cobas® CMV

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

For in vitro diagnostic use

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

Table of contents

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 (eg, 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; ie, the risk of CMV disease increases significantly after CMV viral load reaches a "critical threshold." For example, when using a laboratory-developed whole blood CMV DNA assay to test liver transplant recipients, the critical threshold was ≥ 5 $\log_{10}$ copies/mL of CMV DNA.[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 $\log_{10}$) to represent biologically important changes. Since variability is greatest at low concentrations, viral load changes may need to be more than 5-fold (0.7 $\log_{10}$) when the titer values are near the assay's lower limit of quantification, to be considered significant.[11,12]

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.[8-14] 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).[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 real-time PCR methods potentially offers a wide dynamic range, precision, and high sensitivity.

Explanation of the test

cobas® CMV is a quantitative test that is run on the **cobas**® 6800 System and **cobas**® 8800 System. **cobas**® CMV enables the detection and quantitation of CMV DNA in EDTA plasma of infected patients. The viral load is quantified against a non-CMV DNA quantitation standard (DNA-QS), which is introduced into each specimen during sample processing. The DNA-QS also functions to monitor for the entire sample preparation and PCR amplification process. In addition, the test utilizes three external controls: a high titer positive, a low titer positive, and a negative control.

Principles of the procedure

cobas® CMV is based on fully automated sample preparation (nucleic acid extraction and purification) followed by PCR amplification and detection. The **cobas**® 6800/8800 Systems consist of the sample supply module, the transfer module, the processing module, and the analytic module. Automated data management is performed by the **cobas**® 6800/8800 software which assigns test results for all tests as either target not detected, CMV DNA detected < LLoQ (lower limit of quantitation), CMV DNA detected > ULoQ (upper limit of quantitation), or a value in the linear range LLoQ < x < ULoQ. Results can be reviewed directly on the system screen, exported, or printed as a report.

Nucleic acid from patient samples and added lambda DNA-QS molecules is simultaneously extracted. In summary, viral nucleic acid is released by addition of proteinase and lysis reagent to the sample. The released nucleic acid binds to the silica surface of the added magnetic glass particles. Unbound substances and impurities, such as denatured protein, cellular debris and potential PCR inhibitors are removed with subsequent wash reagent steps and purified nucleic acid is eluted from the glass particles with elution buffer at elevated temperature.

Selective amplification of target nucleic acid from the sample is achieved by the use of target virus-specific forward and reverse primers which are selected from highly-conserved regions of the CMV DNA polymerase (UL54) gene. Selective amplification of DNA-QS is achieved by the use of sequence-specific forward and reverse primers which are selected to have no homology with the CMV genome. A thermostable DNA polymerase enzyme is used for amplification. The target and DNA-QS sequences are amplified simultaneously utilizing a universal PCR amplification profile with predefined temperature steps and number of cycles. The master mix includes deoxyuridine triphosphate (dUTP), instead of deoxythimidine triphosphate (dTTP), which is incorporated into the newly synthesized DNA (amplicon).[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.

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.[26,27] The fluorescent signal of the intact probes is suppressed by the quencher dye. During the PCR amplification step, hybridization of the probe to the specific single-stranded DNA templates results in cleavage by the 5'-to-3' nuclease activity of the DNA polymerase resulting in separation of the reporter and quencher dyes and the generation of a fluorescent signal. With each PCR cycle, increasing amounts of cleaved probes are generated and the cumulative signal of the reporter dye is concomitantly increased. Real-time detection and discrimination of PCR products is accomplished by measuring the fluorescence of the released reporter dyes for the viral targets and DNA-QS.

Reagents and materials

cobas® CMV reagents and controls

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

Table 1 cobas® CMV

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

Kit components	Reagent ingredients	Quantity per kit 96 tests
Proteinase Solution (PASE)	Tris buffer, < 0.05% EDTA, calcium chloride, calcium acetate, 8% proteinase EUH210: Safety data sheets available on request. EUH208: May produce an allergic reaction. Contains: Subtilisin, 9014-01-1	13 mL
DNA Quantitation Standard (**DNA-QS**)	Tris buffer, < 0.05% EDTA, < 0.001% non-CMV DNA construct containing non-CMV primer binding and a unique probe region (non-infectious DNA),< 0.002% Poly rA RNA (synthetic), < 0.1% sodium azide	13 mL
Elution Buffer (EB)	Tris buffer, 0.2% methyl-4 hydroxybenzoate	13 mL
Master Mix Reagent 1 (MMX-R1)	Manganese acetate, potassium hydroxide, < 0.1% sodium azide	5.5 mL
CMV Master Mix Reagent 2 (CMV MMX-R2)	Tricine buffer, potassium acetate, < 18% dimethyl sulfoxide, glycerol, < 0.1% Tween 20, EDTA, < 0.12% dATP, dCTP, dGTP, dUTPs, < 0.01% upstream and downstream CMV primers, < 0.01% Quantitation Standard forward and reverse primers, < 0.01% fluorescent-labeled oligonucleotide probes specific for CMV and the CMV Quantitation Standard,< 0.01% oligonucleotide aptamer, < 0.1% ZO5D DNA polymerase, < 0.10% AmpErase (uracil-N- glycosylase) enzyme (microbial), < 0.1% sodium azide	6 mL

Table 2 **cobas**® CMV Control Kit

cobas® CMV Control Kit
Store at 2–8°C
(P/N 07001037190)

Kit components	Reagent ingredients	Quantity per kit	Safety symbol and warning
CMV Low Positive Control (CMV L(+)C)	< 0.001% synthetic (plasmid) CMV DNA encapsulated in Lambda bacteriophage coat protein, normal human plasma, non-reactive by licensed tests for antibody to HCV, antibody to HIV-1/2, HBsAg, antibody to HBc; HIV-1 RNA, HIV-2 RNA, HCV RNA, HBV DNA, HEV RNA, WNV RNA and CMV DNA not detectable by PCR methods. 0.1% ProClin® 300 preservative	4 mL (8 x 0.5 mL)	  Warning H317: May cause an allergic skin reaction. P261: Avoid breathing dust/fumes/gas/mist/ vapors/spray. P272: Contaminated work clothing should not be allowed out of the workplace. P280: Wear protective gloves. P302 + P352: IF ON SKIN wash with plenty of soap and water. P333 + P313: If skin irritation or rash occurs: Get medical advice/attention. P363: Wash contaminated clothing before reuse.
CMV High Positive Control (CMV H(+)C)	< 0.001% high titered synthetic (plasmid) CMV DNA encapsulated in Lambda bacteriophage coat protein, normal human plasma, non-reactive by licensed tests for antibody to HCV, antibody to HIV-1/2, HBsAg, antibody to HBc; HIV-1 RNA, HIV-2 RNA, HCV RNA, HBV DNA, HEV RNA, WNV RNA and CMV DNA not detectable by PCR methods. 0.1% ProClin® 300 preservative	4 mL (8 x 0.5 mL)	  Warning H317: May cause an allergic skin reaction. P261: Avoid breathing dust/fumes/gas/mist/ vapors/spray. P272: Contaminated work clothing should not be allowed out of the workplace. P280: Wear protective gloves. P302 + P352: IF ON SKIN wash with plenty of soap and water. P333 + P313: If skin irritation or rash occurs: Get medical advice/attention. P363: Wash contaminated clothing before reuse.

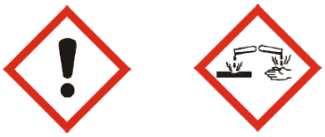
Table 3 **cobas**® NHP Negative Control Kit

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

Kit components	Reagent ingredients	Quantity per kit	Safety symbol and warning
Normal Human Plasma Negative Control (NHP-NC)	Normal human plasma, non-reactive by licensed tests for antibody to HCV, antibody to HIV-1/2, HBsAg, antibody to HBc; HIV-1 RNA, HIV-2 RNA, HCV RNA, HBV DNA, HEV RNA, WNV RNA, and CMV DNA not detectable by PCR methods. < 0.1% ProClin® 300 preservative	16 mL (16 x 1 mL)	 Warning H317: May cause an allergic skin reaction. P261: Avoid breathing dust/fumes/gas/mist/vapors/spray. P272: Contaminated work clothing should not be allowed out of the workplace. P280: Wear protective gloves. P302+ P352: IF ON SKIN wash with plenty of soap and water. P333 + P313: If skin irritation or rash occurs: Get medical advice/attention. P363: Wash contaminated clothing before reuse.

cobas omni reagents for sample preparation

Table 4 cobas omni reagents for sample preparation*

Reagents	Reagent ingredients	Quantity per kit	Safety symbol and warning
cobas omni MGP Reagent (MGP) Store at 2–8°C (P/N 06997546190)	Magnetic glass particles, Tris buffer, 0.1% methyl-4 hydroxybenzoate, < 0.1% sodium azide	480 tests	Not applicable
cobas omni Specimen Diluent (SPEC DIL) Store at 2–8°C (P/N 06997511190)	Tris buffer, 0.1% methyl-4 hydroxybenzoate, < 0.1% sodium azide	4 x 875 mL	Not applicable
cobas omni Lysis Reagent (LYS) Store at 2–8°C (P/N 06997538190)	42.56% (w/w) guanidine thiocyanate, 5% (w/v) polydocanol, 2% (w/v) dithiothreitol, dihydro sodium citrate	4 x 875 mL	 Danger H302: Harmful if swallowed. H318: Causes serious eye damage. H412: Harmful to aquatic life with long lasting effects. EUH032: Contact with acids liberates very toxic gas. P301 + P312: IF SWALLOWED: Call a POISON CENTER or doctor/physician if you feel unwell. P264: Wash skin thoroughly after handling. P270: Do not eat, drink or smoke when using this product. P273: Avoid release to the environment. P280: Wear protective gloves/eye protection/face protection. P305 + P351 + P338: IF IN EYES Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. P310: Immediately call a POISON CENTER or doctor/Physician if you feel unwell. P330: Rinse mouth
cobas omni Wash Reagent (WASH) Store at 15–30°C (P/N 06997503190)	Sodium citrate dihydrate, 0.1% methyl-4 hydroxybenzoate	4.2L	Not applicable

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

Reagent storage and handling requirements

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

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

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

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

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

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

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

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

Additional materials required

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

Material	P/N
cobas omni Processing Plate	05534917001
cobas omni Amplification Plate	05534941001
cobas omni Pipette Tips	05534925001
cobas omni Liquid Waste Container	07094388001
cobas omni Lysis Reagent	06997538190
cobas omni MGP Reagent	06997546190
cobas omni Specimen Diluent	06997511190
cobas omni Wash Reagent	06997503190
Solid Waste Bag	07435967001
Solid Waste Container	07094361001

Instrumentation and software required

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

Table 8 Instrumentation

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

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

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

Precautions and handling requirements

Warnings and precautions

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

- For *in vitro* diagnostic use only.
- **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 **cobas**® 6800/8800 Systems should perform this procedure.
- All human-sourced materials should be considered potentially infectious and should be handled with universal precautions. If spillage occurs, immediately disinfect with a freshly prepared solution of 0.5% sodium hypochlorite in distilled or deionized water (dilute household bleach 1:10) or follow appropriate site procedures.
- **cobas**® CMV Control Kit and **cobas**® NHP Negative Control Kit contain plasma derived from human blood. The source material has been tested by 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.
- **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.

Reagent handling

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

Good laboratory practice

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

Sample collection, transport, and storage

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

Store all samples at specified temperatures.

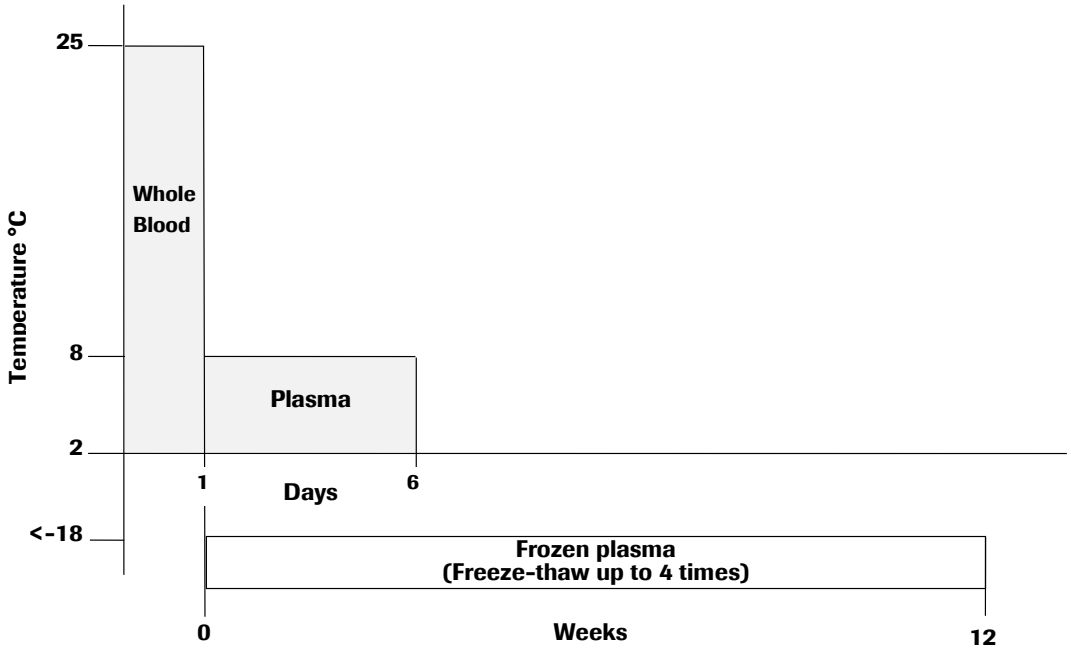
Sample stability is affected by elevated temperatures.

If using frozen samples in secondary tubes, place the samples at room temperature (15-30ºC) until completely thawed and then centrifuge to collect all sample volume at the bottom of the tube.

Samples

- Blood should be collected in BD Vacutainer® PPT™ Plasma PreparationTubes for Molecular Diagnostic Test Methods or in sterile tubes using EDTA as the anticoagulant. Follow the sample collection tube manufacturer instructions. Refer to Figure 1.
- Whole blood collected in BD Vacutainer® PPT™ Plasma PreparationTubes for Molecular Diagnostic Test Methods or in sterile tubes using EDTA as the anticoagulant may be stored and/or transported for up to 24 hours at 2-25°C prior to plasma preparation. Centrifugation should be performed according to manufacturer instructions.
- Upon separation plasma samples may be stored for up to 6 days at 2-8°C or up to 12 weeks at ≤ -18°C.
- Plasma samples are stable for up to four freeze/thaw cycles when frozen at ≤ -18°C.

Figure 1 Sample storage conditions



- If samples are to be shipped, they should be packaged and labeled in compliance with applicable country and/or international regulations covering the transport of samples and etiologic agents.

Note: Alternatively, whole blood collected in in BD Vacutainer® PPT™ Plasma PreparationTubes for Molecular Diagnostic Test Methods or in sterile tubes using EDTA as the anticoagulant may be stored and/or transported for up to 36 hours at 2-25°C prior to plasma preparation, but then separated plasma cannot be stored for longer and needs to be analyzed directly.

Instructions for use

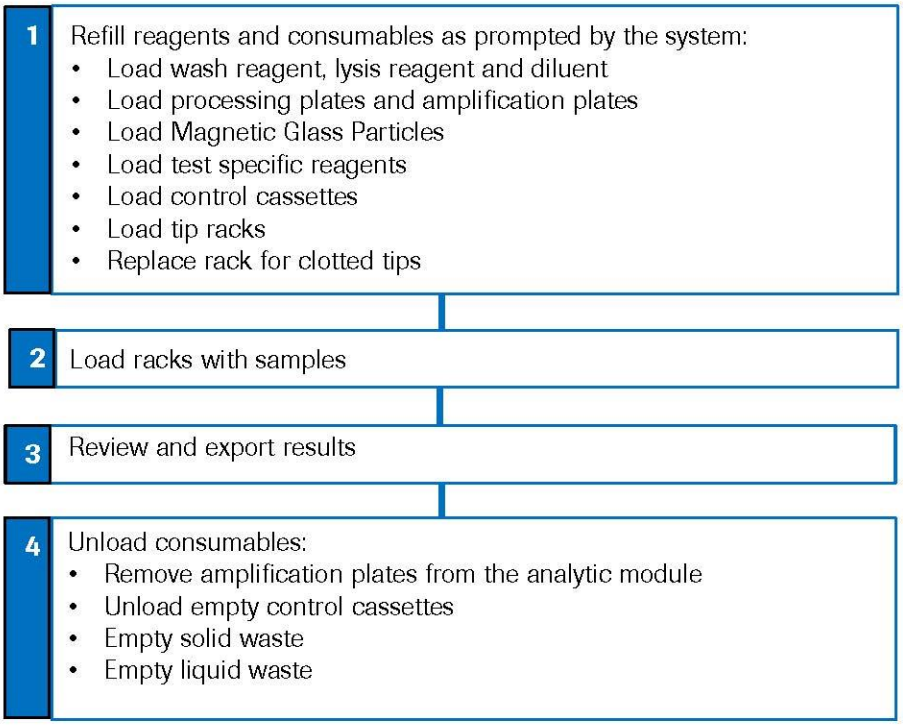
Procedural notes

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

Running cobas® CMV

cobas® CMV can be run with one required sample volume of 500 µL. The test procedure is described in detail in the **cobas**® 6800/8800 Systems Operator's Manual. Figure 2 below summarizes the procedure.

Figure 2 cobas® CMV procedure



Results

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

Quality control and validity of results

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

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

Control flags

Table 9 Control flags for negative and positive controls

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

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

Interpretation of results

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

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

Table 10 Target results for individual target result interpretation

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

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

Procedural limitations

- **cobas**® CMV has been evaluated only for use in combination with the **cobas**® CMV Control Kit, **cobas**® NHP Negative Control Kit, **cobas omni** MGP Reagent, **cobas omni** Lysis Reagent, **cobas omni** Specimen Diluent, and **cobas omni** Wash Reagent for use on the **cobas**® 6800/8800 Systems.
- Reliable results depend on proper sample collection, storage and handling procedures.
- This test has been validated only for use with EDTA plasma. Testing of other sample types with **cobas**® CMV may result in inaccurate results. Plasma viral load measurements are not directly comparable to those of other sample types.
- Quantitation of CMV DNA may be affected by sample collection methods, patient factors (i.e., age, presence of symptoms), and/or stage of infection.
- Though rare, 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.
- 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.
- The **cobas**® CMV test is not intended for use as a screening test for the presence of CMV in blood or blood products and has not been evaluated as a diagnostic test to confirm the presence of CMV infection.

Non-clinical performance evaluation

Key performance characteristics

Limit of Detection (LoD)

WHO International Standard

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

The results for EDTA plasma are shown in Table 11. The study demonstrates that **cobas**® CMV detected CMV DNA at a concentration of 23 IU/mL or greater with a hit rate of ≥ 95%.

Table 11 Limit of detection in EDTA plasma

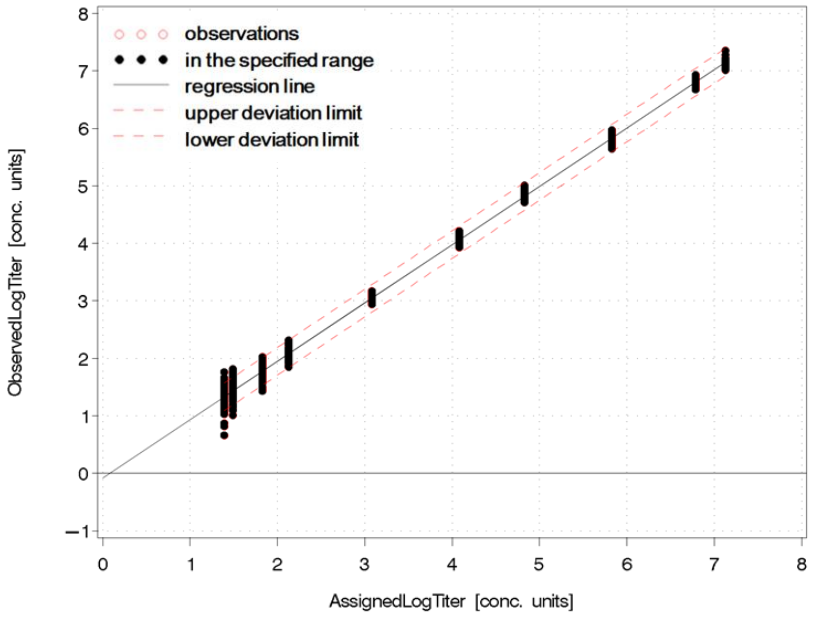
Input titer concentration (CMV DNA IU/mL)	Number of valid replicates	Number of positives	Hit rate in %
92.0	189	189	100.00
46.0	189	188	99.47
34.5	188	187	99.47
23.0	189	181	95.77
11.5	189	158	83.60
5.8	189	117	61.60
2.9	189	66	34.92
1.4	189	28	14.81
0.0	189	0	0.00
LoD by PROBIT at 95% hit rate	20.6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

Linear range

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

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

Figure 3 Linear range determination in EDTA plasma



Precision – within laboratory

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

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

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

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

Genotype verification

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

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

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

CMV cell culture supernatants for three different Glycoprotein B genotypes (gB-2, gB-3 and gB-4) were diluted to three different concentration levels in CMV negative EDTA plasma. The hit rate determination was performed with 63 replicates for each level. Testing was conducted with three lots of **cobas**®CMV reagents. The results are shown in Table 13. These results verify that **cobas**® CMV detected CMV DNA for three different genotypes at concentrations of 34.5 IU/mL with a hit rate of ≥ 95%.

Table 13 CMV DNA genotype verification of limit of detection

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

* Upper one-sided 95% confidence interval

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

The dilution series used in the verification of genotypes linearity study of **cobas**® CMV consistsed of seven panel members spanning the assay linear range. Testing was conducted with two lots of **cobas**® CMVreagent; 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 Forscarnet)
- Verification of the linear range for drug resistant CMV specimens (resistant against Ganciclovir, Valganciclovir, Cidofovir or Forscarnet)

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

Cell culture supernatants for two different drug resistant CMV specimens (resistant against Foscarnet or Ganciclovir, Valganciclovir and Cidofovir) were diluted to three different concentration levels in CMV negative EDTA plasma. The hit rate determination was performed with 63 replicates for each level. Testing was conducted with three lots of **cobas**® CMV reagents. The results are shown in Table 14. These results verify that **cobas**® CMV detected CMV DNA for two different specimens resistant against Forscarnet or Ganciclovir, Valganciclovir and Cidofovir at concentrations of 34.5 IU/mL with a hit rate of ≥ 95%.

Table 14 Drug Resistant CMV Specimens verification of limit of detection

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

* Upper one-sided 95% confidence interval

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

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

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

Specificity

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

Analytical specificity

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

Table 15 Microorganisms tested for cross-reactivity

Viruses	Bacteria	Yeast
Adenovirus type 5	Propionibacterium acnes	Aspergillus niger
BK Polyomavirus	Staphylococcus aureus	Candida albicans
Epstein-Barr Virus	Chlamydia trachomatis	Cryptococcus neoformans
Hepatitis B Virus	Clostridium perfringens	
Hepatitis C Virus	Enterococcus faecalis	
Herpes Simplex Virus Type1	Escherichia coli	
Herpes Simplex Virus Type 2	Klebsiella pneumonia	
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 pyrogenes	
Human Papillomavirus	Mycoplasma pneumonia	
JC virus	Salmonella typhimurium	
Parvovirus B19	Streptococcus pneumonia	
Varicella-Zoster Virus		

Analytical specificity – interfering substances

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

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

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

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

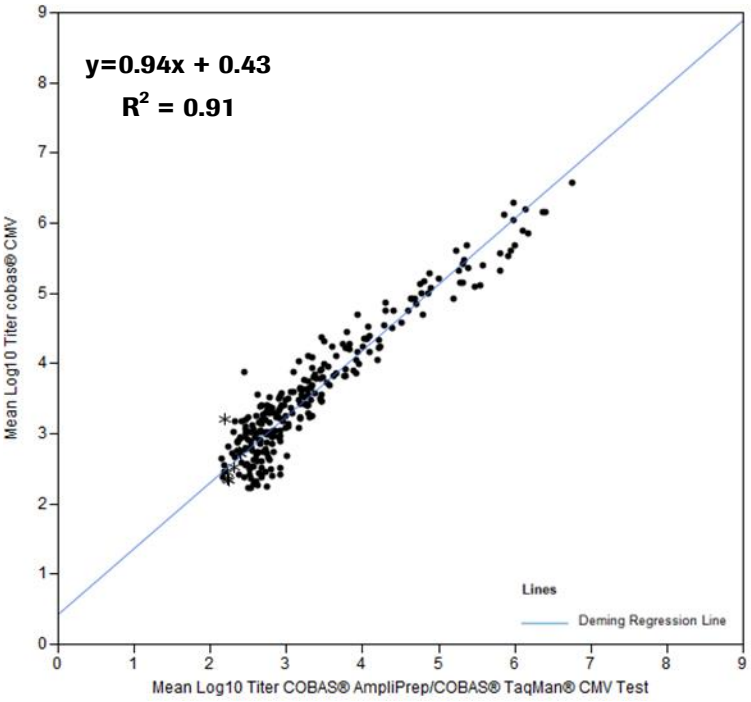
Class of drug	Generic drug name	
Antibimicrobial	Cefotetan	Sulfamethoxazole
	Clavulanate potassium	Ticarcillin disodium
	Fluconazole	Trimethoprim
	Piperacillin	Vancomycin
	Tazobactam sodium	
Compounds for Treatment of Herpes Viruses	Ganciclovir	Cidofovir
	Valganciclovir	Foscarnet
Immune suppressant	Azathioprine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

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

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

The Deming regression results are shown in Figure 4.

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



Whole system failure

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

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

Cross contamination

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

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

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

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

Table 17 Symbols used in labeling for Roche PCR diagnostics products

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

US Customer Technical Support 1-800-526-1247

Manufacturer and distributors

Table 18 Manufacturer and distributors

Manufactured in the United States

Roche Diagnostics GmbH
Sandhofer Straße 116
68305 Mannheim, Germany

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

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:
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Estrada Nacional, 249-1
2720-413 Amadora, Portugal

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Document revision

Document Revision Information	
Doc Rev. 1.0 10/2014	First Publishing.