# 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 diagnosis and management of CMV in solid organ transplant patients and in hematopoietic stem cell transplant patients. The test can be used in these populations to assess the need to initiate antiviral treatment. In patients receiving anti-CMV therapy, serial DNA measurements can be used to assess viral response to treatment.

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

# Summary and explanation of the test

### Background

Human cytomegalovirus (CMV) is a viral pathogen belonging to the herpes virus family found ubiquitously in communities worldwide.[1,2] In immunocompetent hosts, infections with CMV are often asymptomatic but primary lytic infection can present as an acute mononucleosis-like syndrome. Once acquired, CMV usually persists as a lifelong latent infection that may reactivate intermittently. Peripheral blood mononuclear cells of the myeloid lineage (but not lymphocytes) and endothelial cells appear to be the major sites of CMV infection.[3] CMV remains in a latent stage in monocytes/macrophages in humans.[2] Latently infected individuals may asymptomatically shed the virus in their body fluids (e.g., urine, saliva) and thus infect others. Immunocompromised individuals, including neonates, transplant recipients, and AIDS patients, are at high risk for developing severe primary CMV infections or reactivations of latent CMV that lead to a high rate of morbidity and mortality.[4] Severe manifestations of CMV disease include retinitis, polyradiculopathy, gastroenteritis, hepatitis, encephalitis, esophagitis, enterocolitis, pancreatitis, nephritis, donor organ rejection, pneumonitis, and CMV viral syndrome.[5-7]

Our current understanding of clinically-relevant thresholds for the development of CMV disease comes from a variety of studies using different technologies, study populations, and end-points.[8-13] In general; higher viral loads are more closely associated with the risk of development of CMV disease. The relationship between viremia and disease is sigmoidal; i.e., the risk of CMV disease increases significantly after CMV viral load reaches a "critical threshold." For example, when using a laboratory-developed whole blood CMV DNA assay to test liver transplant recipients, the critical threshold was $\geq 5 \log_{10}$ copies/mL of CMV DNA.[11] In patients with HIV/AIDS, CMV DNA levels have been correlated with the risk of CMV disease and overall mortality.[14-17]

However, current laboratory-developed methods of CMV DNA quantification are limited by a lack of standardized results, which can lead to a high degree of inter-laboratory and inter-assay variability.[18] Validating the reproducibility of CMV DNA viral load is critical to ensuring consistency of results for the management of patients with CMV disease. Current guidelines based on the precision of PCR tests suggest that the changes in serial viral load measurements should be at least 3-fold ($0.5 \log_{10}$) to represent biologically important changes. Since variability is greatest at low concentrations, viral load changes may need to be more than 5-fold ($0.7 \log_{10}$) when the titer values are near the assay's lower limit of quantification, to be considered significant.[10]

While the exact threshold is still a subject of debate due to assay-to-assay variability, the critical threshold concept appears valid and has been reported in natural history studies showing that higher viral load values correlate with increased risk for the development of CMV disease.[8-12] One study using the COBAS® AMPLICOR CMV MONITOR Test established a cutoff for predicting disease between 2,000 and 5,000 copies/mL in CMV seropositive liver transplant recipients.[10]

## Rationale for NAT testing

Laboratory methods for diagnosing disseminated infection and active visceral disease for human CMV include isolation of virus by culture from peripheral blood leukocytes (PBL), histology on biopsies, serologic methods, measurement of pp65 antigenemia, and detection of CMV DNA by polymerase chain reaction (PCR).[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 systems. **cobas**® CMV enables the detection and quantitation of CMV DNA in EDTA plasma of infected patients. The viral load is quantified against a non-CMV DNA quantitation standard (DNA-QS), which is introduced into each specimen during sample processing. The DNA-QS also functions to monitor for the entire sample preparation and PCR amplification process. In addition, the test utilizes three external controls: a high titer positive, a low titer positive, and a negative control. The high positive and low positive external controls are manufactured by dilution from stock material with a titer traceable to 1st CMV WHO International Standard (NIBSC code: 09/162). Each Amplification/Detection kit lot is calibrated traceable to 1st CMV WHO International Standard (NIBSC code: 09/162).

## 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 systems 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 9 through Table 11.

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

**Table 1 cobas**® CMV

**(CMV)**

Store at 2-8°C
192 test cassette (P/N 09040897190)

| Kit components | Reagent ingredients | Quantity per kit<br>192 tests |
|---|---|---|
| **Proteinase Solution (PASE)** | Tris buffer, < 0.05% EDTA, calcium chloride, calcium acetate, 8% proteinase, glycerol<br>EUH210: Safety data sheets available on request.<br>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, < 0.10% 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, and the **cobas**® 6800/8800 systems with software version 2.0 or higher (P/N 09040919190)

For use on the **cobas**® 6800/8800 systems with software version 1.4 (P/N 07001037001 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.)<br><0.1% ProClin® 300 preservative** | 4 mL (8 x 0.5mL) |   <br>**WARNING**<br>H317: May cause an allergic skin reaction.<br>H412: Harmful to aquatic life with long lasting effects.<br>P261: Avoid breathing dust/fume/gas/ mist/vapours/spray.<br>P273: Avoid release to the environment.<br>P280: Wear protective gloves.<br>P333 + P313: If skin irritation or rash occurs: Get medical advice/ attention.<br>P362 + P364: Take off contaminated clothing and wash it before reuse.<br>P501: Dispose of contents/ container to an approved waste disposal plant.<br>55965-84-9 Reaction mass of: 5-chloro-2-methyl-4-isothiazolin-3-one [EC no. 247-500-7]and 2-methyl-2H -isothiazol-3- one [EC no. 220-239-6] (3:1) |
| **CMV High Positive Control (CMV H(+)C)** | < 0.001% synthetic (plasmid) CMV DNA encapsulated in Lambda bacteriophage coat protein, normal human plasma (CMV DNA not detectable by PCR methods.)<br><0.1% ProClin® 300 preservative** | 4 mL (8 x 0.5mL) |   <br>**WARNING**<br>H317: May cause an allergic skin reaction.<br>H412: Harmful to aquatic life with long lasting effects.<br>P261: Avoid breathing dust/fume/gas/ mist/vapours/spray.<br>P273: Avoid release to the environment.<br>P280: Wear protective gloves.<br>P333 + P313: If skin irritation or rash occurs: Get medical advice/ attention.<br>P362 + P364: Take off contaminated clothing and wash it before reuse.<br>P501: Dispose of contents/ container to an approved waste disposal plant.<br>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

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

**(NHP-NC)**

Store at 2-8°C

For use on the **cobas**® 5800 system, and the **cobas**® 6800/8800 systems with software version 2.0 or higher (P/N 09051554190)

For use on the **cobas**® 6800/8800 systems with software version 1.4 (P/N 07002220190 and P/N 09051554190)

| Kit components | Reagent ingredients | Quantity per kit | Safety symbol and warning* |
|---|---|---|---|
| **Normal Human Plasma Negative Control (NHP-NC)** | Normal human plasma (CMV DNA not detectable by PCR methods.)<br>0.1% ProClin® 300 preservative** | 16 mL (16 x 1 mL) |  <br>**WARNING**<br>H317: May cause an allergic skin reaction.<br>P261: Avoid breathing dust/fume/gas/mist/vapours/spray.<br>P272: Contaminated work clothing should not be allowed out of the workplace.<br>P280: Wear protective gloves.<br>P333 + P313: If skin irritation or rash occurs: Get medical advice/attention.<br>P362 + P364: Take off contaminated clothing and wash it before reuse.<br>P501: Dispose of contents/container to an approved waste disposal plant.<br>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)**<br>Store at 2–8°C<br>(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)**<br>Store at 2–8°C<br>(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)**<br>Store at 2–8°C<br>(P/N 06997538190) | 43% (w/w) guanidine thiocyanate**, 5% (w/v) polydocanol**, 2% (w/v) dithiothreitol**, dihydro sodium citrate<br>EUH032: Contact with acids liberates very toxic gas. | 4 x 875 mL |   <br>**DANGER**<br>H302 + H332: Harmful if swallowed or if inhaled.<br>H314: Causes severe skin burns and eye damage.<br>H412: Harmful to aquatic life with long lasting effects.<br>P261: Avoid breathing dust/ fume/ gas/ mist/ vapours/ spray.<br>P273: Avoid release to the environment.<br>P280: Wear protective gloves/ protective clothing/ eye protection/ face protection.<br>P303 + P361 + P353: IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water.<br>P304 + P340 + P310: IF INHALED: Remove person to fresh air and keep comfortable for breathing. Call a POISON CENTER/doctor.<br>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.<br>593-84-0 Guanidinium thiocyanate<br>9002-92-0 Polidocanol<br>3483-12-3 (R*,R*)-1,4-dimercaptobutane-2,3-diol |
| **cobas® omni Wash Reagent (WASH)**<br>Store at 15–30°C<br>(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 requirements

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

When reagents are not loaded on the **cobas**® 5800 system 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 and cobas® 6800/8800 systems

Reagents loaded onto the **cobas**® 5800 system or **cobas**® 6800/8800 systems are stored at appropriate temperatures and their expiration is monitored and enforced by the system. The system allows reagents to be used only if all of the reagent handling conditions shown in Table 6, Table 7 and Table 8 are met. The system automatically prevents use of expired reagents. Remaining open-kit stability and number of kit uses information for assay specific reagents is accessible through the system user interface.

**Table 6** Reagent expiry conditions monitored and enforced by the **cobas**® 5800 system

| Reagent | Open-kit stability | Number of kit uses | On-board stability |
|---|---|---|---|
| **cobas**® CMV | 90 days from first usage | 40 | 36 days from loading |
| **cobas**® CMV Control Kit | single use vial | 8 | 36 days from loading |
| **cobas**® NHP Negative Control Kit | single use vial | 16 | 36 days from loading |

**Table 7** Reagent expiry conditions monitored and enforced by the **cobas**® 6800/8800 systems

| Reagent | Open-kit stability | Number of kit uses | On-board stability (outside on board refrigerator) |
|---|---|---|---|
| **cobas**® CMV | 90 days from first usage | 40 | 40 hours from loading |
| **cobas**® CMV Control Kit | single use vial | 8 | 8 hours from loading |
| **cobas**® NHP Negative Control Kit | single use vial | 16 | 10 hours from loading |

Table 8 shows the open-kit stability of the **cobas**® **omni** reagents. Prior to each run, the system verifies the open-kit stability and ensures sufficient fill volume. Therefore, these reagents have no number of kit uses or on-board stability assigned.

**Table 8** **cobas**® **omni** reagent expiry condition monitored and enforced by the **cobas**® 5800/6800/8800 systems

| Reagent | Open-kit stability |
|---|---|
| **cobas**® **omni** Lysis Reagent | 30 days from loading |
| **cobas**® **omni** MGP Reagent | 30 days from first usage |
| **cobas**® **omni** Specimen Diluent | 30 days from loading |
| **cobas**® **omni** Wash Reagent | 30 days from loading |

## Additional materials required for cobas® 5800/6800/8800 systems

**Table 9** Material for use on the **cobas**® 5800/6800/8800 systems

| Material | P/N |
|---|---|
| **cobas**® **omni** Lysis Reagent | 06997538190 |
| **cobas**® **omni** MGP Reagent | 06997546190 |
| **cobas**® **omni** Specimen Diluent | 06997511190 |
| **cobas**® **omni** Wash Reagent | 06997503190 |

**Table 10** Consumables for use on **cobas**® 5800 system*

| Material |
|---|
| **cobas**® **omni** Processing Plate 24 |
| **cobas**® **omni** Amplification Plate 24 |
| **cobas**® **omni** Liquid Waste Plate 24 |
| Tip CORE TIPS with Filter, 1mL |
| Tip CORE TIPS with Filter, 300µL |
| **cobas**® **omni** Liquid Waste Container |
| Solid Waste Bag or Solid Waste Bag With Insert |

* For Part Numbers please refer to the **cobas**® 5800 system User Assistance.

**Table 11** Consumables for use on **cobas**® 6800/8800 systems*

| Material |
|---|
| **cobas**® **omni** Processing Plate |
| **cobas**® **omni** Amplification Plate |
| **cobas**® **omni** Pipette Tips |
| **cobas**® **omni** Liquid Waste Container |
| Solid Waste Bag and Solid Waste Container or Solid Waste Bag With Insert and Kit Drawer |

* For Part Numbers please refer to the **cobas**® 6800/8800 systems User Assistance

## Instrumentation and software required

The **cobas**® 5800 software, the **cobas**® 6800/8800 systems software and **cobas**® CMV analysis package (ASAP) for the **cobas**® 5800/6800/8800 systems shall be installed.

For **cobas**® 5800 and the **cobas**® 6800/8800 systems software version 2.0 or higher, the x800 Data Manager software and PC (or server) will be provided with the system.

For the **cobas**® 6800/8800 systems software version 1.4, the Instrument Gateway (IG) server will be provided with the system.

**Table 12** Instrumentation

| Equipment | P/N |
|---|---|
| **cobas**® 5800 system | 08707464001 |
| **cobas**® 6800 system | 05524245001 and 09575154001 |
| **cobas**® 8800 system | 05412722001 and 09575146001 |
| Sample Supply Module for **cobas**® 6800/8800 systems | 06301037001 and 09936882001 |

Refer to the **cobas**® 5800 system or **cobas**® 6800/8800 systems User Assistance for additional information.

# Precautions and handling requirements

## Warnings and precautions

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

- For in vitro diagnostic use only.
- **cobas**® CMV has not been evaluated for use as a screening test for the presence of CMV in blood or blood products.
- All patient samples should be handled as if infectious, using good laboratory procedures as outlined in Biosafety in Microbiological and Biomedical Laboratories and in the CLSI Document M29-A4.[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 should be considered potentially infectious and should be handled with universal precautions. If spillage occurs, immediately disinfect with a freshly prepared solution of 0.5% sodium 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 on request from your local Roche representative.
- Closely follow procedures and guidelines provided to ensure that the test is performed correctly. Any deviation from the procedures and guidelines may affect optimal test performance.
- False positive results may occur if carryover of samples is not adequately controlled during sample handling and processing.
- **Do not allow cobas**® **omni Lysis Reagent, which contains guanidine thiocyanate, to contact sodium or potassium hypochlorite solution or other highly reactive reagents such as acids or bases**. **This mixture can produce a highly toxic gas.** If liquid containing guanidine hydrochloride is spilled, clean with suitable laboratory detergent and water. If the spilled liquid contains potentially infectious agents, **FIRST** clean the affected area with laboratory detergent and water, and then with 0.5% sodium or potassium hypochlorite.
- Inform your local competent authority and manufacturer about any serious incidents which may occur when using this assay.

## Reagent handling

- Handle all reagents, controls, and samples according to good laboratory practice in order to prevent carryover of samples or controls.
- Before use, visually inspect each reagent cassette, diluent, lysis reagent, and wash reagent to ensure that there are no signs of leakage. If there is any evidence of leakage, do not use that material for testing.
- **cobas**® **omni** Lysis Reagent contains guanidine thiocyanate, a potentially hazardous chemical. Avoid contact of reagents with the skin, eyes, or mucous membranes. If contact does occur, immediately wash with generous amounts of water; otherwise, burns can occur.
- **cobas**® CMV test kits, **cobas**® **omni** MGP Reagent, and **cobas**® **omni** Specimen Diluent contain sodium azide as a preservative. Avoid contact of reagents with the skin, eyes, or mucous membranes. If contact does occur,

immediately wash with generous amounts of water; otherwise, burns can occur. If these reagents are spilled, dilute with water before wiping dry.
- Dispose of all materials that have come in contact with samples and reagents in accordance with country, state, and local regulations.

## Good laboratory practice

- Do not pipette by mouth.
- Do not eat, drink, or smoke in designated work areas.
- Wear laboratory gloves, laboratory coats, and eye protection when handling samples and reagents. Gloves must be changed between handling samples and **cobas**® CMV kits and **cobas**® **omni** reagents to prevent contamination. Avoid contaminating gloves when handling samples and controls.
- Wash hands thoroughly after handling samples and kit reagents, and after removing the gloves.
- Thoroughly clean and disinfect all laboratory work surfaces with a freshly prepared solution of 0.5% sodium 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 instruments, follow the instructions in the **cobas**® 5800 system or **cobas**® 6800/8800 systems User Assistance 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

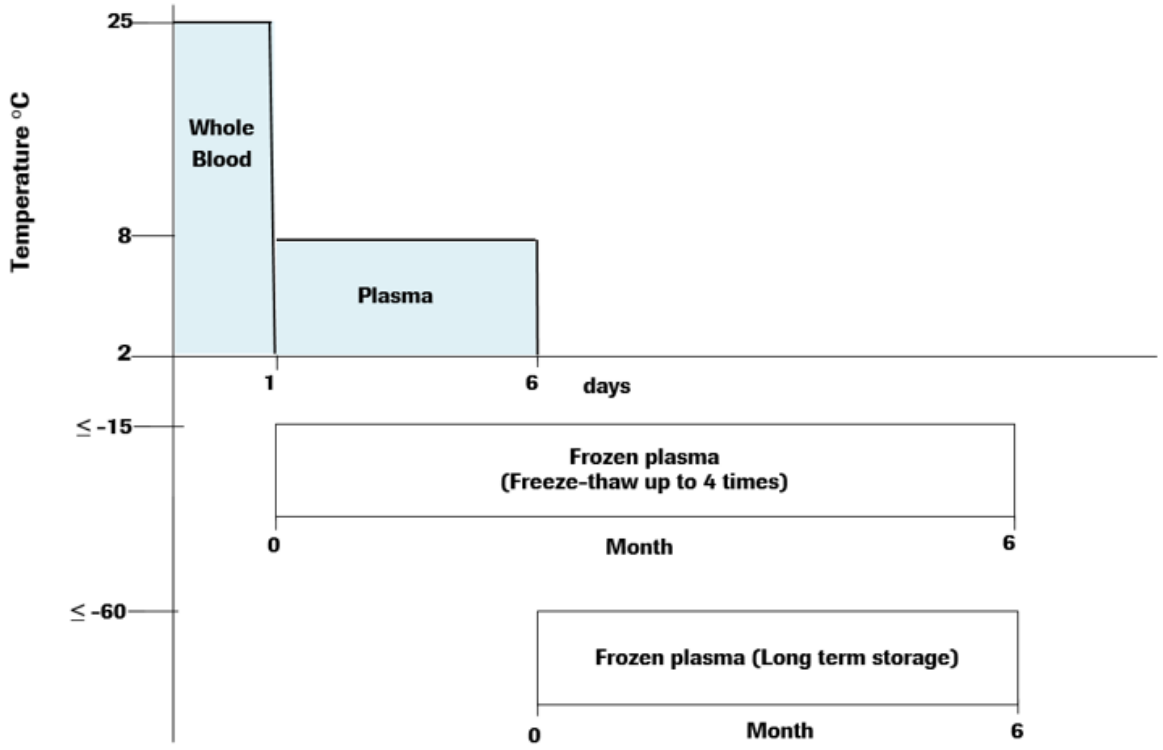
Sample stability is affected by elevated temperatures.

If using frozen samples in secondary tubes, place the samples at room temperature (15-30°C) 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 when immediately separated and tested and not further stored as separated EDTA-plasma.
- 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 6 months 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 ≤ -18°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.
- **cobas**® CMV can be run with a minimum sample volume of 500 µL of which 350 µL is processed.

## Running cobas® CMV on the cobas® 5800/6800/8800 systems

- The operation of the instruments is described in detail in the **cobas**® 5800 system or **cobas**® 6800/8800 systems User Assistance.
- Refer to the **cobas**® 5800 system or **cobas**® 6800/8800 systems User Assistance for proper maintenance of instruments.
- Ensure that specimen barcode labels on sample tubes are visible through the openings on the side of RD5 or MPA sample racks. Refer to the **cobas**® 5800 system or **cobas**® 6800/8800 systems User Assistance for proper barcode specifications and additional information on loading sample tubes.
- Figure 2 and Figure 3 summarize the procedure.

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

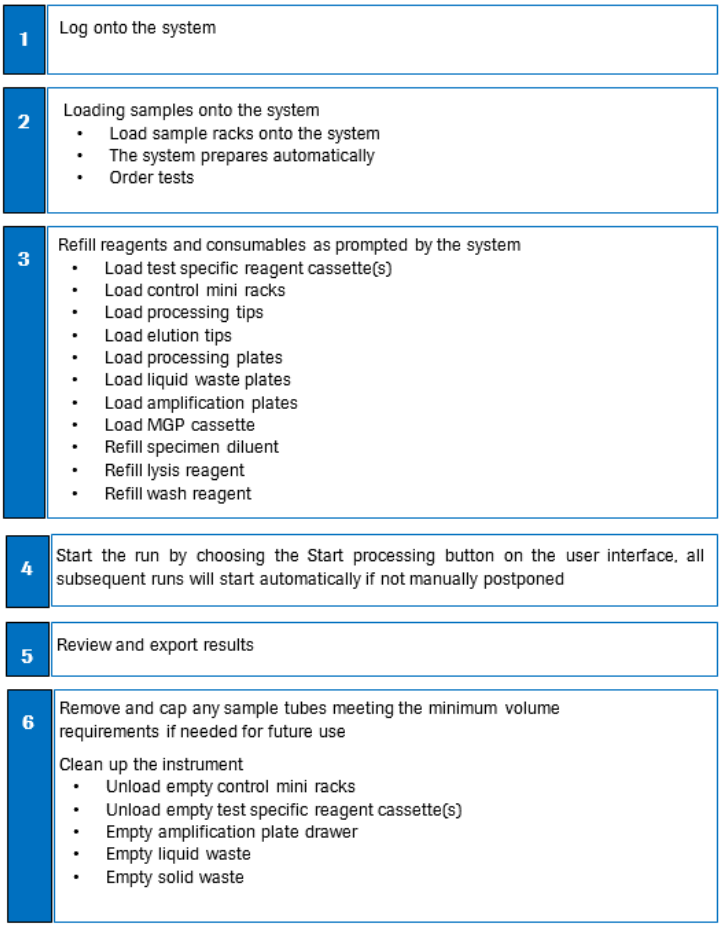
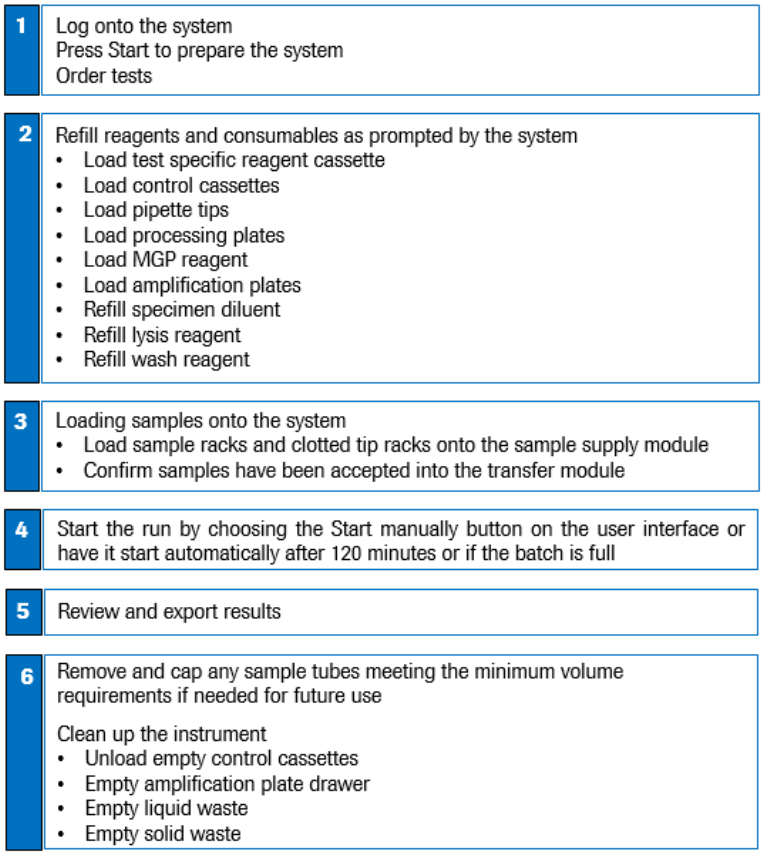


**Figure 3** **cobas**® CMV test procedure on the **cobas**® 6800/8800 systems



# Results

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

## Quality control and validity of results on the cobas® 5800 system and cobas® 6800/8800 systems with software version 2.0 or higher

- One **cobas**® NHP Negative Control [(–) C], and two **cobas**® CMV Positive Controls, a low positive control [CMV L (+) C] and a high positive control [CMV H (+) C] are processed at least every 72 hours and with every new kit lot. Positive and/or negative controls can be scheduled more frequently based on laboratory procedures and/or local regulations.
- The results of the controls are shown in the "Controls" app.
- In the software and/or report, check for flags to ensure the validity of the corresponding test results (refer to the x800 Data Manager User Assistance for a 'List of flag codes').
- Controls are marked with "Valid" in the column "Control result" if the respective target of the controls are reported valid. Controls are marked with 'Invalid' in the column "Control result" if the respective 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 controls is invalid, repeat testing of all controls and all associated samples is required.

Validation of results is performed automatically by the instrument software based on control results.

**NOTE:** The **cobas**® 5800 system and the **cobas**® 6800/8800 systems with software version 2.0 or higher 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.

## Quality control and validity of results on the cobas® 6800/8800 systems software version 1.4

- One **cobas**® NHP Negative Control [(–) C] and two **cobas**® CMV Positive Controls, a low positive control [CMV L (+) C ] and a high positive control [CMV H (+) C] are processed with each batch.
- In the software and/or report, check for flags and their associated results to ensure the batch validity.
- All flags are described in the **cobas**® 6800/8800 systems User Assistance.
- The batch is valid if no flags appear for all controls. If the batch is invalid, repeat testing of the entire batch is required.

Validation of results is performed automatically by the instrument software based on control results.

## Control flags on the cobas® 6800/8800 systems with software version 1.4

**Table 13** Control flags for negative and positive controls

| **Negative Control** | **Flag** | **Result** | **Interpretation** |
|---|---|---|---|
| (–) C | Q02<br>(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<br>(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<br>(Control batch failed) | Invalid | An invalid result or the calculated titer result for the high positive control is not within the assigned range. |

## Interpretation of results for cobas® 5800/6800/8800 systems

For a valid batch, check each individual sample for flags in the **cobas**® 5800 system and **cobas**® 6800/8800 system software and/or reports. The result interpretation should be as follows:

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

**Table 14** Target results for individual target result interpretation

| **Results** | **Interpretation** |
|---|---|
| Target Not Detected | CMV DNA not detected.<br>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).”<br>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.<br>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).”<br>Titer max = 1.0E+07 IU/mL |

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

### Interpretation of results on the cobas® 5800 system and cobas® 6800/8800 with software version 2.0 or higher

The results of the samples are shown in the "Results" app of the software.

For a valid control batch, check each individual sample for flags in the 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 shown in Table 14 above.
- If one or more sample targets are marked with "Invalid" the 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 with software version 1.4

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 shown in Table 14 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.
- Reliable results depend on proper sample collection, storage and handling procedures.
- This test has been validated only for use with EDTA plasma. Testing of other sample types with **cobas**® CMV may result in inaccurate results. Plasma viral load measurements are not directly comparable to those of other sample types.
- Quantitation of CMV DNA may be affected by sample collection methods, patient factors (i.e., age, presence of symptoms), and/or stage of infection.
- Mutations within the highly-conserved regions of the CMV DNA polymerase (UL54) gene covered by **cobas**® CMV, may affect primers and/or probe binding resulting in the under-quantitation of virus or failure to detect the presence of virus. **cobas**® CMV mitigates this risk, through the use of redundant amplification primers.
- Due to inherent differences between technologies, it is recommended that, prior to switching from one technology to the next, users perform method correlation studies in their laboratory to qualify technology differences. Users should follow their own specific policies/procedures.

- **cobas**® CMV is not intended for use as a screening test for the presence of CMV in blood or blood products and has not been evaluated as a diagnostic test to confirm the presence of CMV infection.

# Non-clinical performance evaluation

## System equivalency

System equivalency of the **cobas**® 5800, **cobas**® 6800 and **cobas**® 8800 systems was demonstrated via performance studies. The data presented in this Instructions for Use support equivalent performance for all systems.

## Key performance characteristics

## Limit of Detection (LoD)

### WHO International Standard

The limit of detection of **cobas**® CMV was determined by analysis of serial dilutions of the 1st WHO International Standard for Human Cytomegalovirus DNA for Nucleic Acid Amplification Technology Assays (1st HCMV WHO International Standard) obtained from NIBSC (NIBSC code: 09/162), 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 15. The study demonstrates that **cobas**® CMV detected CMV DNA at a concentration of 23 IU/mL or greater with a hit rate of ≥ 95%.

**Table 15** Limit of detection in EDTA plasma

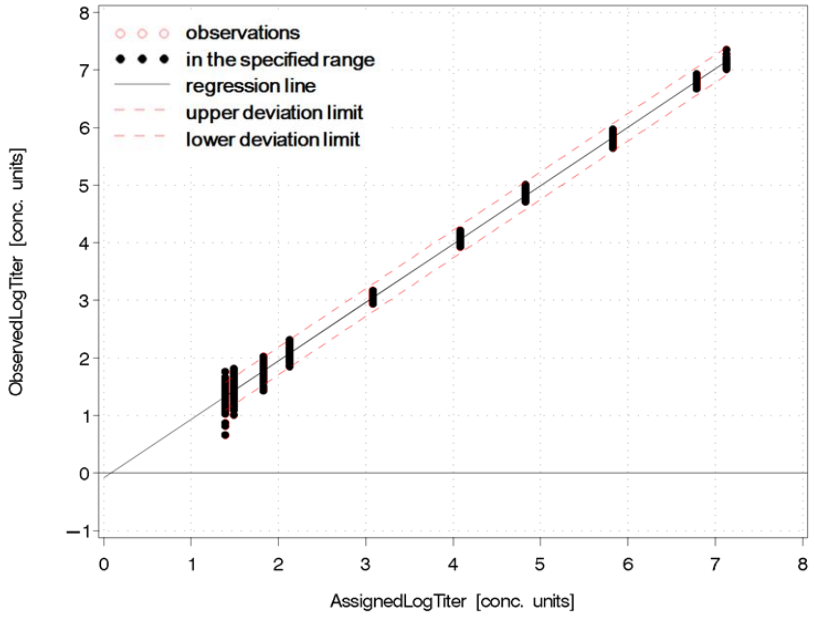
| Input titer concentration (CMV DNA IU/mL) | Number of valid replicates | Number of positives | Hit rate in % |
|---|---|---|---|
| 92.0 | 189 | 189 | 100.00 |
| 46.0 | 189 | 188 | 99.47 |
| 34.5 | 188 | 187 | 99.47 |
| 23.0 | 189 | 181 | 95.77 |
| 11.5 | 189 | 158 | 83.60 |
| 5.8 | 189 | 117 | 61.60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

## Linear range

Linearity of the **cobas**® CMV was evaluated using a dilution series consisting of 10 panel members with CMV genotype gB-1 DNA concentrations spanning the assay linear range (2.45E+01 IU/mL to 1.34E+07 IU/mL). Each panel member was tested in 48 replicates across three lots of **cobas**® CMV test reagents and the results of the study are presented in Figure 4.

**cobas**® CMV was demonstrated to be linear from 2.45E+01 IU/mL to 1.34E+07 IU/mL.

**Figure 4** Linear range determination in EDTA plasma



## Precision – within laboratory

Precision of **cobas**® CMV was determined by analysis of serial dilutions of high titer cultured Virus (Merlin, gB-1 genotype) in CMV negative EDTA plasma. Ten dilution levels were tested in 48 replicates for each level across three lots of **cobas**® CMV test reagents using three 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 are shown in Table 16.

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

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

## Genotype verification

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

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

### Verification of limit of detection for the Glycoprotein B genotypes gB-2, gB-3 and gB-4

CMV cell culture supernatants for three different Glycoprotein B genotypes (gB-2, gB-3 and gB-4) were diluted to three different concentration levels in CMV negative EDTA plasma. The hit rate determination was performed with 63 replicates for each level. Testing was conducted with three lots of **cobas**® CMV reagents. The results are shown in Table 17. These results verify that **cobas**® CMV detected CMV DNA for three different genotypes at concentrations of 34.5 IU/mL with a hit rate of ≥ 95%.

**Table 17** CMV DNA genotype verification of limit of detection

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

* Upper one-sided 95% confidence interval

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

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

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

## Drug resistant CMV specimens verification

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

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

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

Cell culture supernatants for two different drug resistant CMV specimens (resistant against Foscarnet or Ganciclovir, Valganciclovir and Cidofovir) were diluted to three different concentration levels in CMV negative EDTA plasma. The hit rate determination was performed with 63 replicates for each level. Testing was conducted with three lots of **cobas**® CMV reagents. The results are shown in Table 18. These results verify that **cobas**® CMV detected CMV DNA for two different specimens resistant against Foscarnet or Ganciclovir, Valganciclovir and Cidofovir at concentrations of 34.5 IU/mL with a hit rate of ≥ 95%.

**Table 18** Drug resistant CMV specimens verification of limit of detection

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

* Upper one-sided 95% confidence interval

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

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

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

## Specificity

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

## Analytical specificity

The analytical specificity of **cobas**® CMV was evaluated by diluting a panel of microorganisms to a concentration of 1.00E+06 particles, copies, IU, genome equivalents or CFU/mL with CMV DNA positive and CMV DNA negative EDTA plasma. The specific organisms tested are listed in Table 19. Each panel member was evaluated with **cobas**® CMV. None of the non-CMV pathogens were shown to interfere with test performance.

**Table 19** Microorganisms tested for cross-reactivity

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

## Analytical specificity – interfering substances

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

The impact of the presence of autoimmune diseases such as systemic lupus erythematosus (SLE), rheumatoid arthritis (RA) and antinuclear antibody was also evaluated in the presence and absence of CMV DNA. In addition, drug compounds listed in Table 20 were tested at three times the $C_{max}$ in presence and absence of CMV DNA.

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

**Table 20** Drug compounds tested for interference with the quantitation of CMV DNA by **cobas**® CMV

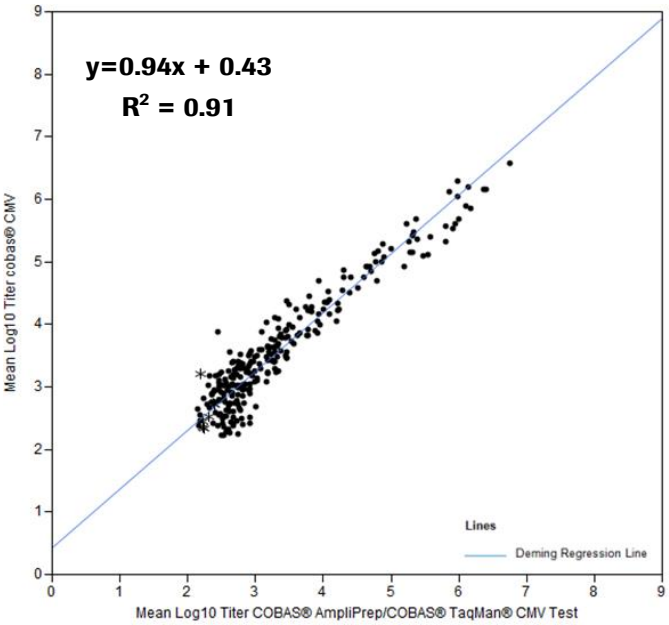
| Class of drug | Generic drug name | Generic drug name |
|---|---|---|
| Antimicrobial | Cefotetan | Sulfamethoxazole |
| Antimicrobial | Clavulanate potassium | Ticarcillin disodium |
| Antimicrobial | Fluconazole | Trimethoprim |
| Antimicrobial | Piperacillin | Vancomycin |
| Antimicrobial | Tazobactam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

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

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

The Deming regression results are shown in Figure 5.

**Figure 5** Regression analysis of **cobas**® CMV vs CAP/CTM CMV Quantitative Test



## Whole system failure

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

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

## Cross contamination

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

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

# Clinical performance evaluation

## 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 21 through Table 23 below.

Table 21 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 21** 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%<br>(2.97)<br>0.0129 | 6%<br>(6.49)<br>0.0282 | 0%<br>(0.00)<br>0.0000 | 3%<br>(4.47)<br>0.0194 | 90%<br>(25.15)<br>0.1076 | 0.114 | 26.61 |
| 3.26 | 3.27 | 322 | 10%<br>(4.29)<br>0.0186 | 13%<br>(4.85)<br>0.0210 | 3%<br>(2.50)<br>0.0109 | 0%<br>(0.00)<br>0.0000 | 74%<br>(11.71)<br>0.0507 | 0.059 | 13.64 |
| 3.86 | 3.90 | 324 | 23%<br>(7.26)<br>0.0315 | 0%<br>(0.00)<br>0.0000 | 0%<br>(0.22)<br>0.0010 | 0%<br>(0.00)<br>0.0000 | 77%<br>(13.50)<br>0.0584 | 0.066 | 15.36 |
| 6.70 | 6.74 | 324 | 15%<br>(5.16)<br>0.0224 | 3%<br>(2.31)<br>0.0100 | 1%<br>(1.52)<br>0.0066 | 0%<br>(0.00)<br>0.0000 | 81%<br>(11.98)<br>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 22 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 22** 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 23 below presents the reproducibility results for the negative panel member for the **cobas**® 6800 system.

**Table 23** Reproducibility results for the negative panel member

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

[a] Negative Percent Agreement = (number of negative results / total valid tests in negative panel member)*100%.

[b] Calculated using the Clopper-Pearson exact binomial confidence interval method.

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

## Clinical performance evaluation: solid organ transplant (SOT) population

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

The demographic characteristics of the patient population are presented in Table 24.

**Table 24** 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 25 through Table 28 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 25** 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 26** 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 27** 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 28** 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 29 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 29** 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 Day 49 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 30).

**Table 30** 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 31 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 31** 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 contain a 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 32 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 32** 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** 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 2.7$log_{10}$ 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.

Table 33 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 33** Summary of concordance analyses (paired samples at timepoints of interest post anti-CMV therapy initiation) in the SOT population

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

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

$log_{10}$ (1.37E+02) = 2.137; $log_{10}$ (5.0E+02) = 2.699; $log_{10}$ (1.8E+03) = 3.255; $log_{10}$ (7.943E+03) = 3.899.

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

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

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

Table 34 below shows the summary of concordance of viral load results by different thresholds (Target Not Detected, 137 IU/mL, 500 IU/mL, and 1800 IU/mL) for all paired samples evaluable at Day14, Day 21, Day 28, Day 35, or Day 49 post anti-CMV therapy initiation in the SOT population.

**Table 34** 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** 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 35 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 35** 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]<br>95% CI[b] | r |
|---|---|---|---|---|---|---|
| Clinical and Spiked | 543 | Intercept | 0.348<br>0.407* | 0.033 | (0.283, 0.413)<br>(0.356, 0.462) | 0.98 |
| Clinical and Spiked | 543 | Slope | 0.961<br>0.945* | 0.009 | (0.944, 0.979)<br>(0.933, 0.957) | 0.98 |
| Clinical | 445 | Intercept | 0.193<br>0.229* | 0.037 | (0.120, 0.266)<br>(0.160, 0.301) | 0.97 |
| Clinical | 445 | Slope | 1.023<br>1.010* | 0.010 | (1.002, 1.044)<br>(0.992, 1.030) | 0.97 |
| Spiked | 98 | Intercept | 0.012<br>N/A | 0.063 | (-0.114, 0.138)<br>N/A | 0.99 |
| Spiked | 98 | Slope | 0.985<br>N/A | 0.013 | (0.960, 1.010)<br>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.

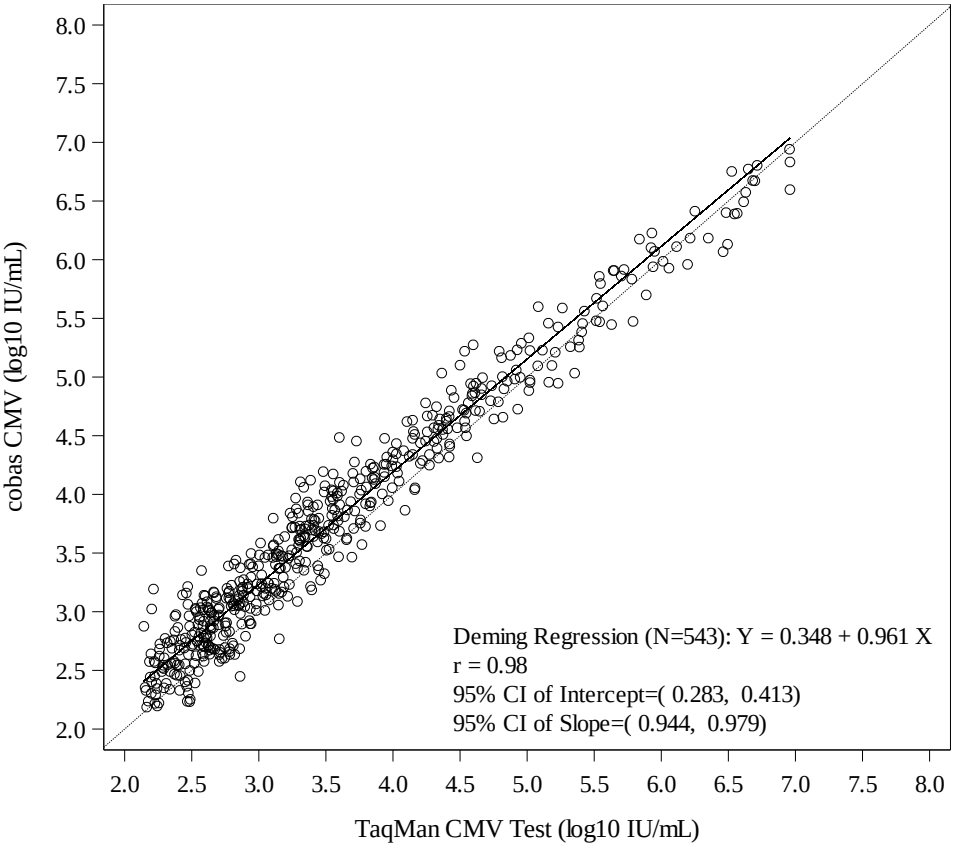
[a] Assumed independence between all samples.

[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)

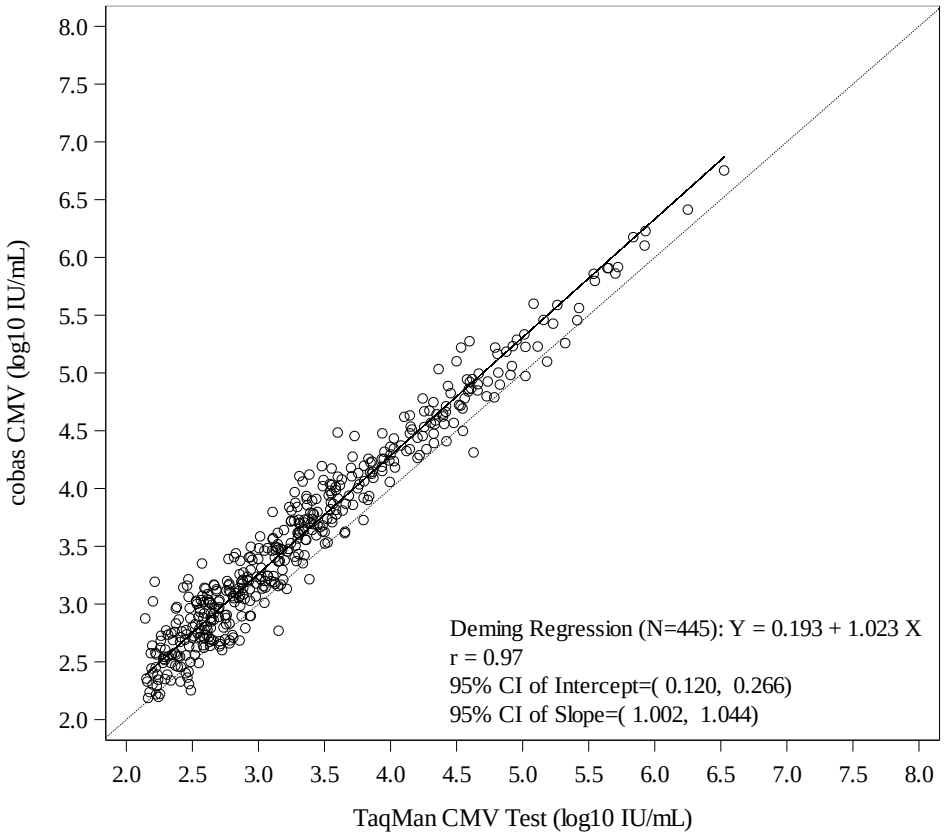



Note: Twenty-six samples from nine subjects were excluded from method comparison analyses due to impactful sequence mismatch.

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)

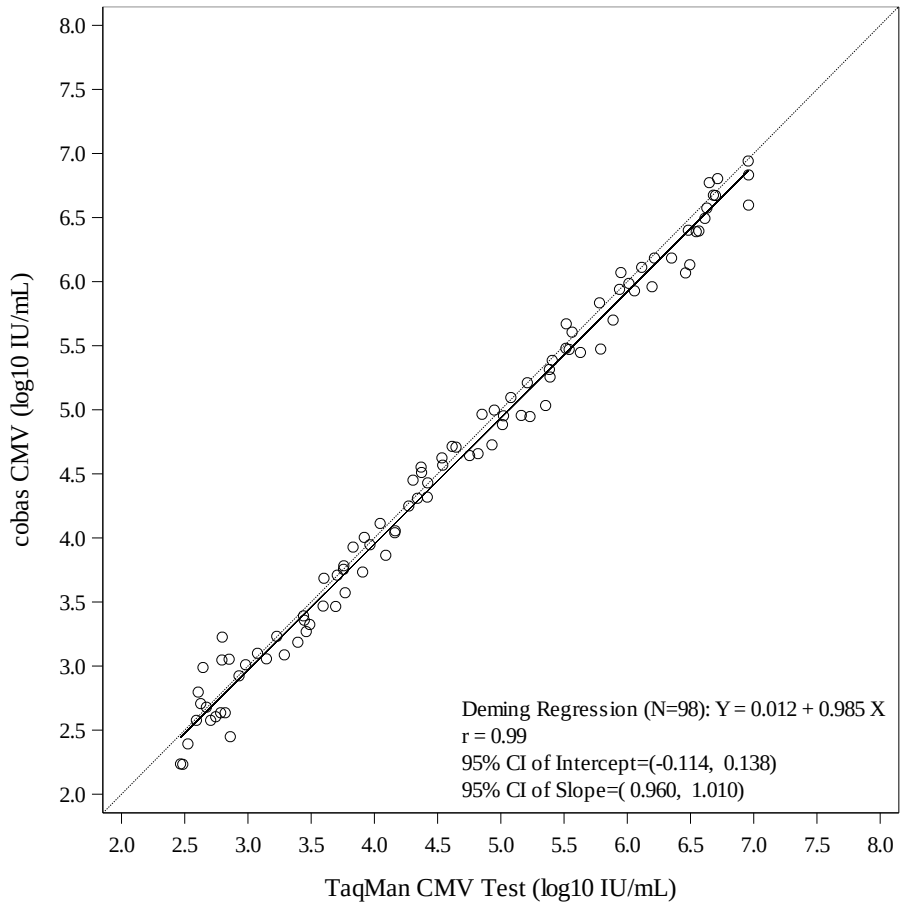



Note: Twenty-six samples from nine subjects were excluded from method comparison analyses due to impactful sequence mismatch.

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 36 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 36** 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(**cobas**® CMV estimate $log_{10}$ IU/mL) - 10(TaqMan® CMV Test Viral Load Level $log_{10}$ IU/mL).

## Mean paired difference

Table 37 below presents the mean paired difference between **cobas**® CMV and the TaqMan® CMV Test at representative thresholds and associated 95% CIs calculated using the paired t-test.[28]

**Table 37** 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 38 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 38** 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) |

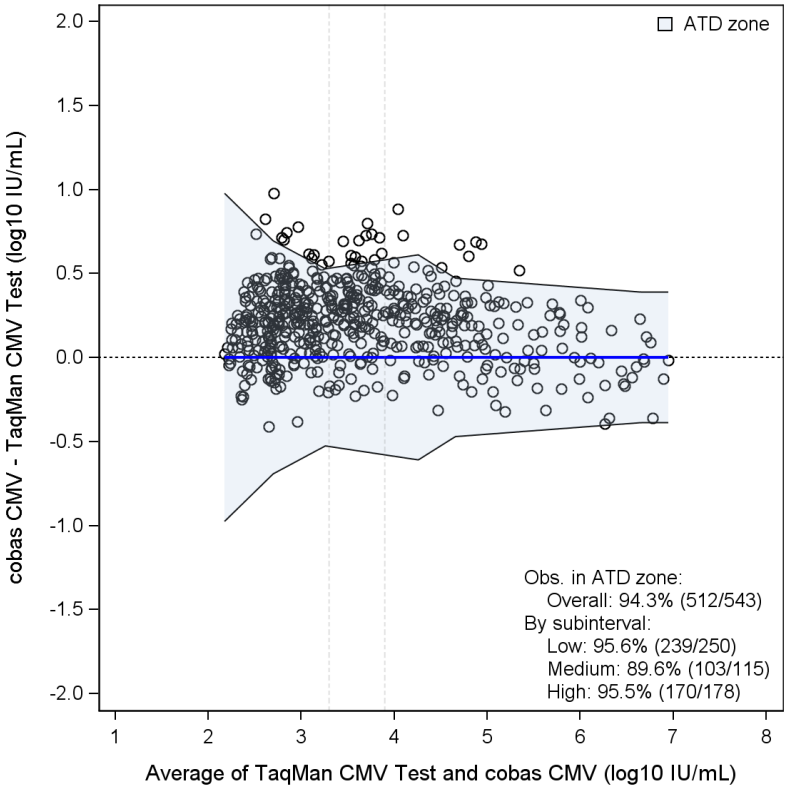
Note: Twenty-six samples from nine subjects were excluded from method comparison analyses due to impactful sequence mismatch. The table only includes paired samples with paired results that were each within 1.37E+02 IU/mL to 9.1E+06 IU/mL, the overlapping linear range of both assays. Paired results were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

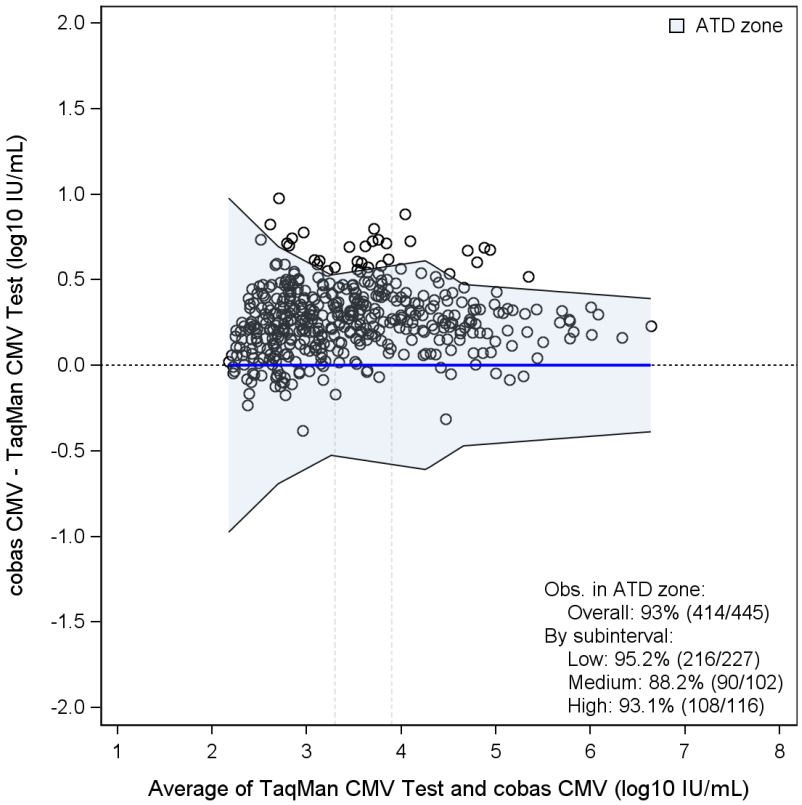
**Figure 9** Allowable Total Difference (ATD) plot of individual viral load differences versus their average ($log_{10}$ IU/mL) in the SOT population (**cobas**® CMV versus TaqMan® CMV Test; clinical and spiked samples)




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).

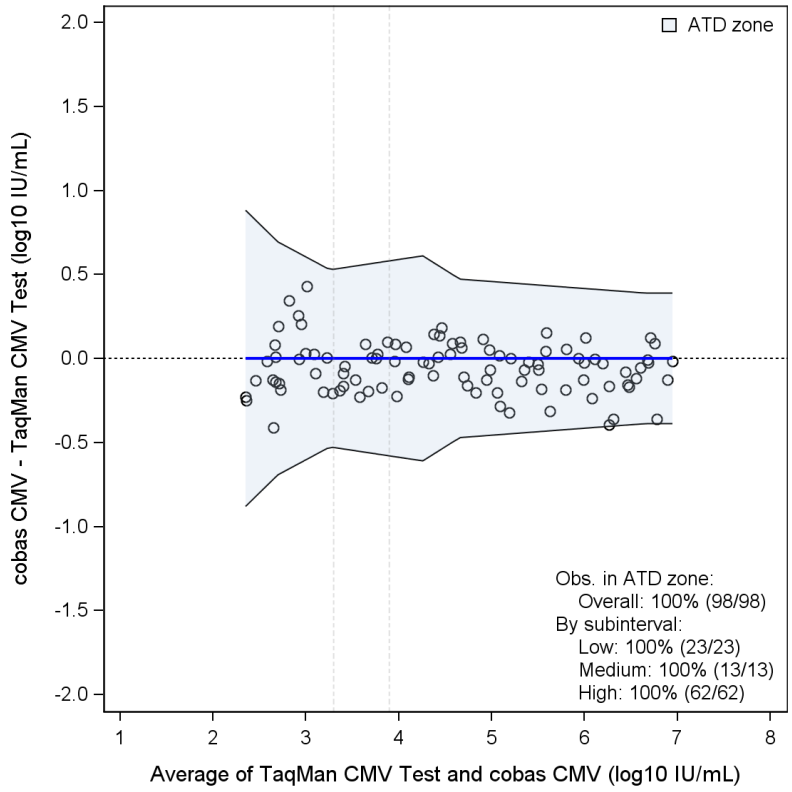
**Figure 10** Allowable Total Difference (ATD) plot of individual viral load differences versus their average ($log_{10}$ IU/mL) in the SOT population (**cobas**® CMV versus TaqMan® CMV Test; clinical samples)




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 39.

**Table 39** Results of CMV IgG-negative specimens (**cobas**® CMV versus TaqMan® CMV Test)

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

Note: The lower limit of quantitation (LLoQ) is 1.37E+02 IU/mL for TaqMan® CMV Test.

CMV = cytomegalovirus; IgG = immunoglobulin G.

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

The study was designed to evaluate the concordance between **cobas®** CMV and the COBAS® AmpliPrep/COBAS® TaqMan® CMV Test in a hematopoietic stem cell transplant (HSCT) population. Residual samples from a Phase 2, randomized, double blind, placebo-controlled dose-ranging multicenter clinical trial of brincidofovir for CMV prophylaxis[21] were tested.

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

Table 40 below summarizes the demographics and baseline clinical characteristics of the 258 subjects.

**Table 40** 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 41 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 41** 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 42 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 42** 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 43 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 43** 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 44 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 44** 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 45 below shows the concordance analyses of CMV episode resolution by time point for viremic subjects who initiated anti-CMV therapy.

**Table 45** Concordance analysis of CMV episode resolution by time point for viremic HSCT subjects who initiated anti-CMV therapy

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

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

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

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

CMV = cytomegalovirus. NC = not calculable.

Table 46 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 46** 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 47 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 47** 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 48 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 48** 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 49 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 49** 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 50 below shows the summary concordance of viral load results for paired samples at time points of interest post anti-CMV therapy initiation from HSCT patients using different thresholds (Target Not Detected, 137 IU/mL, 500 IU/mL and 1800 IU/mL).

**Table 50** Summary concordance of viral load results for HSCT Patients Using Different Thresholds (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 51 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 51** 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]<br>95% Bootstrap CI[b] | r |
|---|---|---|---|---|---|---|
| **Clinical and Spiked** | 204 | Intercept | 0.145<br>0.172* | 0.041 | (0.064, 0.227)<br>(0.132, 0.219) | 0.99 |
| **Clinical and Spiked** | 204 | Slope | 0.990<br>0.982* | 0.009 | (0.972, 1.008)<br>(0.972, 0.990) | 0.99 |
| **Clinical** | 107 | Intercept | -0.146<br>-0.188* | 0.106 | (-0.356, 0.064)<br>(-0.462, -0.008) | 0.96 |
| **Clinical** | 107 | Slope | 1.110<br>1.125* | 0.034 | (1.041, 1.178)<br>(1.066, 1.217) | 0.96 |
| **Spiked** | 97 | Intercept | -0.097<br>N/A | 0.063 | (-0.223, 0.028)<br>N/A | 0.99 |
| **Spiked** | 97 | Slope | 1.025<br>N/A | 0.012 | (1.000, 1.049)<br>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.

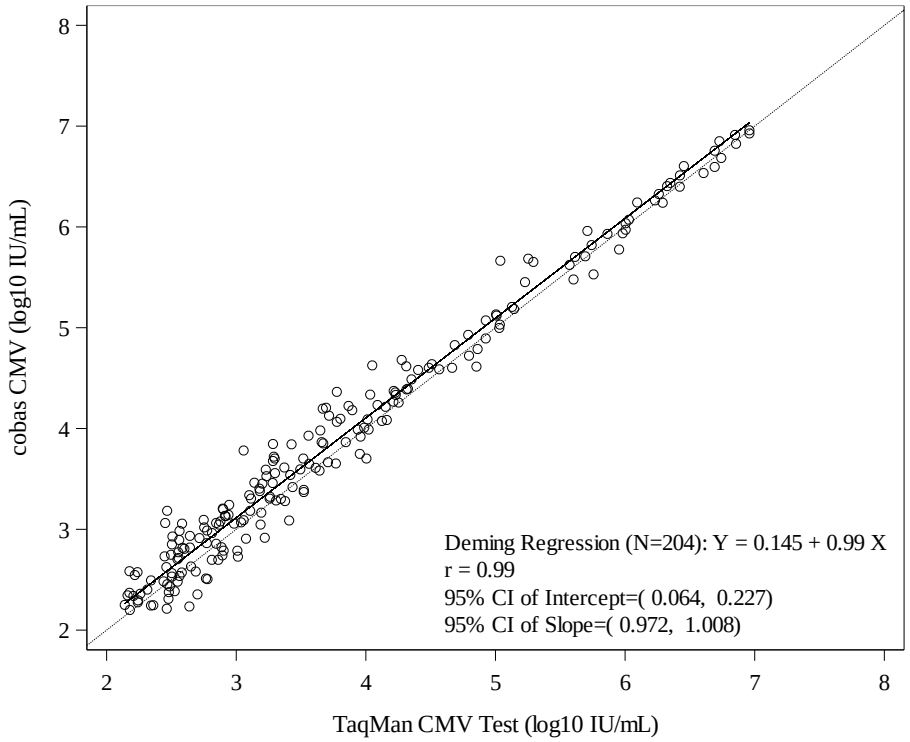
[b] Adjusted correlation between samples from the same subject by the bootstrap method with 500 iterations.

* 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)

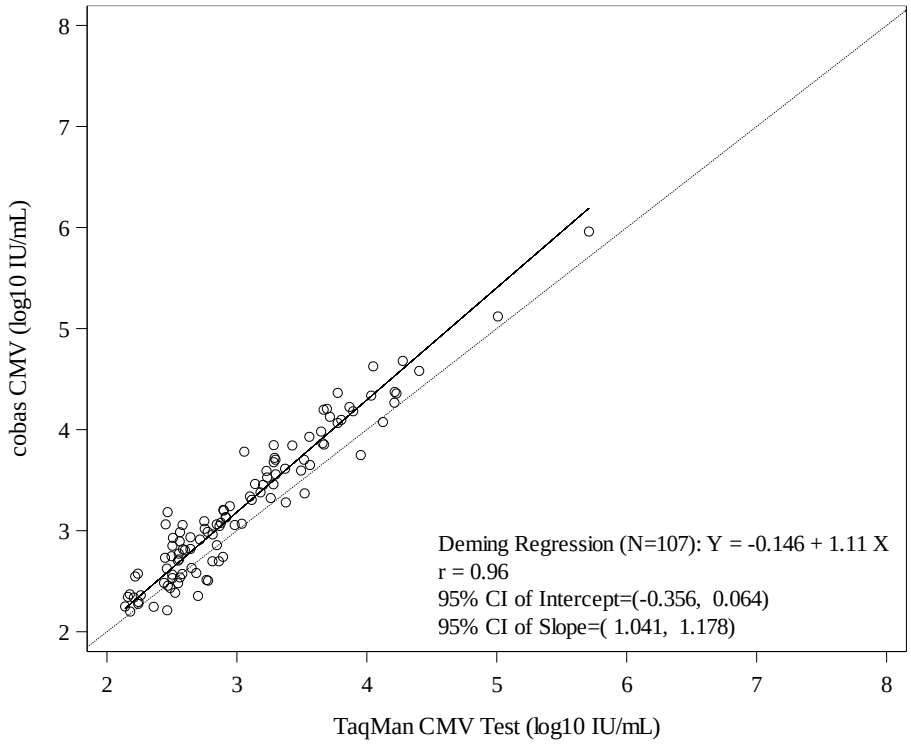



Note: Seven samples from three subjects were excluded from method comparison analyses due to impactful sequence mismatch.

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)

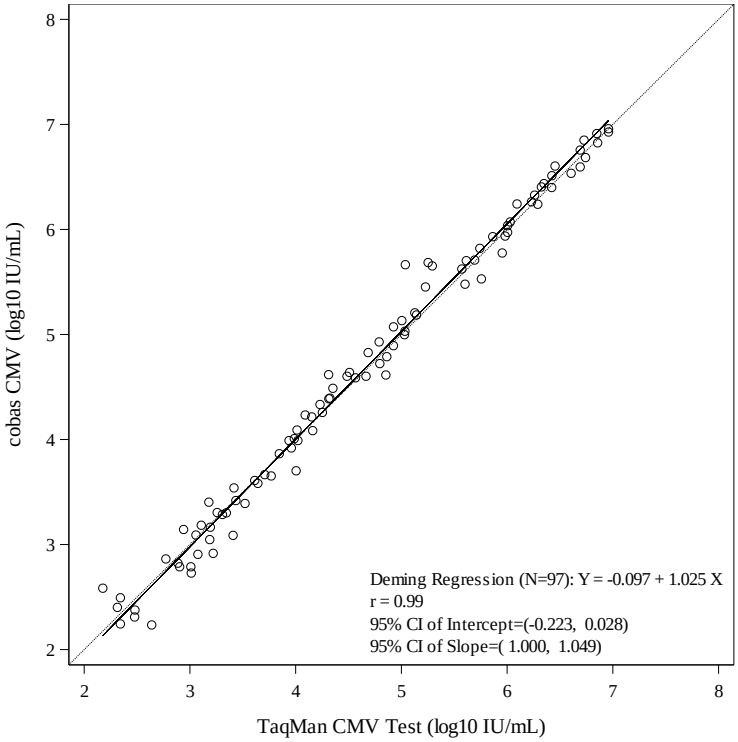



Note: Seven samples from three subjects were excluded from method comparison analyses due to impactful sequence mismatch.

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 52 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 52** 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 53 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 53** 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 54 below shows the percentage of results within low, medium and high intervals of the Allowable Total Difference zone by sample type.

**Table 54** 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) |

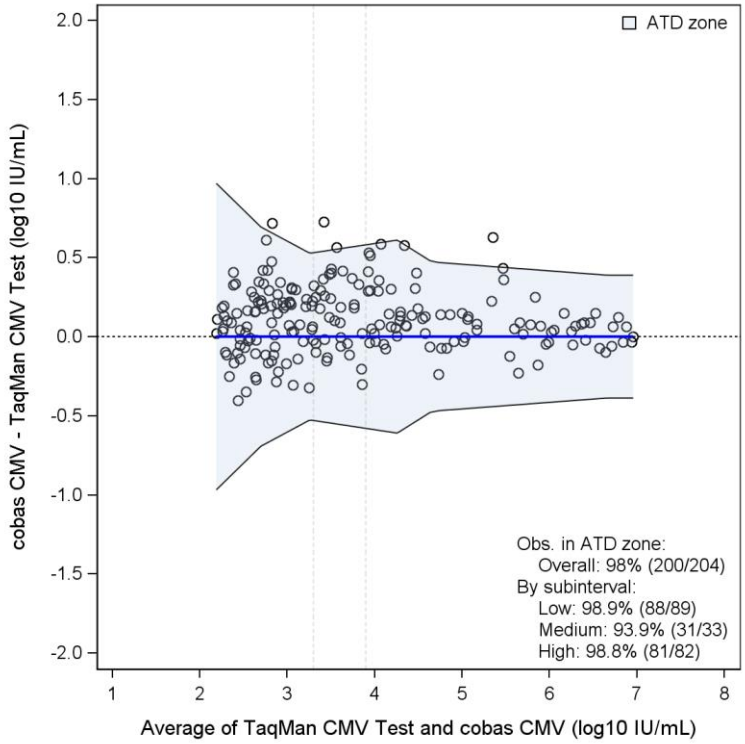
Note: Seven samples from three subjects were excluded from method comparison analyses due to impactful sequence mismatch. The table only included paired samples with results each within 1.37E+02 to 9.1E+06 IU/mL, the common linear range of both assays. Paired results were categorized into the intervals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

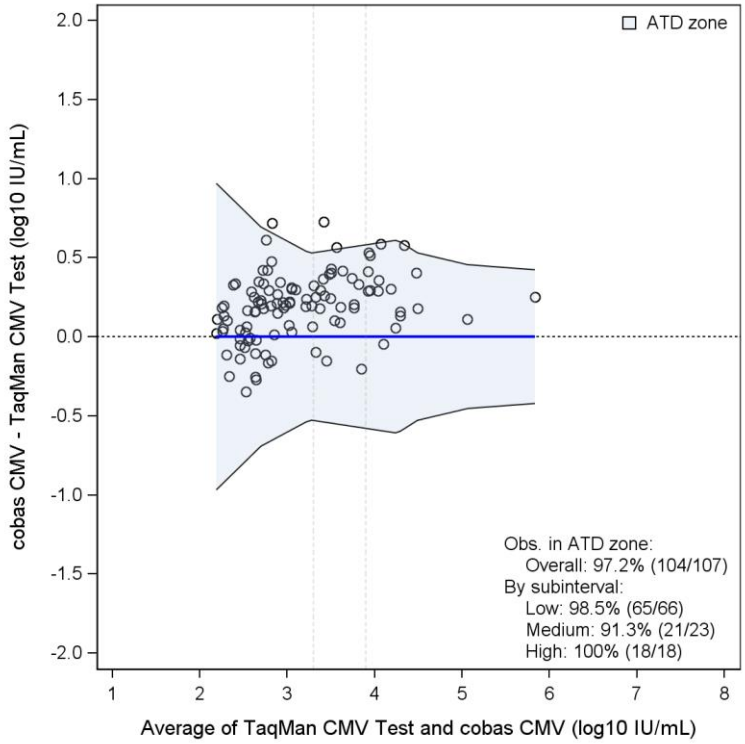
**Figure 15** Allowable Total Difference (ATD) plot of viral load difference ($log_{10}$ IU/mL) in the HSCT population (clinical and spiked samples)




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.

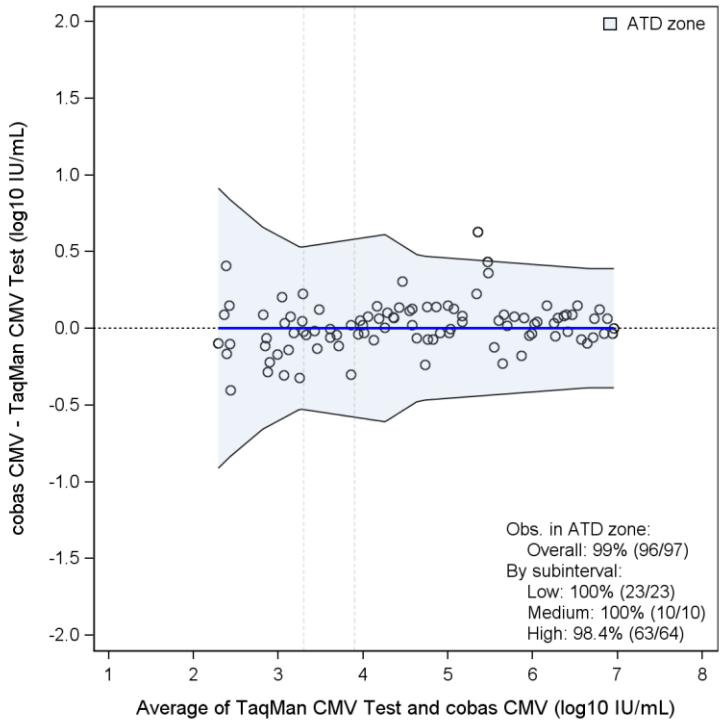
**Figure 16** Allowable Total Difference (ATD) plot of viral load difference ($log_{10}$ IU/mL) in the HSCT population (clinical samples)




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 55 below.

**Table 55** Results of CMV IgG-Negative Specimens Tested on **cobas**® CMV and TaqMan® CMV Test

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

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

IgG = immunoglobulin G.

## Conclusion

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

# Additional information

## Key test features

| | |
|---|---|
| **Sample type** | EDTA plasma |
| **Minimum amount of sample required** | 500 µL* |
| **Sample processing volume** | 350 µL |
| **Analytical sensitivity** | 34.5 IU/mL |
| **Linear range** | 34.5 IU/mL to 1E+07 IU/mL |
| **Specificity** | 100% |
| **Genotypes detected** | CMV Glycoprotein B Genotype 1-4 |
| **Drug resistant CMV specimens detected** | CMV specimens resistant against Ganciclovir, Valganciclovir, Cidofovir and Foscarnet |

*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) 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 56** 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 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 For USA: Caution: 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 importer

**Table 57** Manufacturer and importer

| | |
|---|---|
| | Manufactured in United States |
|  | Roche Molecular Systems, Inc.<br>1080 US Highway 202 South<br>Branchburg, NJ 08876, USA<br>www.roche.com |
| | Made in USA |
|  | Roche Diagnostics GmbH<br>Sandhofer Strasse 116<br>68305 Mannheim, Germany |

## Trademarks and patents

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

## Copyright




Roche Diagnostics GmbH
Sandhofer Str. 116
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# Document revision

| Document Revision Information | |
|---|---|
| Doc Rev. 5.0<br>12/2024 | Added system software version 2.0 information for **cobas**® 6800/8800 systems.<br>Added NIBSC code: 09/162 for WHO International Standard.<br>P/Ns of consumables removed, detailed information on consumables are referenced in the **cobas**® 5800 and **cobas**® 6800/8800 systems User Assistance.<br>Removed Rx Only from front page.<br>Updated the harmonized symbol page.<br>Added IVD symbol.<br>Please contact your local Roche Representative if you have any questions. |
| Doc Rev. 6.0<br>04/2025 | **Table 10 and Table 11**: consumables information corrected.<br>**Table 12**: P/Ns for **cobas**® 6800 and **cobas**® 8800 systems corrected.<br>Please contact your local Roche Representative if you have any questions. |

The summary of safety and performance report can be found using the following link: https://ec.europa.eu/tools/eudamed.