cobas® CMV

Quantitative nucleic acid test

for use on the cobas® 4800 System

For in vitro diagnostic use

cobas® CMV	120 Tests	P/N: 07865970190
cobas® CMV Control Kit	10 Sets	P/N: 07865988190
cobas® 4800 System Sample Preparation Kit 2	240 Tests 960 Tests	P/N: 06979513190 P/N: 06979521190
cobas® 4800 System Wash Buffer Kit	240 Tests 960 Tests	P/N: 05235863190 P/N: 05235871190
cobas® 4800 System Lysis Kit 2	240 Tests 960 Tests	P/N 06979530190 P/N 06979548190

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Additional information

Intended use

cobas® CMV is an in-vitro nucleic acid amplification test for the quantitative measurement 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 human 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 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.[8-15] 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 so the risk of CMV disease increases significantly after CMV viral load reaches a "critical threshold." For example, when using a laboratory-developed whole blood CMV DNA assay to test liver transplant recipients, the critical threshold was ≥ 5 $\log_{10}$ copies/mL of CMV DNA.[13] In patients with HIV/AIDS, CMV DNA levels have been correlated with the risk of CMV disease and overall mortality.[16-19]

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.[20] 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 $\log_{10}$ copies/mL) to represent biologically important changes. Since variability is greatest at low concentrations, values near the assay's lower limit of quantification viral load changes may need to be more than 5-fold (0.7 $\log_{10}$ copies/mL) to be considered significant.[11,12]

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.[8-15] One study using the COBAS® AMPLICOR CMV MONITOR Test (CACM Test, Research Use Only status in the United States and CE-IVD approved) established a cutoff for predicting disease between 2,000 and 5,000 copies/mL in CMV seropositive liver transplant recipients.[10]

Rationale for CMV 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).[21] 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.[22] The pp65 assay is also difficult to perform on neutropenic patients. Direct detection of CMV DNA by e.g., 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**® 4800 System. **cobas**® CMV enables the detection and quantitation of CMV DNA traceable to the 1st HCMV WHO International Standard 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.

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**® 4800 System consists of the **cobas x** 480 instrument and the **cobas z** 480 analyzer. Automated data management is performed by the **cobas**® 4800 software which assigns test results for all tests as target not detected, < LLoQ (below lower limit of quantitation), > ULoQ (above upper limit of quantitation) or CMV DNA detected, 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 acids from patient samples, external controls and added lambda DNA QS molecules are simultaneously extracted. In summary, viral nucleic acids are released by addition of proteinase and lysis reagent to the sample. The released nucleic acids bind to the silica surface of the added magnetic glass particles. Unbound substances and impurities, such as denatured proteins, cellular debris and potential PCR inhibitors are removed with subsequent wash buffer steps and purified nucleic acids are eluted from the magnetic 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).[23-25] 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.

cobas® CMV master mix contains one detection probe specific for the 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 detection channels.[26,27] When not bound to the target sequence, the fluorescent signals of the intact probes are suppressed by a quencher dye. During the PCR amplification step, hybridization of the probes to the specific single-stranded DNA template results in cleavage of the probe by the 5'-to-3' 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 increases concomitantly. 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.

Materials and reagents

Reagents

All unopened reagents and controls shall be stored as recommended in the Reagent storage and handling requirements table.

Kit	Components and Reagent Ingredients	Quantity per Kit	Safety Symbol and Warning
cobas® CMV 120 Tests (P/N: 07865970190)	**MMX R1** (**cobas**® Master Mix Reagent 1) Manganese acetate, potassium hydroxide, < 0.1% sodium azide	10 × 1.75 mL	N/A
	CMV MMX R2 (**cobas**® CMV Master Mix Reagent 2) Tricine buffer, potassium acetate, 18% dimethyl sulfoxide, glycerol, < 0.1% Tween 20, EDTA, < 0.12% dATP, dCTP, dGTP, dUTP, < 0.01% CMV forward and reverse primers, < 0.01% Quantitation Standard forward and reverse primers, < 0.01% fluorescent-labeled oligonucleotide probes specific for CMV and the Quantitation Standard,< 0.01% oligonucleotide aptamer, < 0.01% Z05D DNA polymerase (microbial), < 0.01% AmpErase (uracil-N- glycosylase) enzyme (microbial), < 0.1% sodium azide	10 × 0.5 mL	N/A
	DNA QS (**cobas**® DNA Quantitation Standard) 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	10 × 1.75 mL	N/A

Kit	Components and Reagent Ingredients	Quantity per Kit	Safety Symbol and Warning[a]
cobas® CMV Control Kit 10 Sets (P/N: 07865988190)	**CMV L(+)C** (**cobas**® CMV Low Positive Control) < 0.001% synthetic (plasmid) CMV DNA encapsulated in Lambda bacteriophage coat protein, normal human plasma, non- reactive by licensed tests for antibody to HCV, antibody to HIV-1/2, HBsAg, antibody to HBc; HIV-1 RNA, HIV-2 RNA, HCV RNA, HBV DNA, HEV RNA, WNV RNA and CMV DNA not detectable by PCR methods. 0.1% ProClin® 300 preservative	10 × 0.75 mL	  Warning H317: May cause an allergic skin reaction. P261: Avoid breathing dust/ fume/ gas/ mist/ vapours/ spray. P272: Contaminated work clothing should not be allowed out of the workplace. P280: Wear protective gloves. P333 + P313: If skin irritation or rash occurs: Get medical advice/attention. P362 + P364: Take off contaminated clothing and wash it before reuse. P501: Dispose of contents/container to an approved waste disposal plant.
	CMV H(+)C (**cobas**® CMV High Positive Control) < 0.001% high titered synthetic (plasmid) CMV DNA encapsulated in Lambda bacteriophage coat protein, normal human plasma, non-reactive by licensed tests for antibody to HCV, antibody to HIV-1/2, HBsAg, antibody to HBc; HIV-1 RNA, HIV-2 RNA, HCV RNA, HBV DNA, HEV RNA, WNV RNA and CMV DNA not detectable by PCR methods. 0.1% ProClin® 300 preservative	10 × 0.75 mL	
	(–)C2 (**cobas**® Negative Control 2) Normal human plasma, non-reactive by licensed tests for antibody to HCV, antibody to HIV-1/2, HBsAg, antibody to HBc; HIV-1 RNA, HIV-2 RNA, HCV RNA, HBV DNA, HEV RNA, WNV RNA, and CMV DNA not detectable by PCR methods. < 0.1% ProClin® 300 preservative	10 × 0.75 mL	

[a] Product safety labeling primarily follows EU GHS guidance

Kit	Components and Reagent Ingredients	Quantity per Kit	Safety Symbol and Warning
cobas® 4800 System Sample Preparation Kit 2 240 Tests (P/N: 06979513190)	**MGP 2** (**cobas**® 4800 MGP Reagent 2) Magnetic glass particles, Tris buffer, 0.1% methyl-4 hydroxybenzoate, < 0.1% sodium azide	10 × 8 mL	N/A
	EB 2 (**cobas**® 4800 Elution Buffer 2) Tris buffer, 0.2% methyl-4 hydroxybenzoate	10 × 17 mL	
cobas® 4800 System Sample Preparation Kit 2 960 Tests (P/N: 06979521190)	**MGP 2** (**cobas**® 4800 MGP Reagent 2) Magnetic glass particles, Tris buffer, 0.1% methyl-4 hydroxybenzoate, < 0.1% sodium azide	10 × 16 mL	N/A
	EB 2 (**cobas**® 4800 Elution Buffer 2) Tris buffer, 0.2% methyl-4 hydroxybenzoate	10 × 17 mL	
cobas® 4800 System Wash Buffer Kit 240 Tests (P/N: 05235863190)	**WB** Sodium citrate dihydrate, 0.05% N-Methyl isothiazolone HCl	10 × 55 mL	N/A
cobas® 4800 System Wash Buffer Kit 960 Tests (P/N: 05235871190)	**WB** Sodium citrate dihydrate, 0.05% N-Methyl isothiazolone HCl	10 × 200 mL	N/A

Kit	Components and Reagent Ingredients	Quantity per Kit	Safety Symbol and Warning[a]
cobas® 4800 System Lysis Kit 2 240 Tests (P/N: 06979530190)	**P 2** (**cobas**® 4800 Protease 2) Tris buffer, < 0.05% EDTA, calcium chloride, calcium acetate, 8% (w/v) proteinase	10 × 1.0 mL	 Danger H302+H332: Harmful if swallowed or if inhaled. H317: May cause an allergic skin reaction. H318: Causes serious eye damage. H334: May cause allergy or asthma symptoms or breathing difficulties if inhaled. H412: Harmful to aquatic life with long lasting effects. EUH032: Contact with acids liberates very toxic gas. P261: Avoid breathing dust/ fume/ gas/ mist/ vapours / spray. P280: Wear protective gloves/ eye protection/ face protection. P284 Wear respiratory protection. P304 + P340 + P312: IF INHALED: Remove person to fresh air and keep comfortable for breathing. Call a POISON CENTER/doctor if you feel unwell. P305 + P351 + P338 + P310: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Immediately call a POISON CENTER/ doctor. P342 + P311: If experiencing respiratory symptoms: Call a POISON CENTER/doctor.
	LYS 2 (**cobas**® 4800 Lysis Buffer 2) 43% (w/w) guanidine thiocyanate, 5% (w/v) polydocanol, 2% (w/v) dithiothreitol, dihydro sodium citrate	10 × 27 mL	

[a] Product safety labeling primarily follows EU GHS guidance

Kit	Components and Reagent Ingredients	Quantity per Test	Safety Symbol and Warning[a]
cobas® 4800 System Lysis Kit 2 960 Tests (P/N: 06979548190)	**P 2** (**cobas**® 4800 Protease 2) Tris buffer, < 0.05% EDTA, calcium chloride, calcium acetate, 8% (w/v) proteinase	10 × 1.0 mL	 Danger H302+H332: Harmful if swallowed or if inhaled. H317: May cause an allergic skin reaction. H318: Causes serious eye damage. H334: May cause allergy or asthma symptoms or breathing difficulties if inhaled. H412: Harmful to aquatic life with long lasting effects. EUH032: Contact with acids liberates very toxic gas. P261: Avoid breathing dust/ fume/ gas/ mist/ vapours / spray. P280: Wear protective gloves/ eye protection/ face protection. P284: Wear respiratory protection. P304 + P340 + P312: IF INHALED: Remove person to fresh air and keep comfortable for breathing. Call a POISON CENTER/doctor if you feel unwell. P305 + P351 + P338 + P310: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Immediately call a POISON CENTER/doctor. P342 + P311: If experiencing respiratory symptoms: Call a POISON CENTER/doctor.
	LYS 2 (**cobas**® 4800 Lysis Buffer 2) 43% (w/w) guanidine thiocyanate, 5% (w/v) polydocanol, 2% (w/v) dithiothreitol, dihydro sodium citrate	10 × 84 mL	

[a] Product safety labeling primarily follows EU GHS guidance

Reagent storage and handling requirements

Reagent	Storage Temperature	Storage Time
cobas® CMV	2–8°C	Stable until the expiry date indicated
cobas® CMV Control Kit*	2–8°C	Stable until the expiry date indicated
cobas® 4800 System Sample Preparation Kit 2	2–8°C	Stable until the expiry date indicated
cobas® 4800 System Wash Buffer Kit	15–25°C	Stable until the expiry date indicated
cobas® 4800 System Lysis Kit 2	2–8°C	Stable until the expiry date indicated

* Store Control Kit box in an upright position

Do not freeze reagents.

Additional materials required

Materials	P/N
cobas® 4800 System Extraction (deepwell) Plate 2.0 mL	06884008001
cobas® 4800 System AD (microwell) Plate 0.3 mL	05232724001
Sealing foil applicator	04900383001
CORE Tips, 1000 µL, rack of 96	04639642001
200 mL Reagent Reservoir	05232759001
50 mL Reagent Reservoir	05232732001
24-position carrier	04639502001
32-position carrier	04639529001
Solid waste bag	05530873001 (small) or 04691989001 (large)
Hamilton STAR Plastic Chute	04639669001
Disposable gloves, powderless	Any powderless disposable gloves are acceptable.
Vortex Mixer (single tube)	Any vortex mixer is acceptable.
Centrifuge equipped with a swinging bucket rotor with minimum RCF of 1500	Any appropriate centrifuge is acceptable.

For more information regarding the materials sold separately, contact your local Roche representative.

Instrumentation and software required but not provided

Required Instrumentation and Software, Not Provided
cobas® 4800 System **cobas x** 480 instrument **cobas z** 480 analyzer Control Unit
cobas® 4800 System Application Software (Core) Version 2.2 or higher
cobas® 4800 System **cobas**® CMV AP v1.0.0 or higher

Note: Contact your local Roche representative for a detailed order list for sample racks, tip racks, reagent racks and plate carriers accepted on the instruments.

Supported sample tubes

The Test accepts commonly used primary and secondary sample tubes.

The following sample tubes are supported:

Primary tubes (processing volume 400 µL)

Nominal Diameter (mm)	Specimen input volume – processed (centrifuged) whole blood	EDTA Plasma tube
11–14	1800 µL or more	With or without gel
14.5–16	More than 4000 µL	With or without gel

For specific sample tube order information, and minimum specimen input volumes for specific primary tubes, contact your local Roche representative.

Secondary tubes (processing volume 400 µL)

Nominal Diameter (mm)	Specimen input volume
11–16	1000 µL or more (specific secondary tubes have a minimum input volume of less than 1000 µL)

For specific sample tube order information, and minimum specimen input volumes for specific secondary tubes, contact your local Roche representative.

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 analytical sensitivity of this test, care should be taken to keep reagents, specimens 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.[28,29] Only personnel proficient in handling infectious materials and the use of **cobas**® CMV and the **cobas**® 4800 System should perform this procedure.
- All human-sourced materials should be considered potentially infectious and should be handled with universal precautions. **cobas**® CMV Control Kit contains plasma derived from human blood. The source material has been tested by licensed antibody tests and found non-reactive for the presence of antibody to HCV, antibody to HIV-1/2, HBsAg, and antibody to HBc. Testing of normal human plasma by PCR methods also showed no detectable HIV-1 (Groups M and O) RNA, HIV-2 RNA, HCV RNA, HBV DNA, HEV RNA, WNV RNA, or CMV DNA. No known test method can offer complete assurance that products derived from human blood will not transmit infectious agents.
- Prevent exposure of MGP to sources of magnetic field.
- **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.

- Store the **cobas**® CMV Control Kit box in an upright position.
- False positive results may occur if carryover of samples is not adequately controlled during sample handling and processing.
- For additional warnings, precautions and procedures to reduce the risk of contamination for the **cobas x** 480 instrument or **cobas z** 480 analyzer, consult the **cobas**® 4800 System - User Assistance (formerly known as System Manual). If contamination is suspected, perform cleaning and weekly maintenance as described in the **cobas**® 4800 System - User Assistance.

Note: For specific instructions, see "Specimen collection, transport, and storage".

Good laboratory practice

- Do not pipette by mouth.
- Do not eat, drink or smoke in laboratory work areas.
- Wash hands thoroughly after handling specimens and kit reagents, and after removing the gloves.
- Wear eye protection, laboratory coats and disposable gloves when handling any reagents. Avoid contact of these materials with the skin, eyes or mucous membranes. If contact does occur, immediately wash with large amounts of water. Burns can occur if left untreated. If spills occur, dilute with water before wiping dry.
- Thoroughly clean and disinfect all laboratory work surfaces with a freshly prepared solution of 0.5% sodium hypochlorite in distilled or deionized water (dilute household bleach 1:10). Follow by wiping the surface with 70% ethanol.
- Maintain a consistent temperature in the laboratory that conforms to the environmental specifications of the system, as provided in the **cobas**® 4800 System - User Assistance

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 bottle and vial to ensure that there are no signs of leakage. If there is any evidence of leakage, do not use that material for testing.
- **cobas**® 4800 Lysis Buffer 2 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, **cobas**® 4800 Sample Preparation Kit 2 contain sodium azide as a preservative. Avoid contact of reagents with the skin, eyes, or mucous membranes. If contact does occur, immediately wash with generous amounts of water; otherwise, burns can occur. If these reagents are spilled, dilute with water before wiping dry.
- Do not allow **cobas**® 4800 Lysis Buffer 2, which contains guanidine thiocyanate, to contact sodium hypochlorite (bleach) solution. This mixture can produce a highly toxic gas.

Contamination

- Gloves must be worn and must be changed between handling specimens and **cobas**® CMV reagents to prevent contamination. Avoid contaminating gloves when handling specimens and controls. Wear lab gloves, laboratory coats, and eye protection when handling specimens and kit reagents.
- Avoid microbial and ribonuclease contamination of reagents.
- False positive results may occur if carryover of specimens is not prevented during specimen handling.

Integrity

- Do not use kits after their expiration dates.
- Do not pool reagents.
- Do not use disposable items after their expiration date.
- All disposable items are for one time use. Do not reuse.
- All equipment should be properly maintained according to the manufacturer's instructions.

Disposal

- **cobas**® CMV, **cobas**® 4800 System Sample Preparation Kit 2 contain sodium azide (see "**Warnings and precautions**"). Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. While disposing of solutions containing sodium azide down laboratory sinks, flush the drains with a large volume of cold water to prevent azide buildup.
- Dispose of unused reagents and waste in accordance with country, federal, state and local regulations.

Note: For disposal of liquid waste, refer to the cobas® 4800 System - User Assistance.

Spillage and cleaning

- **cobas**® 4800 Lysis Buffer 2 contains guanidine thiocyanate. If liquid containing guanidine thiocyanate 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 hypochlorite.
- If spills occur on the **cobas x** 480 instrument follow the instructions in the **cobas**® 4800 System - User Assistance to clean.
- Do not use sodium hypochlorite solution (bleach) for cleaning the **cobas x** 480 instrument or the **cobas z** 480 analyzer. Clean the **cobas x** 480 instrument or the **cobas z** 480 analyzer according to procedures described in the **cobas**® 4800 System - User Assistance.

Specimen collection, transport, and storage

- 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.

Note: Handle all specimens as if they are capable of transmitting infectious agents.

Specimen collection

Blood should be collected in BD Vacutainer® PPT™ Plasma Preparation Tubes or Lavender Top Tubes for Molecular Diagnostic Test Methods or in sterile tubes using EDTA as the anticoagulant.

Note: The user must follow the guidance provided by the tube manufacturer for plasma preparation.

Specimen transport storage and stability

- Whole blood collected in BD Vacutainer® PPT™ Plasma Preparation Tubes or Lavender Top Tubes or in sterile tubes using EDTA as the anticoagulant for Molecular Diagnostic Test Methods may be stored and/or transported for up to 36 hours at 2°C to 25°C prior to centrifugation and subsequent testing.
- Alternatively plasma samples may be stored in primary tubes for up to 36 hours at 2°C to 25°C or 6 days at 2°C to 8°C.
- Plasma samples may be stored in secondary tubes for up to 36 hours at 2°C to 30°C, up to 6 days at 2°C to 8°C or up to 6 weeks at -15°C to -25°C. Separated plasma samples in secondary tubes are stable for up to three freeze/thaw cycles when stored frozen at -15°C to -25°C.
- If samples are to be shipped, they should be packaged and labeled in compliance with applicable country and/or international regulations covering the transport of samples and etiologic agents.

Instructions for use

Running the test

The sample processing volume for **cobas**® CMV is 400 µL.

Figure 1: cobas® CMV workflow

1	Start the system
2	Perform instrument maintenance
3	Remove samples and reagents from storage
4	Start run
5	Scan parameter cards
6	Load samples
7	With LIS: confirm work order Without LIS: create work order
8	Load consumables (deepwell plate, microwell plate, tip racks)
9	Load reagents
10	Start sample preparation run
11	Unload and seal microwell plate
12	Load microwell plate into analyzer
13	Remove samples, used reagents, and deepwell plate
14	Review results
15	With LIS: send results to LIS
16	Unload analyzer

Note: Refer to the cobas® 4800 System - User Assistance for detailed operating instructions.

Run Size

The generic sample preparation reagents (**cobas**® 4800 System Sample Preparation Kit 2, **cobas**® 4800 System Lysis Kit 2 and **cobas**® 4800 System Wash Buffer Kit) are available in two kit sizes, each sufficient for 10 runs of up to either 24 or 96 samples, which include the controls and specimens to be run. **cobas**® CMV is available in a single kit size sufficient to test up to 120 (10×12) samples, including controls and specimens. The **cobas**® CMV Control Kit is available in a single kit size and can support all run configurations. For each test batch, one CMV Low Positive Control, one CMV High Positive

Control and one Negative Control must be used. For a single test run, the maximum number of samples allowed is 93 specimens and 3 controls.

Figure 1 summarizes the procedure.

Note: For optimal use of reagents, the generic sample preparation reagents can be used for a run containing 1-21 total specimens (10×24 test kit size) or 1-93 total specimens (10×96 test kit size). However, different kit sizes of the cobas® 4800 System Wash Buffer Kit, cobas® 4800 System Sample Preparation Kit 2 and cobas® 4800 System Lysis Kit 2 cannot be combined. For example, if a 96-test Wash Buffer reagent bottle is scanned at the start of the run, 96-test size reagents from the other sample preparation reagent kits must also be used.

Workflow

cobas® CMV is performed using the full workflow within the **cobas**® 4800 Software. It consists of sample preparation on the **cobas x** 480 instrument followed by amplification/detection on the **cobas z** 480 analyzer. **cobas**® CMV may be performed alone, or in mixed-batch mode with tests that share the same automated specimen extraction process and PCR profile for amplification and detection. At the test selection step the software will display tests that are compatible with **cobas**® CMV for mixed batch mode. Refer to the **cobas**® 4800 System – User Assistance software for details.

1. Perform the system startup by following the instructions in the **cobas**® 4800 System - User Assistance.
2. Perform maintenance procedures by following the instructions in the **cobas**® 4800 System - User Assistance.
3. Collect all reagents and consumables needed. All reagents except CMV MMX R2 and MMX R1 must be at ambient temperature prior to loading on the **cobas x** 480 instrument. The CMV MMX R2 and MMX R1 reagents may be taken directly from 2- 8°C storage as they will equilibrate to ambient temperature on board the **cobas x** 480 instrument by the time they are used in the process.

Note: All reagents and reagent reservoirs are barcoded and designed for one time use. The cobas® 4800 Software tracks the use of the reagents and reagent reservoirs and rejects previously used reagents or reagent reservoirs.

4. Start a new run and select the workflow type as CMV. To perform a mixed batch run, select other applicable workflow types (i.e., HIV-1, HCV or HCV GT) in addition to CMV.
5. Follow the software wizard guide and scan the barcode on the control ranges and calibration coefficients parameter cards.

Note: Scan parameter cards from unexpired reagents. The software does not check reagent expiry dates in parameter cards. Check the expiry date printed in the parameter card or in the reagent kits before scanning the corresponding barcode ID.

6. Load the samples. Primary or secondary sample tubes can be loaded and minimum sample volume depends on the tube type and size.
7. Create the work order. There are three ways to create a work order:
   - By using the sample editor before any sample rack is loaded into the **cobas x** 480 instrument ("Editor" button on the right of the main menu). Work orders can be saved, edited and reloaded if necessary.
     When selecting the requested results, select "CMV".
   - By following the software wizard for the new run and loading specimens into **cobas x** 480 instrument when prompted. The specimen barcodes will be automatically scanned, and the requested results for each specimen must be defined.
     When selecting the requested results, select "CMV".
   - By using your institution's LIS system.

   Refer to the **cobas**® 4800 System - User Assistance for more details. Load samples and define/select work order or use LIS as appropriate.

8. Load the consumables as instructed by the software wizard. Do not load or remove individual tips into a partially used tip rack, as the software tracks the number of tips that are left. If there are not enough tips for the run to be conducted, the software will alert the user.
9. Load the reagents.

   Load the sample preparation reagents into the barcoded reagent reservoirs. The reagent reservoirs are available in two sizes: 200 mL and 50 mL. Follow the software wizard guide to select the correct reagent reservoir size. The reagent reservoir barcodes must face to the right of the carrier. Use the "scan-scan-pour-place" method to load sample preparation reagents:

   - Scan the reagent bottle barcode
   - Scan the reagent reservoir barcode
   - Pour the reagent into the reservoir
   - Place the filled reagent reservoir into the designated position on the reagent carrier

Note: The cobas® 4800 System has an internal clock to monitor the length of time the reagents are on-board. Once LYS 2 or WB is scanned, 1 hour is allowed to complete the loading process and click on the Start button. A countdown timer is displayed on the "Workplace" tab. The system will not allow the run to start if the on-board timer has expired.

Note: To assure the accurate transfer of MGP, vortex or vigorously shake the MGP vial <u>immediately prior</u> to dispensing into the reagent reservoir.

10. Load amplification/detection reagent vials (CMV MMX R2, MMX R1 and DNA QS), control vials [CMV L(+)C, CMV H(+)C and (–)C] and generic reagent vials (P2 as required) directly onto the reagent carrier.

Note: In order to prevent unnecessary run aborts and contamination, it is required to flick down the reagent vials to avoid formation of bubbles/liquid films. Controls should be opened starting with the ones closest to you (from position 24 to 1). Change gloves after handling positive controls.

11. Start sample preparation run. After a successful sample preparation run, the "Sample Preparation results" button and the Unload button become available. If desired, select "Sample Preparation results" button to review the results then select "Unload" to unload the plate carriers. Alternatively, select "Unload" to unload the plate carrier without reviewing the results. See the **cobas**® 4800 System - User Assistance.
12. After unloading the microwell plate, follow the instructions in the **cobas**® 4800 System - User Assistance for sealing and transferring the plate to the **cobas z** 480 analyzer.
13. Load the microwell plate into the analyzer and start the amplification and detection run as instructed in the **cobas**® 4800 System - User Assistance.

Note: The cobas® 4800 System has an internal clock to monitor the length of time after addition of the prepared samples to activated master mix. Amplification and detection should be started as soon as possible but no later than 40 minutes after the end of the cobas x 480 instrument run. A countdown timer is displayed on the "Workplace" tab. The system will abort the run if the timer has expired.

14. Remove samples, used reagents, and deepwell plate as instructed in the **cobas**® 4800 System - User Assistance.
15. After the amplification and detection run is completed, follow the instructions in the **cobas**®4800 System - User Assistance software to review and accept results.
16. If working with LIS, send results to the LIS.
17. Follow the instructions in the **cobas**® 4800 System - User Assistance software to unload the microwell plate from the **cobas z** 480 analyzer.

Results

The **cobas**® 4800 System automatically determines the CMV DNA concentration for the samples and controls. The CMV DNA concentration is expressed in International Units per milliliter (IU/mL).

Quality control and validity of results

- One negative control (–)C2 and two positive controls, a low positive control CMV L(+)C and a high positive control CMV H(+)C, are processed with each batch.
- In the **cobas**® 4800 Software and/or report, check for batch validity.
- Invalidation of results is performed automatically by the **cobas**® 4800 Software based on negative and positive control failures.

Control result interpretation

Table 1: Control result interpretation for negative and positive controls

Negative Control	**Result**	**Interpretation**
(–)C2	Target Not Detected	Control is valid. CMV DNA not detected.
	Invalid	An invalid result or the calculated titer result for the negative control is not negative.
Positive Control	**Result**	**Interpretation**
CMV L(+)C	Titer	Control is valid. Calculated titer is within the control range.
	Invalid	An invalid result or the calculated titer result for the low positive control is not within the assigned range.
CMV H(+)C	Titer	Control is valid. Calculated titer is within the control range.
	Invalid	An invalid result or the calculated titer result for the high positive control is not within the assigned range.

Interpretation of results

Note: All assay and batch validation is determined by the cobas® 4800 Software.

Note: A valid batch may include both valid and invalid specimen results.

For a valid batch, specimen results are interpreted as shown in Table 2.

Table 2: Target results for individual target result interpretation

cobas® CMV	Result Report and Interpretation
Target Not Detected	CMV DNA not detected. Report results as "CMV not detected."
< Titer Min	Calculated titer is below the Lower Limit of Quantitation (LLoQ) of the assay. Report results as "CMV detected less than (Titer Min)." Titer min = 3.45E+01 IU/mL
Titer	Calculated titer is within the Linear Range of the assay – greater than or equal to Titer Min and less than or equal to Titer Max. Report results as "(Titer) of CMV detected".
> Titer Max[a]	Calculated titer is above the Upper Limit of Quantitation (ULoQ) of the assay. Report results as "CMV detected, greater than (Titer Max)." Titer max = 1.00E+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 EDTA plasma, depending on the type of the original sample, and the test should be repeated. Multiply the reported result by the dilution factor.

List of flags

The following table lists all flags which are relevant for result interpretation.

Table 3: List of flags

Flag code	Description	Recommended action
R4800	The target is invalid due to calculation failure.	The target is invalid due to calculation failure. 1. Rerun the sample. 2. If the problem persists, contact Roche Service.
R4801	The quantitation standard is invalid.	The quantitation standard is invalid for a sample. 1. Rerun the sample. 2. If the problem persists, contact Roche Service.
R4802	An external control is invalid.	An external control is invalid.[a] 1. Repeat entire run with fresh reagents. 2. If the problem persists, contact Roche Service.
R4803	The quantitation standard is invalid.	The quantitation standard is invalid for an external control 1. Repeat entire run with fresh reagents. 2. If the problem persists, contact Roche Service.
R4804	The external control is out of range.	The external control is out of range.[b] 1. Repeat entire run with fresh reagents. 2. If the problem persists, contact Roche Service
X3	Error: Clot was detected. Sample was not processed.	Make sure that the samples were handled according to the workflow description. 1. Check the sample for clots. 2. Rerun the sample.
X4	Error: Pipetting error occurred. Sample was not processed.	Insufficient sample volume or mechanical error during pipetting is the most likely reason. 1. Make sure that there is enough sample volume. 2. Check whether the tip eject plate is placed correctly. 3. Rerun the sample.

[a] This is a sample flag and it occurs when an external control in the run is called invalid.
[b] This flag includes all scenarios in which the external control is invalid (target calling or titer).

***Note:** For descriptions of the remaining system flags please refer to the cobas® 4800 System - User Assistance.*

Procedural limitations

1. **cobas**® CMV has been evaluated only for use in combination with the **cobas**® CMV Control Kit, **cobas**® 4800 System Sample Preparation Kit 2, **cobas**® 4800 System Lysis Kit 2 and **cobas**® 4800 System Wash Buffer Kit.
2. Reliable results are dependent on adequate specimen collection, transport, storage and processing. Follow the procedures in this Instructions For Use document (also referred to as a Package Insert) and the **cobas**® 4800 System – User Assistance.
3. This test has been validated only for use with EDTA plasma. Testing of other sample types may result in inaccurate results.
4. Quantitation of CMV DNA is dependent on the number of virus particles present in the samples and may be affected by sample collection methods, patient factors (i.e., age, presence of symptoms), and/or stage of infection.
5. Though rare, mutations within the highly conserved regions of a viral genome 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.
6. The predictive value of an assay depends on the prevalence of the disease in any particular population.
7. The addition of AmpErase enzyme into the **cobas**® CMV Master Mix enables selective amplification of target nucleic acid; however, good laboratory practices and careful adherence to the procedures specified in this Instructions For Use document are necessary to avoid contamination of reagents and amplification mixtures.
8. Use of this product must be limited to personnel trained in the techniques of PCR and the use of the **cobas**® 4800 System.
9. Only the **cobas x** 480 instrument and **cobas z** 480 analyzer have been validated for use with this product. No other sample preparation instrument or PCR System can be used with this product.
10. 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.
11. Cross-contamination can cause false positive results. The sample to sample cross-contamination rate of **cobas**® CMV has been determined in a non-clinical study to be 0.0%. Run to run cross-contamination has not been observed.
12. **cobas**® CMV is not intended for use as a screening test for the presence of CMV in blood or blood products or as a diagnostic test to confirm the presence of CMV infection.

Non-clinical performance evaluation

Key performance characteristics

Limit of Detection (LoD)

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

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[30]) obtained from NIBSC, in CMV-negative human EDTA plasma. Panels of seven concentration levels plus a blank were tested over three lots of **cobas®** CMV test reagents, multiple runs, days, operators, and instruments.

The results are shown in Table 4. The study demonstrates that **cobas®** CMV detected CMV DNA at a concentration of 20.5 IU/mL with a hit rate of ≥ 95% by PROBIT.

Table 4: Limit of detection

Input titer concentration (CMV DNA IU/mL)	Number of valid replicates	Number of positives	Hit rate in %
60.0	126	126	100.0
46.0	126	126	100.0
34.5	124	124	100.0
23.0	126	122	96.8
15.0	126	111	88.1
10.0	126	97	77.0
5.0	126	63	50.0
0.0	72	0	0.0
LoD by PROBIT at 95% hit rate	20.5 IU/mL (95% CI: 16.9 – 23.3 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 (1.55E+01 to 3.11E+07 IU/mL). Two lots of **cobas®** CMV test reagents were used and each panel member was tested in 12 replicates per lot and the results of the study are presented in Figure 2 and Figure 3 for representative results.

The data demonstrated a linear behavior from 1.55E+01 to 3.11E+07 IU/mL. The claimed linear range for the **cobas®** CMV is 34.5 to 1.0E+07 IU/mL

Figure 2: Linearity Lot 1

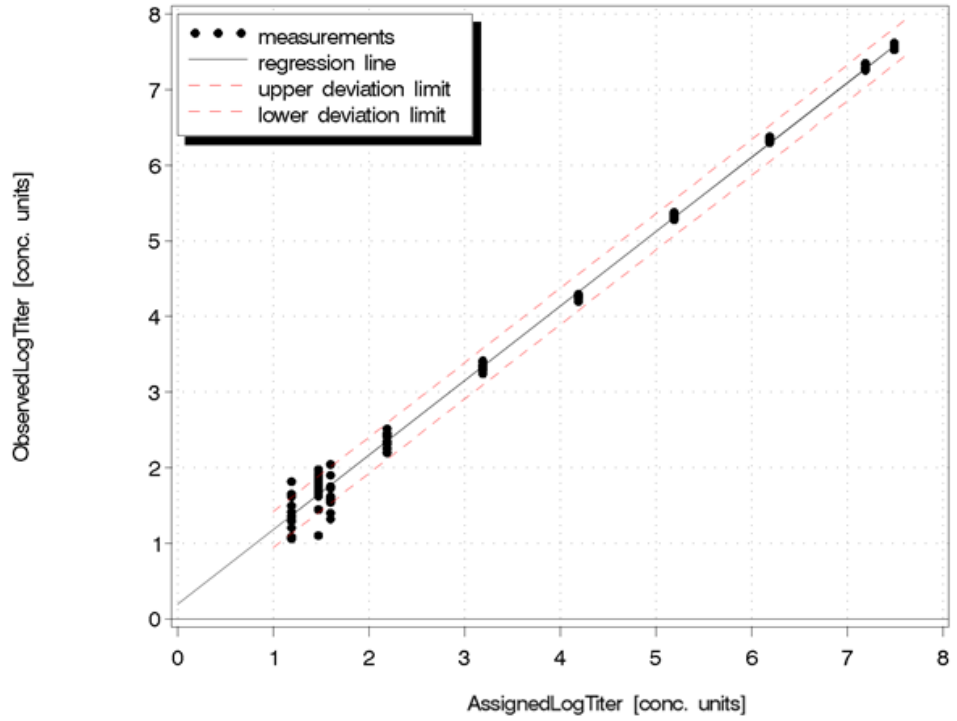
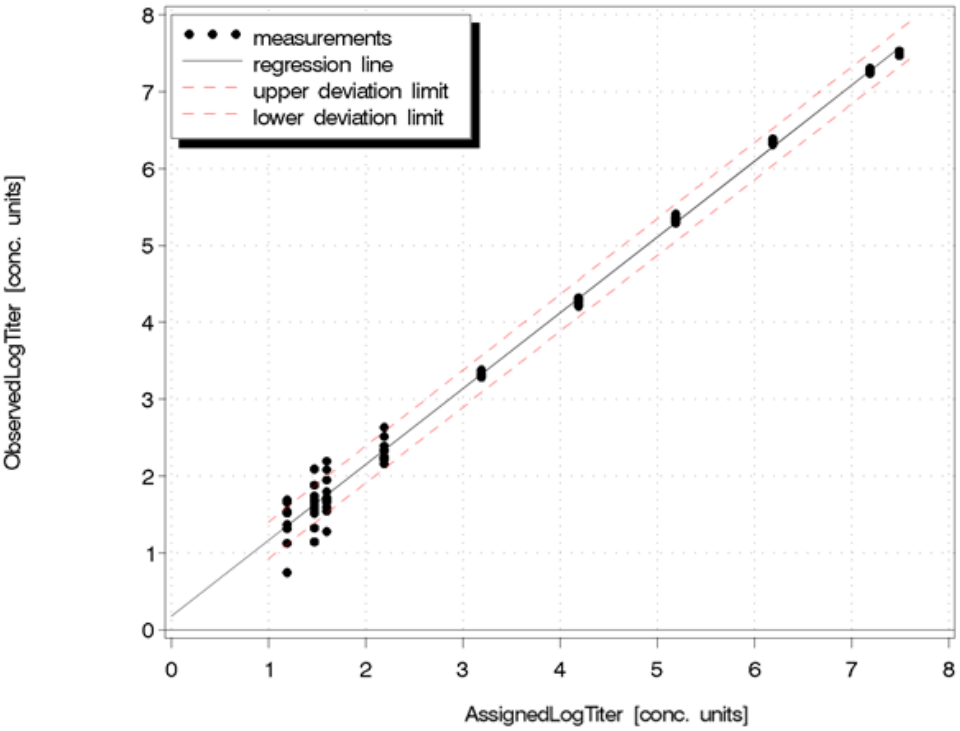


Figure 3: Linearity Lot 2



Precision – within laboratory

Precision of **cobas**® CMV was determined by analysis of serial dilutions of CMV genotype gB-1 DNA. Six dilution levels were tested in 90 replicates for each level across three lots of **cobas**® CMV reagents using two instruments and four operators over 15 days. Each sample was carried through the entire **cobas**® CMV procedure on the **cobas**® 4800 System. Therefore, the precision reported here represents all aspects of the test procedure. The results are shown in Table 5.

cobas® CMV showed high precision for three lots of reagents tested across a concentration range of 3.90E+01 IU/mL to 1.52E+06 IU/mL.

Table 5: Within laboratory precision of cobas® CMV*

Nominal concentration (IU/mL)	Assigned concentration (IU/mL)	Lot 1	Lot 2	Lot 3	All Lots
		SD	SD	SD	Pooled SD
1.80E+06	1.55E+06	0.04	0.04	0.04	0.04
1.80E+05	1.55E+05	0.05	0.03	0.05	0.04
1.80E+04	1.55E+04	0.06	0.04	0.06	0.05
1.80E+03	1.55E+03	0.06	0.05	0.04	0.05
1.80E+02	1.55E+02	0.13	0.10	0.13	0.12
4.60E+01	3.97E+01	0.17	0.15	0.24	0.19

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

Genotype verification

The performance of **cobas**® CMV on CMV 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 two different Glycoprotein B genotypes (gB-2 and gB-3) CMV DNA plasmid for Glycoprotein B genotype 4 (gB-4) were diluted in CMV negative EDTA plasma. The hit rate determination was performed with 42 replicates at one concentration level. Testing was conducted with one lot of **cobas**® CMV reagents. The results are shown in Table 6. 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 6: LoD verification of CMV Glycoprotein B Genotypes gB-2, gB-3 and gB-4

Glycoprotein B Genotype	Hit rate at 34.5 IU/mL
gB-2	100.0%
gB-3	100.0%
gB-4	100.0%

Verification of linear range for Glycoprotein B Genotypes gB-2, gB-3 and gB-4

The dilution series used to verify the linear range (as determined with CMV Glycoprotein B genotype 1) for all claimed CMV glycoprotein B genotypes (gB2, gB3 and gB4) consists of seven panel members spanning the intended linear range. Testing was conducted with one lot of **cobas**® CMV reagent; 12 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). The maximum deviation between the linear regression and the better fitting non-linear regression was equal to or less than 0.06 $\log_{10}$.

Drug resistant CMV specimen verification

The performance of **cobas**® CMV on drug resistant CMV 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)

CMV cell culture supernatants for two different drug resistant CMV specimens (resistant against foscarnet or ganciclovir, valganciclovir and cidofovir) were diluted in CMV negative EDTA plasma. The hit rate determination was performed with 42 replicates for at one concentration level. Testing was conducted with one lot of **cobas**® CMV reagents. The results are shown in Table 7. These results verify that **cobas**® CMV detected CMV DNA for all tested specimens resistant to the common CMV drugs at concentrations of 34.5 IU/mL with a hit rate of ≥ 95%.

Table 7: LoD verification of CMV for drug resistant specimens

Resistance Phenotype	Hit rate at 34.5 IU/mL
foscarnet	100.0%
ganciclovir, valganciclovir, cidofovir	100.0%

Verification of linear range for the drug resistant CMV specimens (resistant against foscarnet or ganciclovir, valganciclovir and cidofovir)

The dilution series used to verify the linear range (as determined with CMV Glycoprotein B genotype 1) on specimens resistant to the common CMV drugs consists of seven panel members spanning the intended linear range. Testing was conducted with one lot of **cobas**® CMV reagent; 12 replicates per level were tested in EDTA plasma.

The linear range of **cobas**® CMV was verified for all tested specimens resistant to the common CMV drugs. For both drug resistant specimens tested the linear regression was the best fitting model.

Specificity

The specificity of **cobas**® CMV was determined by analyzing CMV-negative EDTA plasma samples from individual donors. Six hundred eleven individual EDTA plasma samples were tested with three lots of **cobas**® CMV reagents. Six hundred eleven 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 pathogens (Table 8) with CMV DNA positive and CMV DNA negative EDTA plasma. The pathogens were added to negative EDTA plasma and tested with and without CMV DNA. Negative results were obtained with **cobas**® CMV for all pathogen samples without CMV target and positive results were obtained on all of the pathogen samples with CMV target. Furthermore, the mean $\log_{10}$ titer of each of the positive CMV samples containing potentially cross-reacting organisms was within ± 0.10 $\log_{10}$ of the mean $\log_{10}$ titer of the respective positive spike control.

Table 8: Pathogens tested for cross-reactivity

Viruses	Bacteria	Fungi
Epstein-Barr Virus (EBV)	*Staphylococcus aureus*	*Candida albicans*
Hepatitis B Virus (HBV)	*Staphylococcus epidermis*	*Cryptococcus neoformans*
Hepatitis C Virus (HCV)	*Streptococcus pneumoniae*	*Aspergillus niger*
Human Immunodeficiency Virus Type 1 (HIV-1)	*Streptococcus pyogenes*	
Human Immunodeficiency Virus Type 2 (HIV-2)	*Enterococcus faecalis*	
Herpes Simplex Virus Type 1 (HSV-1)	*Escherichia coli*	
Herpes Simplex Virus Type 2 (HSV-2)	*Klebsiella pneumoniae*	
Human Herpes Virus Type 6 (HHV-6)	*Salmonella typhimurium*	
Human Herpes Virus Type 7 (HHV-7)	*Mycoplasma pneumoniae*	
Human Herpes Virus Type 8 (HHV-8)	*Chlamydia trachomatis*	
Adenovirus Type 5	*Listeria monocytogenes*	
JC Virus	*Propionibacterium acnes*	
Parvovirus B19	*Neisseria gonorrhoeae*	
BK Polyomavirus	*Mycobacterium avium*	
Varicella Zoster Virus (VZV)	*Clostridium perfringens*	
Human Papilloma Virus (HPV)		

Analytical specificity – interfering substances

Elevated levels of triglycerides (33.0 g/L), conjugated bilirubin (0.2 g/L), unconjugated bilirubin (0.2 g/L), albumin (60.0 g/L), hemoglobin (2.0 g/L) and human DNA (2 mg/L) in samples were tested in presence and absence of CMV DNA. The tested substances were shown not to interfere with the test performance of **cobas**® CMV. Moreover, the presence of markers for the autoimmune diseases systemic lupus erythematosus (SLE), rheumatoid arthritis (RA) and antinuclear antibody (ANA) were tested.

In addition, drug compounds listed in Table 9 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. Negative results were obtained with **cobas**® CMV for all samples without CMV target and positive results were obtained on all of the samples with CMV target. Furthermore, the mean $\log_{10}$ titer of each of the positive CMV samples containing potentially interfering substances was within ± 0.36 $\log_{10}$ of the mean $\log_{10}$ titer of the respective positive spike control.

Table 9: Drug compounds tested for interference with the quantitation of CMV DNA by cobas® CMV

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

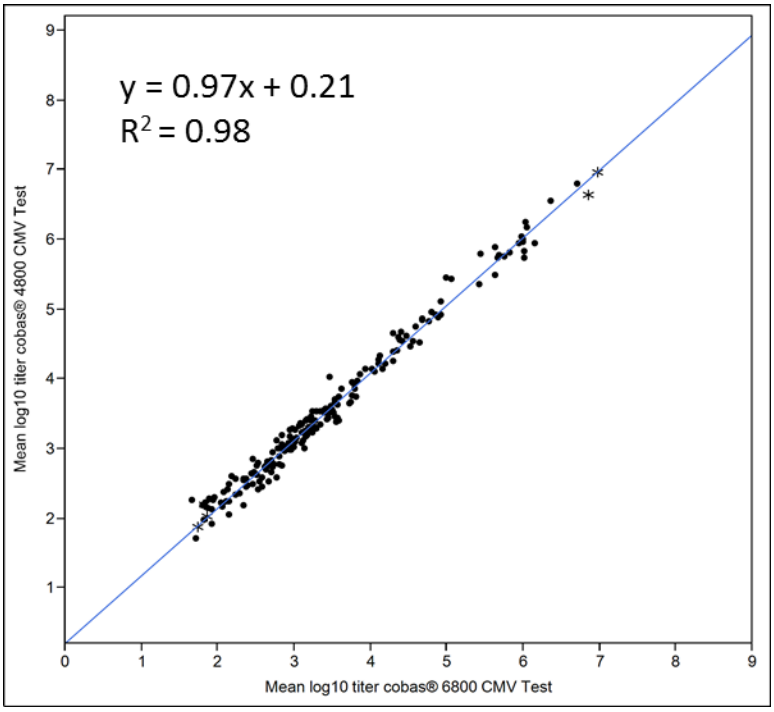
Method correlation

Performance evaluation of cobas®CMV test for use on the cobas® 4800 System compared to the cobas® CMV test for use on the cobas® 6800/8800 Systems

The performance of **cobas®** CMV test for use on the **cobas®** 4800 System and the **cobas®** CMV test for use on the **cobas®** 6800/8800 Systems was compared by analysis of EDTA plasma specimens from CMV-infected patients. A total of 197 EDTA plasma specimens across all CMV genotypes, analyzed in duplicate, were valid and within the quantitation range of both tests. The Deming regression analysis was performed. The mean titer deviation of the samples tested with the two tests was 0.11 $\log_{10}$ (95% Confidence Interval: 0.09; 0.13).

The Deming regression results are shown in Figure 4. The symbol * in the figures shows single determination.

Figure 4: Regression analysis of cobas® CMV for use on the cobas® 4800 vs cobas® CMV for use on the cobas® 6800/8800



Whole system failure

The whole system failure rate for **cobas**® CMV was determined by testing 100 replicates of EDTA plasma spiked with CMV target. These samples were tested at a target concentration of approximately 3 times LLoQ (104 IU/mL).

The results of this study determined that all replicates were valid and positive for the CMV resulting in a whole system failure rate of 0.0%. The two-sided 95% exact confidence interval was 0.0% for the lower bound and 3.6% for the upper bound [0.0%:3.6%].

Cross contamination

The cross-contamination rate for **cobas**® CMV was determined by testing 230 replicates of CMV-negative EDTA-plasma samples and 233 replicates of a high titer CMV sample at 1.55E+07 IU/mL. In total, five runs were performed with positive and negative samples in a checkerboard configuration.

All 230 replicates of the negative samples were valid and detected negative, resulting in a cross-contamination rate of 0.0% with a one-sided 95% confidence interval of 1.3%.

Additional information

Key assay features

Sample type	EDTA plasma
Sample processing volume	400 µL
Analytical sensitivity	34.5 IU/mL
Linear range	34.5 IU/mL – 1.0E+07 IU/mL
Specificity	100 %
Genotypes detected	CMV Glycoprotein B Genotype 1-4
Drug resistant CMV specimens detected	CMV specimens resistant against ganciclovir, valganciclovir, cidofovir and foscarnet

Symbols

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

Table 10: Symbols used in labeling for Roche PCR diagnostic products

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

Manufacturer and distributors

Table 11: Manufacturer and distributors

Manufactured in the United States

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

Roche Diagnostics (Schweiz) AG
Industriestrasse 7
6343 Rotkreuz, Switzerland

Roche Diagnostics GmbH
Sandhofer Strasse 116
68305 Mannheim, Germany

Roche Diagnostics, SL
Avda. Generalitat, 171-173
E-08174 Sant Cugat del Vallès
Barcelona, Spain

Roche Diagnostica Brasil Ltda.
Av. Engenheiro Billings, 1729
Jaguaré, Building 10
05321-010 São Paulo, SP Brazil

Roche Diagnostics
201, Boulevard Armand-Frappier
H7V 4A2 Laval, Québec, Canada
(For Technical Assistance call:
Pour toute assistance technique,
appeler le: 1-877-273-3433)

Roche Diagnostics
2, Avenue du Vercors
38240 Meylan, France

Distributore in Italia:
Roche Diagnostics S.p.A.
Viale G. B. Stucchi 110
20052 Monza, Milano, Italy

Distribuidor em Portugal:
Roche Sistemas de Diagnósticos Lda.
Estrada Nacional, 249-1
2720-413 Amadora, Portugal

Trademarks and patents

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

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Document revision

Document Revision Information	
Doc Rev. 1.0 03/2017	First Publishing.
Doc Rev. 2.0 12/2017	Deleted duplicate paragraph from **Background** section, and updated reference numbers accordingly. Corrected typo in **Table 4**, LoD. Corrected copy-paste error in **Verification of limit of detection for the drug resistant CMV specimens (resistant against foscarnet or ganciclovir, valganciclovir and cidofovir)** section. Corrected caption **Figure 4** to be compliant with **cobas**® CMV branding. Please contact your local Roche Representative if you have any questions.