altona DIAGNOSTICS

Instructions for Use

RealStar®
CMV PCR Kit 1.0

09/2023 EN

RealStar®

RealStar®

CMV PCR Kit 1.0

For use with

Mx 3005P™ QPCR System (Stratagene)

VERSANT® kPCR Molecular System AD (Siemens Healthcare)

ABI Prism® 7500 SDS (Applied Biosystems)

ABI Prism® 7500 Fast SDS (Applied Biosystems)

Rotor-Gene® 6000 (Corbett Research)

Rotor-Gene® Q5/6 plex Platform (QIAGEN)

LightCycler® 480 Instrument II (Roche)

CFX96™ Real-Time PCR Detection System (Bio-Rad)

CFX96™ Deep Well Real-Time PCR Detection System (Bio-Rad)

CE 0483

IVD

REF 021013

Σ 96

09 2023

altona Diagnostics GmbH • Mörkenstr. 12 • D-22767 Hamburg

MAN-021010-EN-S03

Content

1. Intended Use

The RealStar® CMV PCR Kit 1.0 is an *in vitro* diagnostic test, based on real-time PCR technology, for the detection and quantification of cytomegalovirus (CMV) specific DNA in human EDTA plasma and EDTA whole blood.

2. Kit Components

Lid Color	Component	Number of Vials	Volume [µl/Vial]
Blue	Master A	8	60
Purple	Master B	8	180
Green	Internal Control	1	1000
Red	QS1-4*	4	250
White	Water (PCR grade)	1	500

* The RealStar® CMV PCR Kit 1.0 contains Quantification Standards (QS) at four different concentrations (see Chapter 6. Product Description)

3. Storage

- The RealStar® CMV PCR Kit 1.0 is shipped on dry ice. The components of the kit should arrive frozen. If one or more components are not frozen upon receipt, or if tubes have been compromised during shipment, contact altona Diagnostics GmbH for assistance.
- All components should be stored between -25°C and -15°C upon arrival.
- Repeated thawing and freezing of Master reagents (more than twice) should be avoided, as this might affect the performance of the assay. The reagents should be frozen in aliquots, if they are to be used intermittently.
- Storage between +2°C and +8°C should not exceed a period of two hours.
- Protect Master A and Master B from light.

4. Material and Devices required but not provided

- Appropriate real-time PCR instrument (see chapter 6.1 Real-Time PCR Instruments)
- Appropriate nucleic acid extraction system or kit (see chapter 8.2 Sample Preparation)
- Desktop centrifuge with a rotor for 2 ml reaction tubes
- Centrifuge with a rotor for microtiter plates, if using 96 well reaction plates
- Vortex mixer
- Appropriate 96 well reaction plates or reaction tubes with corresponding (optical) closing material
- Pipettes (adjustable)
- Pipette tips with filters (disposable)
- Powder-free gloves (disposable)

NOTE

Please ensure that all instruments used have been installed, calibrated, checked and maintained according to the manufacturer´s instructions and recommendations.

It is highly recommended to use the 72-well rotor with the appropriate 0.1 ml reaction tubes, if using the Rotor-Gene® 6000 (Corbett Research) or the Rotor-Gene® Q 5/6 plex (QIAGEN).

5. Background Information

The *human cytomegalovirus* (CMV) is a member of the family *Herpesviridae* and belongs to the subfamily *Betaherpesvirinae*. The virus consists of an icosahedral capsid with a linear double-stranded DNA genome of approximately 230 kbp, a surrounding integument and an outer envelope.

CMV has a worldwide distribution and infects humans of all ages, with no seasonal or epidemic patterns of transmission. The seroprevalence of CMV increases with age in all populations and ranges from 40 to 100%. Similar to infections with other herpesviruses, primary infection with CMV results in the establishment of a persistent or latent infection. Reactivation of the virus can occur in response to different stimuli, particularly immunosuppression. The vast majority of CMV infections are asymptomatic or subclinical, but congenital infections and infections in immunocompromised patients may be symptomatic and serious. In immunocompromised hosts, such as transplant recipients, HIV-infected or cancer patients, a CMV infection or reactivation may become a life-threatening disseminated disease.

6. Product Description

The RealStar® CMV PCR Kit 1.0 is an *in vitro* diagnostic test, based on real-time PCR technology, for the detection and quantification of cytomegalovirus (CMV) specific DNA in human EDTA plasma and EDTA whole blood.

The assay includes a heterologous amplification system (Internal Control) to identify possible PCR inhibition and to confirm the integrity of the reagents of the kit.

Real-time PCR technology utilizes polymerase chain reaction (PCR) for the amplification of specific target sequences and target specific probes for the detection of the amplified DNA. The probes are labelled with fluorescent reporter and quencher dyes.

Probes specific for CMV DNA are labelled with the fluorophore FAM™. The probe specific for the Internal Control (IC) is labelled with the fluorophore JOE™.

Using probes linked to distinguishable dyes enables the parallel detection of CMV specific DNA and the Internal Control in corresponding detector channels of the real-time PCR instrument.

The test consists of two processes in a single tube assay:

- PCR amplification of target DNA and Internal Control
- Simultaneous detection of PCR amplicons by fluorescent dye labelled probes

The RealStar® CMV PCR Kit 1.0 consists of:

- Two Master reagents (Master A and Master B)
- Internal Control (IC)
- Four Quantification Standards (QS1 - QS4)
- PCR grade water

Master A and Master B contain all components (PCR buffer, DNA polymerase, magnesium salt, primers and probes) to allow PCR mediated amplification and detection of CMV specific DNA and Internal Control in one reaction setup.

The Quantification Standards contain standardized concentrations of CMV specific DNA. They were calibrated against the 1st WHO International Standard for Human Cytomegalovirus for Nucleic Acid Amplification Techniques (NIBSC code: 09/162). The Quantification Standards can be used individually as positive controls, or together to generate a **standard curve**, which can be used to determine the concentration of CMV specific DNA in a sample.

The Quantification Standards have the following concentrations:

Quantification Standard	Concentration [IU/µl]
QS1	1.00E+04
QS2	1.00E+03
QS3	1.00E+02
QS4	1.00E+01

6.1 Real-Time PCR Instruments

The RealStar® CMV PCR Kit 1.0 was validated for EDTA plasma in combination with the QIAamp® MiniElute® Virus Spin Kit (QIAGEN) on the following real-time PCR instruments:

- Mx 3005P™ QPCR System (Stratagene)
- VERSANT® kPCR Molecular System AD (Siemens Healthcare)
- ABI Prism® 7500 SDS (Applied Biosystems)
- ABI Prism® 7500 Fast SDS (Applied Biosystems)
- Rotor-Gene® 6000 (Corbett Research)
- Rotor-Gene® Q5/6 plex Platform (QIAGEN)
- LightCycler® 480 Instrument II (Roche)
- CFX96™ Real-Time PCR Detection System (Bio-Rad)
- CFX96™ Deep Well Real-Time PCR Detection System (Bio-Rad)

The RealStar® CMV PCR Kit 1.0 was validated for EDTA plasma and EDTA whole blood in combination with the VERSANT® kPCR Molecular System SP (Siemens Healthcare) on the following real-time PCR instrument:

- VERSANT® kPCR Molecular System AD (Siemens Healthcare)

7. Warnings and Precautions

Read the Instructions for Use carefully before using the product.

- Before first use check the product and its components for:
  - Integrity
  - Completeness with respect to number, type and filling (see chapter 2. Kit Components)
  - Correct labelling
  - Frozenness upon arrival
- Use of this product is limited to personnel specially instructed and trained in the techniques of real-time PCR and *in vitro* diagnostic procedures.
- Specimens should always be treated as infectious and/or biohazardous in accordance with safe laboratory procedures.
- Wear protective disposable powder-free gloves, a laboratory coat and eye protection when handling specimens.
- Avoid microbial and nuclease (DNase/RNase) contamination of the specimens and the components of the kit.
- Always use DNase/RNase-free disposable pipette tips with aerosol barriers.
- Always wear protective disposable powder-free gloves when handling kit components.
- Use separated and segregated working areas for (i) sample preparation, (ii) reaction setup and (iii) amplification/detection activities. The workflow in the laboratory should proceed in unidirectional manner. Always wear disposable gloves in each area and change them before entering a different area.
- Dedicate supplies and equipment to the separate working areas and do not move them from one area to another.
- Store positive and/or potentially positive material separated from all other components of the kit.
- Do not open the reaction tubes/plates post amplification, to avoid contamination with amplicons.

- Additional controls may be tested according to guidelines or requirements of local, state and/or federal regulations or accrediting organizations.
- Do not autoclave reaction tubes after the PCR, since this will not degrade the amplified nucleic acid and will bear the risk to contaminate the laboratory area.
- Do not use components of the kit that have passed their expiration date.
- Discard sample and assay waste according to your local safety regulations.

8. Procedure

8.1 Specimen Collection, Transport and Storage

Blood has to be withdrawn with commercially available standard EDTA blood collection systems (e.g. Sarstedt, Becton Dickinson, Greiner or equivalent). Tubes should be mixed directly after sample collection. The blood samples should be shipped cooled (2–8°C). Transport should occur following the local and national instructions for the transport of biological material.

For generation of EDTA plasma, EDTA whole blood should be centrifuged according to the instructions provided by the manufacturer of the collection system within 24 hours after collection. EDTA plasma and EDTA whole blood should be stored at 2–8°C for no longer than 14 days (Abdul-Ali et al. 2011).

8.2 Sample Preparation

DNA extracted from human EDTA plasma or EDTA whole blood is the starting material for the RealStar® CMV PCR Kit 1.0.

The quality of the extracted DNA has a profound impact on the performance of the entire test system. It has to be ensured that the system used for nucleic acid extraction is compatible with real-time PCR technology.

For **EDTA plasma** the following nucleic acid extraction methods were validated for use with the RealStar® CMV PCR Kit 1.0:

- QIAamp® MinElute® Virus Spin Kit (QIAGEN)
- VERSANT® kPCR Molecular System SP in combination with the VERSANT® Sample Preparation 1.2 Reagents Kit (Siemens Healthcare)

In order to increase the sensitivity of the system, the protocol of the QIAamp® MinElute® Virus Spin Kit (QIAGEN) can be modified according to the specifications listed in Table 3: Adaptations of the QIAamp® MinElute® Virus Spin Kit (QIAGEN) protocol (see chapter 11.1.2 Analytical Sensitivity for EDTA Plasma Samples).

If using a spin column based sample preparation procedure including washing buffers containing ethanol, it is highly recommended to perform an additional centrifugation step for 10 min at approximately 17000 x g (~ 13000 rpm), using a new collection tube, prior to the elution of the nucleic acid.

For **EDTA whole blood** the following nucleic acid extraction method was validated for use with the RealStar® CMV PCR Kit 1.0:

- VERSANT® kPCR Molecular System SP in combination with VERSANT® Sample Preparation 1.2 Reagents Kit (Siemens Healthcare)

For EDTA whole blood samples the sample preparation protocol for the VERSANT® Sample Preparation 1.2 Reagents (SMN 10629800 and 10629801) should be modified as follows: EDTA whole blood samples should be mixed with PRE buffer in a 1:1 ratio (350 µl + 350 µl) rather than the ratio stated in the instructions for use.

CAUTION	
	***If your sample preparation system is using washing buffers containing ethanol, make sure to eliminate any traces of ethanol prior to elution of the nucleic acid. Ethanol is a strong inhibitor of real-time PCR.***

CAUTION

The use of carrier RNA is crucial for extraction efficiency and stability of the extracted nucleic acid.

For additional information and technical support regarding pre-treatment and sample preparation please contact our Technical Support (see chapter 14. Technical Assistance).

8.3 Master Mix Setup

All reagents and samples should be thawed completely, mixed (by pipetting or gentle vortexing) and centrifuged briefly before use.

The RealStar® CMV PCR Kit 1.0 contains a heterologous Internal Control (IC), which can either be used as a PCR inhibition control or as a control of the sample preparation procedure (nucleic acid extraction) and as a PCR inhibition control.

► If the IC is used as a PCR inhibition control, but not as a control for the sample preparation procedure, set up the Master Mix according to the following pipetting scheme:

Number of Reactions (rxns)	1	12
Master A	5 µl	60 µl
Master B	15 µl	180 µl
Internal Control	1 µl	12 µl
Volume Master Mix	**21 µl**	**252 µl**

► If the IC is used as a control for the sample preparation procedure and as a PCR inhibition control, add the IC during the nucleic acid extraction procedure.

► No matter which method/system is used for nucleic acid extraction, the IC **must not** be added directly to the specimen. The IC should always be added to the specimen/lysis buffer mixture. The volume of the IC which has to be added, always and only depends on the elution volume. It represents 10% of the elution volume. For instance, if the nucleic acid is going to be eluted in 60 µl of elution buffer or water, 6 µl of IC per sample must be added into the specimen/lysis buffer mixture.

► If the IC was added during the sample preparation procedure, set up the Master Mix according to the following pipetting scheme:

Number of Reactions (rxns)	1	12
Master A	5 µl	60 µl
Master B	15 µl	180 µl
Volume Master Mix	**20 µl**	**240 µl**

CAUTION

If the IC (Internal Control) was added during the sample preparation procedure, at least the negative control must include the IC.

No matter which method/system is used for nucleic acid extraction, never add the IC directly to the specimen.

8.4 Reaction Setup

- ► Pipette 20 µl of the Master Mix into each required well of an appropriate optical 96-well reaction plate or an appropriate optical reaction tube.
- ► Add 10 µl of the sample (eluate from the nucleic acid extraction) or 10 µl of the controls (Quantification Standard, Positive or Negative Control).

Reaction Setup	
Master Mix	20 µl
Sample or Control	10 µl
Total Volume	**30 µl**

- ► Make sure that at least one Positive (QS) and one Negative Control is used per run.
- ► For quantification purposes all Quantification Standards (QS1 to QS4) should be used.
- ► Thoroughly mix the samples and controls with the Master Mix by pipetting up and down.
- ► Close the 96-well reaction plate with appropriate lids or optical adhesive film and the reaction tubes with appropriate lids.
- ► Centrifuge the 96-well reaction plate in a centrifuge with a microtiter plate rotor for 30 seconds at approximately 1000 x g (~ 3000 rpm).

9. Programming the Real-Time PCR Instrument

For basic information regarding the setup and programming of the different real-time PCR instruments, please refer to the user manual of the respective instrument.

For detailed programming instructions regarding the use of the RealStar® CMV PCR Kit 1.0 on specific real-time PCR instruments please contact our Technical Support (see chapter 14. Technical Assistance).

9.1 Settings

► Define the following settings:

Settings	
Reaction Volume	30 µl
Ramp Rate	Default
Passive Reference	ROX™

9.2 Fluorescence Detectors (Dyes)

► Define the fluorescence detectors (dyes):

Target	Detector Name	Reporter	Quencher
CMV specific DNA	CMV	FAM™	(None)
Internal Control (IC)	IC	JOE™	(None)

9.3 Temperature Profile and Dye Acquisition

► Define the temperature profile and dye acquisition:

	Stage	Cycle Repeats	Acquisition	Temperature [°C]	Time [min:sec]
Denaturation	Hold	1	-	95	10:00
Amplification	Cycling	45	-	95	00:15
			yes	58	01:00

10. Data Analysis

For basic information regarding data analysis on specific real-time PCR instruments, please refer to the user manual of the respective instrument.

For detailed instructions regarding the analysis of the data generated with the RealStar® CMV PCR Kit 1.0 on different real-time PCR instruments please contact our Technical Support (see chapter 14. Technical Assistance).

10.1 Validity of Diagnostic Test Runs

10.1.1 Valid Diagnostic Test Run (qualitative)

A **qualitative** diagnostic test run is **valid**, if the following control conditions are met:

Control ID	Detection Channel	
	FAM™	JOE™
Positive Control (QS)	+	+/-*
Negative Control	-	+

* The presence or absence of a signal in the JOE™ channel is not relevant for the validity of the test run.

10.1.2 Invalid Diagnostic Test Run (qualitative)

A **qualitative** diagnostic test run is **invalid**, (i) if the run has not been completed or (ii) if any of the control conditions for a **valid** diagnostic test run are not met.

In case of an **invalid** diagnostic test run, repeat testing by using the remaining purified nucleic acids or start from the original samples again.

10.1.3 Valid Diagnostic Test Run (quantitative)

A **quantitative** diagnostic test run is **valid**, if all control conditions for a **valid qualitative** diagnostic test run are met [see chapter 10.1.1 Valid Diagnostic Test Run (qualitative)]. The **quantification** results are **valid** if the generated **standard curve** reaches the following control parameter value:

Control Parameter	Valid Value
R square (R^2)	> 0.98

NOTE

Not all real-time PCR instruments display the R square (R^2) value. For detailed information, please refer to the user manual of the respective instrument.

10.1.4 Invalid Diagnostic Test Run (quantitative)

A **quantitative** diagnostic test run is **invalid**, (i) if the run has not been completed or (ii) if any of the control conditions for a **valid quantitative** diagnostic test run are not met.

In case of an **invalid** diagnostic test run, repeat testing by using the remaining purified nucleic acids or start from the original samples again.

10.2 Interpretation of Results

10.2.1 Qualitative Analysis

Detection Channel		Result Interpretation
FAM™	JOE™	
+	+*	CMV specific DNA detected.
-	+	No CMV specific DNA detected. Sample does not contain detectable amounts of CMV specific DNA.
-	-	PCR inhibition or reagent failure. Repeat testing from original sample or collect and test a new sample.

* Detection of the Internal Control in the JOE™ detection channel is not required for positive results in the FAM™ detection channel. A high CMV DNA load in the sample can lead to a reduced or absent Internal Control signal.

A CMV specific positive result can be expected with a positivity rate of 95%, if the analysed sample contains at least 265 IU of CMV per ml EDTA plasma [95% confidence interval: 50 – 2278 IU/ml] and 835 IU of CMV per ml EDTA whole blood [90% confidence interval: 614 to 1274 IU/ml].

As with any diagnostic test, results obtained with the RealStar® CMV PCR Kit 1.0 should be interpreted in consideration of all clinical and laboratory findings.

10.2.2 Quantitative Analysis

The RealStar® CMV PCR Kit 1.0 includes four Quantification Standards (QS). In order to generate a **standard curve** for quantitative analysis, these have to be defined as **standards** with appropriate concentrations (see chapter 6. Product Description). Using **standards** of known concentrations a standard curve for quantitative analysis can be generated.

$$C_t = m \cdot \log (N_0) + b$$

C_t = Threshold Cycle
m = Slope
N_0 = Initial Concentration
b = Intercept

Derived from the standard curve positive samples of unknown concentrations can be quantified.

$$N_0 = 10^{(C_t - b)/m}$$

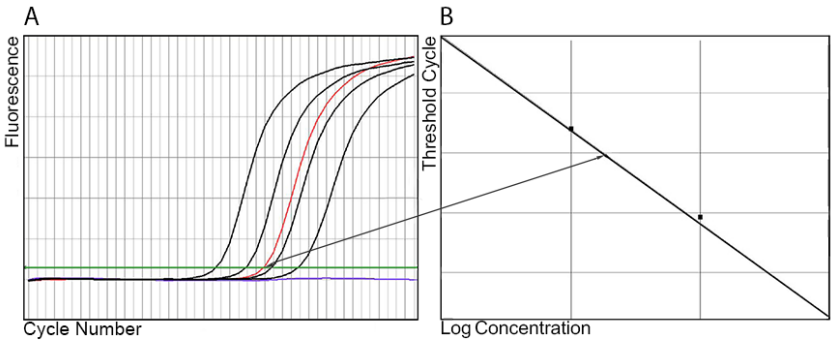

Figure 1: Quantification Standards (black), a positive (red) and a negative sample (blue) displayed in the Amplification Plot [**A**] and Standard Curve analysis [**B**]

NOTE

The concentration of the "Sample" is displayed in IU/µl and refers to the concentration in the eluate.

To determine the **viral load of the original sample**, the following formula has to be applied:

$$\text{Viral load (}\textbf{Sample}\text{) [IU/ml]} = \frac{\text{Volume (}\textbf{Eluate}\text{) [µl]} \cdot \text{Viral load (}\textbf{Eluate}\text{) [IU/µl]}}{\text{Sample Input [ml]}}$$

11. Performance Evaluation

The analytical performance evaluation without a selected nucleic acid extraction method was done using quantified CMV specific DNA. The analytical performance evaluation with selected nucleic acid extraction methods was done using the 1st WHO International Standard for Human Cytomegalovirus for Nucleic Acid Amplification Techniques (NIBSC code: 09/162).

11.1 Analytical Sensitivity

The analytical sensitivity (Limit of Detection - LoD) of the RealStar® CMV PCR Kit 1.0 is defined as the concentration of CMV DNA molecules that can be detected with a positivity rate of 95%. The analytical sensitivity was determined with and without a selected nucleic acid extraction method.

11.1.1 Analytical Sensitivity excluding Nucleic Acid Extraction

A dilution series of CMV DNA was set up from 1.21 IU/µl to nominal 0.0004 IU/µl and analysed using the RealStar® CMV PCR Kit 1.0 in combination with the following real-time PCR instruments:

- Mx 3005P™ QPCR System (Stratagene)
- ABI Prism® 7500 Fast SDS (Applied Biosystems)
- LightCycler® 480 Instrument II (Roche)
- Rotor-Gene® 6000 (Corbett Research)/Rotor-Gene® Q 5/6 plex Plattform (QIAGEN)
- CFX96™ Real-Time PCR Detection System (Bio-Rad)
- CFX96™ Deep Well Real-Time PCR Detection System (Bio-Rad)

Testing was carried out on two days with at least eight replicates per concentration at a time. The results were determined by probit analysis.

Table 1: PCR results used for the calculation of the analytical sensitivity [Mx 3005P™ QPCR System (Stratagene)]

Input Conc. [IU/µl]	Number of Replicates	Number of Positives	Hit Rate [%]
1.2100	16	16	100
0.3826	16	16	100
0.1210	16	13	81
0.0383	16	4	25
0.0121	16	2	13
0.0038	16	0	0
0.0012	16	0	0
0.0004	16	0	0

Table 2: Analytical sensitivity determined by probit analysis using different real-time PCR instruments

Real-time PCR Instrument	Limit of Detection [95%]	Confidence Interval [95%]
ABI Prism® 7500 Fast SDS	0.668 IU/µl	0.323 - 2.258 IU/µl
Rotor-Gene® 6000/Q 5/6 plex	0.249 IU/µl	0.160 - 0.644 IU/µl
LightCycler® 480 Instrument II	0.238 IU/µl	0.149 - 0.624 IU/µl
Mx 3005P™ QPCR System	0.257 IU/µl	0.150 - 0.704 IU/µl
CFX96™ Deep Well Real-Time PCR Detection System	0.943 IU/µl	0.083 - 16.884 IU/µl
CFX96™ Real-Time PCR Detection System	0.702 IU/µl	0.396 - 1.985 IU/µl

11.1.2 Analytical Sensitivity for EDTA Plasma Samples

The analytical sensitivity in consideration of a selected nucleic acid extraction method for EDTA plasma samples was determined using a dilution series of the 1st WHO International Standard for Human Cytomegalovirus for Nucleic Acid Amplification Techniques (NIBSC code: 09/162) ranging from 316 IU/ml to nominal 0.03 IU/ml in CMV negative EDTA plasma.

On two days eight aliquots per concentration at a time were subjected to nucleic acid extraction using the QIAamp® MinElute® Virus Spin Kit (QIAGEN). The protocol of the QIAamp® MinElute® Virus Spin Kit (QIAGEN) was adapted according to the following table.

Table 3: Adaptations of the QIAamp® MinElute® Virus Spin Kit (QIAGEN) protocol

	QIAGEN Protocol [µl]	Adaptation [µl]
Sample	200	400
Protease	25	50
Lysisbuffer (AL)	200	400
Ethanol[1] (abs.)	250	500
Washbuffer (AW1)	500	700
Washbuffer (AW2)	500	700
Ethanol[2] (abs.)	500	700

[1] added to the Sample/Lysisbuffer Mix

[2] Washstep 3

Each eluate was analysed using the RealStar® CMV PCR Kit 1.0 in combination with the following real-time PCR instruments:

- Mx 3005P™ QPCR System (Stratagene)
- ABI Prism® 7500 Fast SDS (Applied Biosystems)
- LightCycler® 480 Instrument II (Roche)
- Rotor-Gene® 6000 (Corbett Research) / Rotor-Gene® Q 5/6 plex Platform (QIAGEN)
- CFX96™ Real-Time PCR Detection System (Bio-Rad)
- CFX96™ Deep Well Real-Time PCR Detection System (Bio-Rad)

The results were determined by probit analysis.

Table 4: PCR results used for the calculation of the analytical sensitivity for EDTA plasma samples [LightCycler® 480 Instrument II (Roche)]

Input Conc. [IU/ml]	Number of Replicates	Number of Positives	Hit Rate [%]
316.23	16	16	100
100.00	16	15	94
31.62	16	13	81
10.00	16	6	38
3.16	16	3	19
1.00	16	0	0
0.32	16	0	0
0.10	16	0	0
0.03	16	0	0

Table 5: Analytical sensitivity for EDTA plasma samples determined by probit analysis using different real-time PCR instruments

Real-time PCR Instrument	Limit of Detection [95%]	Confidence Interval [95%]
ABI Prism® 7500 Fast SDS	92.10 IU/ml	46.94 - 288.29 IU/ml
Rotor-Gene® 6000 / Q 5/6 plex	106.29 IU/ml	51.05 - 356.08 IU/ml
LightCycler® 480 Instrument II	91.38 IU/ml	49.16 - 271.13 IU/ml
Mx 3005P™ QPCR System	85.14 IU/ml	45.09 - 256.13 IU/ml
CFX96™ Deep Well Real-Time PCR Detection System	116.32 IU/ml	59.67 - 357.43 IU/ml
CFX96™ Real-Time PCR Detection System	264.98 IU/ml	49.54 - 2278.24 IU/ml

11.1.3 Analytical Sensitivity for EDTA Whole Blood Samples

The analytical sensitivity in consideration of a selected nucleic acid extraction method for EDTA whole blood samples was determined using a dilution series of the 1st WHO International Standard for Human Cytomegalovirus for Nucleic Acid Amplification Techniques (NIBSC code: 09/162) ranging from 10000 IU/ml to nominal 20 IU/ml in CMV negative EDTA whole blood.

In three independent runs eight aliquots per concentration at a time were subjected to nucleic acid extraction using the VERSANT® kPCR Molecular System SP in combination with the VERSANT® Sample Preparation 1.2 Reagents Kit (Siemens Healthcare).

The results were determined by probit analysis.

Table 6: PCR results used for the calculation of the analytical sensitivity for EDTA whole blood samples [VERSANT® kPCR Molecular System AD (Siemens)]

Input Conc. [IU/ml]	Number of Replicates	Number of Positives	Hit Rate [%]
10000	24	24	100
3162	24	24	100
1500	24	24	100
1000	24	24	100
750	24	22	92
500	24	20	83
250	24	16	67
100	24	10	42
20	22	1	5

Table 7: Analytical sensitivity for EDTA whole blood samples determined by probit analysis using the VERSANT® kPCR Molecular System AD (Siemens)

Real-time PCR Instrument	Limit of Detection [95%]	Confidence Interval [90%]
VERSANT® kPCR Molecular System AD	835 IU/ml	614 - 1274 IU/ml

11.2 Analytical Specificity

The analytical specificity of the RealStar® CMV PCR Kit 1.0 is ensured by the thorough selection of the oligonucleotides (primers and probes). The oligonucleotides were checked by sequence comparison analysis against publicly available sequences to ensure that all relevant CMV genotypes will be detected.

Over a hundred different CMV negative EDTA plasma and over thirty different CMV negative EDTA whole blood specimens were analysed with the RealStar® CMV PCR Kit 1.0. None of these showed a positive CMV specific signal, but all showed a valid IC signal. In addition, the specificity of the RealStar® CMV PCR Kit 1.0 was evaluated by testing a panel of genomic DNA/RNA extracted from other herpesviruses and other pathogens significant in immunocompromised patients.

The RealStar® CMV PCR Kit 1.0 did not cross-react with any of the following pathogens:

- Herpes simplex virus 1
- Herpes simplex virus 2
- Varicella-zoster virus
- Epstein-Barr virus
- Human herpesvirus 6A
- Human herpesvirus 6B
- Human herpesvirus 7
- Human herpesvirus 8
- Human parvovirus B19
- BK virus
- JC virus
- Simian virus 40
- Hepatitis A virus
- Hepatitis B virus
- Hepatitis C virus
- Human immunodeficiency virus 1

11.3 Linear Range

The linear range of the RealStar® CMV PCR Kit 1.0 was evaluated by analysing a logarithmic dilution series of CMV specific DNA (concentrations ranging from 1.21E+09 to 1.21E+00 IU/µl) using the following real-time PCR instruments:

- Mx 3005P™ QPCR System (Stratagene)
- ABI Prism® 7500 Fast SDS (Applied Biosystems)
- LightCycler® 480 Instrument II (Roche)
- Rotor-Gene® 6000 (Corbett Research)/Rotor-Gene® Q 5/6 plex Plattform (QIAGEN)
- CFX96™ Real-Time PCR Detection System (Bio-Rad)
- CFX96™ Deep Well Real-Time PCR Detection System (Bio-Rad)

Each concentration was analysed in eight replicates per real-time PCR instrument. The linear range of the RealStar® CMV PCR Kit 1.0 extends over an interval of at least nine orders of magnitude on all real-time PCR instruments used.

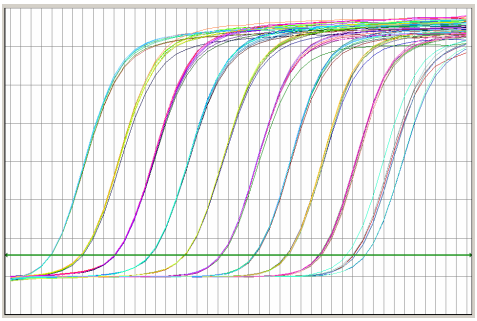

Figure 2: Amplification curves on the ABI Prism® 7500 Fast SDS (Applied Biosystems)

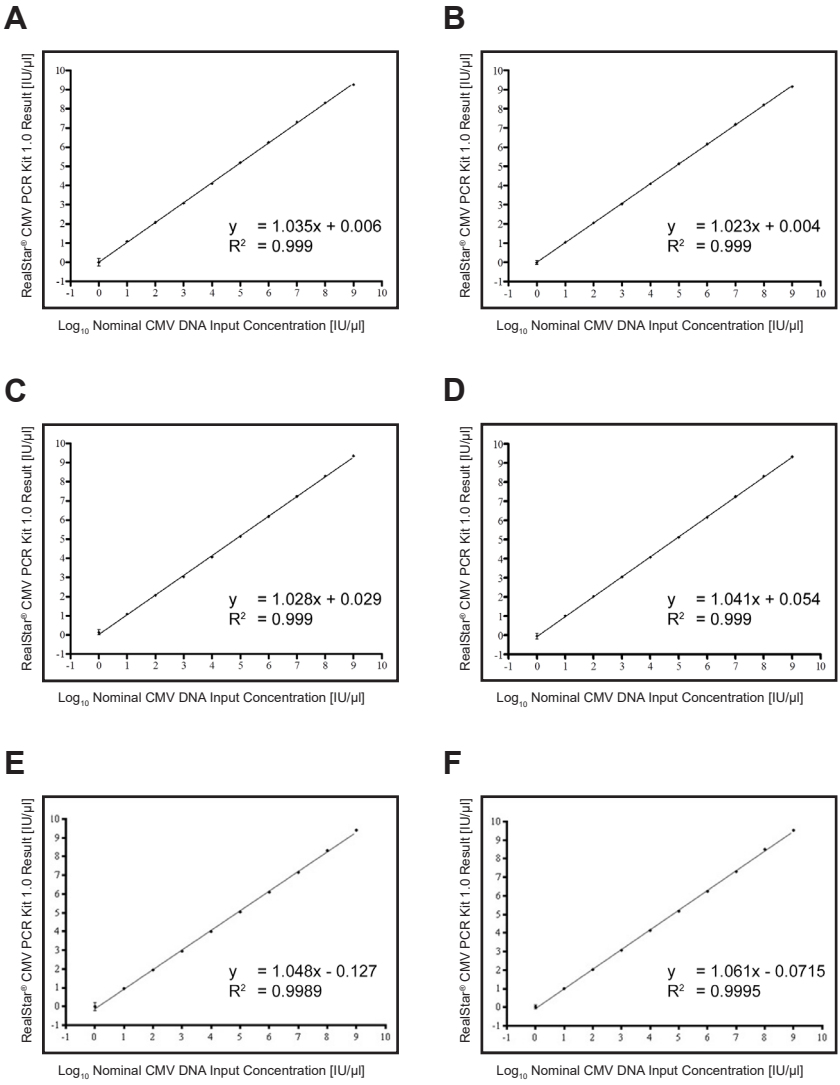

Figure 3: Linear regression of the analysed dilution series on the ABI Prism® 7500 Fast SDS (Applied Biosystems) [**A**], the Mx 3005P™ QPCR System (Stratagene) [**B**], the LightCycler® Instrument 480 II (Roche) [**C**], the Rotor-Gene™ 6000 (Corbett Research) / Rotor-Gene™ Q 5/6 plex Platform (QIAGEN) [**D**], the CFX96™ Real-Time PCR Detection System (Bio-Rad) [**E**], and the CFX96™ Deep Well Real-Time PCR Detection System (Bio-Rad) [**F**].

11.4 Precision

Precision of the RealStar® CMV PCR Kit 1.0 was determined as intra-assay variability (variability within one experiment), inter-assay variability (variability between different experiments) and inter-lot variability (variability between different production lots). Total variability was calculated by combining the three analyses.

Variability data are expressed in terms of the coefficient of variation of the total variability. The data are based on quantification analysis of a high positive control (121 IU/µl) and on threshold cycle (C_t) value in terms of a low positive control (1.8 IU/µl) and the Internal Control (IC). At least eight replicates per sample were analysed.

Table 8: Precision in terms of Coefficient of Variation of the Total Variability using different real-time PCR instruments

Real-time PCR Instrument	Total Variability / Coefficient of Variation [%]		
	High Positive Control	Low Positive Control	Internal Control
ABI Prism® 7500 Fast SDS	4.89	1.49	0.91
LightCycler® 480 Instrument II	6.62	1.13	0.12
Rotor-Gene® 6000 / Q 5/6 plex	10.79	4.20	0.65
Mx 3005P™ QPCR System	19.77	1.22	0.60
CFX96™ Deep Well Real-Time PCR Detection System	5.33	1.85	1.74
CFX96™ Real-Time PCR Detection System	7.28	1.59	3.36

11.5 Diagnostic Evaluation

11.5.1 Specimen Type: EDTA Plasma

The RealStar® CMV PCR Kit 1.0 was evaluated in a comparative study with the CE marked Abbott RealTime CMV kit from Abbott Diagnostics.

124 EDTA plasma specimens sent in for routine CMV testing were handled using the m2000sp nucleic acid extraction system (Abbott Diagnostics) and analysed with the CE marked Abbott RealTime CMV kit on a m2000rt instrument (Abbott Diagnostics). The DNA eluates were stored at -20°C and reanalysed using the RealStar® CMV PCR Kit 1.0 on a m2000rt Instrument.

Table 9: Results of the evaluation of the diagnostic sensitivity and specificty

		RealStar® CMV PCR Kit 1.0	
		+	-
Abbott RealTime CMV	+	103	1
	-	0	20

The diagnostic sensitivity and specificity of the RealStar® CMV PCR Kit 1.0 compared to the Abbott RealTime CMV test was 99.04% and 100%, respectively.

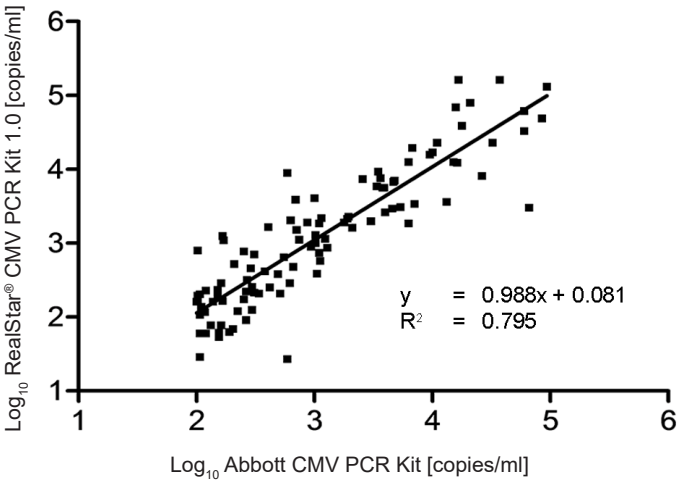

Figure 4: Correlation of the quantification results between the RealStar® CMV PCR Kit 1.0 and the Abbott RealTime CMV test (n=102).

There was a good correlation between the quantitative results of the RealStar® CMV PCR Kit 1.0 and of the Abbott RealTime CMV kit. No systematic or proportional differences between the two methods were identified.

11.5.2 Specimen Type: EDTA Whole Blood

The RealStar® CMV PCR Kit 1.0 was evaluated in a comparative study with the CE marked Q - CMV Real Time Complete Kit from ELITech Molecular Diagnostics.

90 CMV positive EDTA whole blood specimens from routine CMV monitoring were tested in parallel using the NucliSENS® easyMAG® (Biomerieux) nucleic acid extraction system with the CE marked Q - CMV Real Time Complete Kit on the ABI 7300 Real Time PCR System (Applied Biosystems) and using the kPCR Molecular System (Siemens) with the RealStar® CMV PCR Kit 1.0. For the quantitative correlation all samples tested negative with one or both of the assays as well as two outliers were removed from the analysis. Results from the remaining 71 samples were used for further analysis. Quantitative correlation of the results was determined by Passing-Bablok regression analysis.

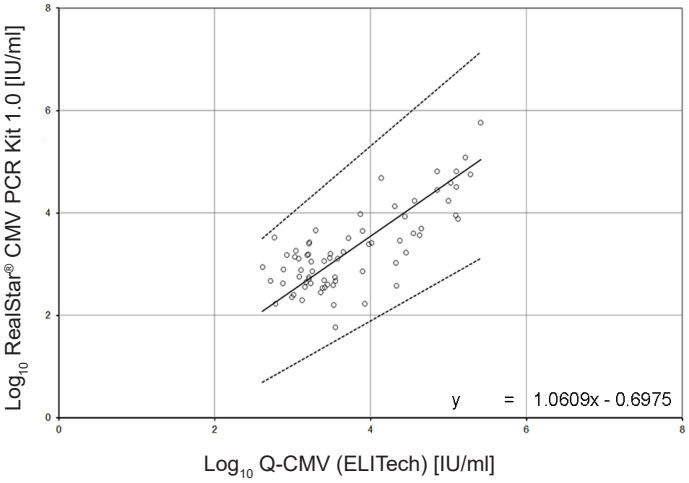

Figure 5: Correlation of the quantification results between the RealStar® CMV PCR Kit 1.0 and the Q-CMV Real Time Complete Kit from ELITech Molecular Diagnostics

There was a good correlation between the quantitative results of the RealStar® CMV PCR Kit 1.0 and the Q - CMV Real Time Complete Kit. No systematic or proportional differences between the two methods were identified.

12. Limitations

- Strict compliance with the Instructions for Use is required for optimal results.
- Use of this product is limited to personnel specially instructed and trained in the techniques of real-time PCR and *in vitro* diagnostic procedures.
- Good laboratory practice is essential for proper performance of this assay. Extreme care should be taken to preserve the purity of the components of the kit and reaction setups. All reagents should be closely monitored for impurity and contamination. Any suspicious reagents should be discarded.
- Appropriate specimen collection, transport, storage and processing procedures are required for the optimal performance of this test.
- This assay must not be used on the specimen directly. Appropriate nucleic acid extraction methods have to be conducted prior to using this assay.
- The presence of PCR inhibitors (e.g. heparin) may cause underquantification, false negative or invalid results.
- Potential mutations within the target regions of the CMV genome covered by the primers and/or probes used in the kit may result in underquantification and/or failure to detect the presence of the pathogens.
- As with any diagnostic test, results of the RealStar® CMV PCR Kit 1.0 need to be interpreted in consideration of all clinical and laboratory findings.

13. Quality Control

In accordance with the altona Diagnostics GmbH EN ISO 13485-certified Quality Management System, each lot of RealStar® CMV PCR Kit 1.0 is tested against predetermined specifications to ensure consistent product quality.

14. Technical Assistance

For technical advice, please contact our Technical Support:

e-mail: **support@altona-diagnostics.com**

phone: **+49-(0)40-5480676-0**

15. Literature

[1] 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.

[2] Pellett PE, Roizman B. Herpesviridae. In: Knipe DM, Howley PM, et al., eds. Fields Virology, 6th ed., Lippincott Williams & Willkins, Philadelphia. 2013:1803-1822.

[3] Mocarski, Jr ES, Shenk T, Griffiths PD, Pass RF. Cytomegaloviruses. In: Knipe DM, Howley PM, et al., eds. Fields Virology, 6th ed., Lippincott Williams & Willkins, Philadelphia. 2013:1961-2014.

[4] Hodinka RL. Human Cytomegalovirus. In: Versalovic J, Carroll KC, Funke G, Jorgensen JH, Landry ML, Warnock DW, eds. Manual of Clinical Microbiology, 10th ed., American Society for Microbiology, Washington. 2011:1558-1574.

[5] Abdul-Ali D, Kraft CS, Ingersoll J, Frempong M, Caliendo AM., Cytomegalovirus DNA stability in EDTA anti-coagulated EDTA whole blood and plasma samples., J Clin Virol. 2011 November ; 52(3): 222–224.

16. Trademarks and Disclaimers

RealStar® (altona Diagnostics); ABI Prism® (Applied Biosystems); CFX96™ (Bio-Rad); FAM™, JOE™, ROX™ (Life Technologies); LightCycler® (Roche); Mx 3005P™ (Stratagene); Rotor-Gene®, QIAamp®, MinElute® (QIAGEN); VERSANT® (Siemens Healthcare).

Registered names, trademarks, etc. used in this document, even if not specifically marked as such, are not to be considered unprotected by law.

The RealStar® CMV PCR Kit 1.0 is a CE-marked diagnostic kit according to the European *in vitro* diagnostic directive 98/79/EC.

Product not licensed with Health Canada and not FDA cleared or approved.

Not available in all countries.

17. Explanation of Symbols

Symbol	Explanation
IVD	*In vitro* diagnostic medical device
LOT	Batch code
CAP	Cap color
REF	Product number
CONT	Content
NUM	Number
COMP	Component
GTIN	Global trade identification number
	Consult instructions for use
	Contains sufficient for “n” tests/reactions (rxns)
	Temperature limit
	Use-by date
	Manufacturer
	Caution
	Note
	Version

Notes:

Notes:

Notes:

always a drop ahead.

altona Diagnostics GmbH
Mörkenstr. 12
22767 Hamburg, Germany

phone +49 40 548 0676 0
fax +49 40 548 0676 10
e-mail info@altona-diagnostics.com

www.altona-diagnostics.com