Sacace BIOTECHNOLOGIES

CE 1023

IVD For *inVitro* Diagnostic Use

For Professional Use Only

CMV Real-TM Quant

Handbook

Real Time PCR test for quantitative detection of Cytomegalovirus (CMV)

REF V7-100/2FRT

Σ **100**

1. INTENDED USE

CMV Real-TM QuantPCR kitis an *in vitro* nucleic acid amplification test for qualitative detection and quantification of human cytomegalovirus(*CMV*)DNAin the clinical materials (peripheral blood plasma, amniotic fluid, cerebrospinal fluid (liquor), saliva, oropharyngeal swabs, urine samples, bronchoalveolar lavage, whole human blood, white blood cells, and viscera biopsy material) by using real-time hybridization-fluorescence detection.

The results of PCR analysis are taken into account in complex diagnostics of disease.

2. PRINCIPLE OF PCR DETECTION

*CMV*determination by the polymerase chain reaction (PCR) with hybridization fluorescent detection includes three stages: DNA extraction from clinical samples, PCR-amplification of pathogen genome specific region, and real-time hybridization fluorescent detection. DNA is extracted from peripheral blood plasma, amniotic fluid, cerebrospinal fluid (liquor), saliva, oropharyngeal swabs, urine samples, bronchoalveolar lavage, whole human blood, white blood cells, and viscera biopsy material in presence of Internal Control (IC), which allows monitoringof analysis of each sample. Endogenous internal control (IC Glob - β-globin gene DNA) allows monitoringof PCR analysis stages (DNA extraction and PCRamplification), material sampling and storage adequacy. Then, *CMV* DNA is amplified using specific primers and polymerase (TaqF).

β-globin gene DNA is a part of human genome DNA and it should be present in an adequate amount in DNA sample, obtained from the cells. There must be no less than 20 000 genomes per sample (DNA from 10 000 cells). Internal Control (IC), added during the sample preparation from plasma, liquor, amniotic liquid, sputum, bronchial lavages and other cell free or low in DNA content materials, serves as an amplification control for each individually processed specimen and to identify possible reaction inhibition, while endogenous IC (β-globine gene), present in all samples obtained from cells (whole blood, leucocytes, biopsy and autopsy material, saliva, swabs)allows not only to control analysis steps, but also to estimate sample handling and storage.

In real-time PCR, the amplified product is detected using fluorescent dyes. These dyes are linked to oligonucleotide probes which bind specifically to the amplified product during thermocycling. The real-time monitoring of the fluorescence intensities during the real-time PCR allows the detection of accumulating product without re-opening the reaction tubes after the PCR run.**CMV Real-TM Quant** PCR kit uses “hot-start”, which greatly reduces the frequency of nonspecifically primed reactions. “Hot-start” is guaranteed by separation of nucleotides and Taq-polymerase by using a chemically modified polymerase (TaqF). Chemically modified polymerase (TaqF) is activated by heating at 95ºC for 15 min.

3. CONTENT

Reagent	*Description*	*Volume (ml)*	*Quantity*
PCR-mix-1-FRT*CMV*	colorless clear liquid	0.6	2 tubes
PCR-mix-2-FRT	colorless clear liquid	0.3	2 tubes
Polymerase (TaqF)	colorless clear liquid	0.03	2 tubes
RNA-buffer	colorless clear liquid	0.6	1 tube
DNA calibrator QS1	colorless clear liquid	0.2	1 tube
DNA calibrator QS2	colorless clear liquid	0.2	1 tube
RNA-buffer	colorless clear liquid	1.2	1 tube
Negative Control (C–)*	colorless clear liquid	1.2	2tubes
**Positive Control DNA *CMV*/ human DNA ** **	colorless clear liquid	0.1	2 tubes
Internal Control (IC)***	colorless clear liquid	0.6	2 tubes

* *must be used in the extraction procedure as Negative Control of Extraction: add 100 µl of C– (Negative Control) to labeled NCE;*

*** must be used in the extraction procedure as Positive Control of Extraction (PCE): add 90 µl of C– (Negative Control) and 10 µl of DNA CMV/human DNA Rec Pos controlto the tubes labeled PCE;*

****add 10 µl of Internal Control STI-87 during the DNA extraction procedure directly to the sample/lysis mixture (see DNA/RNA Prep **REF** K2-9 and DNA-sorb-B **REF** K-1-1/B protocols).*

4. ADDITIONAL REQUIREMENTS

- DNA extraction kit.
- Disposable powder-free gloves and laboratory coat.
- Automatic adjustable pipettes (from 5 to 20 µl and from 20 to 200 µl).
- Disposable tips with aerosol barriers (100 or 200 µl) in tube racks.
- Tube racks.
- Vortex mixer/desktop centrifuge.
- PCR box.
- Real Time PCR instrument.
- Disposable polypropylene microtubes for PCR or PCR-plate
- Refrigerator for 2–8 °C.
- Deep-freezerfor ≤ –16 °C.
- Waste bin for used tips.

5. GENERAL PRECAUTIONS

IVD **_In Vitro_ Diagnostic Medical Device**
For _In Vitro_ Diagnostic Use Only

The user should always pay attention to the following:

- Use sterile pipette tips with aerosol barriers and use new tip for every procedure.
- Store and handle amplicons away from all other reagents.
- Thaw all components thoroughly at room temperature before starting detection.
- When thawed, mix the components and centrifuge briefly.
- Use disposable gloves, laboratory coats, protect eyes while samples and reagents handling. Thoroughly wash hands afterward.
- Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in laboratory work areas.
- Do not use a kit after its expiration date.
- Dispose of all samples and unused reagents in compliance with local authorities requirements.
- Samples should be considered potentially infectious and handled in a biological cabinet in accordance with appropriate biosafety practices.
- Clean and disinfect all sample or reagent spills using a disinfectant such as 0.5% sodium hypochlorite, or other suitable disinfectant.
- Avoid contact with the skin, eyes, and mucous membranes. If skin, eyes, and mucous membranes contact, immediately flush with water, seek medical attention.
- Material Safety Data Sheets (MSDS) are available on request.
- Use of this product should be limited to personnel trained in the techniques of DNA amplification.
- The laboratory process must be one directional, it should begin in the Extraction Area and then move to the Amplification and Detection Areas. Do not return samples, equipment and reagents to the area in which the previous step was performed.

Some components of this kit contain sodium azide as a preservative. Do not use metal tubing for reagent transfer.

Sampling of biological materials for PCR-analysis, transportation, and storage are described in details in the handbook of the manufacturer. It is recommended that this handbook is read before beginning of the work.

6. SAMPLING AND HANDLING

CMV Real-TM Quant can analyze DNA extractedfrom:

- *Plasma*:
  - EDTA tubes may be used with the **CMV Real-TM Quant**. Follow sample tube manufacturer's instructions.
  - Whole blood collected in EDTA should be separated into plasma and cellular components by centrifugation at 800-1600 x g for 20 min within six hours. The isolated plasma has to be transferred into a sterile polypropylene tube. Plasma may be stored at 2-8°C for an additional 3 days. Alternatively, plasma may be stored at -18°C for up to one month or 1 year when stored at -70°C.
  - Do not freeze whole blood.
  - Specimens anti-coagulated with heparin are unsuitable for this test.
  - Thaw frozen specimens at room temperature before using.
  - Whole blood must be transported at 2-25°C and processed within 6 hours of collection. Plasma may be transported at 2-8°C or frozen.
- *Liquor (CSF)* collected in the sterile "Eppendorf" tube:
  - Liquor may be stored at 2-8°C for 1 days. Alternatively, may be stored at -18°C for up to one month or 1 year when stored at -70°C
- *Amnioticliquid* collected in the sterile "Eppendorf" tube:
  - centrifuge 1,0 ml of sample at 8000-9000 x g for 10 min. Discard the supernatant and leave about 200 µl of solution. Vortex the the tube and use 100 µl for DNA extraction.
- *White blood cells.* Collect 2.5-10 ml blood samples according to standard procedures in tubes containing anticoagulant (recommended anticoagulant is EDTA). Centrifuge samples at ~1500-2000 *X* g for 10-15 min. This will separate the blood into an upper plasma layer, a lower red blood cell (RBC) layer, and a thin interface containing the WBCs, also called the buffy coat. Remove the plasma with a transfer pipet, being careful not to disturb the WBCs. Samples with exceptionally high WBC counts will have a thicker buffy coat. Use transfer pipet to carefully aspirate the exposed WBC layer in a volume of about 0.5 ml or less. Aspirate slowly, using a circular motion, to pull all the visible buffy coat material into the transfer pipet. Some contamination of the WBCs with the underlying RBCs is expected.

Add 300 µl of Solution for Lysis to the tube with the obtained leukocyte sample (for **DNA/RNA-Prep** protocol).

Specimens can be stored at +2-8°C for no longer than 12 hours, or freeze at -20°C to -80°C. Transportation of clinical specimens must comply with country, federal, state and local regulations for the transport of etiologic agents.

7. WORKING CONDITIONS

CMV Real-TM QuantPCR kitshould be used at 18–25 °C.

8. PROTOCOL

8.1. DNA Extraction

Any commercial RNA/DNA isolation kit, if IVD-CE validated for the specimen types indicated herein at the “SAMPLING AND HANDLING” paragraph, could be used.
Sacace Biotechnologies recommends to use the following kits:

- DNA/RNA-prep, **REF** K-2-9;
- DNA-sorb-B, **REF** K-1-1/B;
- SaMag Viral Nucleic Acids Extraction Kit, **REF** SM003 (for plasma, liquor).

Extract DNA according to the manufacturer’s instructions.

DNA is extracted from each clinical sample in the presence of internal control sample, **Internal Control IC** (**10µl** of **IC** is added to each sample). Transfer **100µl** of **Negative Control** to the tube labeled C–. Transfer **90µl** of **Negative Control** and**10µl** of **Pos DNA *CMV*/human DNA** to the tube labeled PCE.

8.2. Preparing PCR

The total reaction volume is **25 µl**, the volume of DNA sample is **10µl**.

8.2.1 Preparing tubes for PCR

1. Prepare the mixture of **PCR-mix-2-FRT** and **polymerase (TaqF)**. For this purpose transfer the content of the tube with **polymerase (TaqF) (30 µl)** into the tube with **PCR-mix-2-FRT(300 µl)** and mix by vortexing without foam forming.

The prepared mixture is intended for 60 samples analysis. Mixture is to be stored at the temperature between 2 ºC and 8 ºC for 3 months. Use when needed.

If the mixture can’t be used up for 3 months it’s necessary to prepare mixture for smaller number of reactions. For example, mix **150 µl of PCR-mix-2-FRT** and**15 µl of polymerase (TaqF).** The obtained mixture is intended for 30 reactions.

2. Prepare the reaction mixture. Note that for analysis of even one clinical DNA sample in the qualitative format, it is necessary to run two controls of PCR amplification stage: positive control (DNA calibrator QS2) and negative control of amplification (RNA-buffer). For analysis of even one clinical DNA sample in the quantitative format, it is necessary to run five controls of PCR amplification stage: two calibrators (QS1 and QS2) in two replicates and the negative control of amplification (RNA-buffer). In addition, you should take reagents for one extra reaction.

3. Mix**PCR-mix-1-FL*CMV*** and the mixture of **PCR-mix-2-FRT** and **polymerase (TaqF)**prepared before in a new tube in the following proportion:
   - **10 µl of PCR-mix-1-FRT*CMV*,**
   - **5 µl of PCR-mix-2-FRTandpolymerase (TaqF).**

   Calculate the required number of reactions with allowance forthe clinical and control samples. See Appendix 1.

If 60 samples are analyzed simultaneously, you can use a simplified version of mixture preparation: transfer the content of one tube with PCR-mix-2-Flu and the content of one tube with polymerase (TaqF) to the tube with PCR-mix-1-FL *CMV* screen/monitor.

4. Take the required quantity of tubes for amplification of clinical and control DNA samples. Transfer **15 µl** of the prepared mixture to each tube.
5. Add **10 µl** of **DNA**obtained from clinical or control samples to the tubes with the reaction mixture.
6. <u>For qualitative analysis:</u>

NCA - Add **10 µl** of **RNA-buffer** to the tube labeled NCA (Negative Control of Amplification).

C+ - Add **10 µl** of DNA calibratorQS2 to the tube labeled C+ (Positive Control of Amplification).

<u>For quantitative analysis:</u>

NCA - Add **10 µl** of **RNA-buffer** to the tube labeled NCA (Negative Control of Amplification).

Standards QS1&QS2 - Add **10 µl** ofQS1to two tubes and **10 µl** ofQS2to two other tubes.

8.2. 2. Amplification

1. Program the thermocycleraccording to **Manufacturer's manual**, **Guidelines** and Tables 1 and 2.
2. Create a temperature profile on your instrument as follows:

Table 1

Programfor rotor-type instruments[1]

Step	***Temperature, °C***	***Time***	***Fluorescence detection***	***Cycle repeats***
Hold	95	15 min	–	1
Cycling 1	95	5 s	–	5
	60	20 s	–	
	72	15 s	–	
Cycling 2	95	5 s	–	40
	60	20 s	FAM/Green, JOE/Yellow, ROX/Orange	
	72	15 s	–	

Table 2

Program for plate-or modular type instruments[2]

Step	***Temperature, °C***	***Time***	***Fluorescence detection***	***Cycle repeats***
1	95	15 min	–	1
2	95	5 s	–	5
	60	20 s	–	
	72	15 s	–	
3	95	5 s	–	40
	60	30 s	FAM, HEX/JOE/Cy3, ROX/Texas Red	
	72	15 s	–	

Fluorescence is detected on the 2nd step **(60°C)**in FAM/Green, HEX/JOE/Cy3 Yellow and ROX/Orange/Texas Redfluorometer channels.

[1]For example, Rotor-Gene 6000/Q (Qiagen), or equivalent.

2 For example, SaCycler-96™ (Sacace),iQ5™ (BioRad); Mx3005P™ (Stratagene), ABI® 7300/7500/StepOne (Applied), SmartCycler® (Cepheid), LineGeneK® (Bioer) or equivalent.

9. DATA ANALYSIS

β-Globin gene DNA (IC Glob) is detected in the FAM/Green channel, *CMV* DNA (Positive Control DNA *CMV* and human DNA) is detected in the JOE/HEX/Yellow channel, Internal Control STI-87 (IC) DNA is detected in the ROX/Orange channel.

If total DNA from cell suspension (whole human blood, white blood cells, viscera biopsy material) is isolated, the results are detected in two channels - β-globin gene DNA (IC Glob) in the FAM/Green channel, *CMV* DNA in the JOE/HEX/Yellow/Cy3 channel.

If total DNA from peripheral blood plasma, amniotic fluid, cerebrospinal fluid (liquor), saliva, oropharyngeal swabs, urine samples, and bronchoalveolar lavage are isolated with internal control sample, the results are detected in two channels: *CMV* DNAis detected in the JOE/Yellow/HEX/Cy3 channel, Internal Control (IC) DNAis detected in the ROX/Orange/Texas Red channel.

Interpretation of results

The results are interpreted by the software of thePCR instrument used by the crossing (or not crossing) of the fluorescence curve with the threshold line.

If total DNA from cell suspension (whole human blood, white blood cells, viscera biopsy material) is extracted, the results are interpreted as follows:

1. The sample is considered to be **positive** for *CMV*DNA if a Ct value in the JOE/Yellow/HEX/Cy3 channel, which does not exceed the Ct value of the positive result (Ct < 38), is defined in the results grid. The fluorescence curve should cross the threshold line in the exponential growth region.
2. For qualitative analysis, the sample is considered to be **negative** for *CMV*DNA if its Ct value is not defined in the results grid (the fluorescence curve does not cross the threshold line) in the JOE/HEX/Yellow/Cy3 channel and the Ct value in the results grid in the FAM/Green channel does not exceed 38. For quantitative analysis, the sample is considered to be negative for *CMV*DNA if its Ct value is not defined in the results grid (the fluorescence curve does not cross the threshold line) in the JOE/HEX/Yellow channel and the quantity of IC Glob DNA is greater than 2000 copies per reaction.
3. For qualitative analysis, the analysis result is considered to be **invalid** if the Ct value is not defined in the results grid (the fluorescence curve does not cross the threshold line) in the JOE/HEX/Yellow channel and the Ct value in the results grid in the FAM/Green channel exceeds the Ct 38. For quantitative analysis, the result of analysis is considered to be invalid if the Ct value is not defined in the results grid (the fluorescence curve does not cross the threshold line) in the JOE/HEX/Yellow/cy3channel and the quantity of IC Glob DNA is less than 2000 copies per reaction. In such cases,PCR analysis of the sample should be repeated.

4. For clinical samples whose Ct values in the JOE/Yellow/HEX/Cy3 channel exceed the boundary Ct value (> 38), the result is considered to be **equivocal**. Such DNA samples should be additionally analyzed in duplicate. If a reproducible positive Ct value is obtained, the result should be considered as **positive**. If Ct values are not reproduced in two replicates, the result is considered as **equivocal**.

   For qualitative analysis, if the Ct value in the FAM/Green channel exceeds 38, the negative result is considered to be **invalid**.

   For quantitative analysis, if the quantity of IC Glob DNA is less than 2000 copies/reaction then the quantitative positive or negative result is considered to be **invalid**.

   Results of analysis are accepted as relevant if the results obtained for positive and negative controls of amplification and the negative control of extraction are correct. For quantitative analysis, the results for C+ should fall into the concentration range indicated in the **Data Sheet CMV Real-TM Quant.**

Results for controls if total DNA was isolated from cell suspension (whole human blood, white blood cells, and viscera biopsy material)

Control	Stage for control	Ct in channel				Interpretation
		FAM/Green		JOE/HEX/Yellow		
		Qualitative format	Quantitative format	Qualitative format	Quantitative format	
NCE	DNA extraction, amplification	Neg	Neg	Neg	Neg	OK
PCE	DNAextraction, amplification	Pos (< boundary value)	Pos (< boundary value)	Pos (< boundary value)	Ct value is in the range indicated in Data Sheet	OK
NCA	Amplification	Neg	Neg	Neg	Neg	OK
C+	Amplification	Pos (< boundary value)	–	Pos (< boundary value)	–	OK
QS1,QS2	Amplification	–	Ct value and calculated concentration aredetermined	–	Ct value and calculated concentration are determined	OK

For quantitative analysis, the concentration in log of *CMV* DNA copies per standard cell quantity (10^5) in control and clinical samples (whole human blood, white blood cells, viscera biopsy material) is calculated by following formula:

$$\log \{ \frac{\textit{CMV} \text{ DNA copies in PCR sample}}{\text{Glob DNA copies in PCR sample}} \times 2*10^5 \} = \log \{ \textit{CMV} \text{ DNA copies}/10^5 \text{ cells} \}.$$

To express relative *CMV* DNA concentration in copies per the standard cell quantity (e.g., for 10^5), use the following recalculation ratio:

$$10^5 \text{ cells} = 2*10^5 \text{ human genomic equivalents}$$

If total DNA from peripheral blood plasma, amniotic fluid, cerebrospinal fluid (liquor), saliva, oropharyngeal swabs, urine samples, and bronchoalveolar lavage is isolated together with the internal control sample, the results are interpreted as follows:

1. The sample is considered to be **positive** for *CMV*DNA if a Ct value in the JOE/Yellow/HEX/Cy3 channel, which does not exceed the Ct value of the positive result (< 38), is defined in the results grid. The fluorescence curve should cross the threshold line in the exponential growth region.
2. The sample is considered to be **negative** for *CMV*DNA if its Ct value is not defined in the results grid (the fluorescence curve does not cross the threshold line) in the JOE/HEX/Yellow/Cy3 channel and the Ct value in the results grid in the ROX/Orange/Texas Red channel is < 38.
3. The analysis result is considered to be **invalid** if the Ct value is not defined in the results grid (the fluorescence curve does not cross the threshold line) in the JOE/HEX/Yellow/Cy3channel and the Ct value in the results grid in the ROX/Orange/Texas Red channel is > 38. PCR analysis of such clinical samples should be repeated.
4. For clinical samples whose Ct values in the JOE/Yellow/HEX/Cy3 channel exceed the boundary Ct value (38) the result is considered to be **equivocal**. Such DNA samples should be additionally analyzed in duplicate. If a reproducible positive Ct value is obtained, the result should be considered as **positive**. If Ct values are not reproduced in two replicates, the result is considered as **equivocal.**

   Results of analysis are accepted as relevant if the results obtained for positive and negative controls of amplification and the negative control of extraction are correct. For quantitative analysis, the results for C+ should fall into the concentration range indicated in the **Data Sheet CMV Real-TM Quant**.

Table. Results for controls if total DNA was isolated from peripheral blood, amniotic fluid, cerebrospinal fluid (liquor), saliva, oropharyngeal swabs, urine samples, and bronchoalveolar lavage together with the internal control

Control	Stage for control	Ct in channel				Interpretation
		JOE/HEX/Yellow/Cy3		ROX/Orange/Texas Red		
		Qualitative format	Quantitative format	Qualitative format	Quantitative format	
NCE	DNA extraction, amplification	Neg	Neg	Pos (< boundary value)	Pos (< boundary value)	OK
PCE	DNA extraction, amplification	Pos (< boundary value)	Ct value is in the range indicated in Data Card	Pos (< boundary value)	Pos (< boundary value)	OK
NCA	Amplification	Neg	Neg	Neg	Neg	OK
C+	Amplification	Pos (< boundary value)	–	Pos (< boundary value)	–	OK
QS1,QS2	Amplification	–	Ct value and calculated, concentration determined	–	Ct value and calculated, concentration determined	OK

For quantitative analysis, the concentration of *CMV* DNA (**KP *CMV* DNA**) per ml of sample for peripheral blood plasma, amniotic fluid, cerebrospinal fluid (liquor), saliva, oropharyngeal swabs, urine samples, and bronchoalveolar lavage is calculated by the following formula:

$$K_{P\ CMV\ DNA} = K_{CMV\ DNA} / K_{IC} \times \text{IC coefficient (copies/ml)}$$

$K_{CMV\ DNA}$ is the number of *CMV* DNA copies in DNA sample;

K_{IC} is the number of ICDNA copies in DNA sample;

IC coefficient corresponds to the number of ICDNA copies in DNA sample. It is specified in the Data Sheet CMV Real-TM Quant provided with each lot of the reagent kit. This coefficient is specific for each lot.

CMV Real-TM Quant PCR kit is a test which contains the Internal Control IC (human beta-globine gene), which allows to control the presence of cellular material in the sample. If the sample is not correctly prepared or it is an insufficient quantity of epithelial cells the Internal Control will not be detected.

A negative control of extraction (NCE), positive control of extraction (PCE), negative amplification control (NCA), positive amplification control (C+) are required for every run to verify that the specimen preparation, the amplification and the detection steps are performed correctly.

If the controls are out of their expected range (see table Results for Controls), all of the specimens and controls from that run must be processed beginning from the sample preparation step.

10. TROUBLESHOOTING

Results of analysis are not taken into account in the following cases:

1. The presence of any Ct value in JOE/Yellow/HEX/Cy3, FAM/Green, and ROX/Orange/Texas Red channels in the results grid for the Negative Control of Amplification (NCA) and for the Negative Control of Extraction (C–) in JOE/Yellow/HEX/Cy3 channel indicates contamination of reagents or samples. In this case, PCR analysis should be repeated for all samples where CMV DNA was detected starting from the DNA extraction stage.
2. If, for qualitative analysis, the Ct value in the results grid for the Positive Control of Amplification (QS2) in JOE/Yellow/HEX/Cy3 (CMV), FAM/Green, or ROX/Orange/Texas Red channels is absent, it is necessary to repeat amplification for all samples where CMV DNA was not detected.
3. If the Ct value in the results grid for the Positive Control of Extraction (Positive Control DNA *CMV* and human DNA) in JOE/Yellow/HEX/Cy3 (CMV), FAM/Green, or ROX/Orange/Texas Red channels is absent, the results of analysis of all samples are considered to be **invalid**. It is necessary to repeat PCR analysis for such samples.
4. If the Ct value for the sample in the JOE/Yellow/HEX/Cy3 channel is absent or exceeds the boundary Ct value and the Ct value for the sample is greater than the maximum Ct value for IC in FAM/Green and ROX/Orange channels, it is necessary to repeat the analysis starting from the DNA extraction stage. This error may be due to incorrect treatment of clinical material, which resulted in the loss of DNA, or to the presence of PCR inhibitors.
5. If the Ct value for the sample in the JOE/Yellow/HEX/Cy3 channel is absent or exceeds the boundary Ct value (> 38) and Ct value for the sample is less than the boundary Ct value in FAM/Green and ROX/Orange, the result is considered to be **equivocal**. It is necessary to repeat analysis of such samplesin duplicate. If a reproducible positive Ct value is obtained, the result is considered to be **positive**; otherwise, the result is considered to be **equivocal**.

11. TRANSPORTATION

CMV Real-TM QuantPCR kit should be transported at 2–8 ºC for no longer than 5 days.

12. STABILITY AND STORAGE

All components of the**CMV Real-TM Quant** PCR kit must be be stored at -20ºC when not in use. All components of the **CMV Real-TM Quant** PCR kit are stable until the expiration date on the label.The shelf life of reagents before and after the first use is the same, unless otherwise stated.

PCR-mix-1-FL *CMV* screen/monitor is to be kept away from light.

13. PRODUCT USE LIMITATIONS

All reagents may exclusively be used in in vitro diagnostics. Use of this product should be limited to personnel trained in the techniques of DNA amplification (EN375). Strict compliance with the user manual is required for optimal PCR results. Attention should be paid to expiration dates printed on the box and labels of all components. Do not use a kit after its expiration date.

14. QUALITY CONTROL

In accordance with Sacace's ISO 13485-Certified Quality Management System, each lot is tested against predetermined specifications to ensure consistent product quality.

15. SPECIFICATIONS

15.1 Sensitivity

Linear range of **CMV Real-TM Quant** PCR kit is **500–10.000.000 copies/ml**. If the result is greater than 10.000.000 copies/ml, it is indicated as ***the result is more than 10.000.000 CMV DNA copies/ml***. If the result is less than 500 copies/ml, it is indicated as ***the result is less than 500 CMV DNA copies/ml***.

The analytical sensitivity of **CMV Real-TM Quant** PCR kit is given in the table below.

Type of clinical material	**Nucleic acid extraction kit**	**Sensitivity**
Peripheral blood plasma, amniotic fluid, cerebrospinal fluid (liquor), saliva, oropharyngeal swabs, urine samples, bronchoalveolar lavage	DNA/RNA-prep	400 copies/ml
Whole human blood, white blood cells, viscera biopsy material	DNA/RNA-prep	5 *CMV* DNAcopies per 10^5 cells

15.2 Specificity

CMV Real-TM Quant PCR kit is intended for human cytomegalovirus(*CMV*)DNA detection. Specific activity of **CMV Real-TM Quant** PCR kit was confirmed in studies of the *CMV*reference strain AD 169 as well as by analyzing clinical material with subsequent confirmation of results by sequencing the amplification fragments. The activity of thePCR kit components with respect to DNA of other viruses (Epstein-Barr virus, herpes simplex virus types 1 and 2, human herpes virus types 6 and 8, Varicella Zoster Virus, Parvovirus B19, and others), bacterial pathogens (*Staphylococcus aureus,Streptococcus pyogene, Streptococcus agalactiae,*and others), and human DNA is absent. The clinical specificity of **CMV Real-TM Quant** PCR kit is 100%.

16. REFERENCES

- PCR detection of cytomegalovirus DNA in serum as a diagnostic test for congenital cytomegalovirus infection.C T Nelson, A S Istas, M K Wilkerson, and G J Demmler. J ClinMicrobiol. 1995 December; 33(12): 3317–3318.
- Detection of Cytomegalovirus DNA in Peripheral Blood of Patients Infected with Human Immunodeficiency Virus. D. Shibata, W. John Martin, Maria D. Appleman, Dennis M. Causey, J. M. Leedom, N. Arnheim. J Infect Dis. (1988) 158 (6): 1185-1192.
- Multiplex PCR for six herpesviruses after hematopoietic stem cell transplantation. Sawada A, Koyama-Sato M, Yasui M, Kondo O, Ishihara T, Takeshita Y, Okamura T, Nishikawa M, Inoue M, KawaPediatr Int. 2011 Aug 2. doi: 10.1111/j.1442-200X.2011.03437.
- Cytomegalovirus Infections in Non-immunocompromised and Immunocompromised Patients in the Intensive Care Unit.Florescu DF, Kalil AC. Infect Disord Drug Targets. 2011 Jun 16.
- Comparison of PCR, Antigenemia Assay, and Rapid Blood Culture for Detection and Prevention of Cytomegalovirus Disease after Lung Transplantation. Adriana Weinberg, Tony N. Hodges, Shaobing Li, GuanyungCai, M. R. Zamora. Journal of Clinical Microbiology, February 2000, p. 768-772, Vol. 38, No. 2
- Optimization of Quantitative Detection of Cytomegalovirus DNA in Plasma by Real-Time PCR. Michael Boeckh,MeeiLi Huang, James Ferrenberg, Terry Stevens-Ayers, Laurence Stensland, W. Garrett Nichols, and Lawrence Corey. Journal of Clinical Microbiology, March 2004, p. 1142-1148, Vol. 42, No. 3
- Quantification of Human Cytomegalovirus DNA by Real-Time PCR. ElyanneGault,Yanne Michel, AxelleDehée, ChahrazedBelabani, Jean-Claude Nicolas, Antoine Garbarg-Chenon. J ClinMicrobiol. 2001 February; 39(2): 772
- Definitions of Cytomegalovirus Infection and Disease in Transplant Recipients. Per Ljungman, Paul Griffiths, Carlos Paya, …Clin Infect Dis. (2002) 34 (8): 1094-1097

KEY TO SYMBOLS USED

REF	List Number		Caution!
LOT	Lot Number		Contains sufficient for <n> tests
IVD	For *inVitro* Diagnostic Use	VER	Version
	Store at	**NCA**	Negative Control of Amplification
	Manufacturer	**C–**	Negative control of Extraction
	Consult instructions for use	**C+**	Positive Control of Amplification
	Expiration Date	**IC**	Internal Control

NOTE

*iQ5™ is a registered trademark of Bio-Rad Laboratories
* Rotor-Gene™ Technology is a registered trademark of Qiagen
* MX3005P® is a registered trademark of Agilent Technologies
*ABI® is a registered trademark of Applied Biosystems
* LineGeneK® is a registered trademark of Bioer
* SmartCycler® is a registered trademark of Cepheid
* SaCycler™ is a registered trademark of Sacace Biotechnologies

Sacace Biotechnologies Srl
via Scalabrini, 44 – 22100 – Como – Italy Tel +390314892927 Fax +390314892926
mail: ***info@sacace.com*** *web:* ***www.sacace.com***