COBAS® AmpliPrep/COBAS® TaqMan® CMV Test

cobas®

FOR IN VITRO DIAGNOSTIC USE.

COBAS® AmpliPrep/COBAS® TaqMan® CMV Test	**CMVCAP**	72 Tests	P/N: 04902025 190
COBAS® AmpliPrep/COBAS® TaqMan® Wash Reagent	**PG WR**	5.1 Liters	P/N: 03587797 190

INTENDED USE

The COBAS® AmpliPrep/COBAS® TaqMan® CMV Test is an *in vitro* nucleic acid amplification test for the quantitative measurement of cytomegalovirus (CMV) DNA in human EDTA plasma using the COBAS® AmpliPrep Instrument for automated specimen processing and the COBAS® TaqMan® Analyzer or the COBAS® TaqMan® 48 Analyzer for automated amplification and detection.

The COBAS® AmpliPrep/COBAS® TaqMan® CMV Test is intended for use as an aid in the management of solid-organ transplant patients who are undergoing anti-CMV therapy. In this population serial DNA measurements can be used to assess virological response to antiviral treatment. The results from the COBAS® AmpliPrep/COBAS® TaqMan® CMV Test must be interpreted within the context of all relevant clinical and laboratory findings.

The COBAS® AmpliPrep/COBAS® TaqMan® CMV Test is not intended for use as a screening test for the presence of CMV DNA in blood or blood products.

SUMMARY AND EXPLANATION OF THE TEST

Human CMV (CMV or HCMV) is a human viral pathogen belonging to the herpesvirus family. CMV can be transmitted through blood, oropharyngeal secretions (e.g., saliva), urine, cervical and vaginal excretions, spermatic fluid, breast milk, tears, and feces.[1-8] CMV infection in immunocompetent individuals is usually asymptomatic; however, primary infection is associated with persistent latent infection of monocytes/macrophages.[2] Latently infected individuals may intermittently shed the virus through their body fluids and thus infect others. Immunocompromised individuals, including transplant patients, and AIDS patients, are at risk for reactivation of latent infection and the development of severe CMV-associated disease[7]. Primary infection or secondary reactivation during pregnancy can lead to congenital CMV infection and significant morbidity. Clinical manifestations of CMV disease in immunocompromised hosts include fever, hematological abnormalities, retinitis, gastroenteritis, hepatitis, encephalitis, esophagitis, enterocolitis, pancreatitis, and pneumonia.[1-13]

Studies in transplant recipients have shown the association between CMV viral load in blood and the risk of developing CMV disease; similarly, increases in viral load over time have been associated with worse clinical outcomes.[14-16] Quantitative polymerase chain reaction (PCR) assays allow for assessing the virological response to antiviral treatment.

PRINCIPLES OF THE PROCEDURE

The COBAS® AmpliPrep/COBAS® TaqMan® CMV Test is a nucleic acid amplification test for the quantitation of cytomegalovirus (CMV) DNA in human plasma. The COBAS® AmpliPrep/COBAS® TaqMan® CMV Test is based on two major processes: (1) specimen preparation to isolate CMV DNA and (2) simultaneous PCR amplification and detection of both CMV DNA target and an internal control Quantitative Standard (QS). Specimen preparation is automated using the COBAS® AmpliPrep instrument with amplification and detection automated using the COBAS® TaqMan® Analyzer or the COBAS® Taqman® 48 analyzer.

The COBAS® AmpliPrep/COBAS® TaqMan® CMV Test permits automated specimen preparation followed by PCR amplification and detection of CMV target DNA and CMV QS DNA. The QS compensates for effects of inhibition and controls the preparation and amplification processes, allowing a more accurate quantitation of CMV DNA in each specimen. The CMV QS is a non-infectious DNA construct that contains identical primer binding sites as the CMV target DNA and a unique probe binding region that allows CMV QS amplicon to be distinguished from CMV target amplicon. The CMV QS is added to each specimen at a known copy number and is carried through the subsequent steps of specimen preparation

The Master Mix reagent contains the same primers to amplify both the CMV DNA target and CMV QS DNA. The detection of amplified DNA is performed using a target-specific and a QS-specific oligonucleotide hydrolysis probe, each labeled with a different fluorochrome that permits the simultaneous, independent identification of both the CMV target amplicon and CMV QS amplicon. The quantitation of CMV viral DNA is performed using both the CMV DNA target critical threshold (Ct) value and the QS Ct values. The CMV DNA concentration of each specimen and control is calculated automatically by comparing the difference between the CMV target Ct value and the CMV QS Ct value and using lot-specific calibration coefficients generated by an in-house calibration obviating the need for the laboratory to calibrate.

Target Selection

The CMV target for this test is a highly-conserved, non-drug target region of the CMV DNA polymerase (UL54) gene. Generic silica-based specimen preparation is used to capture the CMV DNA and CMV QS DNA, and defined oligonucleotides are used as primers in amplification of the CMV DNA and CMV QS DNA. A target-specific and a QS-specific dual-labeled oligonucleotide probe permit independent identification of the CMV amplicon and of the CMV QS amplicon. The COBAS® AmpliPrep/COBAS® TaqMan® CMV Test uses two amplification primers for PCR. A fluorescent, signal-generating probe modified with a 5' fluorochrome (FAM) and a 3' quencher hybridizes to one of the two strands and is cleaved by Z05 DNA polymerase during extension of the primers.

Specimen Preparation

The COBAS® AmpliPrep/COBAS® TaqMan® CMV Test uses automated specimen preparation on the COBAS® AmpliPrep Instrument by a generic silica-based capture technique. The procedure requires an EDTA-plasma sample input volume of 500 µL, 350 µL of which is processed by the COBAS® AmpliPrep Instrument. The CMV particles are lysed by incubation at elevated temperature with a protease and chaotropic lysis/binding buffer that releases nucleic acids and protects the released CMV DNA from DNAses in plasma. Protease and a known number of CMV QS DNA molecules are introduced into each specimen along with the lysis reagent and magnetic glass particles. Subsequently, the mixture is incubated and the CMV DNA and CMV QS DNA are bound to the surface of the magnetic glass particles. Unbound substances, such as salts, proteins, and other cellular impurities, are removed by washing the magnetic glass particles. After separating the magnetic glass particles and completing the washing steps, the adsorbed nucleic acids are eluted at an elevated temperature with an aqueous solution. The processed specimen, containing the released CMV DNA and CMV QS DNA, is added to the amplification mixture and transferred to the COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer.

PCR Amplification

The PCR amplification reaction is performed with the thermostable recombinant enzyme *Thermus specie* Z05 DNA Polymerase (Z05). In the presence of magnesium (Mg^{2+}) and under the appropriate buffer conditions, Z05 has DNA polymerase activity.[17] This allows PCR amplification to occur together with real-time detection of the amplicon.

Processed specimens are added to the amplification mixture in amplification tubes (K-tubes) in which PCR amplification occurs. In the presence of Mg^{2+} and excess deoxynucleotide triphosphates (dNTPs), including deoxyadenosine, deoxyguanosine, deoxycytidine, deoxyuridine and deoxythymidine triphosphates, Z05 polymerase extends the annealed primers forming double-stranded DNA.

Target Amplification

The Thermal Cycler in the COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer heats the reaction mixture to denature the double-stranded DNA and to expose the specific primer target sequences. As the mixture cools, the primers anneal to the target DNA. Z05 in the presence of Mg^{2+} and excess deoxynucleotide triphosphates (dNTPs) extends the annealed primers along the target template to produce double-stranded DNA molecules termed an amplicon. The COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer automatically repeats this process for a designated number of cycles, with each cycle intended to double the amount of amplicon DNA. The required number of cycles is preprogrammed into the COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer. Amplification occurs only in the region of the CMV genome between the primers; the entire CMV genome is not amplified.

Target Detection

The COBAS® AmpliPrep/COBAS® TaqMan® CMV Test uses real-time[18,19] PCR technology. The use of fluorescently-labeled hydrolysis probes allows for real-time detection of PCR product accumulation by monitoring of the emission intensity of fluorescent reporter dyes released during the amplification process. The probes consist of CMV target and CMV QS-specific oligonucleotide probes with a reporter dye and a quencher dye. In the COBAS® AmpliPrep/COBAS® TaqMan® CMV Test the CMV target and CMV QS probes are labeled with different fluorescent reporter dyes. When these probes are intact, the fluorescence of the reporter dye is suppressed by the proximity of the quencher dye due to Förster-type energy transfer effects. During PCR, the probe hybridizes to a target sequence and is cleaved by the 5' → 3' nuclease activity of the thermostable Z05 DNA polymerase during the extension phase of the PCR cycle. Once the reporter and quencher dyes are released and separated, quenching no longer occurs, and the fluorescent activity of the reporter dye is increased. The amplification of CMV DNA and CMV QS DNA are measured independently at different wavelengths. This process is repeated for a designated number of cycles, each cycle effectively increasing the emission intensity of the individual reporter dyes, permitting independent identification of CMV DNA and CMV QS DNA. The PCR cycle where a growth curve starts exponential growth is related to the amount of starting material at the beginning of the PCR.

Selective Amplification

Selective amplification of target nucleic acid from the specimen is achieved in the COBAS® AmpliPrep/COBAS® TaqMan® CMV Test by the use of AmpErase (uracil-N-glycosylase) enzyme and deoxyuridine triphosphate (dUTP). The AmpErase enzyme recognizes and catalyzes the destruction of DNA strands containing deoxyuridine,[20] but not DNA containing deoxythymidine. Deoxyuridine is not present in naturally occurring DNA, but is always present in amplicon due to the use of deoxyuridine triphosphate as one of the dNTPs in the Master Mix reagent; therefore, only amplicon contains deoxyuridine. Deoxyuridine renders contaminating amplicon susceptible to destruction by the AmpErase enzyme prior to amplification of the target DNA. Also, any nonspecific product formed after initial activation of the Master Mix by magnesium is destroyed by the AmpErase enzyme. The AmpErase enzyme, which is included in the Master Mix reagent, catalyzes the cleavage of deoxyuridine-containing DNA at the deoxyuridine residues by opening the deoxyribose chain at the C1-position. When heated in the first thermal cycling step, the amplicon DNA chain breaks at the position of the deoxyuridine, thereby rendering the DNA non-amplifiable. The AmpErase enzyme remains inactive for a prolonged period of time once exposed to temperatures above 55°C, i.e., throughout the thermal cycling steps, and therefore does not destroy target amplicon formed during amplification.

CMV DNA Quantitation

The COBAS® AmpliPrep/COBAS® TaqMan® CMV Test quantitates CMV DNA by analyzing the difference between the CMV target and QS Ct values. The CMV QS is a non-infectious DNA construct, containing fragments of CMV sequences with primer binding regions identical to those of the CMV target sequence. The CMV QS contains CMV primer binding regions and generates an amplification product of the same length and base composition as the CMV target DNA. The detection probe binding region of the CMV QS has been modified to differentiate CMV QS amplicon from CMV target amplicon.

During the extension phase of the PCR in the COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer, the specimens are illuminated and excited by filtered light, and filtered emission fluorescence data are collected for each specimen. The readings from each specimen are then corrected for instrumental fluctuations. These fluorescence readings are sent by the instrument to the AMPLILINK software and stored in a database. Pre-Checks are used to determine if the CMV DNA target and CMV QS DNA data represent sets that are valid, and flags are generated when the data lie outside the preset limits. After all Pre-Checks are completed and passed, the fluorescence readings are processed to generate Ct values for the CMV DNA target and the CMV QS DNA. The lot-specific calibration constants provided with the COBAS® AmpliPrep/COBAS® TaqMan® CMV Test are used to calculate the titer value for the specimens and controls based on both the CMV DNA target and CMV QS DNA Ct values. Titer results are reported in International Units/mL (IU/mL).

REAGENTS

COBAS® AmpliPrep/COBAS® TaqMan® CMV Test | **CMVCAP** | **72 Tests**

(P/N: 04902025 190)

CMV CS1 — **1 x 72 Tests**

(Magnetic Glass Particles Reagent Cassette)

Magnetic glass particles

Tris-base buffer

0.09% Sodium azide

0.1% Methylparaben

This reagent contains less than 0.1% of sodium azide as a preservative. As sodium azide may react with lead and copper plumbing to form explosive metal azides, this reagent should be disposed of by flushing with copious amounts of water.

CMV CS2 — **1 x 72 Tests**

(CMV Lysis Reagent Cassette)

Sodium citrate dihydrate

42.5% Guanidine thiocyanate

3.6% Polydocanol

1.8% Dithiothreitol

Xn  42.5% (w/w) Guanidine thiocyanate

Harmful

R20/21/22-R52/53-R32: Harmful by inhalation, in contact with skin and if swallowed. Harmful to aquatic organisms, may cause long-term adverse effects in the aquatic environment. Contact with acids liberates very toxic gas.

S24/25-S36/37/39-S61: Avoid contact with skin and eyes. Wear suitable protective clothing, gloves and eye/face protection. Avoid release to the environment. Refer to special instructions/Safety data sheets.

CMV CS3 **1 x 72 Tests**

CMV Multi-Reagent Cassette containing:

Pase 1 x 3.8 mL

(Proteinase Solution)

Tris buffer

< 0.05% EDTA

Calcium chloride

Calcium acetate

≤ 7.8% Proteinase

Glycerol

Xn  ≤ 7.8% (w/w) Proteinase

Harmful

R36-R42: Irritating to eyes. May cause sensitization by inhalation.

S23-S45: Do not breathe vapor. In case of accident or if you feel unwell seek medical advice immediately (show the label where possible)

EB 1 x 8.1 mL

(Elution Buffer)

Tris-base buffer

Sodium hydroxide

0.09% Sodium azide

This reagent contains less than 0.1% of sodium azide as a preservative. As sodium azide may react with lead and copper plumbing to form explosive metal azides, this reagent should be disposed of by flushing with copious amounts of water.

CMV CS4 **1 x 72 Tests**

CMV Test-Specific Reagent Cassette containing:

CMV QS 1 x 6.2 mL

(CMV Quantitation Standard)

Tris-HCl buffer

EDTA

< 0.005% Poly rA RNA (synthetic)

< 0.001% Non-infectious plasmid DNA (microbial) containing CMV primer binding sequences and a unique probe-binding region

0.05% Sodium azide

This reagent contains less than 0.1% of sodium azide as a preservative. As sodium azide may react with lead and copper plumbing to form explosive metal azides, this reagent should be disposed of by flushing with copious amounts of water.

CMV MMX 1 x 3.2 mL

(CMV Master Mix)

Tricine buffer

Potassium acetate

Potassium hydroxide

< 20% Dimethyl sulfoxide

Glycerol

< 0.05% dATP, dCTP, dGTP, dUTP, dTTP

< 0.01% Upstream and downstream CMV primers

< 0.01% Oligonucleotide aptamer

< 0.01% Fluorescent-labeled oligonucleotide probes specific for CMV and the CMV Quantitation Standard

< 0.05% Z05 DNA Polymerase (microbial)

< 0.1% AmpErase (uracil-N-glycosylase) enzyme (microbial)

0.09% Sodium azide

This reagent contains less than 0.1% of sodium azide as a preservative. As sodium azide may react with lead and copper plumbing to form explosive metal azides, this reagent should be disposed of by flushing with copious amounts of water.

$MgCl_2$ 1 x 9.8 mL

(CAP/CTM Magnesium Solution)

< 0.6% Magnesium chloride

0.09% Sodium azide

This reagent contains less than 0.1% of sodium azide as a preservative. As sodium azide may react with lead and copper plumbing to form explosive metal azides, this reagent should be disposed of by flushing with copious amounts of water.

CMV H(+)C 6 x 0.65 mL

(CMV High Positive Control)

< 0.001% Lambda phage containing CMV DNA

Human plasma, non-reactive by US FDA-approved or licensed tests for antibody to HCV, antibody to HIV-1/2 and HIV p24 antigen or HIV-1 RNA, HBsAg; CMV DNA not detectable by PCR method.

Human Cell Line DNA

0.1% ProClin® 300 preservative

Xi  (3:1) mixture of 5-Chloro-2-methyl-2H-isothiazol-3-one and 2-methyl-2H-isothiazol-3-one

Irritant

S24-S37: Avoid contact with skin. Wear suitable gloves.

R43: May cause sensitization by skin contact.

CMV L(+)C 6 x 0.65 mL

(CMV Low Positive Control)

< 0.001% Lambda phage containing CMV DNA at a mean concentration approximately 100 fold lower than the mean concentration CMV DNA in CMV H(+)C

Human plasma, non-reactive by US FDA-approved or licensed tests for antibody to HCV, antibody to HIV-1/2 and HIV p24 antigen or HIV-1 RNA, HBsAg; CMV DNA not detectable by PCR method.

Human Cell Line DNA

0.1% ProClin® 300 preservative

Xi  (3:1) mixture of 5-Chloro-2-methyl-2H-isothiazol-3-one and 2-methyl-2H-isothiazol-3-one

Irritant

S24-S37: Avoid contact with skin. Wear suitable gloves.

R43: May cause sensitization by skin contact.

CMV (–)C 6 x 0.65 mL

(CMV Negative Control)

Human plasma, non-reactive by US FDA approved or licensed tests for antibody to HCV, antibody to HIV-1/2 and HIV p24 antigen or HIV-1 RNA, HBsAg; CMV DNA not detectable by PCR method.

Human Cell Line DNA

0.1% ProClin® 300 preservative

Xi  (3:1) mixture of 5-Chloro-2-methyl-2H-isothiazol-3-one and 2-methyl-2H-isothiazol-3-one

Irritant

S24-S37: Avoid contact with skin. Wear suitable gloves.

R43: May cause sensitization by skin contact.

CMV H(+)C Clip (CMV High Positive Control Barcode Clip)		1 x 6 Clips
CMV L(+)C Clip (CMV Low Positive Control Barcode Clip)		1 x 6 Clips
CMV (–)C Clip (CMV Negative Control Barcode Clip)		1 x 6 Clips
COBAS® AmpliPrep/COBAS® TaqMan® Wash Reagent (P/N: 03587797 190)	**PG WR**	1 x 5.1 L

PG WR

(COBAS® AmpliPrep/COBAS® TaqMan® Wash Reagent)

Sodium citrate dihydrate

< 0.1% N-Methylisothiazolone-HCl

WARNINGS AND PRECAUTIONS

A. **FOR IN VITRO DIAGNOSTIC USE.**

B. **The COBAS® AmpliPrep/COBAS® TaqMan® CMV Test is not intended for use as a diagnostic test to diagnose CMV infection.**

C. **Due to inherent differences among technologies and patient populations, the user should perform method comparison with their own quantitative CMV test currently used in their clinical practice before switching to the COBAS® AmpliPrep/COBAS® TaqMan® CMV Test.**

D. This test is for use with human plasma collected in the anticoagulant EDTA.

E. Do not pipette by mouth.

F. Do not eat, drink, or smoke in laboratory work areas. Wear protective disposable gloves, laboratory coats, and eye protection when handling specimens and kit reagents. Wash hands thoroughly after handling specimens and test reagents.

G. **Avoid microbial and nuclease contamination of reagents when removing aliquots from control vials.**

H. **The use of sterile disposable pipettes and DNase-free pipette tips is recommended.**

I. Do not pool controls from different lots or from different vials of the same lot.

J. Do not mix reagent cassettes or controls from different kits.

K. Do not open COBAS® AmpliPrep cassettes and exchange, mix, remove, or add bottles.

L. Dispose of unused reagents, waste, and specimens in accordance with country, federal, state, and local regulations.

M. Do not use a kit after its expiration date.

N. Material Safety Data Sheets (MSDS) are available on request from your local Roche office.

O. Specimens and controls should be handled as if infectious, using safe laboratory procedures such as those outlined in the Centers for Disease Control and Prevention publication *Biosafety in Microbiological and Biomedical Laboratories*[21] and in the Clinical and Laboratory Standards Institute (CLSI) document M29-A3.[22] Thoroughly clean and disinfect all work surfaces with a freshly prepared solution of 0.5% sodium hypochlorite in deionized or distilled water.

Note: ***Commercial liquid household bleach typically contains sodium hypochlorite at a concentration of 5.25%. A 1:10 dilution of household bleach will produce a 0.5% sodium hypochlorite solution.***

P. **CAUTION**: **CMV (–)C, CMV L(+)C**, and **CMV H(+)C** contain human plasma derived from human blood. The source material has been tested and found non-reactive for the presence of hepatitis B surface antigen (HBsAg), antibodies to HIV-1/2 and HCV, and HIV p24 antigen or HIV-1 RNA. Testing of negative human plasma by PCR method showed no detectable CMV DNA. No known test methods can offer complete assurance that products derived from human blood will not transmit

infectious agents. Therefore, all human-sourced material should be considered potentially infectious. **CMV (–)C**, **CMV L(+)C**, and **CMV H(+)C** should be handled as if infectious using safe laboratory procedures such as those outlined in *Biosafety in Microbiological and Biomedical Laboratories*[21] and in the CLSI document M29-A3.[22] Thoroughly clean and disinfect all work surfaces with a freshly prepared solution of 0.5% sodium hypochlorite in deionized or distilled water.

Q. **MGP, EB, CMV QS, $MgCl_2$,** and **CMV MMX** contain sodium azide. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. While disposing of sodium azide-containing solutions down laboratory sinks, flush the drains with a large volume of water to prevent azide buildup.

R. Wear eye protection, laboratory coats, and disposable gloves when handling any reagent. 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 of these reagents occur, dilute with water before wiping dry.

S. Do not allow **CMV CS2** and liquid waste from the COBAS® AmpliPrep Instrument, which contain guanidine thiocyanate, to contact sodium hypochlorite (bleach) solution. These mixtures can produce a highly toxic gas.

T. When disposing of used COBAS® AmpliPrep Sample Processing Units (SPUs), which contain guanidine thiocyanate, avoid any contact with sodium hypochlorite (bleach) solution. These mixtures can produce a highly toxic gas.

STORAGE AND HANDLING REQUIREMENTS

A. ***Do not freeze reagents or controls.***

B. Store **CMV CS1**, **CMV CS2**, **CMV CS3**, and **CMV CS4** at 2°C to 8°C. Unopened, these reagents are stable until the expiration date indicated. Once opened, these reagents are stable for 70 days if stored at 2°C to 8°C or until the expiration date, whichever comes first. **CMV CS1**, **CMV CS2**, **CMV CS3**, and **CMV CS4** can be used for a maximum of 6 instrument cycles, up to a maximum of 100 hours cumulative on board the COBAS® AmpliPrep Instrument. Reagents must be stored at 2°C to 8°C between instrument cycles.

C. Store **CMV H(+)C**, **CMV L(+)C**, and **CMV (–)C** at 2°C to 8°C. The controls are stable until the expiration date indicated. Once opened, any unused portion must be discarded.

D. Store Barcode clips [**CMV H(+)C Clip, CMV L(+)C Clip** and **CMV (–)C Clip**] at 2°C to 30°C.

E. Store **PG WR** at 2°C to 30°C. **PG WR** is stable until the expiration date indicated. Once opened, this reagent is stable for 28 days at 2°C to 30°C or until the expiration date, whichever comes first.

MATERIALS PROVIDED

A. **COBAS® AmpliPrep/COBAS® TaqMan® CMV Test** **CMVCAP**
(P/N: 04902025 190)

- **CMV CS1**
  (CMV Magnetic Glass Particles Reagent Cassette)
- **CMV CS2**
  (CMV Lysis Reagent Cassette)
- **CMV CS3**
  (CMV Multi-Reagent Cassette)
- **CMV CS4**
  (CMV Test-Specific Reagent Cassette)
- **CMV H(+)C**
  (CMV High Positive Control)
- **CMV L(+)C**
  (CMV Low Positive Control)
- **CMV (–)C**
  (CMV Negative Control)
- **CMV H(+)C Clip**
  (CMV High Positive Control Barcode Clip)
- **CMV L(+)C Clip**
  (CMV Low Positive Control Barcode Clip)

- **CMV (–)C Clip**
  (CMV Negative Control Barcode Clip)

B. **COBAS® AmpliPrep/COBAS® TaqMan® Wash Reagent** **PG WR**
(P/N: 03587797 190)

MATERIALS REQUIRED BUT NOT PROVIDED

A. Instrumentation and Software

- COBAS® AmpliPrep Instrument
- COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer
- Optional: Docking Station
- Optional: cobas p 630 Instrument
- AMPLILINK Software, Version 3.3 Series
- Data Station for the AMPLILINK software, with printer.
- AMPLILINK Software v3.3 Series Manuals:
  - COBAS® AmpliPrep Instrument Instrument Manual For use with the COBAS® TaqMan® Analyzer, COBAS® TaqMan® 48 Analyzer, COBAS® AMPLICOR® Analyzer, or cobas p 630 Instrument, and the AMPLILINK software, Version 3.2 and 3.3 Series
  - COBAS® TaqMan® analyzer (plus optional docking station) Instrument Manual For use with the AMPLILINK software, Version 3.2 and 3.3 Series Application Manual
  - COBAS® TaqMan® 48 analyzer Instrument Manual For use with the AMPLILINK software, Version 3.2 and 3.3 series Application Manual
  - AMPLILINK software Version 3.3 Series Application Manual Version 1.0 For use with COBAS® AmpliPrep instrument, COBAS® TaqMan® analyzer, COBAS® TaqMan® 48 analyzer, COBAS® AMPLICOR® analyzer, and cobas p 630 instrument
  - Optional: cobas p 630 instrument Operator's Manual Software Version 2.2

B. Disposables

- Sample processing units (SPUs)
- Sample input tubes (S-tubes) with barcode clips
- Racks of K-tips
- Racks of K-tubes

OTHER MATERIALS REQUIRED BUT NOT PROVIDED

- Sample Rack (SK-24 rack)
- Reagent Rack
- SPU Rack
- K-carrier
- K-carrier Transporter
- K-carrier Rack (required for use with COBAS® TaqMan® 48 Analyzer only)
- Pipettors with aerosol barrier or positive displacement DNase-free tips (capacity 1000 µL)*
- Disposable gloves, powderless
- Vortex mixer

** Pipettors should be accurate within 3% of stated volume. Aerosol barrier or positive displacement DNase-free tips must be used where specified to prevent specimen and amplicon cross-contamination.*

SPECIMEN COLLECTION, TRANSPORT AND STORAGE

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

Note: ***This test has been validated for use only with human plasma collected in EDTA anticoagulant. Testing of other specimen types may result in inaccurate results.***

A. Specimen Collection

The COBAS® AmpliPrep/COBAS® TaqMan® CMV Test is for use with plasma specimens. Blood should be collected in sterile tubes using EDTA as the anticoagulant (lavender-top tubes) and mixed adequately according to the tube manufacturer's instructions.

B. Specimen Transport

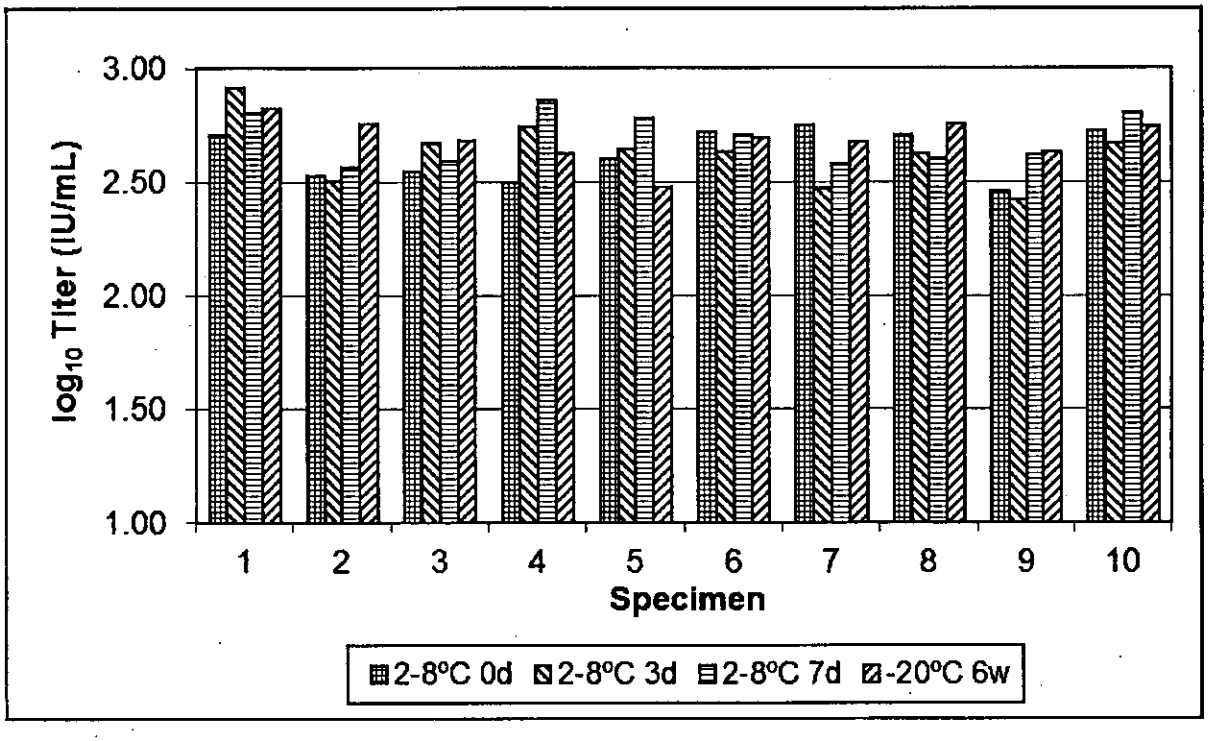
Store whole blood at 2°C to 25°C for no longer than 6 hours. Separate plasma from whole blood within 6 hours of collection by centrifugation at 800-1600 x g for 20 minutes at room temperature. Transfer plasma to a sterile polypropylene tube.

Transportation of whole blood or plasma must comply with country, federal, state, and local regulations for the transport of etiologic agents.[23] Whole blood must be transported at 2°C to 25°C and centrifuged within 6 hours of collection. Plasma must be transported at 2°C to 8°C or frozen at ≤-20°C.

C. Specimen Storage

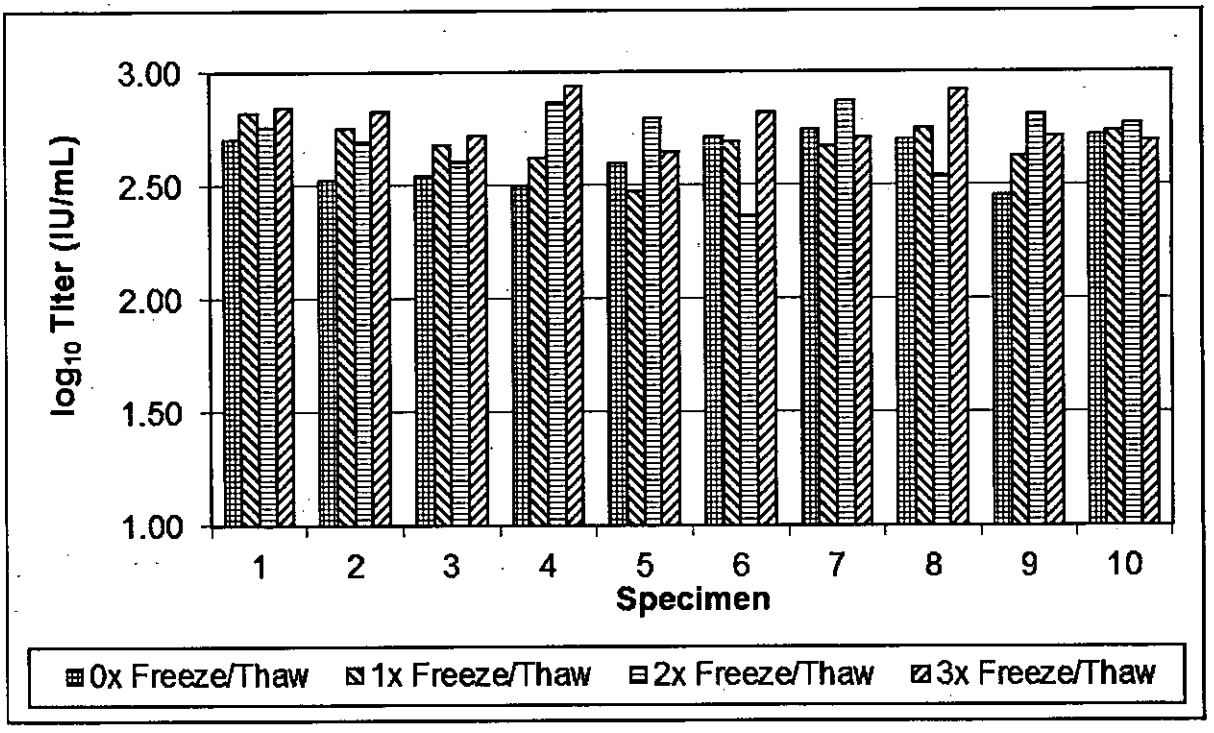
Plasma specimens may be stored at 2°C to 8°C for up to 7 days. Plasma specimens were shown to be stable for up to 6 weeks if frozen at ≤-20°C. It is recommended that specimens be stored in 550 µL to 600 µL aliquots in sterile, 2.0 mL polypropylene screw-cap tubes (such as Sarstedt 72.694.006). Figure 1 shows the specimen stability data from specimen storage studies performed with the COBAS® AmpliPrep/COBAS® TaqMan® CMV Test. The observed $\log_{10}$ differences in titer across the storage conditions ranged from -0.28 to 0.36.

Figure 1
CMV Stability in EDTA Plasma



Plasma specimens may be frozen and thawed up to 3 times without a significant loss of CMV DNA. Figure 2 shows the data from a freeze-thaw study performed with the COBAS® AmpliPrep/COBAS® TaqMan® CMV Test. The observed $\log_{10}$ differences in titer across the freeze/thaw conditions ranged from -0.33 to 0.32.

Figure 2
CMV Results After up to 3 Freeze-Thaw (F-T) Cycles (EDTA plasma)



INSTRUCTIONS FOR USE

Note: ***Due to inherent differences among technologies and patient populations, the user should perform method comparison with their own quantitative CMV test currently used in their clinical practice before switching to the COBAS® AmpliPrep/COBAS® TaqMan® CMV Test.***

Note: ***For detailed operating instructions, a detailed description of the possible configurations, printing results, and interpretation of flags, comments, and error messages, refer to (1) the COBAS® AmpliPrep Instrument Instrument Manual For Use with the COBAS® TaqMan® Analyzer, COBAS® TaqMan® 48 Analyzer, COBAS® AMPLICOR® Analyzer, or cobas p 630 Instrument, and the AMPLILINK software, version 3.3 series; (2) the COBAS® TaqMan® analyzer (plus optional docking station) Instrument Manual For Use with the AMPLILINK software, Version 3.2 and 3.3 Series Application Manual; (3) the COBAS® TaqMan® 48 analyzer Instrument Manual For Use with the AMPLILINK software, Version 3.2 and 3.3 Series Application Manual; (4) the AMPLILINK Software Version 3.3 Series Application Manual Version 1.0 For Use with the COBAS® AmpliPrep instrument, COBAS® TaqMan® analyzer, and COBAS® TaqMan® 48 analyzer, COBAS® AMPLICOR® analyzer, and cobas p 630 instrument; (5) Optional: cobas p 630 instrument Operator's Manual Software Version 2.2.***

Note: ***Use pipettors with aerosol barrier or positive displacement tips where specified. Use extreme care to avoid contamination.***

Batch Size

Each kit contains reagents sufficient for 72 tests, which may be performed in batches of 12 to 24 tests. At least one replicate each of the **CMV (–)C**, **CMV L(+)C**, and **CMV H(+)C** must be included in each batch (see *Quality Control* section).

Workflow

The COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer run must be started within 120 minutes of completion of specimen and control preparation. The instrument will track this time and report invalid results when the transfer time exceeds 120 minutes.

Note: ***Do not freeze or store processed specimens and controls at 2°C to 8°C.***

Specimen and Control Preparation

Note: ***If using frozen specimens, place the specimens at room temperature until completely thawed and vortex for 3 to 5 seconds before use. Controls should be removed from 2°C to 8°C storage and brought to room temperature before use.***

COBAS® AmpliPrep Instrument Setup

Part A. Maintenance and Priming

A1. The COBAS® AmpliPrep Instrument is ready for operation in stand-by mode.

A2. Turn the Data Station for the AMPLILINK software **ON**. Prepare the Data Station as follows:

a. Log onto the Windows® XP operating system.

b. Double-click the AMPLILINK software icon.

c. Log onto the AMPLILINK software by entering the assigned **User ID** and **Password**.

A3. Check the supply of **PG WR** using the **Status** screen and replace if necessary.

A4. Perform all maintenance listed in the **Due** tab. The COBAS® AmpliPrep Instrument will automatically prime the system.

Part B. Loading of Reagent Cassettes

Note: ***All reagent cassettes should be removed from 2°C to 8°C storage, immediately loaded onto the COBAS® AmpliPrep Instrument, and allowed to equilibrate to ambient temperature on the instrument for at least 30 minutes before the first specimen is processed. Do not let reagent cassettes come to ambient temperature outside the instrument as condensation may form on the barcode labels. Do not wipe off condensation if it appears on the barcode labels.***

B1. Place **CMV CS1** onto a reagent rack. Place **CMV CS2**, **CMV CS3**, and **CMV CS4** onto a separate reagent rack.

B2. Load the reagent rack containing **CMV CS1** onto rack position **A** of the COBAS® AmpliPrep Instrument.

B3. Load the reagent rack containing **CMV CS2, CMV CS3**, and **CMV CS4** onto rack position **B**, **C**, **D**, or **E** of the COBAS® AmpliPrep Instrument. See Table 1 for additional information.

Part C. Loading of Disposables

Note: ***Determine the number of COBAS® AmpliPrep reagent cassettes, Sample Processing Units (SPUs), Input Sample tubes (S-tubes), K-tips, and K-tubes needed. One SPU, one Input S-tube, one K-tip, and one K-tube are needed for each specimen or control.***

Multiple workflows for use of the COBAS® AmpliPrep Instrument with the COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer are possible. For reference, see Table 1. Depending on which workflow will be used, load the appropriate number of reagent cassette racks, sample racks with Input S-tubes, SPU-racks, K-tip racks, K-tube racks, and K-carriers on K-carrier racks onto the respective rack positions of the COBAS® AmpliPrep Instrument (see Table 1 for additional information).

C1. Place the SPUs in the SPU rack(s) and load the rack(s) onto rack position **J**, **K**, or **L** of the COBAS® AmpliPrep Instrument.

C2. Depending on which workflow will be used, load full K-tube rack(s) onto rack position **M**, **N**, **O**, or **P** of the COBAS® AmpliPrep Instrument.

C3. Load full K-tip rack(s) onto rack position **M**, **N**, **O**, or **P** of the COBAS® AmpliPrep Instrument.

C4. For Workflow 3 (see Table 1) using the COBAS® TaqMan® 48 Analyzer, load K-carriers on K-carrier rack(s) onto rack position **M**, **N**, **O**, or **P** of the COBAS® AmpliPrep Instrument.

Table 1
Possible Workflows for using the COBAS® AmpliPrep Instrument with the COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer

Workflow		Transfer Mode to COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer	Racks, Carriers, and Disposables	Position on COBAS® AmpliPrep Instrument
1	COBAS® AmpliPrep Instrument plus Docking Station plus COBAS® TaqMan® Analyzer	Automated transfer of K-carrier	K-tubes in full K-tube racks	M – P
			K-tips in full K-tip racks	M – P
			Input S-tubes containing specimens and controls on sample racks	F – H
			SPUs in SPU racks	J – L
			CS1 on Cassette rack	A
			CS2, CS3, CS4 on Cassette rack	B – E
2	COBAS® AmpliPrep Instrument plus COBAS® TaqMan® Analyzer	Manual transfer of K-tubes via sample rack(s) onto COBAS® TaqMan® Analyzer	K-tubes in full K-tube racks	M – P
			K-tips in full K-tip racks	M – P
			Input S-tubes containing specimens and controls on sample racks	F – H
			SPUs in SPU-racks	J – L
			CS1 on Cassette rack	A
			CS2, CS3, CS4 on Cassette rack	B – E
			After specimen processing is finished: K-tubes on sample racks (ready for manual transfer)	F – H
3	COBAS® AmpliPrep Instrument plus COBAS® TaqMan® 48 Analyzer(s)	Manual transfer of K-carrier via K-carrier rack(s) onto COBAS® TaqMan® 48 Analyzer	K-tubes on sample racks	F - H
			K-tips in full K-tip racks	M - P
			Input S-tubes containing specimens and controls on sample racks	F - H
			SPUs in SPU-racks	J - L
			CS1 on Cassette rack	A
			CS2, CS3, CS4 on Cassette rack	B - E
			Empty barcoded K-carrier on K-carrier rack	M - P
			After specimen processing is finished: K-tubes in K-carrier on K-carrier rack	M – P

Part D. Ordering and Loading of Specimens

Note: ***If using the cobas p 630 Instrument for preparation of specimens, refer to the cobas p 630 Instrument Operator's Manual. Vortex all control vials [CMV (–)C, CMV L(+)C, and CMV H(+)C] for 3 to 5 seconds prior to placing the vials onto the cobas p 630 Instrument.***

D1. Prepare sample racks as follows: attach a barcode label clip to each sample rack position where a specimen (S-tube) is to be placed. Attach one of the specific barcode label clips for the controls [**CMV (–)C**, **CMV L(+)C**, and **CMV H(+)C**] to each sample rack position where the controls (S-tube) are to be placed. The barcode label clips for controls should have the same control lot number as the lot number on the control vials in the kit. Take care in assigning the right control to the position with the appropriate control barcode clip. Place one Input S-tube into each position containing a barcode label clip.

D2. Using the AMPLILINK software, create specimen orders for each specimen and control in the **Orders** window **Sample** folder. Select the appropriate test file and complete by saving.

D3. Assign specimen and control orders to sample rack positions in the **Orders** window **Sample Rack** folder. The sample rack number must be for the rack prepared in Step D1.

D4. Print the **Sample Rack Order** report to use as a worksheet.

D5. Prepare specimen and control racks in the designated area for specimen and control addition as follows: Vortex each specimen and control [**CMV (–) C**, **CMV L(+)C**, and **CMV H(+)C**] for 3 to 5 seconds. Avoid contaminating gloves when manipulating the specimens and controls.

D6. Transfer 500 µL of each specimen and control [**CMV (–)C, CMV L(+)C**, and **CMV H(+)C**] to the appropriate barcode labeled Input S-tube, using a micropipettor with an aerosol barrier or positive displacement DNase-free tip. ***Avoid transferring particulates and/or fibrin clots from the original specimen to the Input S-tube***. Specimens and controls should be transferred to tube positions as assigned and recorded on the worksheet in Step D4. The barcode label clips for controls should have the same control lot number as the lot number on the control vials in the kit. Assign the right control to the position with the appropriate control barcode clip. ***Avoid contaminating the upper part of the S-tubes with specimens or controls.***

D7. For Workflows 1 and 2 (see Table 1), load the sample rack(s) filled with Input S-tubes onto rack positions **F**, **G**, or **H** of the COBAS® AmpliPrep Instrument.

D8. For Workflow 3 (see Table 1) using the COBAS® TaqMan® 48 Analyzer, load sample rack(s) with Input S-tubes and K-tubes (one for each Input S-tube, loaded in the right position adjacent to Input S-tubes) onto rack position **F**, **G**, or **H** of the COBAS® AmpliPrep Instrument.

Part E. Start of COBAS® AmpliPrep Instrument Run

E1. Start the COBAS® AmpliPrep Instrument using the AMPLILINK software.

Part F. End of COBAS® AmpliPrep Instrument Run and Transfer to COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer (for Workflow 2 and 3 only)

F1. Check for flags or error messages.

F2. Remove processed specimens and controls from the COBAS® AmpliPrep Instrument on either sample racks (for COBAS® TaqMan® Analyzer without Docking Station) or K-carrier racks (for COBAS® TaqMan® 48 Analyzer), depending on the workflow (for further details see Part G).

F3. Remove waste from the COBAS® AmpliPrep Instrument.

Note: ***Processed specimens and controls should not be exposed to light after completion of specimen and control preparation.***

Amplification and Detection

COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer Setup

The COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer run must be started within 120 minutes following completion of specimen and control preparation. The instrument will track this time and report invalid results when the transfer time exceeds 120 minutes.

Note: ***Do not freeze or store processed specimens and controls at 2°C to 8°C.***

Part G. Loading Processed Specimens

G1. Depending on which workflow will be used (see Table 1), perform the appropriate steps to transfer the K-tubes to the COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer according to the following options:

Workflow 1: Automated transfer of K-carrier via docking station to COBAS® TaqMan® Analyzer. Manual intervention is unnecessary.

Workflow 2: Manual transfer of K-tubes in sample rack(s) to COBAS® TaqMan® Analyzer

Workflow 3: Manual transfer of K-carrier on K-carrier rack(s) to the COBAS® TaqMan® 48 Analyzer. Manual transfer of K-carriers into COBAS® TaqMan® 48 Analyzer using the K-carrier Transporter.

Part H. Start of COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer Run

H1. Depending on which workflow will be used, start the COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer according to one of the following options:

Workflow 1: No intervention necessary.

Workflow 2: Automatic start of the COBAS® TaqMan® Analyzer after insertion of sample rack(s).

Workflow 3: Fill K-carrier with empty K-tubes if there are fewer than 6 K-tubes on the K-carrier. Filling is guided by the AMPLILINK software. Open thermal cycler cover, load K-carrier into thermal cycler and close lid. Start the COBAS® TaqMan® 48 Analyzer run.

Part I. End of COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer Run

I1. At the completion of the COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer run, print the **Results Report**, and check for flags or error messages. Specimens that yield flags and comments are interpreted as described in the Results section below. After acceptance of the Results Report, store data in archive.

I2. Remove used K-tubes from the COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer.

RESULTS

The COBAS® TaqMan® Analyzer or the COBAS® TaqMan® 48 Analyzer automatically determines the CMV DNA concentration for the specimens and controls. The CMV DNA concentration is expressed in International Units (IU) /mL. The conversion factor between CMV DNA copies/mL (as defined by the COBAS® AmpliPrep/COBAS® TaqMan® CMV Test) and International Units (IU)/mL is 1.1 copies/IU [0.91 IU/copy], using the 1st WHO International Standard for Human Cytomegalovirus (HCMV) for Nucleic Acid Amplification (NAT)-based Assays (NIBSC 09/162).[24] **The conversion factor between CMV DNA "copy" and "IU" may be different for other CMV DNA assays.** If needed, results can be converted manually to CMV DNA copies/mL (as defined by the COBAS® AmpliPrep/COBAS® TaqMan® CMV Test) using the following conversion calculation:

CMV DNA concentration in IU/mL x 1.1 copies/IU = CMV DNA in copies/mL

Example: 1.23E+04 IU/mL x 1.1 copies/IU = 1.35E+04 copies/mL

Note: ***The analytical measurement range of analyte values that can be directly measured for a specimen without any dilution using the COBAS® AmpliPrep/COBAS® TaqMan® CMV Test is 1.37E+02 to 9.10E+06 IU/mL.***

AMPLILINK Software:

- Determines the critical threshold (Ct) value for the CMV DNA and the CMV QS DNA.
- Determines the CMV DNA concentration based upon the Ct values for the CMV DNA and CMV QS DNA and the lot-specific calibration coefficients provided on the cassette barcodes.
- Determines that the calculated IU/mL for **CMV L(+)C** and **CMV H(+)C** fall within the fixed ranges.

Batch Validation – AMPLILINK Software Version 3.3 Series

Check the AMPLILINK software results window or printout for flags and comments to ensure that the batch is valid. For control orders, a check is made to determine if the IU/mL value for the control is within its fixed range. If the IU/mL value for the control lies outside of its range, a FLAG is generated to show the control has failed.

The batch is valid if no flags appear for any of the controls [**CMV (–)C**, **CMV L(+)C**, and **CMV H(+)C**].

The batch is <u>not</u> valid if any of the following flags appear for the CMV controls:

Negative Control

Flag	Result	Interpretation
NC_INVALID	Invalid	An invalid result or CMV target DNA detected

CMV Low Positive Control

Flag	Result	Interpretation
LPCINVALID	Invalid	An invalid result or a control out of range

CMV High Positive Control

Flag	Result	Interpretation
HPCINVALID	Invalid	An invalid result or a control out of range

If the batch is invalid, repeat the entire batch including specimen and control preparation, amplification, and detection.

Interpretation of Results

For a valid batch, check each individual specimen for flags or comments on the result printout. A valid batch may include both valid and invalid specimen results depending on whether flags and/or comments are obtained for the individual specimens.

Specimen results are interpreted as follows:

Titer Result	Interpretation
Target Not Detected	Report results as "CMV DNA not detected".
<1. 37E+02 IU/mL	Calculated IU/mL are below the Lower Limit of Quantitation of the test. Report results as "CMV DNA detected, less than 137 IU/mL".
≥1.37E+02 IU/mL and ≤9.10E+06 IU/mL	Calculated CMV DNA result greater than or equal to 137 IU/mL and less than or equal to 9.10E+06 IU/mL are within the Linear Range of the test.
>9.10E+06 IU/mL	Calculated IU/mL is above the linear range of the test. Report results as "CMV DNA is greater than 9.10E+06 IU/mL ". If quantitative results are desired, the original specimen should be diluted with CMV-negative human EDTA-plasma and the test should be repeated. Multiply the reported result by the dilution factor.
Failed	Specimen was not correctly processed. Test should be repeated with another aliquot of the original sample.
Invalid	An invalid result. Test should be repeated with another aliquot of the original sample.

Note: ***Specimens above the range of the test that produce an invalid result with a flag "QS_INVALID" should not be reported as > 9.10E+06 IU/mL. The original specimen should be diluted with CMV-negative EDTA plasma and the test should be repeated. The obtained result should be multiplied by the dilution factor.***

Note: ***The analytical measurement range of analyte values that can be directly measured for a specimen with a maximum dilution of one to one hundred using the COBAS® AmpliPrep/COBAS® TaqMan® CMV Test is 1.37E+02 to 9.10E+08 IU/mL.***

QUALITY CONTROL

One replicate each of the COBAS® TaqMan® Negative Control, the CMV Low Positive Control, and the CMV High Positive Control must be included in each test batch. The batch is valid if no flags appear for any of the controls [**CMV (–)C**, **CMV L(+)C**, and **CMV H(+)C**].

Check the run printout for flags and comments to ensure that the batch is valid.

Negative Control

The **CMV (–)C** must yield a "Target Not Detected" result. If the **CMV (–)C** is flagged as invalid, then the entire batch is invalid. Repeat the entire process (specimen and control preparation, amplification and detection). If **CMV (–)C** is consistently invalid in multiple batches, contact the Roche Response Center for technical assistance.

Positive Controls

The acceptable titer ranges for **CMV L(+)C** and **CMV H(+)C** are provided on the COBAS® AmpliPrep/COBAS® TaqMan® CMV Test reagent cassette barcodes.

The CMV DNA IU/mL for **CMV L(+)C** and **CMV H(+)C** should fall within their acceptable titer ranges. If one or both of the positive controls are flagged as invalid, then the entire batch is invalid. Repeat the entire process (specimen and control preparation, amplification and detection). If the CMV DNA titer of one or both of the positive controls is consistently outside the acceptable ranges in multiple batches, contact the Roche Response Center for technical assistance.

PROCEDURAL PRECAUTIONS

As with any test procedure, good laboratory technique is essential to the proper performance of this test.

LIMITATIONS OF THE PROCEDURE

1. This test has been validated for use only with human plasma collected in EDTA anticoagulant. Testing of other specimen types may result in inaccurate results.
2. Test performance characteristics have been evaluated only for individuals who have undergone kidney transplantation, have been diagnosed with CMV disease and are undergoing purine analogue (guanine) anti-CMV therapy. No information is available on test performance in patients undergoing other types of transplant procedures, neonates or pediatric patients, or AIDS or other immunocompromised patients; nor is information available on test performance in patients who have been diagnosed with CMV disease and are undergoing other therapies, or have life-threatening CMV disease.
3. This test is intended for use as an aid in the management of solid-organ transplant patients who have been diagnosed with CMV disease and are undergoing antiviral therapy. In this population, the test can be used to predict virological response to treatment by measuring the baseline CMV DNA level and to assess the effects of antiviral therapy by measuring CMV DNA during the course of antiviral treatment. Patient management decisions should not be made based solely on the results from this test. Other laboratory and clinical factors must also be considered in making clinical decisions.
4. Reliable results are dependent on adequate specimen collection, transport, storage, and processing procedures.
5. The presence of AmpErase enzyme in the COBAS® AmpliPrep/COBAS® TaqMan® CMV Test Master Mix reduces the risk of amplicon contamination. However, contamination from CMV-positive controls and clinical specimens can be avoided only by good laboratory practices and careful adherence to the procedures specified in this package insert.
6. Use of this product should be limited to personnel trained in the techniques of PCR.
7. This product can only be used with the COBAS® AmpliPrep Instrument and the COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer.
8. Though rare, mutations within the highly conserved regions of the viral genome covered by the COBAS® AmpliPrep/COBAS® TaqMan® CMV Test primers and/or probes may result in the under-quantitation of or failure to detect the virus.
9. Detection of CMV DNA is dependent on the number of virus particles present in the specimen and may be affected by specimen collection methods and patient factors, (i.e., age, presence of symptoms, and/or stage of the infection).
10. Testing for analytical reactivity with various currently known antiviral drug resistance CMV strains was limited. Although the targeted DNA sequence for this test is not known to be involved in some anti-CMV drug resistance pathways, the performance of the test may be affected when other resistance pathways are considered or new variants emerge
11. A specimen with a result of "Target Not Detected" cannot be presumed to be negative for CMV DNA.

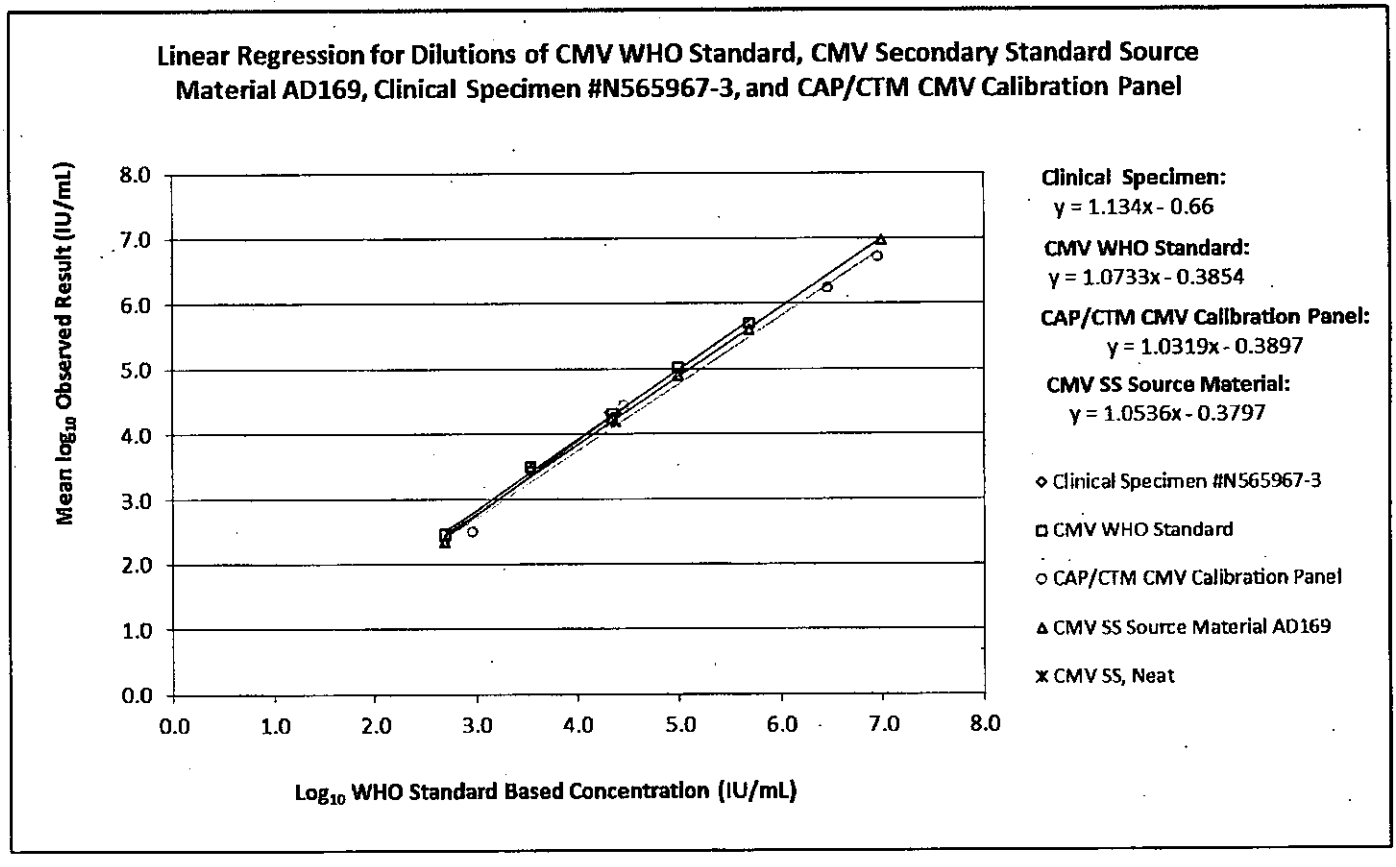
NON-CLINICAL PERFORMANCE EVALUATION

A. Traceability to the 1st WHO International Standard for Human Cytomegalovirus (HCMV) for Nucleic Acid Amplification Techniques (NAT)-based Assays

Several standards and controls have been used during development of this test to provide traceability to the WHO standard [the 1st WHO International Standard for Human Cytomegalovirus (HCMV) for Nucleic Acid Amplification Techniques (NIBSC 09/162).][24] The standards used during development of the test include the CMV WHO Standard, the RMS CMV Secondary Standard, the RMS CMV Secondary Standard Source Material, and the RMS CMV Calibration Panel (Lambda CMA1.2). The Standards, the Calibration Panel, and an independent CMV clinical specimen were tested at similar levels. The concentration range tested for the CMV WHO Standard was from 5.00E+02 IU/mL to 5.00E+05 IU/mL (2.70 – 5.70 $\log_{10}$ IU/mL), the RMS CMV Secondary Standard Source Material was tested from 5.00E+02 IU/mL to 1.00E+07 IU/mL (2.70 – 7.00 $\log_{10}$ IU/mL), the RMS CMV Calibration Panel was tested from 5.23E+02 to 9.30E+06 IU/mL (2.72 – 6.97 $\log_{10}$ IU/mL), and the independent CMV clinical specimen was tested from 5.00E+02 IU/mL to 2.27E+04 IU/mL (2.70 – 4.36 $\log_{10}$ IU/mL).

All materials behaved similarly and demonstrated co-linear dilution performance across the linear range of the COBAS® AmpliPrep/COBAS® TaqMan® CMV Test (CAP/CTM CMV) (Figure 3). Based on these results, the calibration and standardization process of the COBAS® AmpliPrep/COBAS® TaqMan® CMV Test provides quantitation values for the calibration panel, the clinical sample, the source material for the RMS CMV Secondary Standard, and the CMV WHO Standard that are similar to the expected values with deviation of not more than 0.28 $\log_{10}$ IU/mL. The maximum deviation was obtained at the test LLoQ using the regression analyses for the Calibration Panel and the CMV WHO Standard.

Figure 3
Traceability of the COBAS® AmpliPrep/COBAS® TaqMan® CMV Test to the 1st WHO International Standard for Human Cytomegalovirus



B. Limit of Blank, Limit of Detection, and Lower Limit of Quantitation Using the WHO International Standard for Human Cytomegalovirus

The limit of detection (LoD) and lower limit of quantitation (LLoQ) of the COBAS® AmpliPrep/COBAS® TaqMan® CMV Test were determined according to CLSI guideline EP17-A[25] by analysis of 6 low level CMV DNA positive panels. The 6 independent low level CMV DNA panels were prepared using the 1st WHO International Standard for Human Cytomegalovirus (HCMV) (NIBSC 09/162)[1], which represents glycoprotein B genotype 1, and 6 independent pools of EDTA plasma as diluent. Each panel consisted of 6 CMV DNA concentrations, 4.6E+01, 9.1E+01, 1.37E+02, 1.82E+02, 2.70E+02, and 3.64E+02 IU/mL. In addition, the limit of blank (LoB) was determined by analysis of blank samples from 6 unique pools of CMV DNA negative EDTA plasma, which were also used as the diluent for the low level CMV DNA-positive panels. At least 223 valid results per concentration level were obtained from 75 valid runs over 9 days, which were split across 3 lots of COBAS® AmpliPrep/COBAS® TaqMan® CMV Test kit reagents and 3 COBAS® AmpliPrep/COBAS® TaqMan® systems.

All replicates of blank samples reported "Target Not Detected", for a LoB of 0 IU/mL (see Table 2).

Table 2
Limit of Blank (LoB) of the COBAS® AmpliPrep/COBAS® TaqMan® CMV Test using CMV DNA Negative EDTA Plasma Specimen

Negative EDTA Plasma Panel Number	Number of Positives	Number of Results	% Positive
1	0	39	0%
2	0	36	0%
3	0	36	0%
4	0	36	0%
5	0	36	0%
6	0	42	0%
Total	**0**	**225**	**0%**

The study results demonstrate that although 1 kit lot of the COBAS® AmpliPrep/COBAS® TaqMan® CMV Test can detect CMV DNA in EDTA plasma at a concentration of 4.6E+01 IU/mL, with a positivity rate greater than or equal to 95%, 2 additional kit lots can only detect CMV DNA at a concentration of 9.1E+01 IU/mL more than 95% of the time (see Table 3), Therefore, the results of this study support the claimed LoD of 9.1E+01 IU/mL.

Table 3
Limit of Detection (LoD) of the COBAS® AmpliPrep/COBAS® TaqMan® CMV Test using the WHO International Standard for Human Cytomegalovirus (HCMV) (NIBSC 09/162)

Kit Lot	Nominal Concentration (IU/mL)	N	Total Positive	Hit Rate
Lot 16578B	0	75	0	0%
	46	**75**	**75**	**100%**
	91	75	74	98.7%
	137	75	75	100%
	182	75	75	100%
	273	74	74	100%
	364	75	75	100%

Kit Lot	Nominal Concentration (IU/mL)	N	Total Positive	Hit Rate
Lot 16580B	0	78	0	0%
	46	77	73	94.8%
	91	**78**	**78**	**100.0%**
	137	76	76	100%
	182	77	77	100%
	273	78	78	100%
	364	78	78	100%
Lot P11496	0	72	0	0%
	46	72	68	94.4%
	91	**72**	**72**	**100.0%**
	137	72	72	100%
	182	72	72	100%
	273	72	72	100%
	364	71	71	100%

The LLoQ from this study was 9.1E+01 IU/mL using hit rate analysis with a goal for acceptable total analytical error (TAE) of ≤ 1.0 $\log_{10}$, where TAE = |bias| + 2 standard deviations in alignment with the CLSI EP-17A guideline, and TAE = SQUARE ROOT(2) x 2 standard deviations based on the "difference between 2 measurements" approach (see Table 4). Therefore, the results of this study support the claimed LLoQ of 1.37E+02 IU/mL.

Table 4
Lower Limit of Quantitation (LLoQ) of the COBAS® AmpliPrep/COBAS® TaqMan® CMV Test using the WHO International Standard for Human Cytomegalovirus (HCMV) (NIBSC 09/162)

Kit Lot	Nominal CMV Concentration (IU/mL)	$\log_{10}$ Nominal (IU/mL)	N	AVG $\log_{10}$ Titer (IU/mL)	SD $\log_{10}$ Titer (IU/mL)	Bias	TAE = |Bias| + 2 x SD	TAE = SQRT(2) x 2 x SD
All 3 Lots combined	46	1.66	224	1.71	0.37	0.05	0.79	1.05
	91	**1.96**	**225**	**1.99**	**0.31**	**0.04**	**0.66**	**0.88**
	137	2.14	223	2.20	0.29	0.06	0.64	0.82
	182	2.26	224	2.34	0.27	0.08	0.62	0.76
	273	2.44	224	2.55	0.26	0.11	0.63	0.74
	364	2.56	224	2.60	0.27	0.04	0.58	0.76
16578B	46	1.66	75	1.74	0.39	0.09	0.87	1.10
	91	**1.96**	**75**	**2.02**	**0.32**	**0.06**	**0.70**	**0.90**
	137	2.14	75	2.23	0.26	0.10	0.62	0.74
	182	2.26	75	2.37	0.24	0.11	0.59	0.68
	273	2.44	74	2.58	0.25	0.14	0.64	0.71
	364	2.56	75	2.63	0.29	0.07	0.65	0.82

Kit Lot	Nominal CMV Concentration (IU/mL)	$\log_{10}$ Nominal (IU/mL)	N	AVG $\log_{10}$ Titer (IU/mL)	SD $\log_{10}$ Titer (IU/mL)	Bias	TAE = |Bias| + 2 x SD	TAE = SQRT(2) x 2 x SD
16580B	**46**	**1.66**	**77**	**1.63**	**0.34**	**-0.03**	**0.71**	**0.96**
	91	1.96	78	1.92	0.33	-0.03	0.69	0.93
	137	2.14	76	2.13	0.33	0.00	0.66	0.93
	182	2.26	77	2.28	0.29	0.02	0.60	0.82
	273	2.44	78	2.49	0.25	0.05	0.55	0.71
	364	2.56	78	2.55	0.27	-0.01	0.55	0.76
P11496	46	1.66	72	1.75	0.36	0.09	0.81	1.02
	91	**1.96**	**72**	**2.05**	**0.28**	**0.09**	**0.65**	**0.79**
	137	2.14	72	2.23	0.27	0.09	0.63	0.76
	182	2.26	72	2.37	0.25	0.11	0.61	0.71
	273	2.44	72	2.58	0.28	0.14	0.70	0.79
	364	2.56	71	2.64	-0.24	0.08	0.56	0.68

C. LoD and LLoQ Using CMV Glycoprotein B (gB) Genotypes 2-4 Specimens

The LoD and LLoQ of the COBAS® AmpliPrep/COBAS® TaqMan® CMV Test for glycoprotein B (gB) genotypes 2-4 was evaluated by testing at least 49 replicates at 1.37E+02 IU/mL, 9.1E+01 IU/mL, and 2.7E+01 IU/mL for each CMV gB genotype 2-4 clinical specimen. The study was conducted with 25 valid runs tested across 6 days using 2 lots of COBAS® AmpliPrep/COBAS® TaqMan® CMV Test kit reagents and 2 CAP/CTM instrument systems.

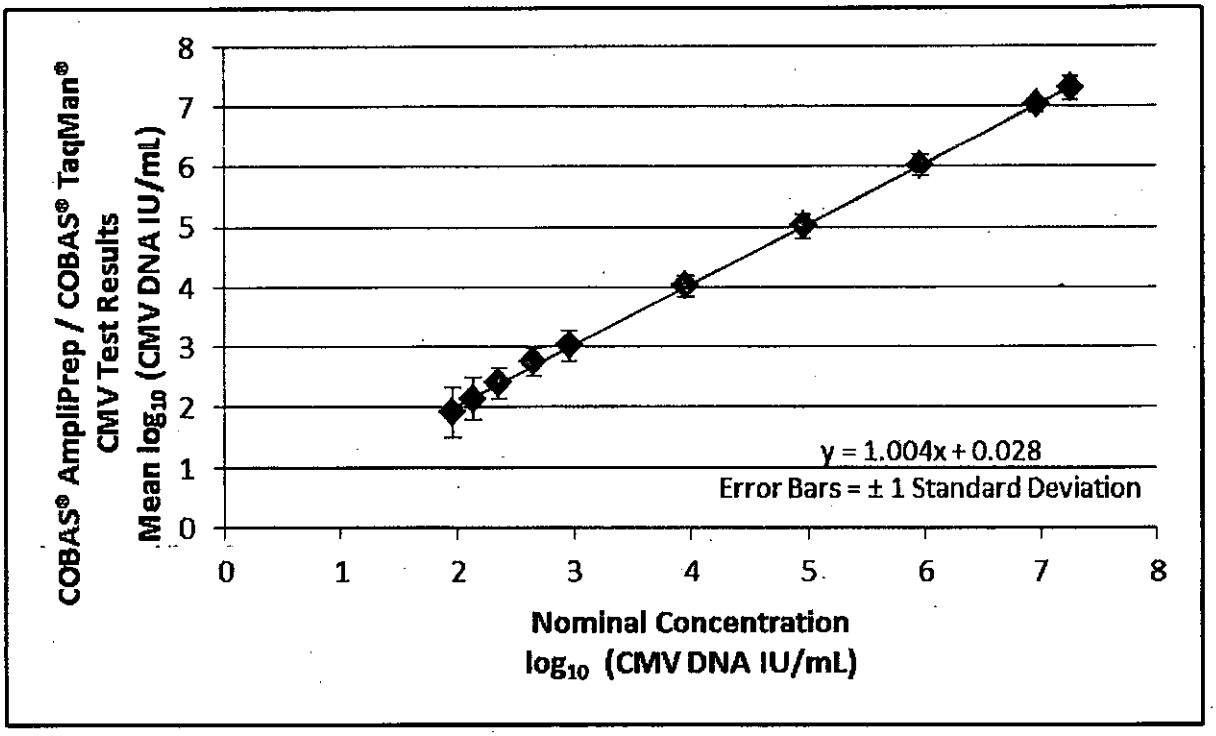
The study results demonstrate that for all 3 gB genotypes (gB genotypes 2-4), the COBAS® AmpliPrep/COBAS® TaqMan® CMV Test can detect CMV DNA in EDTA plasma at a concentration as low as 9.1E+01 IU/mL, with a positivity rate greater than or equal to 95%. At 9.1E+01 IU/mL CMV DNA concentration, a goal for acceptable total analytical error (TAE) of ≤ 1.0 $\log_{10}$, where TAE = |bias| + 2 standard deviations per CLSI EP-17A guideline[25], and TAE = SQUARE ROOT(2) x 2 standard deviations based on the "difference between two measurements" approach, is achieved for each of the 3 gB genotypes 2-4. Therefore, the results of this study support the claimed LLoQ of 1.37E+02 IU/mL.

D. Linear Range Using Cultured CMV AD169 Virus

A 10-member panel was used to evaluate the linear range of the COBAS® AmpliPrep/COBAS® TaqMan® CMV Test in accordance with CLSI Guideline EP06-A.[26] The panel was prepared by diluting cultured CMV virus (strain AD169, glycoprotein B genotype 2) using a pool of EDTA plasma as the diluent. Each panel member was tested with 2 replicates per run, with 3 runs per day, for a minimum of 6 days across 3 lots of kit reagents and 3 CAP/CTM systems. The linear range was evaluated using a minimum of 37 replicates per panel member.

A linear regression of the mean $\log_{10}$ observed titer versus the nominal $\log_{10}$ titer is presented below in Figure 4. The COBAS® AmpliPrep/COBAS® TaqMan® CMV Test was found to give a linear response from 9.1E+01 ($\log_{10}$ = 1.96) CMV DNA IU/mL to at least 9.1E+06 ($\log_{10}$ = 6.96) CMV DNA IU/mL, with maximum deviation between the observed mean $\log_{10}$ titer and the best fitted 1st-order model (i.e., deviation from linearity) of less than 0.08 $\log_{10}$ for each concentration level tested in this interval. Therefore, the results of this study support the claimed LLoQ of 1.37E+02 IU/mL.

Figure 4
Linearity of the COBAS® AmpliPrep/COBAS® TaqMan® CMV Test using CMV AD169 Virus



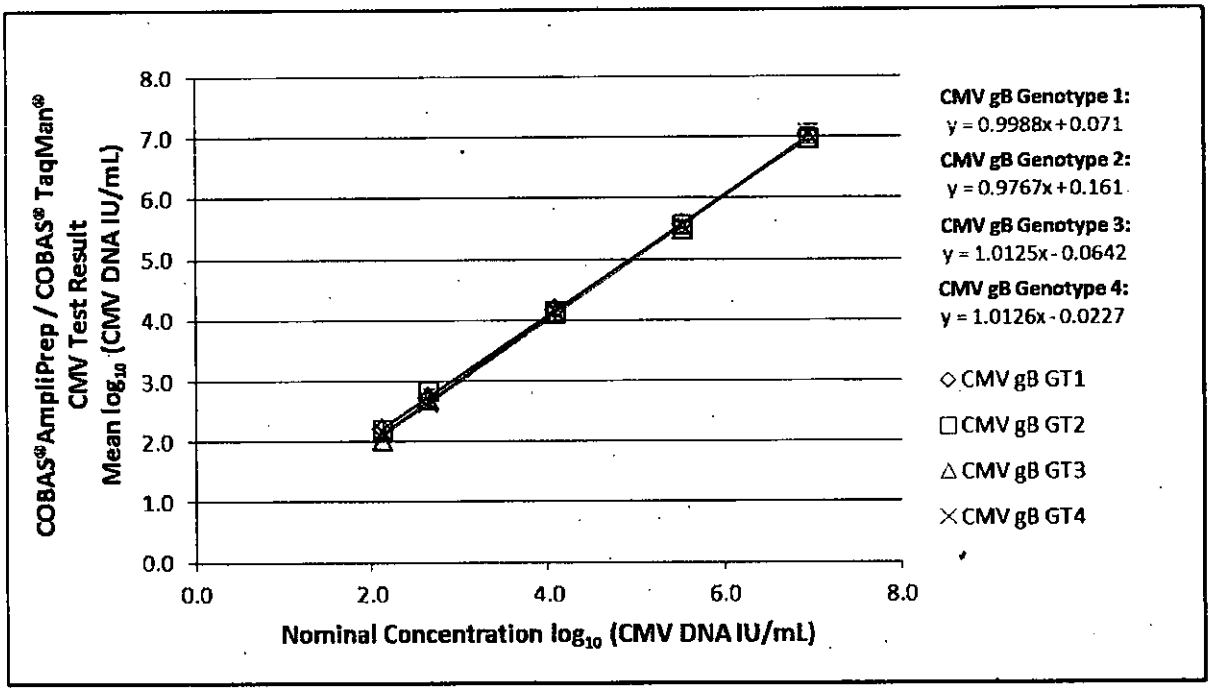
E. Linear Range Using CMV Glycoprotein B (gB) Genotypes 1-4 Specimens

The linear range of the COBAS® AmpliPrep/COBAS® TaqMan® CMV Test was verified by testing the target region of the CMV genome (UL54) from 4 different glycoprotein B (gB) genotype samples (gB 1, 2, 3, and 4) and completed in accordance with CLSI guideline EP6-A.[28] Each of the 4 gB genotype samples (full-length UL54 gene plasmid clones) was tested in replicates of 16 at 9.10E+06, 3.35E+05, 1.24E+04, 4.55E+02, and 1.37E+02 IU/mL.

The COBAS® AmpliPrep/COBAS® TaqMan® CMV Test was found to give a linear response from 1.37E+02 IU/mL (log_{10} = 2.14) CMV DNA to at least 9.1E+06 IU/mL (log_{10} = 6.96) CMV DNA, with maximum deviation between the observed mean log_{10} titer and the best fitted 1st-order model (i.e., deviation from linearity) of less than or equal to 0.08 log_{10} for each gB genotype and each concentration level tested in this interval.

A linear regression of the mean log_{10} observed titer versus the nominal log_{10} titer is presented in Figure 5.

Figure 5
Linearity of the COBAS® AmpliPrep/COBAS® TaqMan® CMV Test using Glycoprotein B (gB) Genotype 1-4 Specimens



Results from this study also demonstrate that the COBAS® AmpliPrep/COBAS® TaqMan® CMV Test is able to quantitate different CMV gB genotypes across the linear range with deviation of not more than 0.11 $\log_{10}$ IU/mL. These analysis results are summarized in Table 5.

Table 5
COBAS® AmpliPrep/COBAS® TaqMan® CMV Test Linearity using CMV Glycoprotein B (gB) Genotypes

CMV gB Genotype	Linear Equation in gB Genotype Linearity Study	Maximum Difference Between gB1 and Corresponding gB Genotype ($\log_{10}$ IU/mL)
1	y = 0.9988x + 0.071	n/a
2	y = 0.9767x + 0.161	0.06
3	y = 1.0125x - 0.0642	0.11
4	y = 1.0126x -0.0227	0.06

* The maximum difference was obtained at the ULoQ or LLoQ of the test.

F. Precision

The precision of the COBAS® AmpliPrep/COBAS® TaqMan® CMV Test was determined according to CLSI guideline EP5-A2[27] by analysis of an 8-member panel. The panel was prepared using a CMV DNA-positive specimen for the lower end of the dynamic range and by diluting cultured CMV (strain AD169) for the mid and high end of the dynamic range. Both source materials were diluted in CMV-negative EDTA plasma. The 8-member panel covered a range from 1.82E+02 CMV DNA IU/mL to 9.10E+06 CMV DNA IU/mL. Each panel member was tested with 2 replicates per run, with 2 runs per day, for 12 days, and for each of 2 system configurations (COBAS® AmpliPrep docked to a COBAS® TaqMan® and COBAS® AmpliPrep linked to a COBAS® TaqMan® 48) for a total 96 replicates per panel member, with replicates evenly distributed across 3 kit lots, 4 COBAS® AmpliPrep/COBAS® TaqMan® systems, and at least 2 operators.

Each sample was carried through the entire COBAS® AmpliPrep/COBAS® TaqMan® CMV Test procedure, including specimen preparation, amplification, and detection. Therefore, the precision reported here represents all aspects of the test procedure. The results of this Precision Study are shown in Table 6 and Table 7.

Table 6
Precision of the COBAS® AmpliPrep/COBAS® TaqMan® CMV Test (EDTA-Plasma in IU/mL)

Level	Average Observed CMV DNA Titer (IU/mL)	N	Within-Run %CV	Between-Run / Operator %CV*	Between-Lot %CV	Between-Workflow %CV	Between Instrument %CV	Between Day %CV	Total %CV
1	8.29E+01	96	50%	0%	18%	0%	6%	0%	55%
2	1.66E+02	96	33%	12%	14%	0%	14%	0%	41%
3	2.83E+02	96	54%	0%	0%	0%	15%	18%	60%
4	5.20E+02	96	19%	16%	14%	3%	12%	0%	32%
5	1.49E+04	96	29%	10%	4%	3%	3%	0%	31%
6	8.00E+04	96	17%	13%	10%	0%	7%	5%	25%
7	8.05E+05	96	15%	0%	11%	0%	12%	6%	23%
8	7.62E+06	96	20%	0%	18%	3%	15%	7%	32%

* Between-Run is confounded with Between-Operator and therefore, is presented as Between Run/Operator.

Table 7
Precision of the COBAS® AmpliPrep/COBAS® TaqMan® CMV Test (EDTA-Plasma in Log $_{10}$ IU/mL)

Level	Average Observed CMV DNA Titer (log_{10} IU/mL)	N	Within-Run SD	Between-Run / Operator* SD	Between-Lot SD	Between-Workflow SD	Between Instrument SD	Between Day SD	Total SD
1	1.92	96	0.21	0.00	0.08	0.00	0.03	0.00	0.22
2	2.22	96	0.14	0.05	0.06	0.00	0.06	0.00	0.17
3	2.45	96	0.22	0.00	0.00	0.00	0.06	0.08	0.24
4	2.72	96	0.08	0.07	0.06	0.01	0.05	0.00	0.14
5	4.17	96	0.12	0.04	0.02	0.01	0.01	0.00	0.13
6	4.90	96	0.07	0.06	0.04	0.00	0.03	0.02	0.11
7	5.91	96	0.07	0.00	0.05	0.00	0.05	0.03	0.10
8	6.88	96	0.08	0.00	0.08	0.01	0.06	0.03	0.13

*Between-Run is confounded with Between-Operator and, therefore, is presented as Between Run/Operator.

G. Performance with CMV DNA-Negative Samples

The performance of the COBAS® AmpliPrep/COBAS® TaqMan® CMV Test with CMV DNA-negative samples was determined by testing 227 anti-CMV IgG seronegative EDTA plasma specimens. The specimens were obtained from an FDA-registered Donor Testing Laboratory, were de-identified, and had been collected from patients under routine diagnostic care.

For CMV IgG seronegative specimens, all 227 specimens tested negative for CMV DNA by the COBAS® AmpliPrep/COBAS® TaqMan® CMV Test, yielding a 100% negativity rate with 95% CI: 98.3% to 100%.

H. Analytical Specificity (Cross-Reactivity)

Various pathogenic organisms were evaluated for cross-reactivity with the COBAS® AmpliPrep/COBAS® TaqMan® CMV Test by adding cultured organisms (viruses, bacteria, fungi) or positive clinical specimens at 1.0E+06 particles/mL input concentration into CMV DNA-negative human EDTA plasma and into CMV DNA-positive EDTA plasma at 6.82E+02 IU/mL CMV. Each sample was tested in triplicate using the COBAS® AmpliPrep/COBAS® TaqMan® CMV Test (see Table 8).

None of the organisms tested showed cross-reactivity with the COBAS® AmpliPrep/COBAS® TaqMan® CMV Test. CMV-positive specimens returned titer results that were within ± 0.3 $\log_{10}$ from a CMV-positive control.

Table 8
Analytical Specificity Specimens

Human Herpesviruses	**Other Viruses**
Herpes simplex virus types 1 and 2	BK Polyomavirus
Varicella-Zoster virus	JC Polyomavirus
Epstein-Barr virus	Hepatitis virus A, B, and C
Human herpesvirus 6, 7, and 8	HIV type 1
	Adenovirus 5
	Parvovirus B19
Bacteria	**Fungi**
Mycoplasma pneumoniae	*Aspergillus niger*
Propionibacterium acnes	*Candida albicans*
Salmonella typhimurium	*Cryptococcus neoformans*
Staphylococcus aureus	
Streptococcus pneumoniae	

I. Interfering Substances

Elevated levels of triglycerides (up to 3300 mg/dL), conjugated bilirubin (up to 20 mg/dL), unconjugated bilirubin (up to 20 mg/dL), hemoglobin (up to 200 mg/dL), human albumin (up to 6,000 mg/dL), and human DNA (up to 0.4 mg/dL) in specimens as well as the presence of autoimmune diseases or respective markers such as Systemic Lupus Erythematosus (SLE), Rheumatoid Arthritis (RA), and Antinuclear Antibody (ANA) did not interfere with the quantitation of CMV DNA nor impact the specificity of the COBAS® AmpliPrep/COBAS® TaqMan® CMV Test. The evaluation was performed according to CLSI guideline EP7-A2[42] using one lot of COBAS® AmpliPrep/COBAS® TaqMan® CMV Test reagents. Potential interference was also evaluated against the commonly used drugs in the transplant patient population. The drug compounds summarized in Table 9 were tested at the test levels recommended in CLSI guideline EP7-A2[28] or at 3 times the Peak Plasma Concentration Level (Cmax; Reference: www.drugs.com), whichever was greater, and did not interfere with the quantitation of CMV DNA nor impact the specificity of the COBAS® AmpliPrep/COBAS® TaqMan® CMV Test.

Table 9
Tested Interfering Substances

Immunosuppressive Drugs	**Antibacterial Drugs**
Azathioprine	Sulfamethoxazole
Cyclosporine	Trimethoprim
Mycophenolate mofetil	Cefotetan
Mycophenolate sodium	Piperacillin
Sirolimus	Tazobactam sodium
Tacrolimus	Clavulanate potassium
Everolimus	Ticarcillin Disodium
Prednisone	Vancomycin
Anti-CMV Drugs	**Anti-Fungal Drugs**
Ganciclovir	Fluconazole
Valganciclovir	
Cidofovir	
Foscarnet	

CLINICAL PERFORMANCE EVALUATION

A. Clinical Reproducibility of the COBAS® AmpliPrep/COBAS® TaqMan® CMV Test

The reproducibility of the COBAS® AmpliPrep/COBAS® TaqMan® CMV Test was evaluated across lot, site/instrument, operator, day, run, and within-run at 3 test sites, each of which was equipped with either the COBAS® AmpliPrep/COBAS® TaqMan® Analyzer system with docked workflow or the COBAS® AmpliPrep/COBAS® TaqMan 48 Analyzer system workflow.

Reproducibility was tested with coded 7-member panels, including a CMV-negative panel member, tested in triplicate. The CMV panel member concentrations covered the linear range of the test, included medical decision points, and were expressed in $\log_{10}$ IU/mL. The CMV-negative panel member was included to assess the reproducibility of negative results and is not summarized in Table 10 and Table 11. Two operators at each of 3 test sites tested 2 runs per day for 3 days for each of 3 reagent lots.

In this reproducibility study, 114 runs were valid out of a total of 122 runs performed. The remaining 8 invalid runs (run invalid rate: 6.5%; 95% CI: 3.4% to 12.4%) were due to instrument error (7 runs) or human error (1 run). Overall, 2,268 tests were performed in valid runs and 1 of 2,268 tests was invalid (invalid rate: 0.04%; 95% CI: 0.008% to 0.25%).

Table 10 summarizes the attributable percentage of total variance and precision standard deviation as determined by the nominal $\log_{10}$ CMV DNA concentration (IU/mL).

Table 10
Attributable Percentage of Total Variance and Precision Standard Deviation (SD) by Nominal $\log_{10}$ CMV DNA Concentration (IU/mL)

CMV DNA Concentration										
$\log_{10}$IU/mL			Contribution to Total Variance (Standard Deviation [SD])						Total Precision	
Nominal	Observed (Average)	No. of Valid Tests	Lot	Site	Oper-ator	Day	Run	Within- Run	SD	Log- normal CV %
2.135	1.924	323*	24% (0.125)	1% (0.027)	0% (0.000)	2% (0.032)	3% (0.042)	71% (0.218)	0.258	65
2.699	2.453	324**	37% (0.109)	7% (0.046)	0% (0.000)	3% (0.033)	1% (0.019)	52% (0.130)	0.180	43
3.260	3.095	324	32% (0.076)	5% (0.030)	0% (0.000)	3% (0.024)	10% (0.043)	50% (0.096)	0.136	32
4.260	4.197	324	3% (0.028)	2% (0.023)	0% (0.000)	8% (0.043)	0% (0.000)	87% (0.145)	0.156	37
4.658	4.605	324	7% (0.033)	5% (0.027)	2% (0.017)	4% (0.023)	2% (0.017)	81% (0.111)	0.123	29
6.658	6.602	324***	2% (0.015)	15% (0.039)	0% (0.000)	2% (0.014)	8% (0.028)	72% (0.084)	0.098	23

Note: Results with detectable viral load are included in this table.

Note: Results <1.37E+2 or >9.10E+6 IU/mL were recalculated based on extrapolation of the calibration curve.

*261 of 323 test results were <1.37E+2 IU/mL and were recalculated based on extrapolation of the calibration curve.

**10 of 324 test results were <1.37E+2 IU/mL and were recalculated based on extrapolation of the calibration curve.

***1 of 324 test results were >9.10E+6 IU/mL and were recalculated based on extrapolation of the calibration curve.

The detectable-fold difference is a clinically informative concept when serially measuring a patient's viral load for statistically significant changes. Variations between measurements that are within the detectable-fold difference could be due to variability in the test's reproducibility. Table 11 summarizes the estimated maximum total variation in standard deviations (SD) and the 95% confidence limits one would theoretically expect for a change between two consecutive CMV DNA determinations in a single patient at various nominal $\log_{10}$ CMV DNA concentrations (IU/mL).

Table 11
Detectable Viral Load Difference by Nominal Log_{10} CMV DNA Concentration (IU/mL)

CMV DNA Concentration (Log_{10}IU/mL)		Result				
Nominal	Mean (Observed)	No. of Tests	Total Precision Standard Deviation (in logs)	Standard Deviation of Difference Between Two Measurements	95% Confidence Limit[1] ($\pm log_{10}$)	Fold Detectable Difference[2]
2.135	1.924	323	0.26	0.36	0.72	5.19
2.699	2.453	324	0.18	0.25	0.50	3.15
3.260	3.095	324	0.14	0.19	0.38	2.38
4.260	4.197	324	0.16	0.22	0.43	2.71
4.658	4.605	324	0.12	0.17	0.34	2.19
6.658	6.602	324	0.10	0.14	0.27	1.87

[1] The 95% confidence limit about the difference between 2 measurements of CMV DNA in the same subject. These measurements do not include within-subject biologic variation and they could be from the same sample tested at different times with different lots, testing sites and/or operators.

[2] The antilog of the 95% confidence limit for the SD of the difference between 2 measurements (eg. 10**0.7151 = 5.20)

Negative agreement of the COBAS® AmpliPrep/COBAS® TaqMan® CMV Test with the negative panel member was 100% (324/324, 95% CI 98.8%-100%) indicating the reproducibility of a negative sample across lot, site/instrument, operator, day, run, and within-run.

B. Clinical Usefulness of the COBAS® AmpliPrep/COBAS® TaqMan® CMV Test

Methods

The clinical usefulness study was a retrospective, longitudinal cohort study of 211 kidney transplant recipients previously diagnosed with CMV disease and treated with anti-CMV drugs (ganciclovir or valganciclovir). The study objective was to assess whether CMV viral load measured with the COBAS® AmpliPrep/COBAS® TaqMan® CMV Test is informative in aiding in the management of CMV disease in kidney transplant recipients with active CMV disease undergoing anti-CMV drug treatment. The usefulness of the test in this clinical setting was assessed on the basis of test performance in predicting resolution of CMV disease when measured at Baseline, and in assessing virological treatment response when measured at subsequent time points.

The original study, the VICTOR Study, was a randomized controlled clinical trial comparing the efficacy of intravenous (IV) versus oral anti-CMV therapy for 21 days followed by oral therapy for an additional 28 days in solid organ transplant recipients diagnosed with CMV disease.[29] The patients were stratified by solid organ transplant type in the VICTOR Study; however, due to the limited number of non-renal solid organ transplant recipients enrolled in the VICTOR Study, this clinical usefulness study design only focused on kidney transplant recipients enrolled in the VICTOR Study.

To determine clinical usefulness, stored VICTOR Study specimens with sufficient sample volume were tested using the COBAS® AmpliPrep/COBAS® TaqMan® CMV Test at available time points (Baseline, Day 7, Day 14, Day 21, Day 28 post-treatment initiation, and Day 49 post-treatment initiation/End of Treatment). Viral load was analyzed alongside available clinical data according to the study design, controlling for relevant baseline clinical characteristics.

Stored VICTOR Study samples were acquired and each sample was assigned a unique identification number. Samples were prepared, amplified, and detected according to the COBAS® AmpliPrep/COBAS® TaqMan® CMV Test Instructions for Use. Each sample was tested at 1 of 3 clinical test sites. Study samples from the same patient across multiple time points in the VICTOR Study were tested at the same randomly assigned test site in the clinical usefulness study. Sites were blinded to the previous viral load results and clinical outcomes. Clinical data (e.g, demographics, CMV disease type upon entry, vital status, physical exam findings, biopsy/tissue sample findings, CMV disease status, clinical laboratory results [CBC, creatinine clearance], randomized treatment received) were extracted from the VICTOR Study database for applicable descriptive summaries or statistical analyses and linked to specimen COBAS® AmpliPrep/COBAS® TaqMan® CMV Test results using the unique identification number.

In the VICTOR Study, there were 237 subjects with kidney transplants and 211 subjects were included in the clinical usefulness study. Since 11% (26/237) of the VICTOR Study subjects were not included in the clinical usefulness study, demographic and clinical characteristics for all kidney transplant participants from the VICTOR Study (237 subjects) were summarized and compared to those evaluable participants with sufficient specimen volume at Baseline (211 subjects) to assess potential selection bias.

The primary clinical outcome measure for this study was the time to CMV disease resolution defined by the number of days from Baseline to clinical resolution of CMV disease. Regular clinical assessments for CMV disease (resolution of signs of viral syndrome and/or signs and symptoms of end-organ damage) took place on study Days 3, 7, 10, 14, 17, 21, 28, 35, 42, and 49. Relevant baseline clinical characteristics (i.e., baseline covariates) included demographics, randomized treatment received, recipient CMV serostatus, previous anti-CMV strategy, previous anti-CMV therapy, prior immunosuppressive regimen, CMV drug resistant status, CMV Glycoprotein B (gB) genotype (if available), and organ donor CMV serostatus (if available).

The time to resolution of CMV disease after the initiation of treatment was assessed for association with viral load results as measured with the COBAS® AmpliPrep/COBAS® TaqMan® CMV Test at Baseline, and the time to resolution of CMV disease after the initiation of treatment was also analyzed with the change in the viral load between Baseline and the respective days of monitoring to determine whether there was a correlation between change in viral load and CMV disease resolution. Specifically, based on existing literature, a Baseline CMV viremia of 18,200 IU/mL[30] (as measured with the COBAS® AmpliPrep/COBAS® TaqMan® CMV Test) and a 1.5 $\log_{10}$ IU/mL decline in CMV viremia from Baseline to Day 14[31] were assessed. Virological suppression (defined as below the LLoQ of the test, including target not detected results) at Day 7, Day 14, and Day 21 post-treatment initiation was also analyzed in relation to the time to resolution of CMV disease. Univariate Cox Proportional Hazards Models were used to assess the relationship between the time to resolution of CMV disease and each of the viral load-based variables of interest (Baseline, 1.5 $\log_{10}$-decline at Day 14, and viral load suppression at Days 7, 14, and 21) and each of the relevant baseline covariates described above. Multivariate Cox Proportional Hazards Models were used to examine the relationship between the time to resolution of CMV disease and each of the viral load-based variables of interest, adjusting for relevant baseline covariates (age, sex, race/ethnicity, recipient CMV serostatus, previous anti-CMV therapy, randomization to ganciclovir or valganciclovir arms, previous immunosuppressive regimen, CMV Glycoprotein B (gB) genotype (if available), and organ donor CMV serostatus (if available).

Results

In the clinical usefulness study, 100% of runs (63 of 63 runs) were valid, from which 12/1,235 test results were invalid (invalid rate: 0.97%; 95% CI: 0.51% to 1.69%).

Demographic characteristic distributions were similar between VICTOR Study kidney transplant recipients and evaluable clinical usefulness study participants (Table 12). Similar clinical characteristic distributions were also observed between VICTOR Study kidney transplant recipients and evaluable clinical usefulness study participants (Table 13).

Table 12
Demographic Characteristics of VICTOR Study Kidney Transplant Recipients and Evaluable Clinical Usefulness Study Participants

		VICTOR Study Kidney Transplant Recipient Set (N=237)	Evaluable Clinical Usefulness Study Participants		
			CMV Disease Type		All CMV Disease (N=211)
			CMV Syndrome (N=113)	Tissue-Invasive CMV Disease (N=98)	
Sex	**Male**	149 (62.9)	75 (66.4)	54 (55.1)	129 (61.1)
	Female	88 (37.1)	38 (33.6)	44 (44.9)	82 (38.9)
Age Category	**18-29**	47 (19.8)	23 (20.4)	22 (22.4)	45 (21.3)
	30-39	52 (21.9)	27 (23.9)	21 (21.4)	48 (22.7)
	40-49	46 (19.4)	18 (15.9)	20 (20.4)	38 (18.0)
	50-59	56 (23.6)	26 (23.0)	26 (26.5)	52 (24.6)
	≥60	36 (15.2)	19 (16.8)	9 (9.2)	28 (13.3)
Race/Ethnicity	**Caucasian/White**	172 (72.6)	91 (80.5)	60 (61.2)	151 (71.6)
	Black	8 (3.4)	3 (2.7)	2 (2.0)	5 (2.4)
	Asian	27 (11.4)	10 (8.8)	15 (15.3)	25 (11.8)
	Hispanic	18 (7.6)	3 (2.7)	15 (15.3)	18 (8.5)
	Other	12 (5.1)	6 (5.3)	6 (6.1)	12 (5.7)
Region	**Asia-Pacific**	77 (32.5)	50 (44.2)	21 (21.4)	71 (33.6)
	Europe	88 (37.1)	29 (25.7)	47 (48.0)	76 (36.0)
	North America	14 (5.9)	8 (7.1)	5 (5.1)	13 (6.2)
	South America	58 (24.5)	26 (23.0)	25 (25.5)	51 (24.2)

Note: Numbers are counts (with percentages) within each column.

Table 13
Clinical Characteristics of VICTOR Study Kidney Transplant Recipients and Evaluable Clinical Usefulness Study Participants

		VICTOR Study Kidney Transplant Recipient Set (N=237)	Evaluable Clinical Usefulness Study Participants		
			CMV Disease Type		All CMV Disease (N=211)
			CMV Syndrome (N=113)	Tissue-Invasive CMV Disease (N=98)	
Organ Donor(D)/ Recipient(R) CMV Serostatus	Missing	70 (29.5)	39 (34.5)	23 (23.5)	62 (29.4)
	D-/R-	13 (5.5)	5 (4.4)	6 (6.1)	11 (5.2)
	D-/R+	16 (6.8)	7 (6.2)	8 (8.2)	15 (7.1)
	D+/R-	37 (15.6)	11 (9.7)	24 (24.5)	35 (16.6)
	D+/R+	101 (42.6)	51 (45.1)	37 (37.8)	88 (41.7)
Recipient(R) CMV Serostatus	Missing	5 (2.1)	3 (2.7)	1 (1.0)	4 (1.9)
	R+	172 (72.6)	93 (82.3)	59 (60.2)	152 (72.0)
	R-	60 (25.3)	17 (15.0)	38 (38.8)	55 (26.1)
Previous Anti-CMV Strategy	Prophylactic	80 (33.8)	50 (44.2)	25 (25.5)	75 (35.5)
	Pre-Emptive	1 (0.4)	1 (0.9)	0 (0.0)	1 (0.5)
	Disease Treatment	25 (10.5)	4 (3.5)	15 (15.3)	19 (9.0)
	None	136 (57.4)	58 (51.3)	62 (63.3)	120 (56.9)
Previous Anti-CMV Therapy	Acyclovir	43 (18.1)	33 (29.2)	7 (7.1)	40 (19.0)
	Ganciclovir	47 (19.8)	19 (16.8)	22 (22.4)	41 (19.4)
	Valacyclovir	4 (1.7)	3 (2.7)	1 (1.0)	4 (1.9)
	Valganciclovir	33 (13.9)	12 (10.6)	17 (17.3)	29 (13.7)
	None	136 (57.4)	58 (51.3)	62 (63.3)	120 (56.9)
Randomized Treatment Received	Ganciclovir	115 (48.5)	61 (54.0)	43 (43.9)	104 (49.3)
	Valganciclovir	122 (51.5)	52 (46.0)	55 (56.1)	107 (50.7)
CMV Disease Sub-Category [a]	CMV Syndrome	124 (52.3)	113 (100.0)	0 (0.0)	113 (53.6)
	TI CMV: CMV GI	19 (8.0)	0 (0.0)	18 (18.4)	18 (8.5)
	TI CMV: Hepatitis	7 (3.0)	0 (0.0)	7 (7.1)	7 (3.3)
	TI CMV: Nephritis	80 (33.8)	0 (0.0)	70 (71.4)	70 (33.2)
	TI CMV: Pneumonia	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	TI CMV: Retinitis	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	Other TI CMV	4 (1.7)	0 (0.0)	3 (3.1)	3 (1.4)
	No CMV in Blood	3 (1.3)	0 (0.0)	0 (0.0)	0 (0.0)

Note: Numbers are counts (with percentages) within each column.

[a] CMV GI = CMV esophagitis, gastroenteritis, or colitis; TI-CMV = Tissue-Invasive CMV Disease; No CMV in Blood = No CMV in Blood (Screening/Baseline).

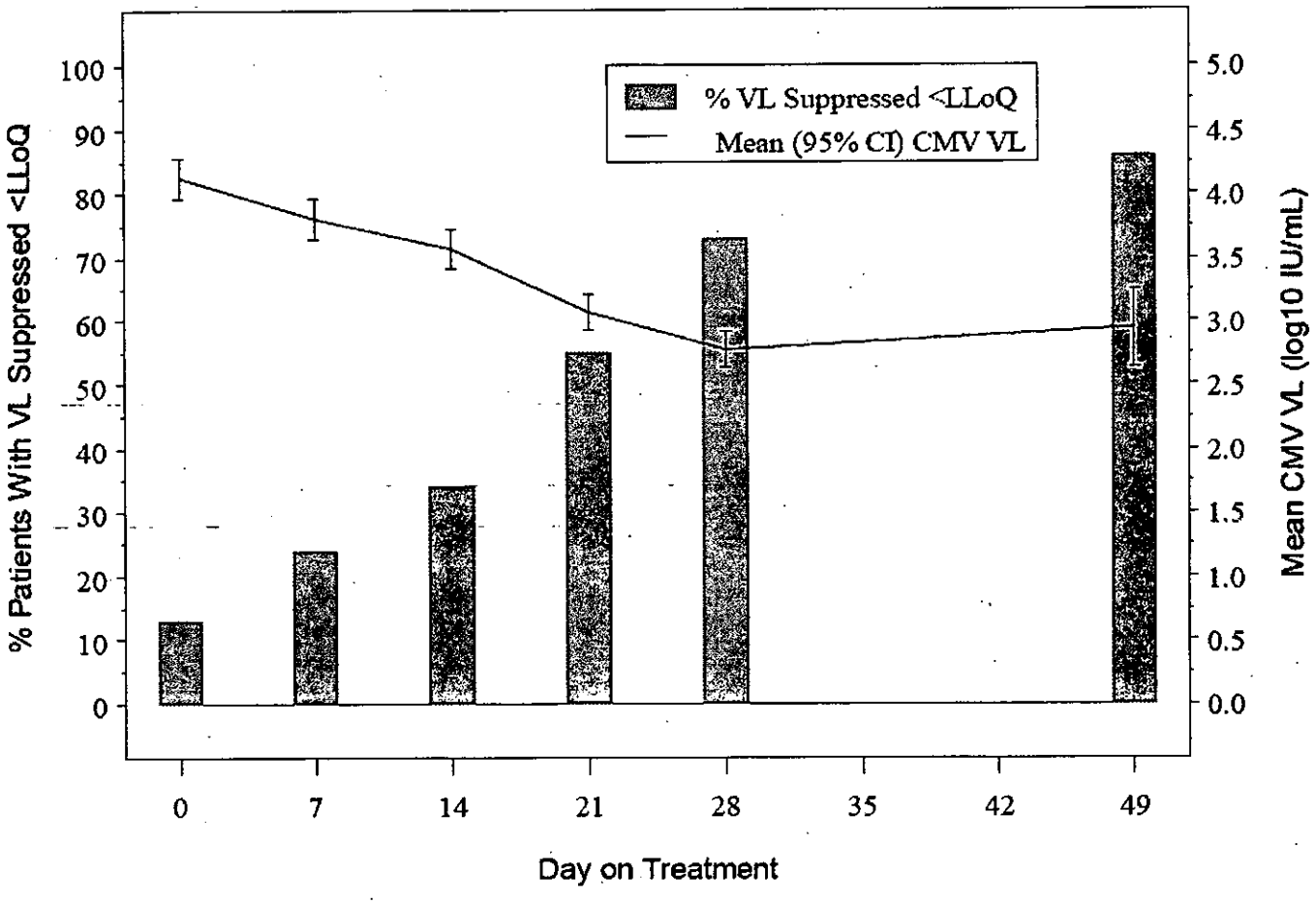
Table 13 (continued)
Clinical Characteristics of VICTOR Study Kidney Transplant Recipients and Evaluable Clinical Usefulness Study Participants

		VICTOR Study Kidney Transplant Recipient Set (N=237)	Evaluable Clinical Usefulness Study Participants		
			CMV Disease Type		All CMV Disease (N=211)
			CMV Syndrome (N=113)	Tissue-Invasive CMV Disease (N=98)	
Previous Immunosuppressive Therapy	ATG	26 (11.0)	13 (11.5)	9 (9.2)	22 (10.4)
	Azathioprine	25 (10.5)	12 (10.6)	9 (9.2)	21 (10.0)
	Basiliximab	17 (7.2)	4 (3.5)	9 (9.2)	13 (6.2)
	Cyclosporine A	128 (54.0)	68 (60.2)	45 (45.9)	113 (53.6)
	Daclizumab	10 (4.2)	4 (3.5)	6 (6.1)	10 (4.7)
	Methylprednisolone	68 (28.7)	29 (25.7)	29 (29.6)	58 (27.5)
	Mycophenolate mofetil	173 (73.0)	80 (70.8)	73 (74.5)	153 (72.5)
	OKT 3	1 (0.4)	1 (0.9)	0 (0.0)	1 (0.5)
	Prednisolone	85 (35.9)	45 (39.8)	28 (28.6)	73 (34.6)
	Prednisone	109 (46.0)	51 (45.1)	50 (51.0)	101 (47.9)
	Sirolimus	20 (8.4)	7 (6.2)	10 (10.2)	17 (8.1)
	Tacrolimus	75 (31.6)	35 (31.0)	35 (35.7)	70 (33.2)
	Other	18 (7.6)	13 (11.5)	4 (4.1)	17 (8.1)
	Not provided	11 (4.6)	6 (5.3)	4 (4.1)	10 (4.7)
CMV Genotype	Not Done*	103 (43.5)	51 (45.1)	36 (36.7)	87 (41.2)
	Genotype 1	43 (18.1)	16 (14.2)	25 (25.5)	41 (19.4)
	Genotype 2	30 (12.7)	16 (14.2)	12 (12.2)	28 (13.3)
	Genotype 3	30 (12.7)	16 (14.2)	10 (10.2)	26 (12.3)
	Genotype 4	13 (5.5)	3 (2.7)	8 (8.2)	11 (5.2)
	Mixed Infection	18 (7.6)	11 (9.7)	7 (7.1)	18 (8.5)
UL54 Resistant	Not Done*	52 (21.9)	18 (15.9)	23 (23.5)	41 (19.4)
	Yes	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	No	185 (78.1)	95 (84.1)	75 (76.5)	170 (80.6)
UL97 Resistant	Not Done*	52 (21.9)	18 (15.9)	23 (23.5)	41 (19.4)
	Yes	1 (0.4)	0 (0.0)	1 (1.0)	1 (0.5)
	No	184 (77.6)	95 (84.1)	74 (75.5)	169 (80.1)

Note: Numbers are counts (with percentages) within each column.
* 'Not Done' due to low sample volume and/or low viral load.

Figure 6 shows a comparison of the mean (with 95% CI) CMV viral load (log_{10} IU/mL) and the percentage of participants with CMV viral load suppressed <LLoQ (including target not detected results) by day on treatment. The percentage of study participants with viral suppression <LLoQ increased with day on treatment. Mean CMV viral load generally decreased with day on treatment from Baseline to Day 49 among those participants who still had quantifiable viral load titers.

Figure 6
Comparison of CMV Viral Load Suppression and Mean CMV Viral Load by Day on Treatment

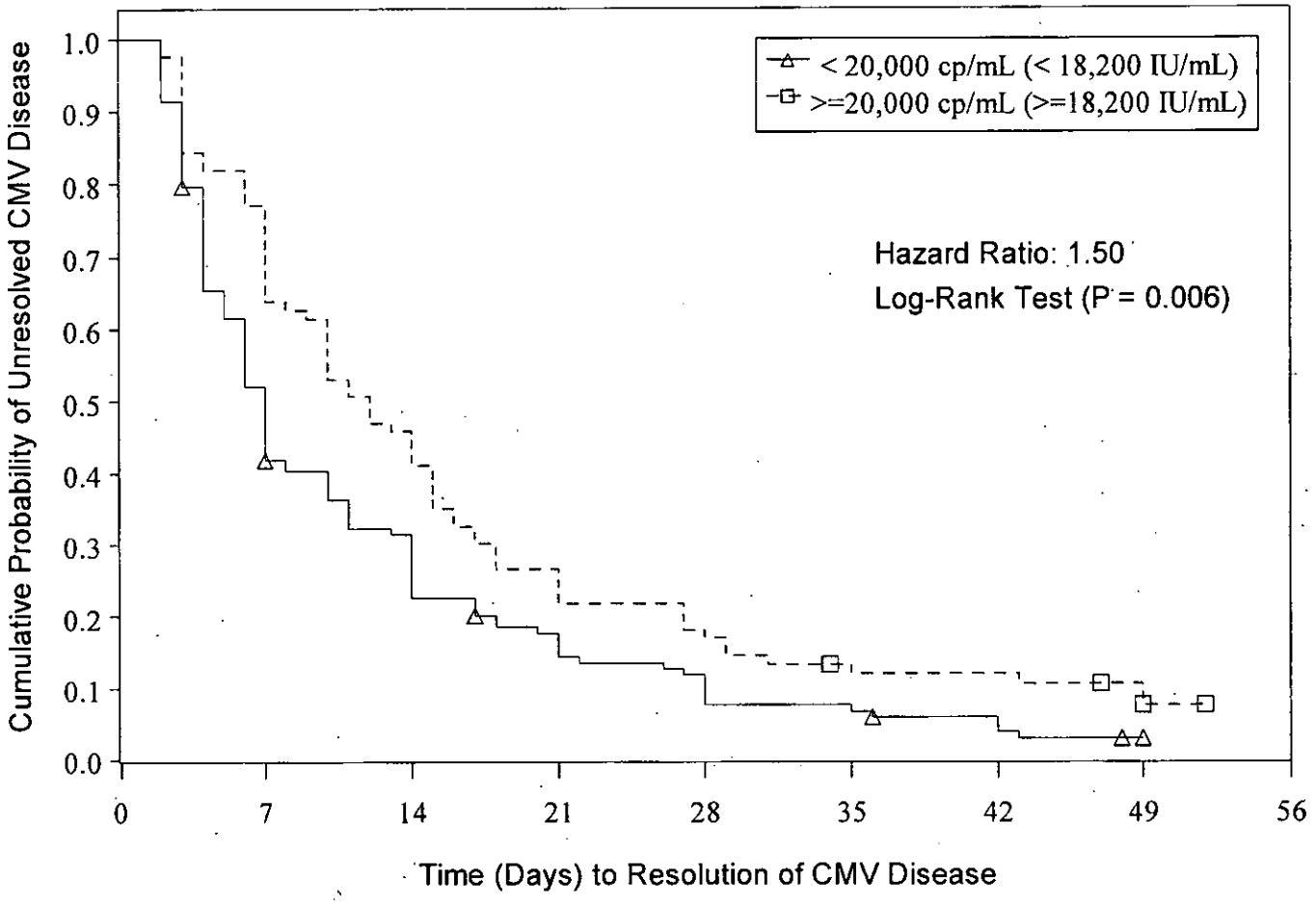


Note: Bars indicate % participants with VL suppressed <LLoQ and line indicates mean CMV VL

Baseline Viral Load and Clinical Resolution of CMV Disease

A Kaplan-Meier survival plot for the time (days) to resolution of CMV disease stratified by baseline CMV viral load (<20,000 copies/mL, ≥20,000 copies/mL; or <18,200 IU/mL, ≥18,200 IU/mL) is shown in Figure 7. There is clear separation between the survival curves, with shorter times to resolution of CMV disease for participants with baseline CMV viral load <20,000 copies/mL (unadjusted hazard ratio [HR] = 1.50, 95% CI of 1.12 to 2.00; Log-Rank Test, $P = 0.006$).

Figure 7
Kaplan-Meier Survival Plot of Time to Resolution of CMV Disease by Baseline COBAS® AmpliPrep®/COBAS® TaqMan® CMV Test Result (<20,000 copies/mL, ≥20,000 copies/mL [<18,200 IU/mL, ≥18,200 IU/mL])



Baseline CAP/CTM CMV Test Result	No. of Participants	Resolved n (%)	Censored n (%)	Median Time to Resolution in Days (95% CI)	Hazard Ratio (HR)
<20,000 copies/mL (<18,200 IU/mL)	128	121 (94.5)	7 (5.5)	7.0 (6.0, 8.0)	1.50
≥20,000 copies/mL (≥18,200 IU/mL)	83	76 (91.6)	7 (8.4)	12.0 (10.0,15.0)	
Total	211	197	14		

Table 14 shows the multivariate Cox proportional hazards model for the relationship between time to resolution of CMV disease and baseline CMV viral load. The HR for baseline CMV viral load was 1.46 (95% CI = 1.08 to 1.99; P = 0.015), indicating a 46% higher chance of resolution of CMV disease at any point in time among participants with baseline CMV viral load <20,000 copies/mL (<18,200 IU/mL) compared to those with baseline CMV viral load ≥20,000 copies/mL (≥18,200 IU/mL), after adjusting for relevant baseline covariates.

Day 14 Decline in Viral Load and Clinical Resolution of CMV Disease

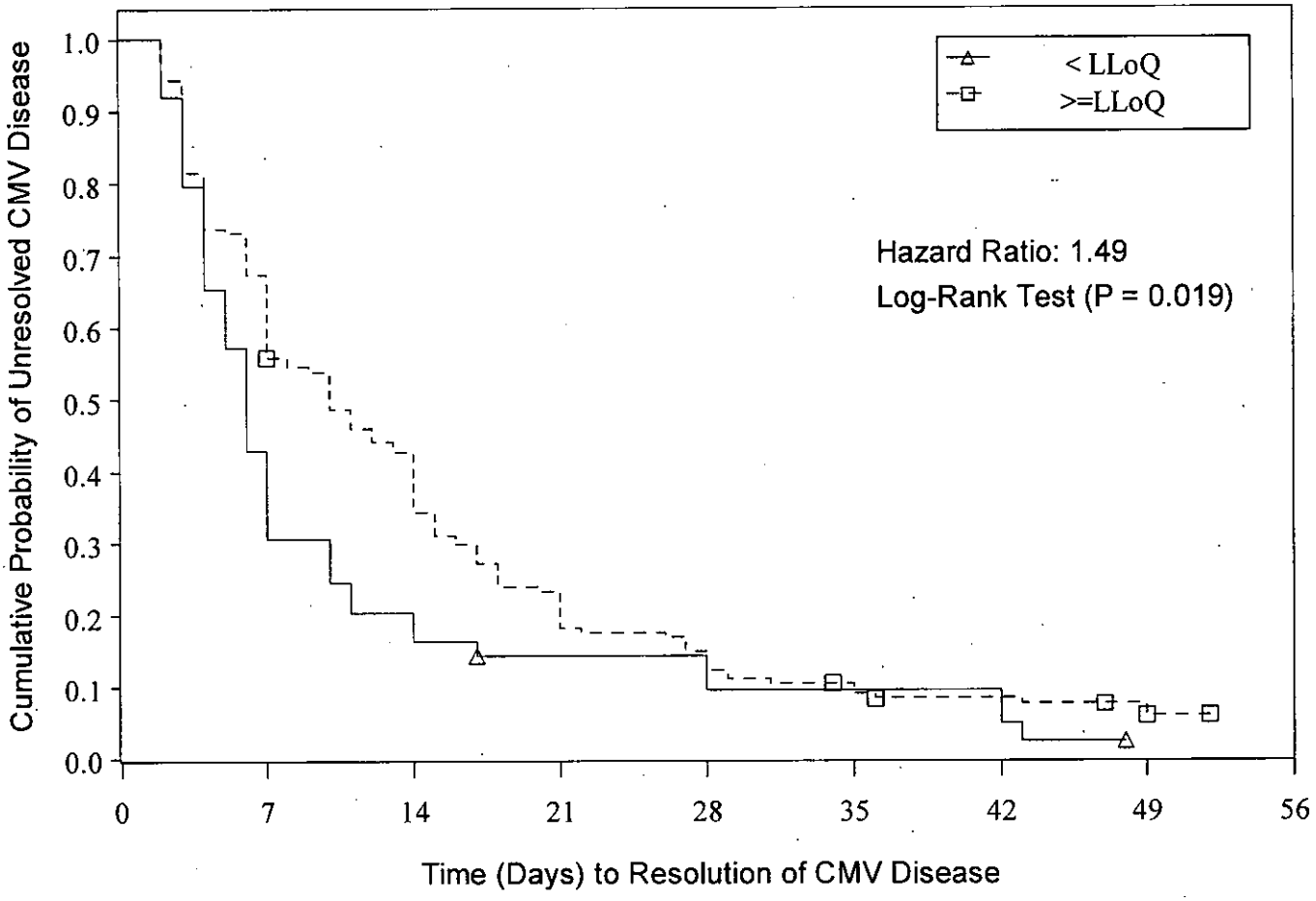
The analysis of 1.5 $\log_{10}$ IU/mL decline in viral load from Baseline at Day 14 calculated an unadjusted hazard ratio of 0.90 (95% CI of 0.67 to 1.20; Log-Rank Test P = 0.460). This finding did not change in multivariate analysis adjusting for baseline covariates (Table 14), indicating that a decline in viral load of greater than 1.5 $\log_{10}$ IU/mL was not associated with time to resolution of CMV disease.

Other degrees of viral load decline (≥1.0, ≥2.0, and ≥2.5 $\log_{10}$ IU/mL) were evaluated (data not shown) and also not found to be predictive of time to resolution of CMV disease.

Day 7, Day 14, and Day 21 Viral Load Suppression (<LLoQ) and Clinical Resolution of CMV Disease

The Kaplan-Meier survival plot for the time to resolution of CMV disease stratified by viral suppression <LLoQ by Day 7 shows wide separation between the survival curves from Days 4 to 27 (Figure 8). The unadjusted HR for the association of viral suppression at Day 7 and the time to resolution of CMV disease was 1.49 (95% CI of 1.07 to 2.07; Log-Rank Test P = 0.019).

Figure 8
Kaplan-Meier Survival Plot of Time to Resolution of CMV Disease by On-Treatment Viral Load Suppression <LLoQ by Day 7

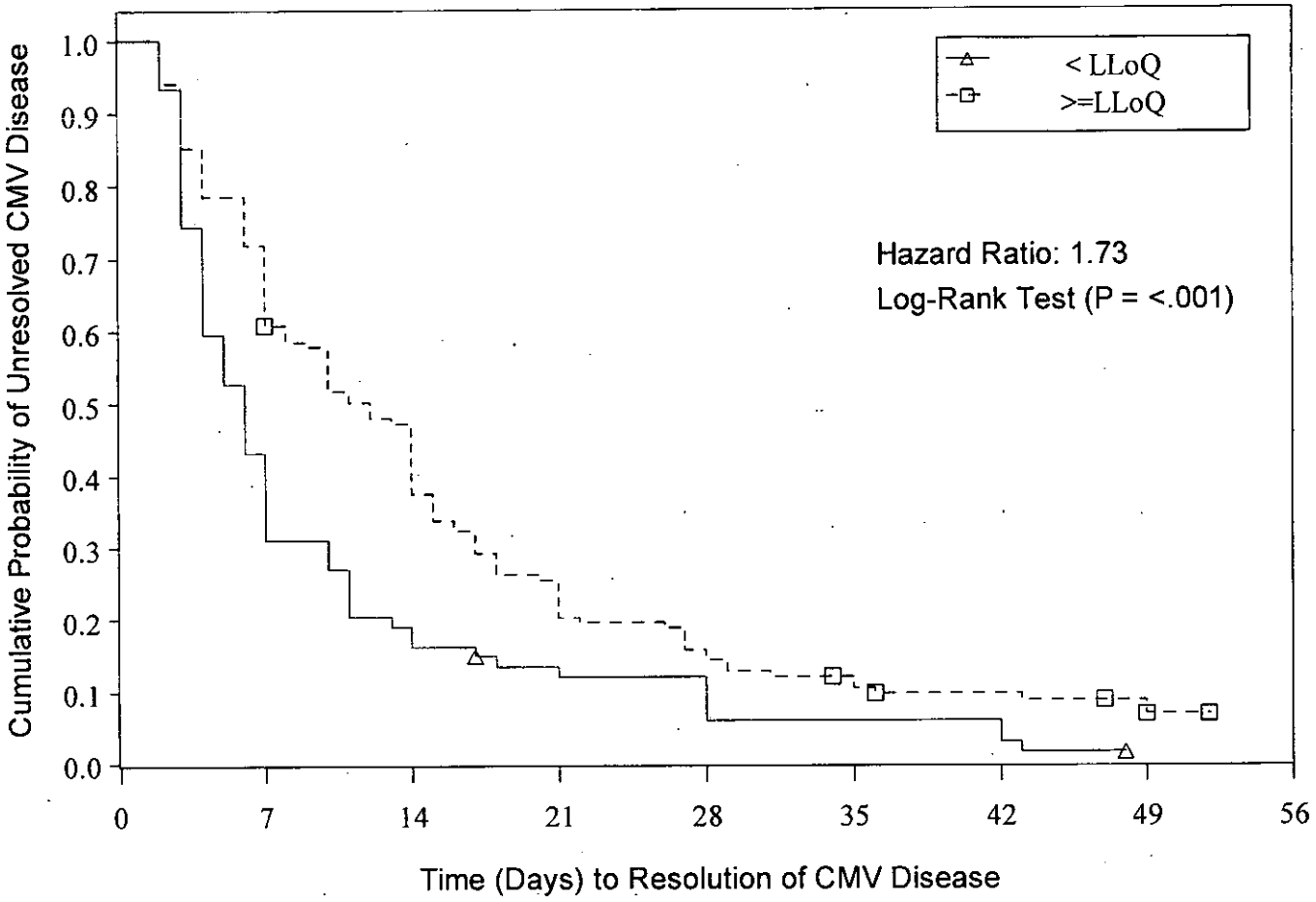


Viral Suppression by Day 7	No. of Participants	Resolved n (%)	Censored n (%)	Median Time to Resolution in Days (95% CI)	Hazard Ratio (HR)
<LLoQ	49	47 (95.9)	2 (4.1)	6.0 (5.0, 7.0)	1.49
≥LLoQ	156	145 (92.9)	11 (7.1)	10.0 (7.0,14.0)	
Total	205	192	13		

After adjusting for relevant baseline covariates, the HR for viral suppression by Day 7 was 1.62 (95% CI = 1.12 to 2.35; $P = 0.010$) from the multivariate Cox proportional hazards model (Table 14). This result indicates that kidney transplant recipients with CMV disease, receiving anti-CMV therapy, with a suppressed viral load by Day 7, have more rapid resolution of their CMV disease.

Shorter times to resolution of CMV disease were observed in study participants who exhibited viral suppression <LLoQ by Day 14 (Figure 9). The log-rank test indicates a strong statistical difference between the survival curves (unadjusted hazard ratio [HR] = 1.73, 95% CI of 1.29 to 2.32; Log-Rank Test $P < 0.001$).

Figure 9
Kaplan-Meier Survival Plot of Time to Resolution of CMV Disease by On-Treatment Viral Load Suppression <LLoQ by Day 14

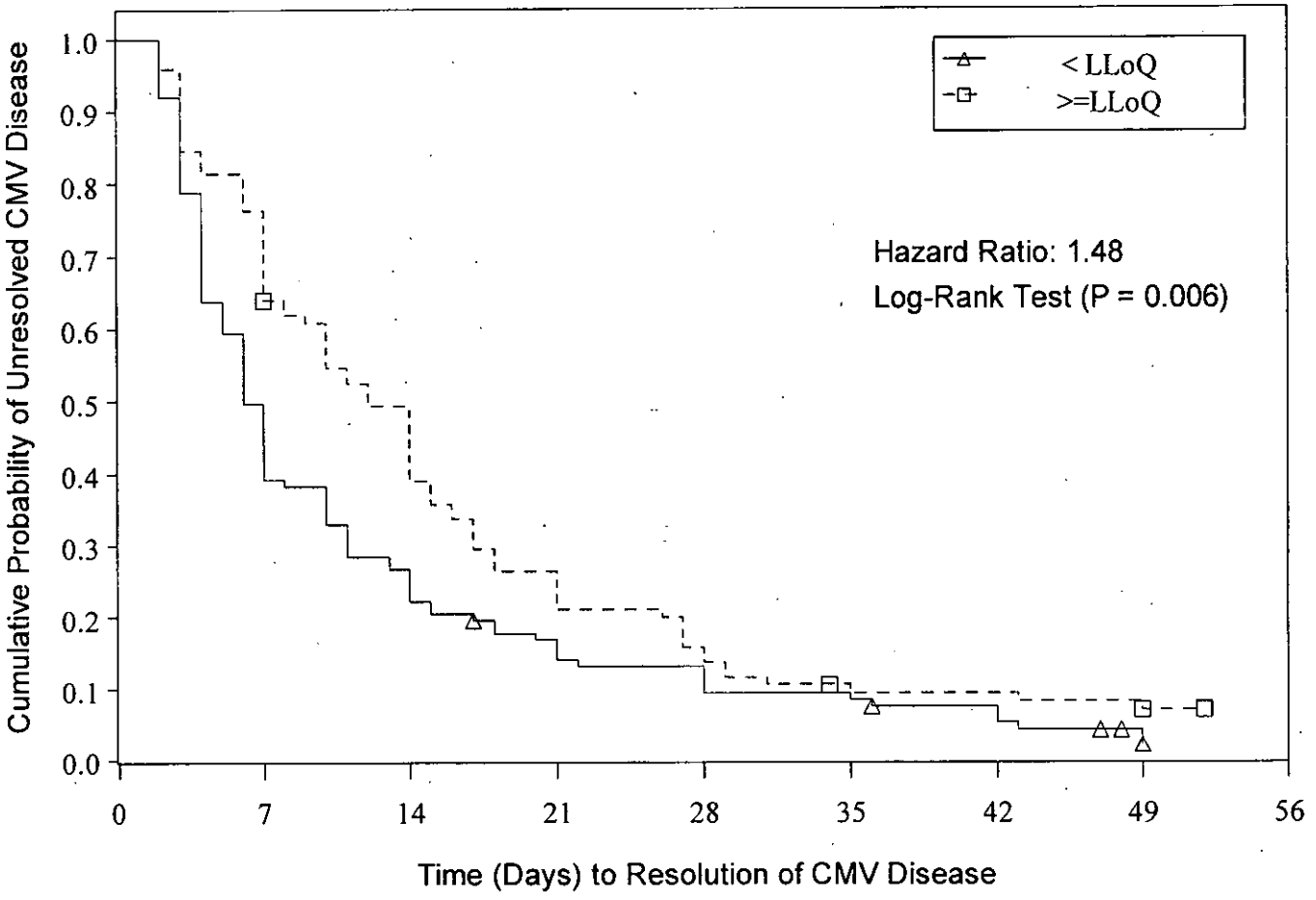


Viral Suppression by Day 14	No. of Participants	Resolved n (%)	Censored n (%)	Median Time to Resolution in Days (95% CI)	Hazard Ratio (HR)
<LLoQ	74	72 (97.3)	2 (2.7)	6.0 (4.0, 7.0)	1.73
≥LLoQ	135	124 (91.9)	11 (8.1)	12.0 (8.0,14.0)	
Total	209	196	13		

The multivariate Cox proportional hazards model for the time to resolution of CMV disease and viral suppression <LLoQ by Day 14 is presented in Table 14. The HR of 1.83 (95% CI = 1.33 to 2.51; $P < 0.001$), indicates that there was an 83% greater chance of resolution of CMV disease at any point in time among study participants with viral suppression <LLoQ by Day 14 compared to those with viral suppression ≥LLoQ by Day 14, after adjusting for relevant baseline covariates.

The Kaplan-Meier survival plot for time to resolution of CMV disease stratified by those study participants who did or did not exhibit viral suppression <LLoQ by Day 21 (Figure 10) also shows clear separation between survival curves (unadjusted hazard ratio [HR] = 1.48, 95% CI of 1.11 to 1.96; Log-Rank Test $P = 0.006$).

Figure 10
Kaplan-Meier Survival Plot of Time to Resolution of CMV Disease by On-Treatment Viral Load Suppression <LLoQ by Day 21



Viral Suppression by Day 21	No. of Participants	Resolved n (%)	Censored n (%)	Median Time to Resolution (95% CI)	Hazard Ratio (HR)
<LLoQ	113	108 (95.6)	5 (4.4)	6.0 (5.0, 7.0)	1.48
≥LLoQ	97	89 (91.8)	8 (8.2)	12.0 (10.0,14.0)	
Total	210	197	13		

From Table 14 the HR for viral suppression <LLoQ by Day 21 is 1.44 (95% CI = 1.07 to 1.92; $P = 0.015$), indicating that there is a 44% greater chance of resolution of disease at any point in time among participants with viral suppression <LLoQ by Day 21 compared to those with viral suppression ≥LLoQ by Day 21, after adjusting for relevant baseline covariates.

Summary

Table 14 summarizes the findings from multivariate Cox proportional hazards models for the association between viral load variables of interest (Baseline, 1.5-$\log_{10}$ decline at Day 14, and suppression at Days 7, 14, and 21) and the time to CMV disease resolution. The multivariate models were adjusted for age, sex, race/ethnicity, recipient CMV serostatus, previous anti-CMV therapy, randomization to ganciclovir or valganciclovir arms, and prior immunosuppressive regimen. Due to incomplete data, the models did not include the variables: donor CMV serostatus and glycoprotein B genotype.

Table 14

Adjusted Hazard Ratios for Viral Load-Based Variables of Interest from Multivariate Cox Proportional Hazards Model for the Time to Resolution of CMV Disease, Adjusted for Relevant Baseline Covariates

			Adjusted Hazard Ratio[a]		
Viral Load-Based Variables of Interest	**Viral Load Category**	**N**	**Estimate**	**95% CI**	**P-value**
Baseline CAP/CTM CMV Test Result	< 18,200 IU/mL	207	1.46	(1.08, 1.99)	0.015
	≥ 18,200 IU/mL		(1.00)		
On-Treatment Viral Load Decline at Day 14	≥ 1.5 $\log_{10}$ IU/mL	205	0.93	(0.68, 1.26)	0.633
	< 1.5 $\log_{10}$ IU/mL		(1.00)		
Viral Suppression by Day 7	< LLoQ	201	1.62	(1.12, 2.35)	0.010
	≥ LLoQ		(1.00)		
Viral Suppression by Day 14	< LLoQ	205	1.83	(1.33, 2.51)	<.001
	≥ LLoQ		(1.00)		
Viral Suppression by Day 21	< LLoQ	206	1.44	(1.07, 1.92)	0.015
	≥ LLoQ		(1.00)		

Note: CI = confidence interval. Note: Rows with hazard ratio estimates within parentheses indicate reference categories.
[a] Adjusted for age (continuous), sex, ethnicity/race, randomized treatment received, recipient (R) CMV serostatus, previous anti-CMV therapy, and prior immunosuppressive regimen (Cyclosporine A, T-Cell Suppressors, and/or corticosteroids).

CONCLUSION

The COBAS® AmpliPrep/COBAS® TaqMan® CMV Test provides clinical value for baseline testing and assessing virological response of patients with CMV disease who are undergoing treatment with anti-CMV drugs (ganciclovir or valganciclovir). Clinicians managing such patients would benefit from knowing that patients with a baseline CMV viral load <18,200 IU/mL (20,000 copies/mL) are likely to resolve CMV disease more rapidly than those who have a higher baseline viral load. Data from this study did not demonstrate that a decline in viral load of 1.5 $\log_{10}$ by Day 14 of treatment is informative to assess treatment response; however, viral load suppression (defined as viral load below LLoQ) at Days 7, 14, and 21 are highly correlated with resolution of CMV disease.

REFERENCES

1. Griffiths PD. 2000. Cytomegalovirus. In Principles and Practice of Clinical Virology. eds. Zuckerman AJ, Banatvala, JE, and Pattison JR. John Wiley and Sons, London. 79-116.
2. Pass RF. 2001. Cytomegalovirus. In: Fields Virology. eds. Knipe D, Howley P. Lippincott, Williams and Wilkins, Philadelphia. 2675-2706.
3. Kapranos N, Petrakou E, Anastasiadou C, Kotronias D. 2003. Detection of herpes simplex virus, cytomegalovirus, and Epstein-Barr virus in the semen of men attending an infertility clinic. Fertility and Sterility **79**:48-52.
4. Forbes BA. 1989. Acquisition of cytomegalovirus infection: an update. Clinical Microbiological Reviews **2**:204-216.
5. Numazaki K. 1997. Human cytomegalovirus infection of breast milk. FEMS Immunology and Medical Microbiology, **18**:2:91-98.
6. Vochem M, Hamprecht K, Jahn G, Speer CP. 1998. Transmission of cytomegalovirus to preterm infants through breast milk. Pediatric Infectious Disease Journal. **17**:55-53.
7. Jordan MC. 1983. Latent infection and the elusive cytomegalovirus. Reviews in Infectious Diseases **5**:205-215.
8. Souza IE, Nicholson D, Matthey S, et al. 1994. Detection of human cytomegalovirus in peripheral blood leukocytes by polymerase chain reaction and a nonradioactive probe. Diagnostic Microbiology Infectious Disease **20**:13-19.
9. Alford CA, Brill WJ. 1990. Cytomegalovirus. In: Virology, Vol. 2, 2nd ed., eds. Fields BN, Knipe DM. Raven Press, New York, NY. 1981-2010.
10. Yasuda A, Kimura H, Hayakawa M, Ohshiro M, et al. 2003. Evaluation of cytomegalovirus infections transmitted via breast milk in preterm infants with a real-time polymerase chain reaction assay. Pediatrics. **111**:1333-6.
11. Drew WL. 1989. Other virus infections in AIDS: I: Cytomegalovirus. Immunology and Serology **44**:507-534.
12. Drew WL. 1992. Nonpulmonary manifestations of Cytomegalovirus infection in immunocompromised patients. Clinical Microbiology Review **5**:204-210.
13. Mocarski ES, Courcelle CT. 2001. Cytomegaloviruses and their replication. In: Fields Virology. eds. Knipe D and Howley P. Lippincott, Williams and Wilkins, Philadelphia. 2629-2674.
14. Hassan-Walker AF, Kidd IM, Sabin C, et al. 1999. Quantity of human cytomegalovirus (CMV) DNAemia as a risk factor for CMV disease in renal allograft recipients: Relationship with donor/recipient CMV serostatus, receipt of augmented methylprednisolone and antithymocyte globulin (ATG). Journal of Medical Virology **58**:182-187.
15. Cope AV, Sweny P, Sabin C, et al. 1997. Quantity of cytomegalovirus viruria is a major risk factor for cytomegalovirus disease after renal transplantation. Journal of Medical Virology **52**:200-205.
16. Gor D, Sabin C, Prentice HG, et al. 1998. Longitudinal fluctuations in cytomegalovirus load in bone marrow transplant patients: relationship between peak virus load, donor/recipient serostatus, acute GVHD and CMV disease. Bone Marrow Transplantation **21**:579-605.
17. Smith ES, Li AK, Wang AM, Gelfand DH, Myers TM. 2003. Amplification of RNA: High-Temperature Reverse Transcription and DNA Amplification with a Magnesium-Activated Thermostable DNA Polymerase. In *PCR Primer: A Laboratory Manual*, 2nd Edition, Dieffenbach C.W. and Dveksler G.S., Eds. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, pp. 211-219.
18. Higuchi R, Dollinger G, Walsh PS, Griffith R. 1992. Simultaneous amplification and detection of specific DNA sequences. Biotechnology (N Y). **10**:413-417.
19. Heid CA, Stevens J, Livak JK, Williams PM. 1996. Real time quantitative PCR. Genome Research **6**:986-994.
20. Longo MC, Berninger MS, Hartley JL. 1990. Use of uracil DNA glycosylase to control carry-over contamination in polymerase chain reactions. Gene **93**:125-128.
21. Richmond JY, McKinney RW. eds. 1999. Biosafety in Microbiological and Biomedical Laboratories. HHS Publication Number (CDC) 93-8395.
22. Clinical and Laboratory Standards Institute (CLSI). Protection of Laboratory Workers from Occupationally Acquired Infections. Approved Guideline-Third Edition. CLSI Document M29-A3 Wayne, PA. CLSI, 2005.
23. International Air Transport Association. Dangerous Goods Regulations, 41st Edition. 2000.

24. Fryer JF, Heath AB, Anderson R, Minor PD and the collaborative study group. 2010 Collaborative Study to evaluate the proposed 1st WHO International Standard for Human Cytomegalovirus (HCMV) for Nucleic Acid Amplification (NAT)-based Assays. WHO ECBS Report WHO/BS/10.2138.

25. Clinical and Laboratory Standards Institute (CLSI). Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline. CLSI document EP17-A, CLSI. 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 2004.

26. Clinical and Laboratory Standards Institute (CLSI). Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline. CLSI Document EP6-A. CLSI, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 2003.

27. Clinical and Laboratory Standards Institute (CLSI). Evaluation of Precision Performance of Quantitative Measurement Methods: Approved Guideline - Second Edition. CLSI Document EP5-A2. CSLI, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 2004.

28. Clinical and Laboratory Standards Institute (CLSI). Interference Testing in Chemistry; Approved Guideline—Second Edition. CLSI Document EP7-A2. CSLI, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 2004.

29. Asberg A, Humar A, Rollag H, Jardine AG, Mouas H, Pescovitz MD, et al. Oral valganciclovir is noninferior to intravenous ganciclovir for the treatment of cytomegalovirus disease in solid organ transplant recipients. Am J Transplant 2007; **7(9)**:2106-13.

30. Razonable RR, Brown RA, Wilson J, Groettum C, Kremers W, Espy M, et al. The clinical use of various blood compartments for cytomegalovirus (CMV) DNA quantitation in transplant recipients with CMV disease. Transplantation 2002;73(6):968-73.

31. Humar A, Gregson D, Caliendo AM, McGeer A, Malkan G, Krajden M, et al. Clinical utility of quantitative cytomegalovirus viral load determination for predicting cytomegalovirus disease in liver transplant recipients. Transplantation 1999;68(9):1305-11.

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