

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

Additional materials required for the cobas® 5800 System

Table 8 Material and consumables for use on the **cobas**® 5800 System

Material	P/N
cobas® **omni** Processing Plate 24	08413975001
cobas® **omni** Amplification Plate 24	08499853001
cobas® **omni** Liquid Waste Plate 24	08413983001
Tip CORE TIPS with Filter, 1 mL	04639642001
Tip CORE TIPS with Filter, 300 µL	07345607001
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 or Solid Waste Bag With Insert	07435967001 or 08030073001

Additional materials required for the cobas® 6800/8800 Systems

Table 9 Materials and consumables for use on the **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 Solid Waste Update	07435967001 and 07094361001 or 08030073001 and 08387281001

Instrumentation and software required

The **cobas**® 5800 software and **cobas**® CMV analysis package for the **cobas**® 5800 System shall be installed on the **cobas**® 5800 instrument. The Data Manager software and PC for the **cobas**® 5800 System will be provided with the system.

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

Table 10 Instrumentation

Equipment	P/N
cobas® 5800 System	08707464001
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**® 5800 System or **cobas**® 6800/8800 Systems – User Assistance and/or User Guides for additional information.

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

Precautions and handling requirements

Warnings and precautions

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

- For in vitro diagnostic use only.
- For prescription use only.
- All patient samples should be handled as if infectious, using good laboratory procedures as outlined in Biosafety in Microbiological and Biomedical Laboratories and in the CLSI Document M29-A4.[26,27] Only personnel proficient in handling infectious materials and the use of **cobas**® CMV and **cobas**® 5800/6800/8800 Systems should perform this procedure.
- All human-sourced materials, such as specimens and controls, should be considered potentially infectious and should be handled with universal precautions. If spillage occurs, immediately disinfect with a freshly prepared solution of 0.6% sodium or potassium hypochlorite in distilled or deionized water 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 upon request from your local Roche representative.
- Closely follow procedures and guidelines provided to ensure that the test is performed correctly. Any deviation from the procedures and guidelines may affect optimal test performance.
- False positive results may occur if carryover of samples is not adequately controlled during sample handling and processing.

Reagent handling

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

Good laboratory practice

- Do not pipette by mouth.
- Do not eat, drink, or smoke in designated work areas.
- Wear laboratory gloves, laboratory coats, and eye protection when handling samples and reagents. Gloves must be changed between handling samples and **cobas**® CMV kits and **cobas**® **omni** reagents to prevent contamination. Avoid contaminating gloves when handling samples and controls.
- Wash hands thoroughly after handling samples and kit reagents, and after removing the gloves.
- Thoroughly clean and disinfect all laboratory work surfaces with a freshly prepared solution of 0.6% sodium or potassium hypochlorite in distilled or deionized water. Follow by wiping the surface with 70% ethanol.
- If spills occur on the **cobas**® 5800/6800/8800 instrument, follow the instructions in the **cobas**® Systems User Assistance and/or User Guides to properly clean and decontaminate the surface of instrument(s).

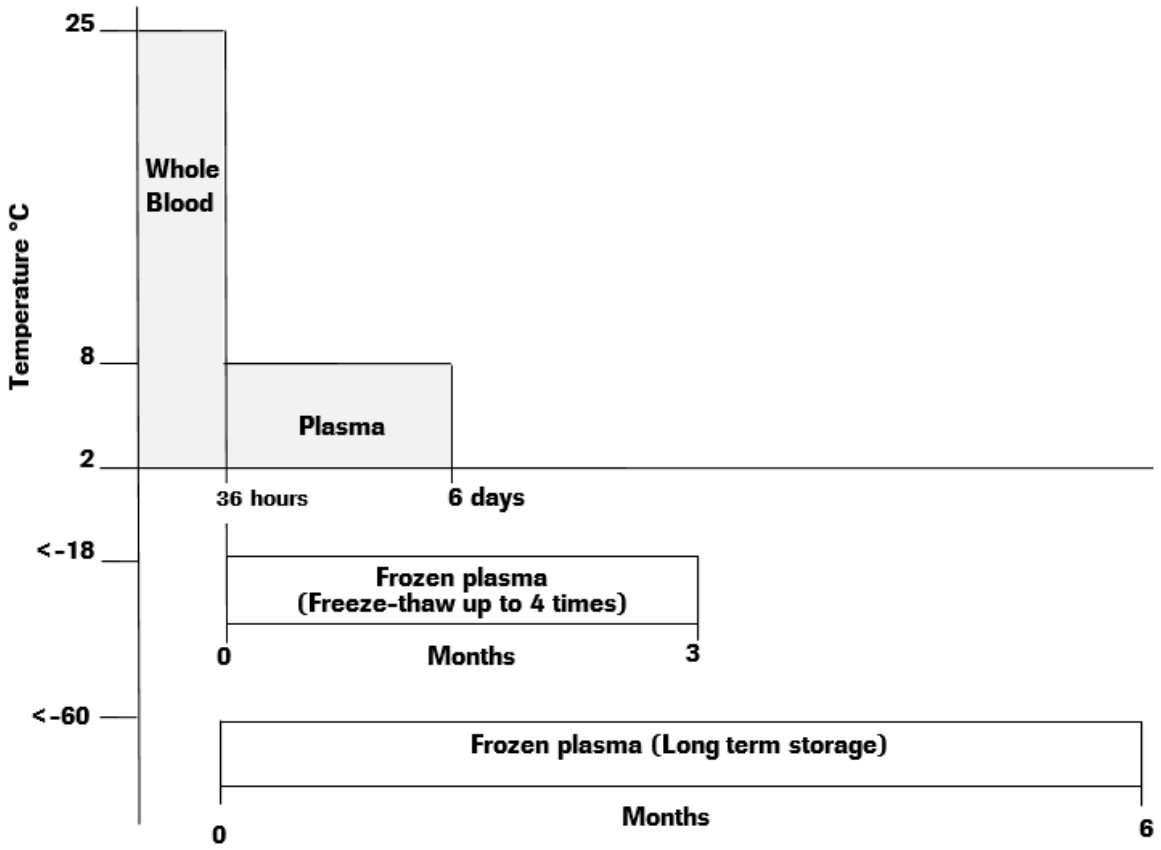
Sample collection, transport, and storage

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

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

Figure 1 Sample storage conditions



Note: Whole blood collected in BD Vacutainer® PPT™ Plasma Preparation Tubes 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

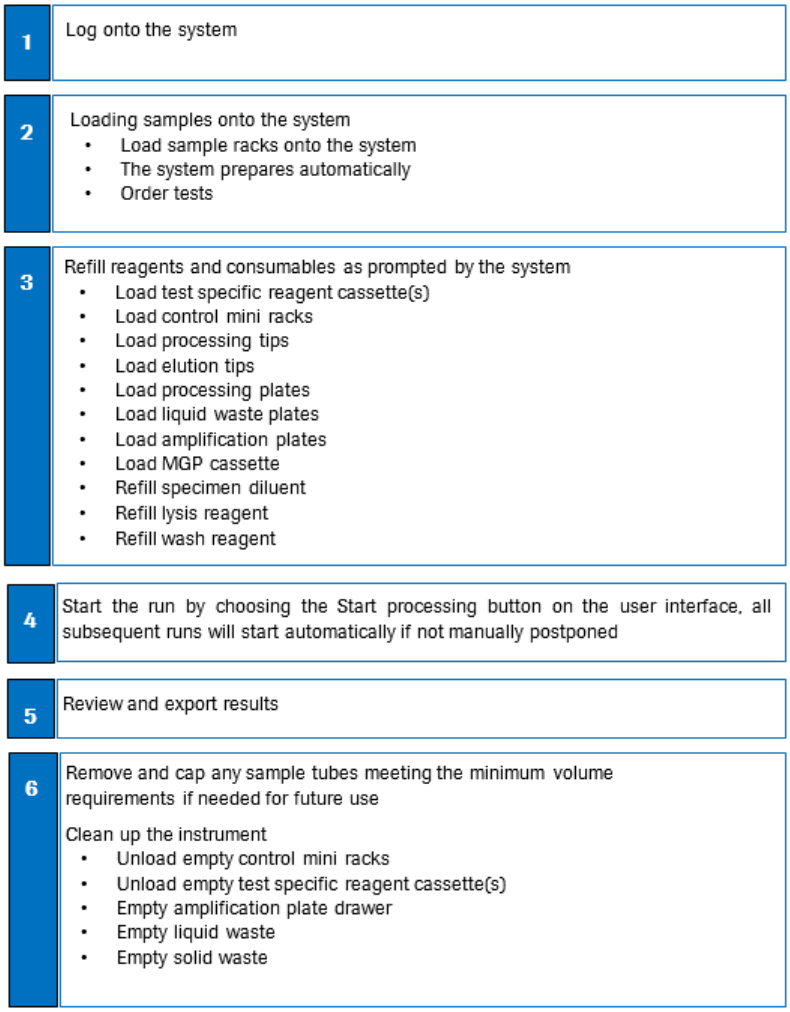
Procedural notes

- 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**® 5800 or **cobas**® 6800/8800 Systems User Assistance and/or User Guide for proper maintenance of instruments.

Running cobas® CMV on the cobas® 5800 System

cobas® CMV can be run with a minimum sample volume of 500 µL of which 350 µL is processed when using the **cobas**® **omni** secondary tube. The test procedure is described in detail in the **cobas**® 5800 System User Assistance and/or User Guide. Figure 2 below summarizes the procedure.

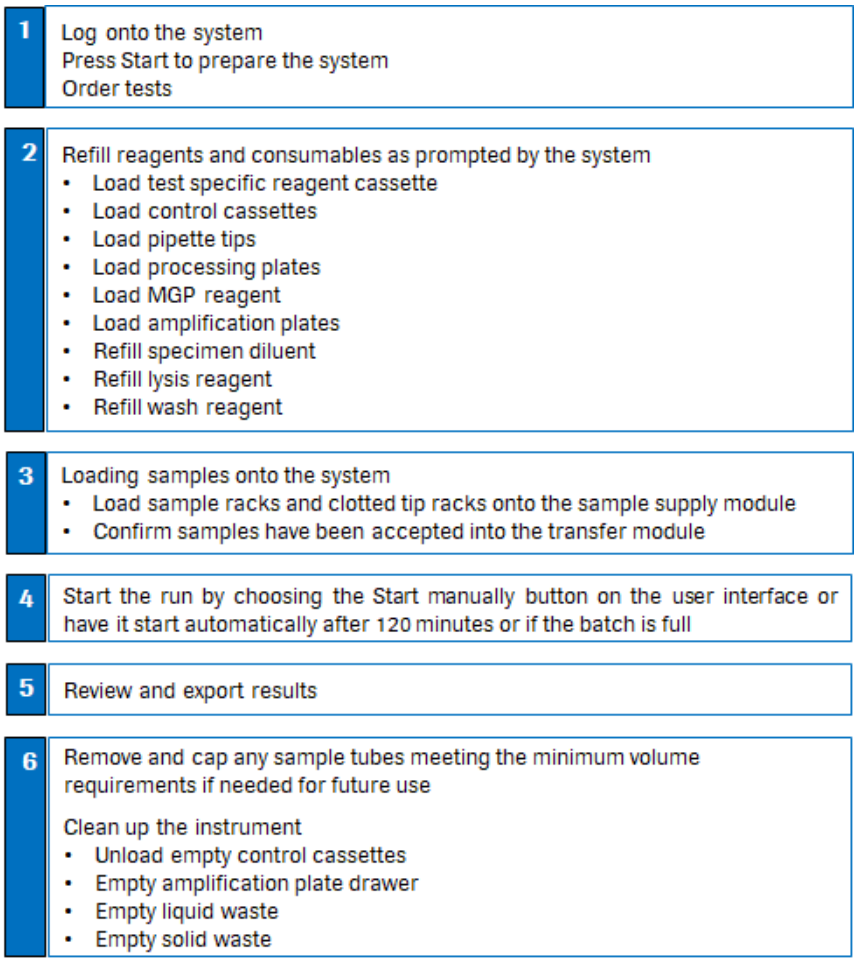
Figure 2 cobas® CMV test procedure on the **cobas**®5800 System



Running cobas® CMV on the cobas® 6800/8800 Systems

cobas® CMV can be run with a minimum sample volume of 500 µL of which 350 µL is processed when using the **cobas**® **omni** secondary tube. The test procedure as described in detail in the **cobas**® 6800/8800 Systems User Assistance and/or User Guide must be followed. Figure 3 below summarizes the procedure.

Figure 3 **cobas**® CMV test procedure on the **cobas**® 6800/8800 Systems



Results

The **cobas**® 5800 System and **cobas**® 6800/8800 System automatically determines the CMV DNA concentration for the samples and controls. The CMV DNA concentration is expressed in International Units per milliliter (IU/mL).

Quality control and validity of results on the cobas® 5800 System

- One negative control [(–) C] and two positive controls, a low positive control [CMV L (+) C] and a high positive control [CMV H (+) C] are processed at least every 72 hours or with every new kit lot. Positive and/or negative controls can be scheduled more frequently based on laboratory procedures and/or local regulations.
- In the **cobas**® 5800 software and/or report, check for flags and their associated results to ensure the result validity.

Invalidation of results is performed automatically by the **cobas**® 5800 software based on negative or positive control failures.

NOTE: The **cobas**® 5800 System will be delivered with the standard setting of running a set of controls (positive and negative) with every run, but can be configured to a less frequent scheduling up to every 72 hours based on laboratory procedures and/or local regulations. Please contact your Roche service engineer and/or Roche customer technical support for more information.

Control results on the cobas® 5800 System

The results of the controls are shown in the **cobas**® 5800 software in the "Controls" app.

- Controls are marked with "Valid" in the column "Control result" if all Targets of the control are reported valid. Controls are marked with 'Invalid' in the column "Control result" if all or one Target of the control are reported invalid.
- Controls marked with 'Invalid' show a flag in the "Flags" column. More information on why the control is reported invalid including flag information is shown in the detail view.
- If one of the positive controls is invalid, repeat testing of the all positive controls and all associated samples. If the negative control is invalid, repeat testing of all controls and all associated samples.

Quality control and validity of results on the cobas® 6800/8800 Systems

- 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 on the cobas® 6800/8800 Systems

Table 11 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**® 5800 and **cobas**® 6800/8800 System software and/or report. The result interpretation should be as follows:

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

Table 12 Target results for individual target result interpretation

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

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

Interpretation of results on the cobas® 5800 System

The results of the samples are shown in the **cobas**® 5800 software in the "Results" app.

For a valid control batch, check each individual sample for flags in the **cobas**® 5800 software and/or report. The result interpretation should be as follows:

- Samples associated with a valid control batch are shown as 'Valid' in the "Control result" column if all Control Target Results reported valid. Samples associated with a failed control batch are shown as 'Invalid' in the "Control result" column if all Control Target Results reported invalid.
- If the associated controls of a sample result are invalid, a specific flag will be added to the sample result as follows:
    - Q05D : Result validation failure because of an invalid positive control
    - Q06D : Result validation failure because of an invalid negative control
- The values in "Results" column for individual sample target result should be interpreted as show in Table 12 above.
- If one or more sample targets are marked with "Invalid" the **cobas**® 5800 software shows a flag in the "Flags" column. More information on why the sample target(s) is reported invalid including flag information is shown in the detail view.

Interpretation of results on the cobas® 6800/8800 Systems

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

- Samples are marked with "Yes" in the column 'Valid' if all requested Target Results reported valid results. Samples marked with "No" in the column 'Valid' may require additional interpretation and action.
- The values for individual sample target result should be interpreted as show in Table 12 above.

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**® 5800/6800/8800 Systems.
- When adopting a new CMV assay for clinical use, laboratories should compare the performance of the new CMV assay to the previously used assay to assess any potentially clinical significant differences in the absolute value of CMV viral load reported.
- Reliable results depend on proper sample collection, storage and handling procedures.
- This test has been validated only for use with EDTA plasma. Testing of other sample types with **cobas**® CMV may result in inaccurate results. Plasma viral load measurements are not directly comparable to those of other sample types.
- Quantitation of CMV DNA may be affected by sample collection methods, patient factors (i.e., age, presence of symptoms), and/or stage of infection.
- Results should be interpreted by qualified healthcare professionals in conjunction with clinical signs and symptoms and all other laboratory findings.
- Mutations within the highly-conserved regions of the CMV DNA polymerase (UL54) gene covered by **cobas**® CMV may affect primers and/or probe binding resulting in the under-quantitation of virus or failure to detect the presence of virus. The **cobas**® CMV mitigates this risk through the use of redundant amplification primers.
- Negative test results do not preclude CMV infection or tissue-invasive CMV disease, and test results should therefore not be the sole basis for patient management decisions.
- Due to potential variability from measurements with different CMV assays, it is recommended that the same device (or assay) be used for the measurement of CMV viral load when managing CMV infection in individual patients.
- **cobas**® CMV is not intended for use as a screening test for the presence of CMV in blood or blood products and has not been evaluated as a diagnostic test to confirm the presence of CMV infection.
- Clinicians should take individual patient risk factors as well as current clinical guidelines into account when using CMV viral load results for the management of transplant patients.

Non-clinical performance evaluation

Key performance characteristics performed on the cobas® 6800/8800 Systems

Limit of detection (LoD)

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

WHO International Standard

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

The results for EDTA plasma are shown in Table 13 through Table 15. The study demonstrates that with the least sensitive lot, the concentration for which 95% hit rate is expected by PROBIT is 30.7 IU/mL with a 95% confidence range of 24.5-40.9 IU/mL in EDTA plasma. The lowest concentration level with a hit rate ≥ 95% is 34.5 IU/mL in EDTA plasma.

Table 13 CMV DNA WHO International Standard Limit of Detection in EDTA plasma, Lot 1

Input titer concentration (IU/mL)	Number of valid replicates (N)	Number of positives (n)	Hit rate (n/N) x100
92.0	63	63	100.0%
46.0	63	63	100.0%
34.5	62	62	100.0%
23.0	63	62	98.4%
11.5	63	57	90.5%
5.8	63	45	71.4%
2.9	63	26	41.3%
1.4	63	11	17.5%
0.0	63	0	0.0%

LoD by PROBIT at 95% hit rate: 14.7 IU/mL, 95% confidence range: 11.7 – 20.0 IU/mL

Table 14 CMV DNA WHO International Standard limit of detection in EDTA plasma, Lot 2

Input titer concentration (IU/mL)	Number of valid replicates (N)	Number of positives (n)	Hit rate (n/N) x100
92.0	63	63	100.0%
46.0	63	62	98.4%
34.5	63	62	98.4%
23.0	63	57	90.5%
11.5	63	43	68.3%
5.8	63	27	42.9%
2.9	63	16	25.4%
1.4	63	4	6.4%
0.0	63	0	0.0%

LoD by PROBIT at 95% hit rate: 30.7 IU/mL, 95% confidence range: 24.5 – 40.9 IU/mL

Table 15 CMV DNA WHO International Standard limit of detection in EDTA plasma, Lot 3

Input titer concentration (IU/mL)	Number of valid replicates (N)	Number of positives (n)	Hit rate (n/N) x100
92.0	63	63	100.0%
46.0	63	63	100.0%
34.5	63	63	100.0%
23.0	63	62	98.4%
11.5	63	58	92.1%
5.8	63	45	71.4%
2.9	63	24	38.1%
1.4	63	13	20.6%
0.0	63	0	0.0%

LoD by PROBIT at 95% hit rate: 14.8 IU/mL, 95% confidence range: 11.6 – 19.9 IU/mL

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

CMV cell culture supernatants for three different Glycoprotein B genotypes (gB-2, gB-3 and gB-4) were diluted to three different concentration levels in CMV negative EDTA plasma. The hit rate determination was performed with 63 replicates for each level. Testing was conducted with three lots of **cobas**® CMV reagents.

The combined results from three lots shown in Table 16 verify that – consistent with a LoD of 34.5 IU/mL – **cobas**® CMV detected CMV DNA for genotype gB-2 at a concentration of 17.25 IU/mL and for gB-3 and gB-4 at a concentration of 34.5 IU/mL with a 95% hit rate. The achieved hit rates at 34.5 IU/mL verify the LoD for each of the three genotypes.

Table 16 CMV DNA genotypes gB-2 through gB-4 verification of limit of detection in EDTA plasma

Genotype	Test concentration	Number of valid replicates (N)	Number of positives (n)	Hit rate (n/N)x100
gB-2	17.25 IU/mL	63	61	96.8%
gB-2	34.50 IU/mL	63	63	100.0%
gB-2	51.75 IU/mL	63	63	100.0%
gB-3	17.25 IU/mL	63	57	90.5%
gB-3	34.50 IU/mL	63	63	100.0%
gB-3	51.75 IU/mL	63	63	100.0%
gB-4	17.25 IU/mL	63	55	87.3%
gB-4	34.50 IU/mL	63	63	100.0%
gB-4	51.75 IU/mL	63	63	100.0%

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

Cell culture supernatants for two different drug resistant CMV specimens (one resistant against foscarnet and one resistant against ganciclovir, valganciclovir and cidofovir) were diluted to three different concentration levels in CMV negative EDTA plasma. The hit rate determination was performed with 63 replicates for each level. Testing was conducted with three lots of **cobas**® CMV reagents. The combined results from three lots shown in Table 17 verify that – consistent with a LoD of 34.5 IU/mL – **cobas**® CMV detected CMV DNA for two different drug resistant specimens at a concentration of 34.5 IU/mL with a 95% hit rate. The achieved hit rates at 34.5 IU/mL verify the LoD for both of the tested drug resistant CMV specimens.

Table 17 Drug resistant CMV specimen verification of limit of detection in EDTA plasma

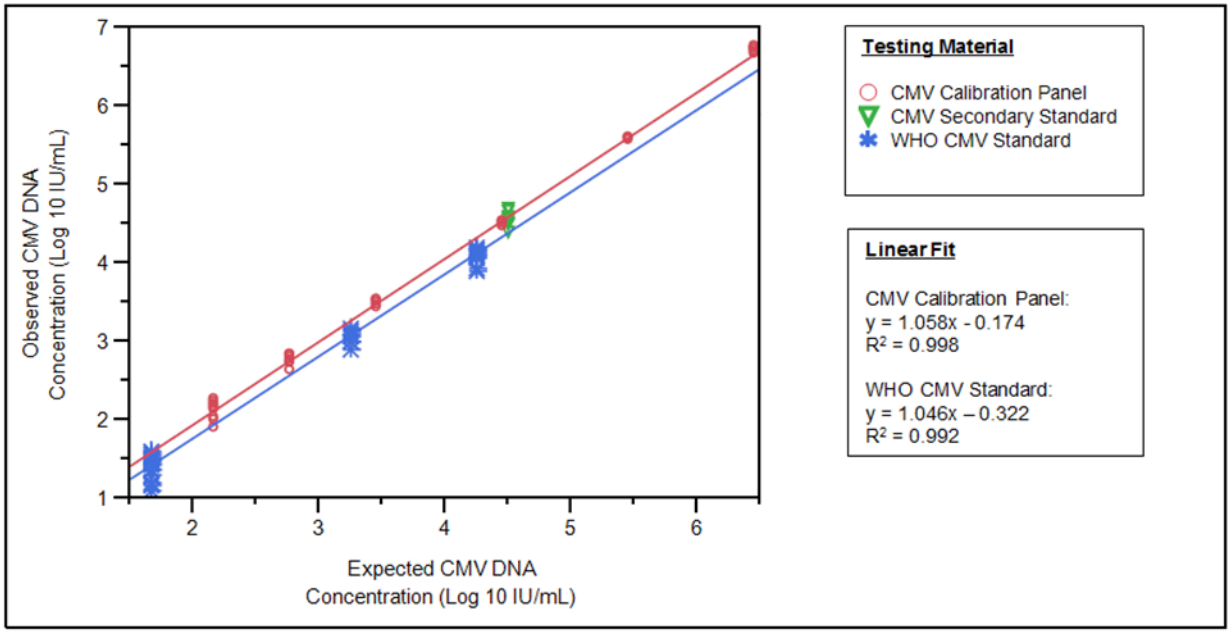
Drug resistance	Mutation site in UL54	Test concentration	Number of valid replicates (N)	Number of positives (n)	Hit rate (n/N)x100
Foscarnet	E756Q	17.25 IU/mL	63	58	92.1%
Foscarnet	E756Q	34.50 IU/mL	63	63	100.0%
Foscarnet	E756Q	51.75 IU/mL	63	63	100.0%
Ganciclovir, Valganciclovir, Cidofovir	L545S	17.25 IU/mL	63	59	93.7%
Ganciclovir, Valganciclovir, Cidofovir	L545S	34.50 IU/mL	63	63	100.0%
Ganciclovir, Valganciclovir, Cidofovir	L545S	51.75 IU/mL	63	63	100.0%

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

Several standards and controls have been used during development of this test to provide traceability to the WHO standard [the 1st WHO International Standard for human Cytomegalovirus DNA for Nucleic Acid Amplification Techniques (NIBSC 09/162)[28]]. The standards used during development of the test include the HCMV WHO Standard, the RMS CMV Secondary Standard, and the RMS CMV Calibration Panel. The Standards and the Calibration Panel were tested. The concentration range tested for the CMV WHO Standard was from 4.60E+01 IU/mL to 1.80E+04 IU/mL (1.66-4.26 $\log_{10}$ IU/mL), the RMS CMV Secondary Standard was tested at 3.16E+04 IU/mL (4.50 $\log_{10}$ IU/mL), and the RMS CMV Calibration Panel was tested from 1.47E+02 to 2.94E+06 IU/mL (2.17-6.47 $\log_{10}$ IU/mL).

The calibration and standardization process of **cobas**® CMV provides quantitation values for the calibration panel, the RMS CMV Secondary Standard, and the CMV WHO Standard that are similar to the expected values with deviation of not more than 0.23 $\log_{10}$ IU/mL (Figure 4). The maximum deviation was obtained around the test LLoQ.

Figure 4 Traceability to WHO International Standard [bivariate fit of observed CMV DNA concentration ($\log_{10}$ IU/mL) by expected CMV DNA concentration ($\log_{10}$ IU/mL)] using **cobas**® CMV



Linear range

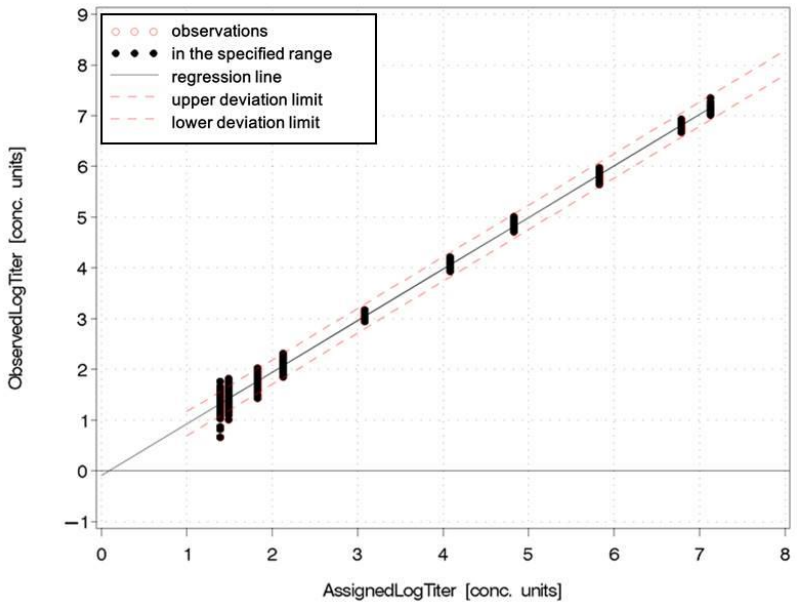
Linearity of the **cobas**® CMV was evaluated using a dilution series consisting of 10 panel members with CMV genotype gB-1 DNA concentrations spanning the assay linear range (2.45E+01 IU/mL to 1.34E+07 IU/mL). Each panel member was tested in 48 replicates across three lots of **cobas**® CMV test reagents and the results of the study are presented in Figure 5.

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

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

Based on the LLoQ and the determined linear range, as well as the medical value the linear measurement range of the test was set to 34.5-1.0E+07 IU/mL. The results of calculation and claimed LLoQ are shown in Table 20.

Figure 5 Linearity in EDTA plasma using CMV Merlin Virus as representative for Glycoprotein B (gB) Genotype 1



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

The dilution series used in the verification of CMV Glycoprotein B genotypes linearity study of **cobas**® CMV consists of seven panel members spanning the intended linear range. Sixteen replicates were tested across two lots of **cobas**® CMV reagent for each level in EDTA plasma. The results of the study are presented in Table 18.

The linearity within the linear range of **cobas**® CMV was verified for all three CMV Glycoprotein B genotypes (gB-2, gB-3 and gB-4). The maximum deviation between the linear regression and the better fitting non-linear regression was equal to or less than ± 0.2 $\log_{10}$.

Table 18 Linearity verification on gB-2, gB-3 and gB-4 Genotypes

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

*The linear regression is the best fitting model.

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

The dilution series used in the linearity study for verification of CMV drug resistant specimens of **cobas**® CMV consists of seven panel members spanning the intended linear range. Sixteen replicates were tested across two lots of **cobas**® CMV reagent for each level in EDTA plasma. The results of the study are presented in Table 19.

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

Table 19 Linearity verification on CMV drug resistant specimens

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

*The linear regression is the best fitting model.

Lower limit of quantitation

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

The results of calculation and claimed LLoQ are shown in Table 20; the lower limit of quantitation (LLoQ) is 34.5 IU/mL.

Table 20 Lower Limit of Quantitation (LLoQ) of **cobas**® CMV using the WHO International Standard for Human Cytomegalovirus (HCMV) (NIBSC 09/162)

Lot	Nominal concentration (IU/mL)	$\log_{10}$ titer nominal	Mean $\log_{10}$ titer observed	SD ($\log_{10}$)	Absolute Bias	TAE (|Bias| + 2x SD)	Difference between Measurements in SD (= SQRT(2) x 2x SD)
1	34.5	1.54	1.28	0.29	0.26	0.83	0.81
1	46.0	1.66	1.43	0.17	0.23	0.57	0.48
1	92.0	1.96	1.76	0.16	0.20	0.53	0.46
2	34.5	1.54	1.42	0.19	0.11	0.50	0.55
2	46.0	1.66	1.63	0.22	0.03	0.47	0.61
2	92.0	1.96	1.84	0.16	0.12	0.44	0.46
3	34.5	1.54	1.32	0.25	0.22	0.71	0.70
3	46.0	1.66	1.48	0.24	0.18	0.66	0.67
3	92.0	1.96	1.80	0.18	0.16	0.52	0.51
3 lots combined	34.5	1.54	1.34	0.25	0.19	0.69	0.70
3 lots combined	46.0	1.66	1.51	0.21	0.15	0.57	0.59
3 lots combined	92.0	1.96	1.80	0.17	0.16	0.50	0.47

Precision – within laboratory

Precision of **cobas**® CMV was determined by analysis of serial dilutions of high titer cultured Virus (Merlin, gB-1 genotype) in CMV negative EDTA plasma. Ten dilution levels were tested in 48 replicates for each level across three lots of **cobas**® CMV test reagents using three instruments and three operators over 12 days. Each sample was carried through the entire **cobas**® CMV procedure on a fully automated **cobas**® 6800/8800 Systems. Therefore, the precision reported here represents all aspects of the test procedure. The results of the within-laboratory precision are shown in Table 21. The results of the variance component estimation are shown on the Table 22.

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

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

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

Table 22 Lognormal Percent Coefficient of Variation (%CV) of **cobas**® CMV by positive panel and contributing components of variance Nominal concentration

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

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

Analytical specificity

The analytical specificity of **cobas**® CMV was evaluated by testing a panel of microorganisms at a concentration of 1.00E+06 particles, copies, IU, genome equivalents or CFU/mL. Microorganisms were diluted into CMV DNA negative human EDTA plasma as well as human EDTA plasma containing (230 IU/mL) CMV DNA (Table 23). Each sample was tested in replicates of three. None of the non-CMV pathogens interfered with test performance. Negative results were obtained with **cobas**® CMV for all microorganism samples without CMV target and positive results were obtained for all of the microorganism samples with CMV target. Furthermore, the mean log_{10} titer of each of the positive CMV samples containing potentially cross-reacting organisms was within ± 0.5 log_{10} of the mean log_{10} titer of the respective positive spike control.

Table 23 Microorganisms tested for cross-reactivity

Viruses	Bacteria	Yeast and Fungi
Adenovirus type 5	*Propionibacterium acnes*	*Aspergillus niger*
BK Polyomavirus	*Staphylococcus aureus*	*Candida albicans*
Epstein-Barr Virus	*Chlamydia trachomatis*	*Cryptococcus neoformans*
Hepatitis B Virus	*Clostridium perfringens*	-
Hepatitis C Virus	*Enterococcus faecalis*	-
Herpes Simplex Virus type1	*Escherichia coli*	-
Herpes Simplex Virus type 2	*Klebsiella pneumoniae*	-
Human Herpes Virus type-6	*Listeria monocytogenes*	-
Human Herpes Virus type-7	*Mycobacterium avium*	-
Human Herpes Virus type-8	*Neisseria gonorrhoeae*	-

Viruses	Bacteria	Yeast and Fungi
Human Immunodeficiency Virus-1	*Staphylococcus epidermidis*	-
Human Immunodeficiency Virus-2	*Streptococcus pyogenes*	-
Human Papillomavirus	*Mycoplasma pneumoniae*	-
JC virus	*Salmonella typhimurium*	-
Parvovirus B19	*Streptococcus pneumoniae*	-
Varicella-Zoster Virus	-	-

Interfering substances

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

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

In addition, drug compounds listed in Table 24 were tested at three times the C_{max} in presence (230 IU/mL) and absence of CMV DNA.

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

Table 24 Drug compounds tested for interference with the quantitation of CMV DNA by **cobas**® CMV

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

Cross contamination

The cross-contamination rate for **cobas**® CMV was determined on the **cobas**® 6800 System by testing 240 replicates of a normal, CMV DNA negative human EDTA-plasma sample and 225 replicates of a high titer CMV sample at 1.00E+06 IU/mL. In total, five runs were performed with positive and negative samples in a checkerboard configuration.

All 240 replicates of the negative sample were negative, resulting in a cross-contamination rate of 0% (95% confidence interval 0%-1.5%).

Clinical performance performed on the cobas® 6800/8800 Systems

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 25 through Table 27 below.

Table 25 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 25 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 26 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 26 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 27 below presents the reproducibility results for the negative panel member for the **cobas**® 6800 System.

Table 27 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 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 28.

Table 28 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 29 through Table 32 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 29 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 30 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 31 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 32 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 33 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 33 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 34).

Table 34 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 35 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 35 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 36 below shows the summary of concordance of viral load results by different thresholds (Target Not Detected, 137 IU/mL, 500 IU/mL, and 1800 IU/mL) for all paired samples in the SOT population.

Table 36 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 37 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 37 Summary of concordance analyses (paired samples at time points 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 38 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 38 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 39 along with Figure 6 through Figure 8 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 39 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.

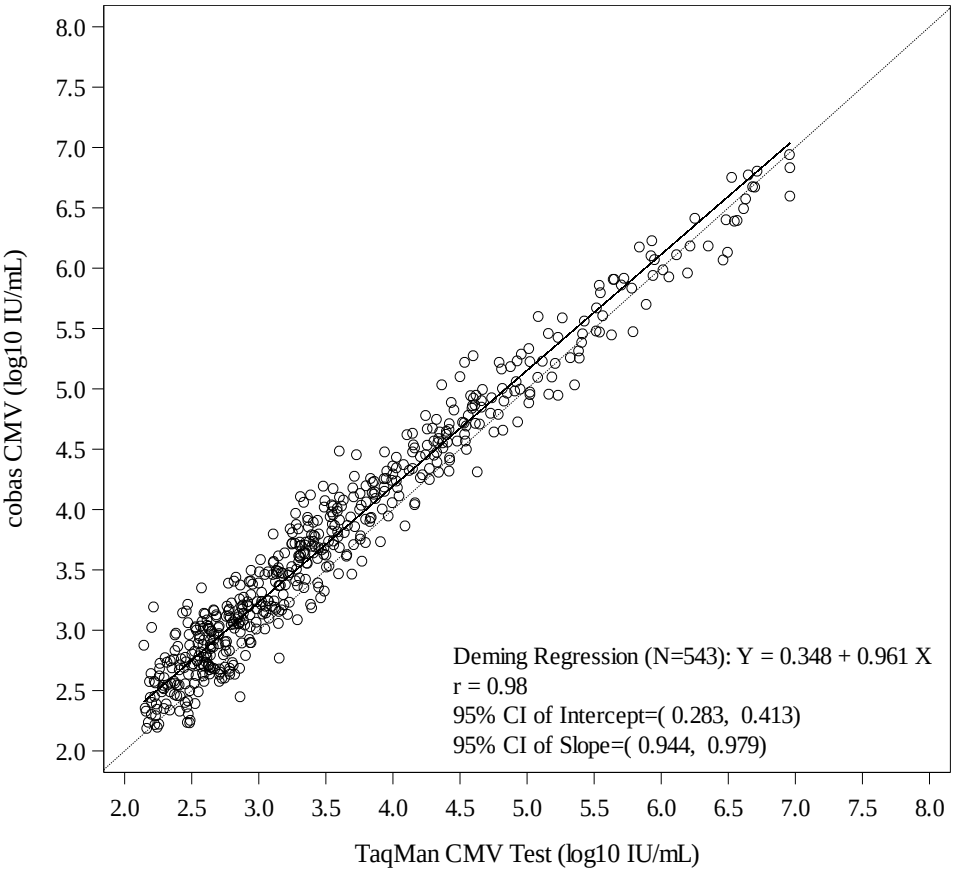
[a] Assumed independence between all samples.

[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.

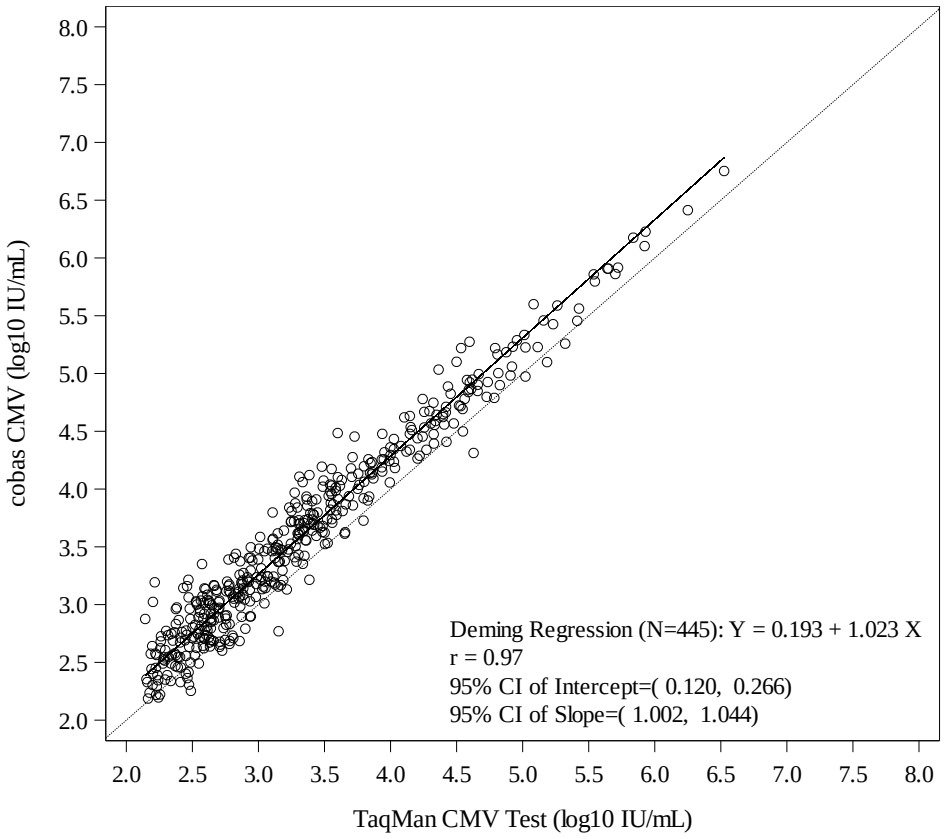
Figure 6 Deming linear regression plot of viral loads (log_{10} IU/mL) in the SOT population (**cobas**® CMV Versus TaqMan® CMV Test; clinical and spiked samples)



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.

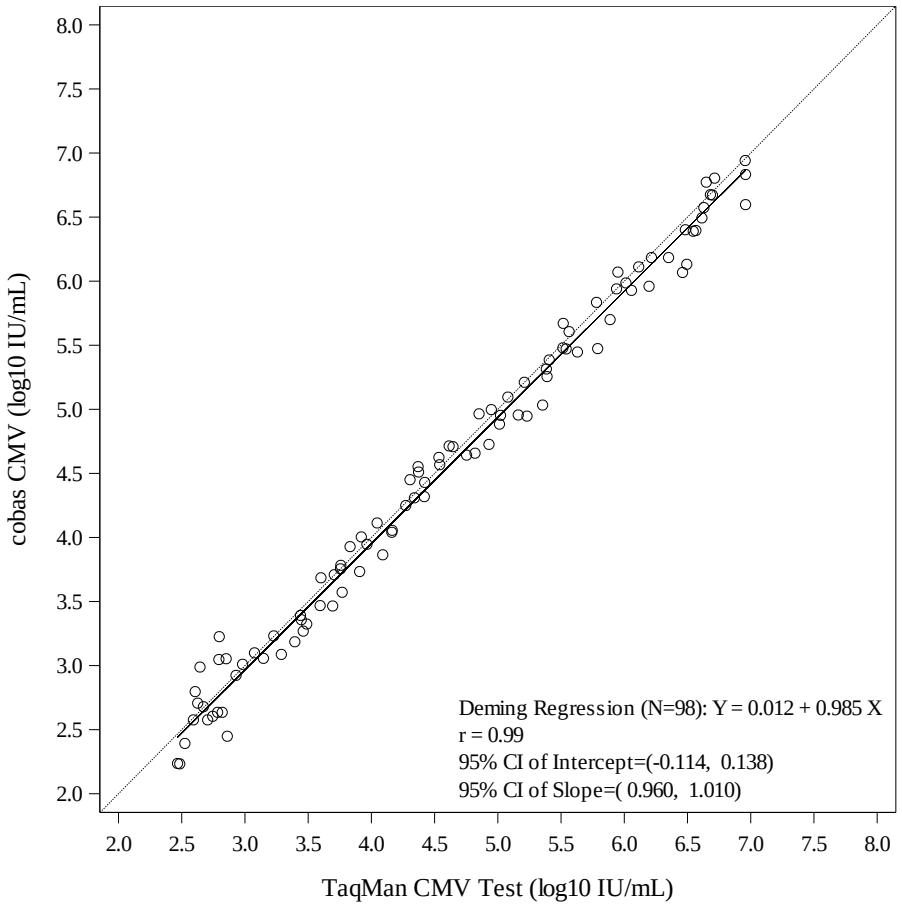
Figure 7 Deming linear regression plot of viral loads (log_{10} IU/mL) in the SOT population (**cobas**® CMV Versus TaqMan® CMV Test; clinical samples)



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 8 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 40 below presents the bias between **cobas**® CMV and the TaqMan® CMV Test at five selected viral load levels from 2.14 $\log_{10}$ IU/mL to 7.00 $\log_{10}$ IU/mL with associated non-transformed equivalents.

Table 40 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 41 below present the mean paired difference between **cobas**® CMV and the TaqMan® CMV Test at representative thresholds and associated 95% CIs calculated using the paired t-test.[29]

Table 41 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 42 along with Figure 9 through Figure 11 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 42 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)

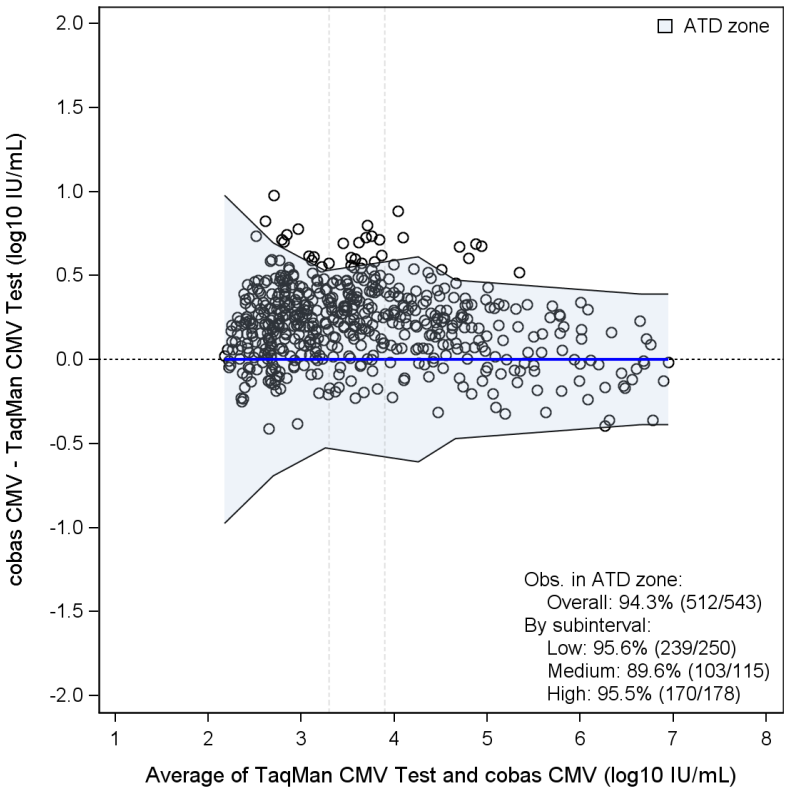
Note: Twenty-six samples from nine subjects were excluded from method comparison analyses due to impactful sequence mismatch. The table only includes paired samples with paired results that were each within 1.37E+02 IU/mL to 9.1E+06 IU/mL, the overlapping linear range of both assays. Paired results were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

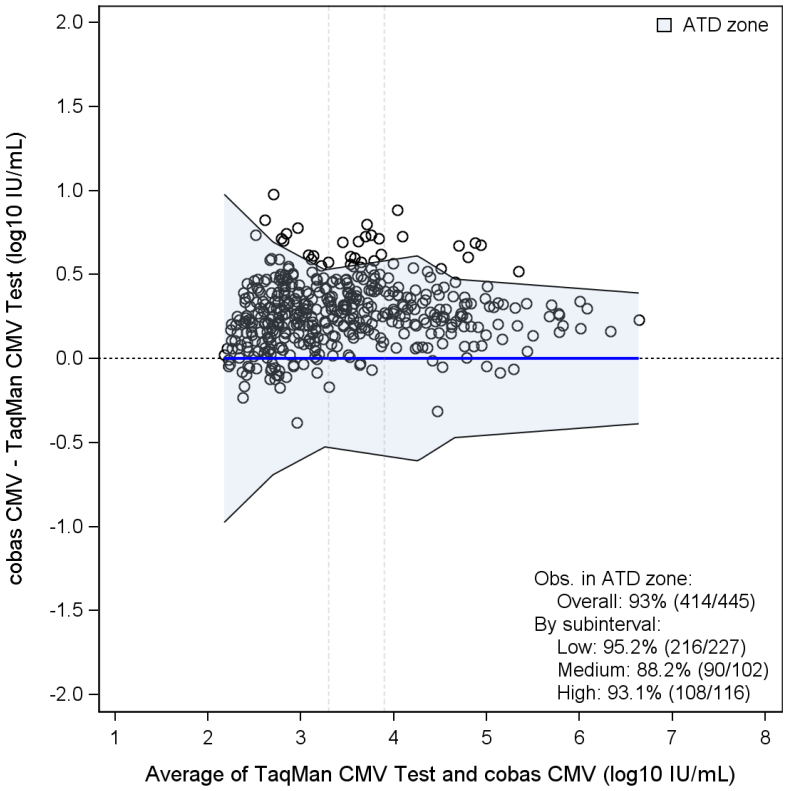
Figure 9 Allowable Total Difference (ATD) plot of individual viral load differences versus their average (log_{10} IU/mL) in the SOT population (**cobas**® CMV versus TaqMan® CMV Test; clinical and spiked samples)



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).

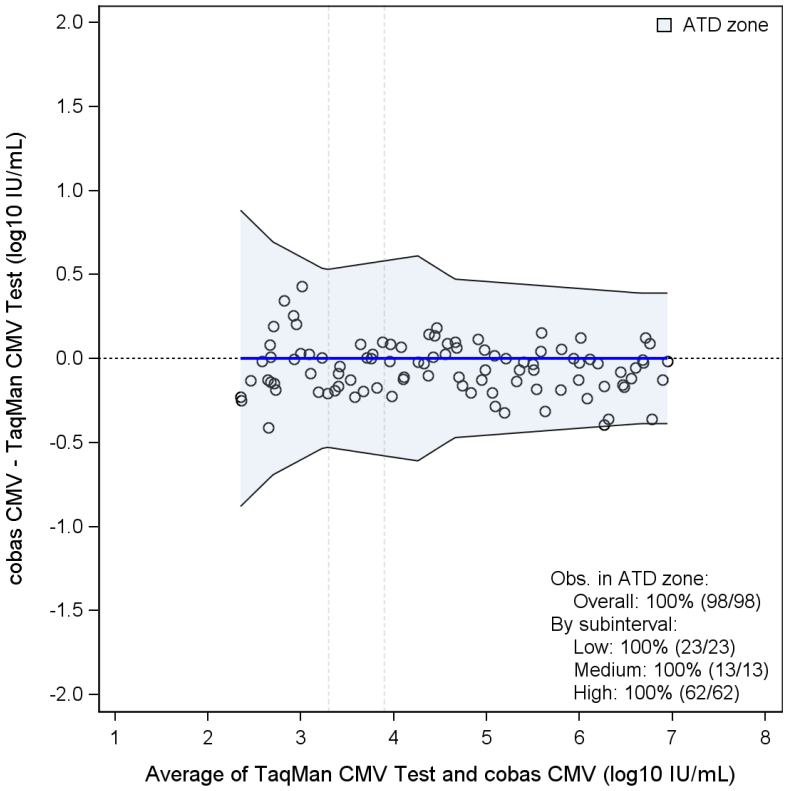
Figure 10 Allowable Total Difference (ATD) plot of individual viral load differences versus their average (log_{10} IU/mL) in the SOT population (**cobas**® CMV versus TaqMan® CMV Test; clinical samples)



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 11 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 43.

Table 43 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[20] 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 44 below summarizes the demographics and baseline clinical characteristics of the 258 subjects.

Table 44 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 45 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 45 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 46 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 46 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 47 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 47 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 48 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 48 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 49 below shows the concordance analyses of CMV episode resolution by time point for viremic subjects who initiated anti-CMV therapy.

Table 49 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%)

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 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%)
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 50 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 50 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 51 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 51 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 52 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 52 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 53 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 53 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 54 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 54 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 55 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 55 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.

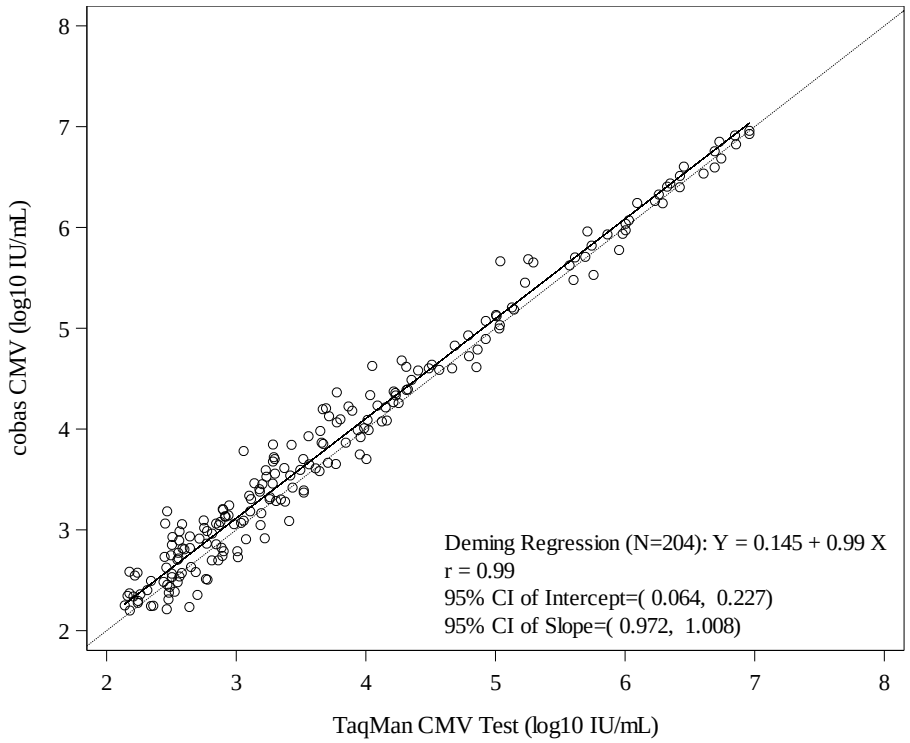
[b] Adjusted correlation between samples from the same subject by the bootstrap method with 500 iterations.

* 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 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 and spiked samples combined.

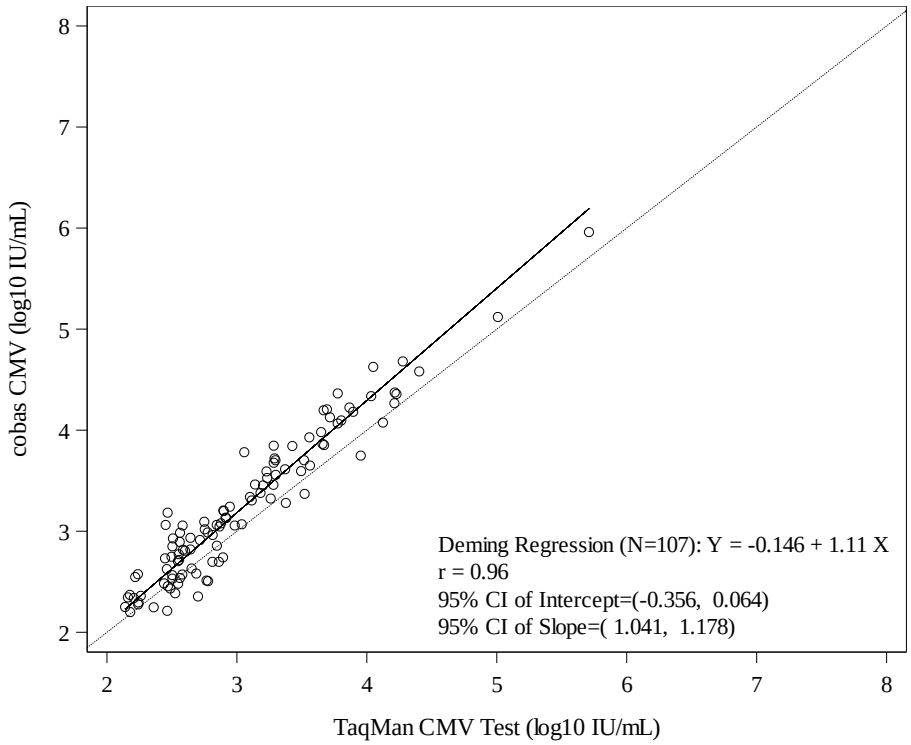
Figure 12 Deming linear regression plot of viral loads (log_{10} IU/mL) in the HSCT population (clinical and spiked samples)



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

Figure 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 clinical samples.

Figure 13 Deming linear regression plot of viral loads (log_{10} IU/mL) in the HSCT population (clinical samples)

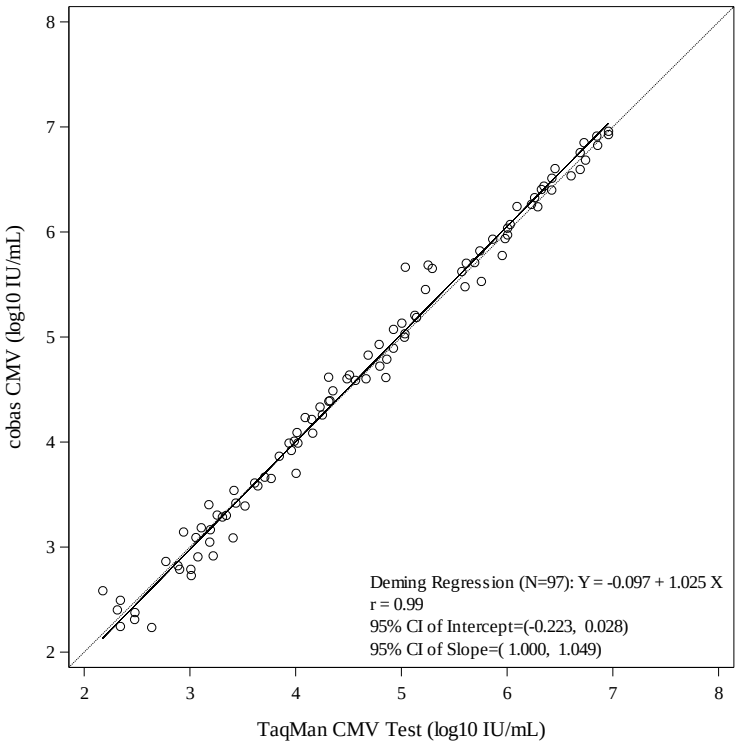


Note: Seven samples from three subjects were excluded from method comparison analyses due to impactful sequence mismatch.

CI = confidence interval; r = correlation coefficient.

Figure 14 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 14 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 56 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 56 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 57 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 57 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 58 below shows the percentage of results within low, medium and high intervals of the Allowable Total Difference zone by sample type.

Table 58 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)

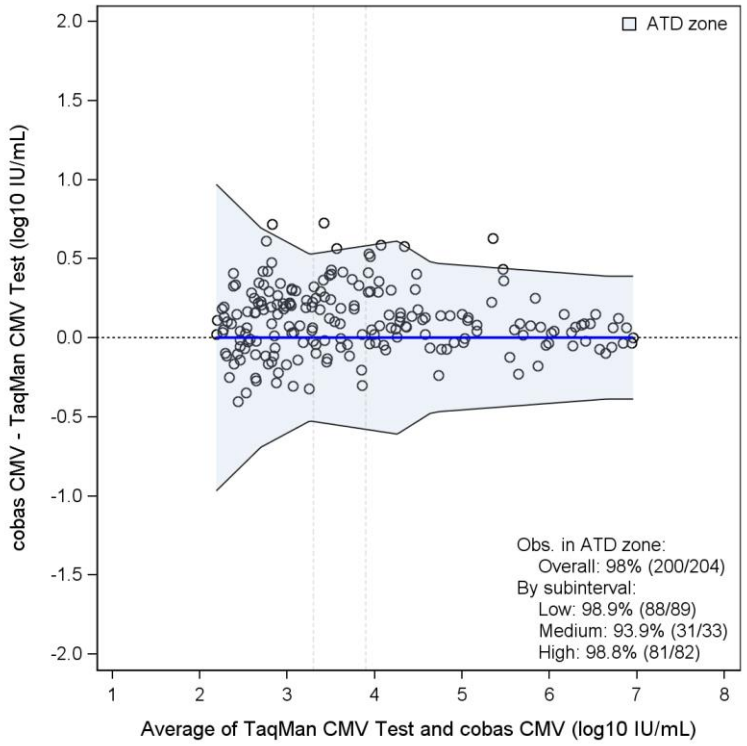
Note: Seven samples from three subjects were excluded from method comparison analyses due to impactful sequence mismatch. The table only included paired samples with results each within 1.37E+02 to 9.1E+06 IU/mL, the common linear range of both assays. Paired results were categorized into the intervals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

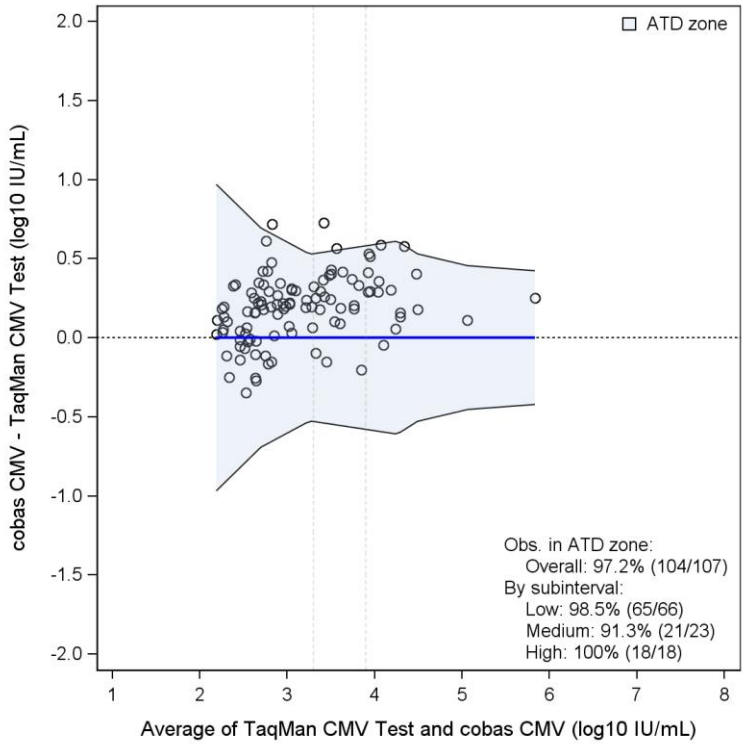
Figure 15 Allowable Total Difference (ATD) plot of viral load difference (log_{10} IU/mL) in the HSCT population (clinical and spiked samples)



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

Figure 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 clinical samples.

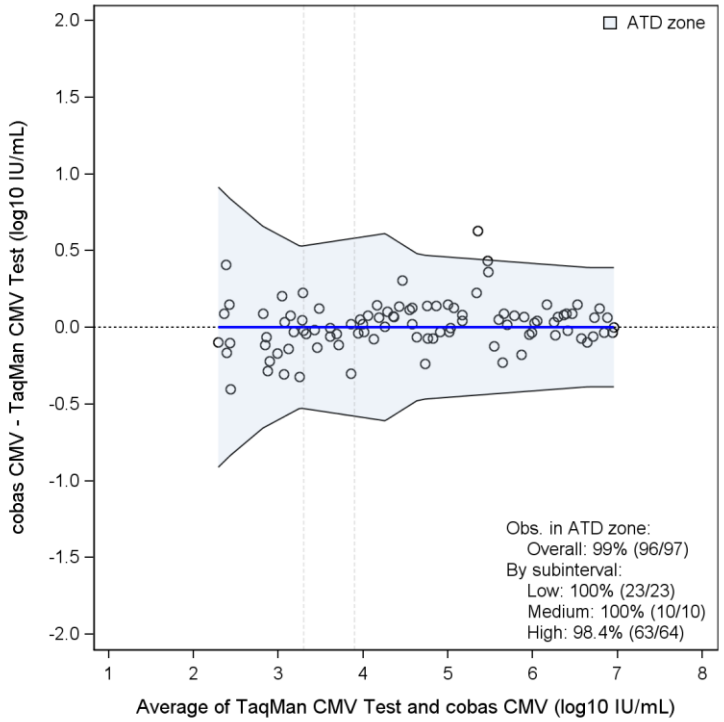
Figure 16 Allowable Total Difference (ATD) plot of viral load difference (log_{10} IU/mL) in the HSCT population (clinical samples)



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

Figure 17 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 17 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 59 below.

Table 59 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.

System equivalency / system comparison

System equivalency of the **cobas**® 5800 and **cobas**® 6800/8800 Systems was demonstrated in the following performance studies: LoD, Linearity (including determination of LLoQ), Reproducibility and Method Comparison.

The results presented in the Instructions for Use support equivalent performance for all systems.

Method comparison

A method comparison study was conducted to evaluate the performance of **cobas**® CMV on the **cobas**® 5800 System to the **cobas**® 6800/8800 Systems using 150 paired samples across 3 sites.

Table 60 below shows the summary of concordance of viral load results by different thresholds (Target Not Detected, 34.5 IU/mL, 137 IU/mL, 500 IU/mL and 1800 IU/mL) for all samples tested on the **cobas**® 6800/8800 Systems versus the **cobas**® 5800 System. Table 61 shows the negative percent agreement (NPA) for all samples tested on the **cobas**® 6800/8800 Systems versus the **cobas**® 5800 System.

Table 60 Summary concordance of viral load results from **cobas**® CMV assay on the **cobas**® 6800/8800 Systems versus **cobas**® 5800 System using different thresholds – all sites combined

	Percent Agreement < Threshold 95% CI (n/N)	**Percent Agreement ≥ Threshold 95% CI (n/N)**	**Overall Percent Agreement 95% CI (n/N)**
Target Not Detected	93.10% (81/87) (87.36%, 97.70%)	99.34%* (450/453) (98.07%, 99.77%)	98.33% (531/540) (97.59%, 99.07%)
34.5 IU/mL (1.5 log10 IU/mL)	100.0%* (90/90) (95.91%, 100.0%)	99.56% (448/450) (99.11%, 100.0%)	99.63% (538/540) (99.26%, 100.0%)
137 IU/mL (2.1 log10 IU/mL)	92.68% (114/123) (89.43%, 95.93%)	100.0%* (417/417) (99.09%, 100.0%)	98.33% (531/540) (97.59%, 99.07%)
500 IU/mL (2.7 log10 IU/mL)	94.74% (162/171) (92.40%, 97.08%)	99.73% (368/369) (99.19%, 100.0%)	98.15% (530/540) (97.41%, 98.89%)
1800 IU/mL (3.3 log10 IU/mL)	100.0%* (240/240) (98.42%, 100.0%)	100.0%* (300/300) (98.74%, 100.0%)	100.0%* (540/540) (99.29%, 100.0%)

*95% CI are based on Wilson score method.

Table 61 Negative percent agreement between the **cobas**® 5800 System and **cobas**® 6800/8800 Systems test results for **cobas**® CMV – all sites combined

cobas® CMV Test Result **cobas® 5800 System**	**cobas® 6800/8800 System** **Positive**	**cobas® 6800/8800 System** **Negative**	**Total**
Positive	0	6	6
Negative	3	81	84
Total	3	87	90
NPA (%) (95% CI)	–	93.103% (81/87) (87.356%, 97.701%)	–

Additional information

Key test features

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

*Dead volume of 0.150 mL is identified for the **cobas**® **omni** Secondary Tubes. Other tubes compatible with **cobas**® 5800/6800/8800 Systems (consult User Assistance and/or User Guides) may have different dead volume and require more or less minimum volume.

Symbols

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

Table 62 Symbols used in labeling for Roche PCR diagnostics products

Age/DOB Age or Date of Birth	Device not for near-patient testing	QS IU/PCR QS IU per PCR reaction, use the QS International Units (IU) per PCR reaction in calculation of the results.
SW Ancillary Software	Device not for self-testing	
Assigned Range [copies/mL] Assigned Range (copies/mL)	Distributor *(Note: The applicable country/region may be designated beneath the symbol)*	SN Serial number
Assigned Range [IU/mL] Assigned Range (IU/mL)	Do not re-use	Site Site
EC REP Authorized representative in the European Community	Female	Procedure Standard Standard Procedure
BARCODE Barcode Data Sheet	For IVD performance evaluation only	STERILE EO Sterilized using ethylene oxide
LOT Batch code	GTIN Global Trade Item Number	Store in dark
Biological risks	Importer	Temperature limit
REF Catalogue number	IVD In vitro diagnostic medical device	TDF Test Definition File
CE marking of conformity; this device is in conformity with the applicable requirements for CE marking of an in vitro diagnostic medical device	LLR Lower Limit of Assigned Range	This way up
	Male	Procedure UltraSensitive Ultrasensitive Procedure
Collect Date Collect date	Manufacturer	UDI Unique Device Identifier
Consult instructions for use	CONTROL − Negative control	ULR Upper Limit of Assigned Range
Σ Contains sufficient for <*n*> tests	NON STERILE Non-sterile	Urine Fill Line Urine Fill Line
CONTENT Content of kit	Patient Name	Rx Only US Only: Federal law restricts this device to sale by or on the order of a physician.
CONTROL Control	# Patient number	Use-by date
Date of manufacture	Peel here	
Device for near-patient testing	CONTROL + Positive control	
Device for self-testing	QS copies / PCR QS copies per PCR reaction, use the QS copies per PCR reaction in calculation of the results.	

Technical support

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

Manufacturer and distributor

Table 63 Manufacturer and distributor

	Roche Molecular Systems, Inc. 1080 US Highway 202 South Branchburg, NJ 08876 USA www.roche.com Made in USA
Distributed by	Roche Diagnostics 9115 Hague Road Indianapolis, IN 46250-0457 USA (For Technical Assistance call the Roche Response Center toll-free: 1-800-526-1247)

Trademarks and patents

See https://diagnostics.roche.com/us/en/about-us/patents

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Document revision

Document Revision Information	
Doc Rev. 3.0 09/2022	Updated front page and table 2 and table 3 with separate information for the Positive and Negative Control Kit use. Updated Patent web address Updated to current economic operators. Updated the harmonized symbol page. Please contact your local Roche Representative if you have any questions.
Doc Rev. 4.0 12/2022	Updated with **cobas**® 5800 information and data Updated Additional Information Updated References Updated hazard information in **Table 2**. Please contact your local Roche Representative if you have any questions.
Doc Rev 5.0 02/2023	Add **cobas**® 5800 to the 3rd bullet point of the **Warnings and precautions** section. Please contact your local Roche Representative if you have any questions.
Doc Rev 6.0 05/2023	Return minimum sample volume to original level in **Instructions for use** and **Key test features** section. Updated **cobas**® branding. Minor wording corrections. Please contact your local Roche Representative if you have any questions.