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Produktinformation

- Forschungsprodukte & Biochemikalien
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See the following pages for more information!

Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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quanty CMV (IEA Region)

REF: RT-12

Detection and quantification of the Cytomegalovirus genome with *Real Time PCR*

INTRODUCTION AND PURPOSE OF USE

The quanty CMV system is a quantitative test that allows the DNA amplification and quantification, by means of *Real Time PCR*, of *IEA* region of CMV genome.
The Procedure allows the detection of the DNA target by means a genomic amplification reaction.
The analysis of the results is made using a Real Time PCR analyzer (thermal cycler integrated with a system for fluorescence detection and a dedicated software).

CONTENT

The kit contains reagents enough to perform 48 amplification tests

	Quantity	Description
R1	3 x 220 µl	**Amplification mMix** dNTPs, Tris-HCl, KCl, $MgCl_2$, Taq Polymerase, *AmpErase* Uracil N-Glycosylase (*UNG)* Nuclease-free water, ROX (Blue Cap)
R2	3 x 130 µl	**CMV probes Mix** Upstream primer, downstream primer, Target probe (FAM), Internal control (β-globin) Probe (VIC) Nuclease-free water (Green Cap)
R3	3 x 35 µl	Cloned DNA corresponding to the *IEA* region at the concentration of 10^5 copies/µl
R4	3 x 35 µl	Cloned DNA corresponding to the *IEA* region at the concentration of 10^4 copies/µl
R5	3 x 35 µl	Cloned DNA corresponding to the *IEA* region at the concentration of 10^3 copies/µl
R6	3 x 35 µl	Cloned DNA corresponding to the *IEA* region at the concentration of 10^2 copies/µl
R7	3 x 100 µl	**Internal control**
R8	1 x 30 µl	**Negative Control**

Instruction for use: **ST. RT12-ENG.10**

MATERIALS AND STRUMENTATION REQUIRED BUT NOT SUPPLIED

Disposable latex powder-free gloves or similar material;
Bench microcentrifuge (12,000 - 14,000 rpm);
Micropipettes and Sterile tips with aerosol filter;
Vortex;
Plastic materials (microplate and optica adhesive cover);
Heat block (only for extraction)
EZ1 Advanced XL DSP Virus Card. - Ref. 9018703 - QIAGEN.
ATL Buffer - Ref. 939016 - QIAGEN.

Reagents
The quanty CMV kit was developed and validated to be used with the following extraction method:

Manual Extraction
Ref. 51304/51306
QIAmp DNA mini kit.
The kit allows the DNA extraction from tested samples. The kit contains reagents for 50/250 samples.(QIAGEN).

Automatic Extraction
Ref. 62724. EZ1 XL DSP Virus Kit
The kit allows the automatic viral DNA from Human samples.
The kit contains reagents for 48 samples. (QIAGEN)

Instruments
The quanty CMV kit was developed and validated to be used with the following instruments:

Extraction System
Ref. 9001492. EZ1 Advanced XL.
Robotic Workstation for the automatic purification of the nucleic acids until 14 samples simultaneously (QIAGEN)

Real Time PCR
The quanty CMV kit was developed and validated to be used with the following real time PCR instruments:

- *7500 Fast* from Lifetechnologies
- *Versant kPCR AD from Siemens o Stratagene MX3005P/MX3000P*
- *Rotor-Gene Q* MDx from QIAGEN
- *CFX96 Real Time PCR System* from *Bio-Rad*
- *LightCycler 480* from Roche

Please ensure that the instruments have been installed, calibrated, checked and maintained according to the manufacturer's instruction and raccommendations

SAMPLES AND STORAGE

The **quanty CMV** system must be used with extracted DNA from the following biological samples: **Blood EDTA Liquor (Cerebrospinal fluid – CSF) and Urine**. Collected material must be shipped and stored at +2 - +8°C. Store the samples at -20°C if not used within 3 days.

PRECAUTIONS USE

This kit is for in *vitro* diagnostic (IVD), for professional use only and not for in vivo use.
After reconstitution, the amplification master mix must be used in one time (16 reactions). Repeat thawing and freezing of 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. At all times follow Good Laboratory Practice (GLP) guidelines.
Wear protective clothing such as laboratory coats and disposable gloves while assaying samples.
Avoid any contact between hands and eyes or nose during specimens collection and testing.
Handle and dispose all used materials into appropriate bio-hazard waste containers. It should be discarded according to local law.
Keep separated the extraction and the reagents preparation.
Never pipette solutions by mouth.
Avoid the air bubbles during the master mix dispensing. Eliminate them before starting amplification.
Wash hands carefully after handling samples and reagents.
Do not mix reagents from different lots.
It is not infectious and hazardous for the health (see Material Safety data Sheet – MSDS).
Do not eat, drink or smoke in the area where specimens and kit reagents are handled.
Read carefully the instructions notice before using this test.
Do not use beyond the expiration date which appears on the package label.
Do not use a test from a damaged protective wrapper.

LIMIT OF THE METHOD

Use only extracted DNA from whole blood EDTA, CSF or Urine
Do not use contaminated DNA with extracted mucoproteins or hemoglobin: the latter inhibit the amplification reaction of nucleic acids and may cause invalid results .
The extreme sensitivity of gene amplification may cause false positives due to cross-contamination between samples and controls. Therefore, you should:

- physically separate all the products and reagents used for amplification reactions from those used for other reactions, as well as from post-amplification products;
- use tips with filters to prevent cross-contamination between samples;
- use disposable gloves and change them frequently;
- carefully open test tubes to prevent aerosol formation;
- close every test tube before opening another one.

The proper functioning of the amplification mix depends on the correct collection, correct transportation, correct storage and correct preparation of a biological sample.
As with any diagnostic device, the results obtained with this product must be interpreted taking in consideration all the clinical data and other laboratory tests done on the patient.
A negative result obtained with this product suggests that the DNA of CMV was not detected in DNA extracted from the sample, but it may also contain CMV-DNA at a lower titre than the detection limit for the product (detection limit for the product, see paragraph on Performance Characteristics); in this case the result would be a false negative.
As with any diagnostic device, with this product there is a residual risk of obtaining invalid, false positives or false negatives results.

STORAGE AND STABILITY

Store the product ***quanty CMV*** (IEA region) at –20°C..
The ***quanty CMV*** kit is shipped on dry ice. The kit components should be frozen.
If one or more components are not frozen upon receipt or if the tubes have been compromised during transport, contact Clonit srl for assistance.
An intact and well stored product has a stability of 12 months from the date of production. Do not use beyond the expiration date which appears on the package label.
Repeat thawing and freezing of 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.

ANALYTICAL PROCEDURE

Manual Extraction
Ref. 51304/51306 - QIAmp DNA mini kit. (QIAGEN).

Procedure to Whole blood
Follow the instructions inside the kit *QIAmp DNA mini kit*.
Elute the sample in 50 µl of buffer AE
Samples are now ready for amplification or storage at -20°C.

Procedure to Urine
Ref. 51304/51306 - QIAmp DNA mini kit (QIAGEN).
Follow the instructions inside the kit *QIAmp DNA Mini Kit.*
After the lysis step at 56°C, add 5 µl of Internal control.
Follow the instructions in the kit.
Elute the sample in 50 µl of buffer AE.
Samples are now ready for amplification or storage at -20°C.

Procedure to Liquor (CSF)
Ref. 51304/51306 - QIAmp DNA mini kit (QIAGEN).
Follow the instructions inside the kit *QIAmp DNA Mini Kit.*
After the lysis step al 56°C add 5 • l of Inhibition Control to the samples.
Follow the instructions inside the kit
Elute the sample in 50 µl of buffer AE
Samples are now ready for amplification or storage at -20°C.

Automatic Extraction
Ref. 62724 - EZ1 XL DSP Virus Kit on *EZ1 Advanced XL.*

Procedure to Whole Blood
Follow the instructions inside the kit *EZ1 XL DSP Virus Kit*.
Volume of sample to be used:

Whole blood [µl]	*ATL [µl]*	*Final volume Samples [µl]*
200	*200*	*400*

Preparation of the Carrier
Solve completely the lyophilize RNA carrier in elution buffer (AVE), from 310 • l, split in aliquots and store to –20 ± 5°C. Not thawing and freezing the aliquots more than 2 times.
For each analyzed sample, dilute 3,6 • l of a original solution include the RNA Carrier in total volume of 60 • l using elution buffer (AVE)
Follow the instructions inside the kit *EZ1 XL DSP Virus Kit*.
Select the protocol starting from 400 • l of samples with the elution of 60 • l.

Procedure to Urine
Before the reaction on EZ1 Advanced XL instrument mix.

Urine [µl]	*ATL [µl]*	*final sample volume [µl]*
300	*100*	*400*

Preparation of the Carrier and the Internal Control (Internal
Solve completely the lyophilize RNA carrier in elution buffer (AVE), from 310 • l split in aliquots and store to –20 ± 5°C. Not thawing and freezing the aliquots more than 2 times.
For each analyzed sample, dilute 3,6 • l of a original solution include the RNA Carrier and 5 • l of *Internal Control* in total volume of 60 • l using elution buffer (AVE)

Follow the instructions inside the kit *EZ1 XL DSP Virus Kit*.
Select the protocol starting from 400 • l of samples with the elution of 60 • l.

Samples are now ready for amplification or storage at -20°C.

Procedure to Liquor (CSF)
Before the reaction on EZ1 Advanced XL instrument mix.

CSF - Liquor [µl]
200

Preparation of the Carrier and the Internal Control (Internal Control)
Solve completely the lyophilize RNA carrier in elution buffer (AVE), from 310 • l split in aliquots and store to –20 ± 5°C. Not thawing and freezing the aliquots more than 2 times.
For each analyzed sample, dilute 3,6 • l of a original solution include the RNA Carrier and 5 • l of *Internal Control* in total volume of 60 • l using elution buffer (AVE)

Follow the instructions inside the kit *EZ1 XL DSP Virus Kit*.
Select the protocol starting from 200 • l of samples with the elution of 60 • l.

Samples are now ready for amplification or storage at -20°C.

SOFTWARE SETTING

Lifetechnologies 7500 fast

Turn the instrument and the computer on and open the control software. Click on "**Advance Setup**": by default the software will shows the page "**experiment properties**". Write in the "**experiment name**" the file name, choose the type of instrument (**7500 o 7500fast),** the type of reaction (**quantitation standard curve),** the type of used reagent (**Taqman®Reagents**) and the reaction time of analysis (**Standard ≈ 2 hours to complete a run**).
Open the page named "**page setup"** (sheet **Define Target and Samples**).
In the window **Define Targets** set:

Target	Reporter	Quencer
CMV probe:	FAM	TAMRA
IC (β-globin) **probe:**	VIC	TAMRA

Set the samples' name in the window "**Define Samples**".
In the same page "**plate setup**" select the sheet "**Assign Target and Samples**". On the screen you will see the microplate draft.
Select an area of the plate where the controls will be placed: select wells of the plate and set both targets (CMV and β-globin). Select "**Assign target to selected wells**" in the blank, the "**task Standard (S)**" for CMV target and set the controls' concentration.
Choose an area in the plate where negative control will be placed: select "**Assign target to selected wells**" in the blank, the "**task Negative (N)**" for the CMV target.
Select an area of the plate where samples will be placed: select the wells and set both targets (CMV and β-globin). Link every well to a sample, through the window "**Assign samples to selected wells**".
For each sample, select in the blank "**Assign targets to selected wells**" the "**task UnKnown (U)**" for the CMV target.
Set ROX as passive reference, using it as normalizer of detecting fluorescence.
Open "**Run Method**" (sheet **Graphic View**) and set the right thermal cycling:

cycles	denaturation	annealing/extension
1	50° C 2 min	
1	95°C 10 min	
45	95° C 15 sec	60° C 1 min

In the window "**Reaction volume plate per well**" set a volume of 25 µl.
After preparing the plate, and correctly inserting it in the instrument, press the button "**Start Run**".

Versant kPCR AD or Stratagene MX3005P/MX3000P

Turn the instrument on and wait until both green lamps have fixed light, turn on the computer and start the control software. In the main screen will appear the window "**New Experiment Options**": select "**Experiment type": quantitative PCR (Multiple Standard).**
Turn the lamp on 20 minutes before doing a new experiment. For turning the lamp on, click on the icon of the lamp in the tool bar or select "**Lamp On**" from the menu **"Instruments".**
Verify the right setting of the gain of the fluorescent reporters: in the menu of settings, choose: **"Instrument"** and then **"Filter set gain setting"**.

Reporter	Gain
FAM	4
HEX	4
ROX	1

Click on button **"setup"** in the tool bar and choose "**Plate Setup".**
Sign the wells corresponding to calibrators. Define the calibrator's positions in right menu, setting:

Well type:	Collect Fluorescent Data:	Reference Dye:	Replicate Symbol:
Standard	FAM/HEX/ROX	ROX	None

Clicking on every single well, the window "**well information**" will appear: choose the name of the calibrator.
In the window "**Select Quantity**", set the concentrations of the 4 calibrators, following the instructions indicated in the paragraph **Interpretation of the results**.
Sign the wells correspondent to Negative control. Define the NTC positions in right menu, setting:

Well type:	Collect Fluorescent Data:	Reference Dye:	Replicate Symbol:
NTC	FAM/HEX/ROX	ROX	None

Clicking on every single well, the window "**well information**" will appear: set NTC as the name.
Sign the wells correspondent to the Samples. Define the samples positions in right menu, setting:

Well type:	Collect Fluorescent Data:	Reference Dye:	Replicate Symbol:
UnKnown	FAM/HEX/ROX	ROX	None

Clicking on every single well, the window "**well information**" will appear: set the name or the code of the sample.
It's possible, indeed, set near the name of fluorescent reporter the name of analyzed targets:

FAM	HEX
CMV	β-Globin

In the tool bar choose the sheet **"Thermal Profile Setup"** and set the correct thermal cycle and reading the fluorescence in the annealing/extension step.

cycles	denaturation	annealing/extension
1	50° C 2 min	
1	95°C 10 min	
45	95° C 15 sec	60° C 1 min

After preparing the plate and inserting it in the instrument, press the button "**Run**", selecting the sheet Thermal profile status and check the correctness of thermal profile.
Select the box **Turn Lamp Off** at the end of execution. Push the button Start: the software will ask you to indicate the name of saved file. The analysis will start.

Rotor-Gene Q MDx

The experiments can be **set using the Quick Start Wizard or the** Advanced Wizard, which appears when the software is started.
Select the wizard "**Advanced**". As a first step, select the model "**Two Step Reaction**" with a double click in the "**New Run**".
In the next window, select the type of rotor installed on the instrument from the list that appears. Check the "**Locking Ring Attached**", check the checkbox and then click "**Next**".
Enter the name of the operator and the reaction volume of 25 µl, and then click "**Next**".
In the next window click on "**edit profile**". Set the following thermal cycle:

cycles	denaturation	annealing/extension
1	50° C 2 min	
1	95°C 10 min	
45	95° C 15 sec	60° C 1 min

Select the annealing/extension from the thermal profile and click on "**Acquiring A to cycling**."
In the next window, select yellow from the available channels and add it to acquiring channel along with the green channel and click "**OK**". In the next window click on "**OK**" and then click "**Next**".
Click on "Edit Gain" button and set the following values for each channel:

Reporter	Gain
Green	5
Yellow	5

To begin the course, click on the button "**Start Run**". You can save the model before you begin your run by clicking on "**Save Template**".
After clicking on the button "**Start R**un" window appears "**Save As**". The stroke can be saved in the desired position by the user.

Once the run started, the window "**Edit Samples**" allows you to set the name of samples and controls in the positions in which they were loaded on the instrument.
Select the locations where they were positioned the controls of known concentration and designate them as CMV Standard. Clicking on the box next to "**Type**" correspondent, in the dropdown menu "**Samples**" you can select the type of sample being analyzed. Select "**Standards**". Enter the concentrations of the controls.
Select the location where you placed the Negative Control and name it as Negative Control. Clicking on the box next to "**Type**" correspondent, in the dropdown menu "**Samples**" you can select the type of sample being analyzed. Select "**Negative Controls**"
Select the location of each sample and enter the name or code of the patient. Clicking on the box next to "**Type**" correspondent, in the dropdown menu "**Samples**" you can select the type of sample being analyzed. Select "**UnKnown**"
At the end of the operation click "**OK**" in the "**edit samples**" and wait until the end of the race for the analysis (see "**Interpretation of Results**").

CFX96 Real Time PCR

Turn the instrument and the computer on and start the control software. In the principal screen will appear the window "**Startup wizard**": select "**CFX96"** and press **"ok".** In the next window push "**create new**" and set the thermal protocol and the reaction volume (25µl):

cycles	denaturation	annealing/extension
1	50° C 2 min	
1	95°C 10 min	
45	95° C 15 sec	60° C 1 min

Save the protocol and click the next button. The software will open in default the sheet "**plate**". Click "**create new**", select **"Fluorophores button"** to choose fluorophores (FAM and VIC). Select the locations where they were positioned the controls of known concentration and choose the **"Sample Type" Standards.** Click "**Load**" check boxes to load fluorophores and Type or select Target Name. In the box "**laod concentration"** set the concentrations of the 4 calibrators, following the instructions indicated in the paragraph **Analysis of the results**.
Select the location where you placed the Negative Control. Choose the **"Sample Type" NTC.** Click "**Load**" check boxes to load fluorophores and Type or select Target Name
Select the location of each sample and enter the name or code of the patient. Choose the **"Sample Type" Unknown.** Click "**Load**" check boxes to load fluorophores and Type or select Target Name
Save the plate clicking the next button and start the experiment.

LightCycler 480

Turn the instrument and the computer on and start the control software. In the principal screen, on "**Experiment Creation**" select the "**Plate type**" and push the "**New experiment**" button. The window "experiment" appears. On the "Run protocol" sheet set: Thermal protocol, Reaction volume (25µl) and Detection format (**dual colour hydrolysis probe**).

cycles	denaturation	annealing/extension
1	50° C 2 min	
1	95°C 10 min	
45	95° C 15 sec	60° C 1 min

Push the "**Subset editor**" button and in this window, select an area of the plate where controls and samples will be placed.
Push the "**Sample editor**" button. Select the correct workflow (Step1: **Abs quantification**), choose the samples Subset created in the step before and insert the name for each well. Choose the correct type for each well: Positive CTR, Negative CTR or Unknown sample.

Push again the "**experiment button**", insert the plate in the instrument and push "**start run**".

PREPARATION OF THE REACTIONS:

Defrost a tube of **Amplification mMix**;
Defrost a tube of **CMV probes Mix;**
Mix carefully by vortex **210µl** of **Amplification mMix** and **126µl** of **CMV probes Mix** (the mix as produced is enough to prepare **16 reactions** of amplification: **4 positive controls, 1 negative control and 11 samples**).
Distribute, in the amplification plate, **20µl** of just reconstituted mix in chosen positions as already set on the instrument software.
Distribute, in the negative control position, **5 µl** of solution taken by the negative control vial.
Distribute, in chosen position for each sample, **5 µl** of corresponding sample.
Distribute, in chosen positions for the positive controls, **5µl** of **10^2 copies/µl** solution**, 10^3 copies/µl** solution**, 10^4 copies/µl** solution **and 10^5 copies/µl** solution.
Seal up accurately the plate using an optical adhesive film and verify that there aren't air bubbles in the mix, to avoid the amplification interferences.
For the Rotor-Gene Q MDx, seal each tube with the appropriate cap. The air bubbles presence is not influently; the rotor centrifugal force will allow automatic deletion.
Transfer the plate in the instrument and push the button "**Start Run**".

QUANTITATIVE ANALYSIS

Lifetechnologies 7500 Fast

At the end of the PCR run, the software automatically opens the "**Analysis**" window in the "**Amplification plot**" sheet on the menu on the left.
Select the wells corresponding to the positive control, negative control and samples for analysis.
Select in the "**Option**" window inside the "**Target**" pop-up menu the **CMV target**. Check the correct setting of the threshold.
Select in the "**Option**" window inside the "**Target**" pop-up menu the **IC Control target**. Check the correct setting of the threshold.

The analysis of the results is made selecting from the menu in the left the page **"Analysis"**. From the page "**Standard Curve**", maintaining open the sheet "**view well plate**" in the right side of the software select the wells containing the points of the curve and verify the parameters described in the paragraph "**Interpretation of the Results**" (coefficient of correlation, slope ecc…).
From the page "**Amplification Plot**" verify the amplification plot for every single sample.
Opening the sheet "**view well table**" in the right side of the software it is possible to verify the data obtained from experiments: Threshold Cycles, emitted fluorescence and the target quantification expressed in copies/reaction or copies/ml, depending on the settings of the calibration curve.
Clicking from the menu file and selecting the box export, the window "**export properties**" will open. Indicate the file name, select the position to save it (**Browse**) and click on button
"Start export". In this way the software will permit to save a excel file with all the data corresponding to selected experiment.

Versant kPCR AD or Stratagene MX3005P/MX3000P

Click on button "**Analysis**" in the toolbar. The software will open in default the sheet "**Analysis Term Setting**". Activate the buttons FAM and HEX in the lower part of the screen and select testing samples.

Click on sheet "**results**"; the software will open in default the page "**Amplification plot**". Check the correct setting of the threshold in the specific window "**Threshold fluorescence**", in the menu on the right of the screen.

Selecting the box "**Standard Curve**" from menu "**Area to Analyze**" it's possible to visualize the data related to the calibration curve and verify the parameters described in paragraph "**Interpretation of Results**" (coefficient of correlation, slope ecc…). Selecting the box **Text report**" from menu "**Area to Analyze**" in the right side of the screen it's possible to verify the data obtained from the experiments: Threshold Cycles, emitted Fluorescences and the target quantification expressed in copies/reaction o copies/ml depending on the settings of the calibration curve.
From the window **Text Report** it's possible to export the results obtained clicking **file, export** on main menu.

Rotor-Gene Q MDx

At the end of the PCR run open the "**Analysis**" window. Select the "**Quantification**" sheet and click on "**cycling A (green)".**
Select from the menu **"Dynamic Tube"** and subsequently **"Slope correct"**.
Check the correct setting of the threshold in the space provided "**CT calculation – Threshold"**.
Open the "**Analysis**" window. Select the "**Quantification**" sheet and click on "**cycling A (yellow)".**
Select from the menu **"Dynamic Tube"** and subsequently **"Slope correct"**.
Check the correct setting of the threshold in the space provided "**CT calculation – Threshold"**.

Also in this case, you can print a report of the analysis by clicking on the **"Report"** window and selecting the file in the first **Quantification cycling A (green)** and then the file **cycling A (yellow).**

CFX96 real time PCR system

At the end of the PCR, select the "**quantitation**" sheet. On the top of the screen, select "**settings**" from the menu and choose "**Baseline Threshold…**" .
You can export the report pushing the paper block figure on the top of the screen.

LightCycler 480

When the run is completed select analysis and choose the correct kind of analysis you want: "**Abs Quant/Fit Points**". Choose the samples subset you want to analyze. Select the "**NoiseBand**" sheet, under the plot you can choose "**NoiseBand (Fluoresc.)**"; and move the line of the NoisBand on the plot with the mouse of your PC. Repeat this action for each fluorophore using the "**Filter comb"** button.
Clicking the sheet "**Analysis**" you can set the threshold choosing the option "Threshold(manual).

After setting parameters push the "**Calculate**" button. Repeat this action for each fluorophore.

INTERPRETATION OF RESULTS

Through Real Time PCR reaction it is possible to give the DNA quantification of CMV DNA, with the correct settings of positive controls values, that compose the calibration curve. This setting must consider all the dilutions and the passages that the sample has to be undergone during extraction and amplification steps.
The Ct values obtained from the amplification of 4 controls of known titre are used by the software for the calculation of the calibration curve from which the unknown samples are interpolated.
A proper functioning of the amplification mix can be verified analyzing these parameters:

Parameter	Ref
RTS conc. 10^5 copies/µl (FAM)	Ct ≤ 25
Correlation Coefficient	0.990 ≤ r^2 ≤ 1
Slope	-3,6 ≤ Slope ≤-3,2
PCR efficiency	90 ≤ efficiency ≤ 100

If the RTS amplification reaction at a concentration of 10^5 copies produces a Ct > 25 or undetermined the session can't be considered valid and must be repeated.
Verify that the correlation coefficient value (r^2), the slope or the reaction efficiency fit to the limited indicated in the above table or do not deviate much from them, which represent the ideal range for a proper PCR reaction.

By correctly setting the standards concentration as a function of the extraction system you can get the quantization of the sample directly in copies / ml:

Manual Extracion

		Ref. 51304/51306 (QIAGEN)
	RTS 1	25.000.000 copies
	RTS 2	2.500.000 copies
	RTS 3	250.000 copies
	RTS 4	25.000 copies

Automatic Extraction

		Whole Blood *Ref. 62724.* *EZ1 XL DSP Virus Kit*
	RTS 1	30.000.000 copies
	RTS 2	3.000.000 copies
	RTS 3	300.000 copies
	RTS 4	30.000 copies
		CSF (Liquor) ***Ref. 62724.*** ***EZ1 XL DSP Virus Kit***
	RTS 1	30.000.000 copies
	RTS 2	3.000.000 copies
	RTS 3	300.000 copies
	RTS 4	30.000 copies
		Urine ***Ref. 62724.*** ***EZ1 XL DSP Virus Kit***
	RTS 1	20.000.000 copies
	RTS 2	2.000.000 copies
	RTS 3	200.000 copies
	RTS 4	20.000 copies

Alternative Extraction

		Alternative Extraction
	RTS 1	500.000 copies
	RTS 2	50.000 copies
	RTS 3	5.000 copies
	RTS 4	500 copies

When alternative systems are used sample concentration expressed in copies/ml will be obtained using the formula:

$$copie/ml = \frac{1000}{Ve} \times \frac{Ev}{Ea} \times C_{reaz}$$

where:
- **Ve:** extracted sample Volume expressed in µl
- **Ev:** eluted sample Volume during extraction step expressed in µl
- **Ea:** extracted sample volume used for amplification expressed in µl
- **C_{reaz}:** copies provided by the instrument.

As with any diagnostic device, the results obtained with this product must be interpreted taking in consideration all the clinical data and the other laboratory tests done on the patient.
As with any diagnostic device, with this product there is a residual risk of obtaining invalid, false positives or false negatives results.
The use of positive and negative controls in each amplification session allow to verify the correct functioning of the amplification mix and the absence of any contamination.
In the amplification reaction of each sample, the Ct values for the internal control (β-globine) specific probe are used to validate the analysis session, from extraction process until detection step.
A good extraction performances presents internal control (β-globin) threshold cycle between 22 and 25.
Be sure that emitted fluorescence from internal control amplification has not a Ct > 28 or undetermined. If a sample shows an undetermined CMV DNA and an internal control Ct >28, this means that there have been problems in the extraction stage or in the amplification stage; therefore the sample could be a false negative.
Repeat the sample.
It can be considered valid the samples with a Ct > 28 for the internal control, and a high concentration of CMV DNA. In this case, the competitive nature of PCR reaction can hide or disadvantage the internal control amplification.

Detector FAM	Detector VIC/Hex	PCR Run	Sample
Ct undetermined	Ct > 28 or undetermined	Not Valid	repeat
Ct undetermined	Ct < 28	Valid	Negative
High Ct	Ct < 28	Valid	Positive
Low Ct	Ct > 28 or undetermined	Valid	HighPositive

PERFORMANCES

Analytical sensitivity:
It is considered as analytical sensitivity the highest dilution (title) to which a positive sample can be diluted without the system losing the ability to detect with a positivity rate of • 95%. The analytical sensitivity of the system was assessed by analyzing plasmid DNA, quantified by spectrophotometric analysis, containing the genomic regions of interest (IEA region) of the virus in serial dilutions.
The analytical sensitivity of ***quanty CMV*** virus is determined by Probit analysis.

Instrument	Copies/ul	95% confidence interval
7500 FAST	1,989 cps/ul	Inf. 1,273 cps/ul Sup. 16,65 cps/ul
Versant kPCR	2,653 cps/ul	Inf. 1,148 cps/ul Sup. 18,707 cps/ul
Rotorgene Q MDx	0,534 cps/ul	Inf. 0,27 cps/ul Sup. 5,65 cps/ul
Biorad CFX96	2,13 cps/ul	Inf. 0,71cps/ul Sup. 19.94 cps/ul
LightCycler 480	1,54 cps/ul	Inf. 0,64cps/ul Sup. 20.45 cps/ul

The analytical sensitivity of ***quanty CMV*** virus is determined by Probit analysis.

Manual Extraction

Average A.Sens.	Whole Blood	CSF	Urine Cps/ml
1,679 cps/ul	420 Cps/ml	420 Cps/ml	420 Cps/ml

Automatic Extraction

Average A.Sens.	Whole Blood	CSF	Urine Cps/ml
1,679 cps/ul	500 Cps/ml	500 Cps/ml	330 Cps/ml

Clinical sensitivity:
It is considered as clinical sensitivity the ability to detect true positive samples in the totality of the samples screened as positive. The analysis was made on CMV positive samples and the test was performed following the method recommendations. Positive samples were confirmed with an other CE approved method available on the market.

Manual Extraction

N.Sample	Whole Blood	CSF	Urine Cps/ml
Positive	32	10	12
Negative	0	0	0
C. Sensitivity	100%	100%	100%

Automatic Extraction

N.Sample	Whole Blood	CSF	Urine Cps/ml
Positive	10	10	10
Negative	0	0	0
C. Sensitivity	100%	100%	100%

Obtained results show a clinical sensitivity of 100% evaluated on each instrument indicated on instruction for use.

Traceability versus NIBSC controls material
The used standard (NIBSC code 09/162, Version 4.0, Dated 20/03/2012) was established as the 1st WHO International Standard for CMV, with an assigned potency of $5x10^6$ IU/ml when reconstituted in 1 ml of nucelase-free water.

Manual Extraction

	Founded Average Copies/ml	Founded Average IU/ml	Awaited conc IU/ml
7500 Fast	14.200.000 (Log_{10} 7.15)	8.460.897 (Log_{10} 6.92)	5.000.000 (Log_{10} 6.7)
MX3000P	7.265.000 (Log_{10} 6.86)	4.709.023 (Log_{10} 6.67)	5.000.000 (Log_{10} 6.7)
RotorGene Q	7.077.000 (Log_{10} 6.84)	4.330.323 (Log_{10} 6.63)	5.000.000 (Log_{10} 6.7)
CFX 96	9.420.000 (Log_{10} 6.97)	5.765.425 (Log_{10} 6.76)	5.000.000 (Log_{10} 6.7)
LightCycler 480	2.830.000 (Log_{10} 6.45)	1.738.991 (Log_{10} 6.23)	5.000.000 (Log_{10} 6.7)

Average conversion factor quanty CMV Kit:

1.63 copies/ml = 1 IU/ml

Automatic Extraction

	Founded Average Copies/ml	Founded Average IU/ml	Awaited conc IU/ml
7500 Fast	7560000 (Log_{10} 6,88)	5518248,18 IU/ml (Log_{10} 6,74)	5.000.000 (Log_{10} 6.7)
MX3000P	9339000 (Log_{10} 6,97)	6816788,32 IU/ml (Log_{10} 6,83)	5.000.000 (Log_{10} 6.7)
RotorGene Q	5647200 (Log_{10} 6,75)	4122043,80 IU/ml (Log_{10} 6,62)	5.000.000 (Log_{10} 6.7)
CFX 96	4824000 (Log_{10} 6,68)	3521167,88 IU/ml (Log_{10} 6,55)	5.000.000 (Log_{10} 6.7)
LightCycler 480	7560000 (Log_{10} 6,88)	5518248,18 IU/ml (Log_{10} 6,74)	5.000.000 (Log_{10} 6.7)

Average conversion factor quanty CMV Kit:

1.37 copies/ml = 1 IU/ml

Traceability versus controls material
Cytomegalovirus was checked versus QCMD Cytomegalovirus DNA

Linearity/Proportionality
System linearity is valued analyzing plasmidic DNA (pCM5018), quantified by spectrophotometric analysis, containing the genomic regions of interest (IEA region) of the virus in serial dilutions (1:10) from 100.000 copiess to 1 copy of DNA in 5µl of extracted material added in the amplification reaction.
The evaluation is performed analyzing 10 calibration curves, that showed these parameters:

RTS conc. 10^5 copie (FAM)	Ct ≤ 25	Medium Ct 23,5254
Correlation Coefficient	0.990 ≤ r^2 ≤ 1	Medium r^2 0,998
Slope	-3,6 ≤ Slope ≤-3,2	Medium slope -3,42
PCR efficiency	90 ≤ Efficienza≤ 100	Medium Eff 97%

Reproducibility and Repeatability:
The reproducibility and repeatability of the system are valued analyzing 3 dilutions of plasmidic DNA containing the IEA region of interest for CMV quantified by spectrophotometric analysis (pCM5018) and 1 negative control (negative DNA). For each session 5 replicates are made for 3 different sessions, made by different workers, with 3 different lots.

Hypothetical value	Lot	N° ripetitions	Med. Reveal. Conc.	Inaccuracy %
100.000 copies	L1	30	85355	19%
100.000 copies	L2	30	77376	11%
100.000 copies	L3	30	76631	10%
100.000 copies	L28	15	100167	11%
100.000 copies	L29	45	349217	14%
100.000 copies	L30	45	323609	11%
100.000 copies	L31	30	266542	22%
1.000 copies	L1	30	746	8%
1.000 copies	L2	30	723	6%
1.000 copies	L3	30	698	5%
1.000 copies	L28	15	976	8%
1.000 copies	L29	45	2779	14%
1.000 copies	L30	45	2835	15%
1.000 copies	L31	30	2930	14%
10 copies	L1	30	5	24%
10 copies	L2	30	4	10%
10 copies	L3	30	4	16%
10 copies	L28	15	10	7%
10 copies	L29	45	27	11%
10 copies	L30	45	29	15%
10 copies	L31	30	32	14%

The medium inaccuracy % is 13%.

Diagnostic Specificity:
For the purposes of this evaluation is considered as diagnostic specificity the skill of the method of determining real negative samples. The diagnostic specificity of the system is valued analyzing human genomic samples tested and confirmed as negative with another disposable system.

Manual Extraction

N.Sample	Whole Blood	CSF	Urine Cps/ml
Positive	1	0	0
Negative	36	10	15
C. Sensitivity	97.2%	100%	100%

Automatic Extraction

N.Sample	Whole Blood	CSF	Urine Cps/ml
Positive	0	0	0
Negative	15	10	15
C. Sensitivity	100%	100%	100%

Analytical Specificity:
Test's specificity was guaranteed by the use of specific primers for CMV. The alignment of the choose regions for specific primers' hybridization for CMV with available sequences of the IEA region present in database, demonstrated: their conservation, the absence of significative mutations and the complete specificity for the analysed target.

Cross-Reactivity:
To check the cross-reactivity of the assay, samples tested as positive for other herpetic viruses were analyzed following the method instructions.

Positive sample	7500 Fast	KVersant MX3005P	Rotorg-Q	CFX96	Light Cycler
HSV- 1	<10copie	<10copie	<10copie	<10copie	<10copie
HSV- 2	<10copie	<10copie	<10copie	<10copie	<10copie
VZV	<10copie	<10copie	<10copie	<10copie	<10copie
EBV	<10copie	<10copie	<10copie	<10copie	<10copie
HHV-6	<10copie	<10copie	<10copie	<10copie	<10copie
HHV-8	<10copie	<10copie	<10copie	<10copie	<10copie

INTERFERENCES

Verify that in the DNA extracted from the sample there is no contamination from mucoproteins and haemoglobin, to exclude possible inhibition of PCR reaction. The interference due to contaminants can be detected through a spectrophotometric analysis, verifying the ratio between the absorbance readings at 260 nm (maximum absorbtion of Nucleic Acids) and 280 nm (maximum absorbtion of Proteins). A pure DNA should have a ratio of approximately 1.8.

QUALITY CONTROL

It is recommended to include in each analytical run, as quality control of every extraction, amplification and detection step, an already tested negative and positive sample, or a reference material with known concentration
In accordance with the Clonit srl ISO EN 13485 Certified quality Management System, each lot of quanty CMV is tested against predetermined specification to ensure consistent product quality.

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TECHNICAL ASSISTANCE

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IVD	*In vitro* diagnostic device
	Read the instruction's manual
	Range of temperature
	Use within (dd/mm/yyyy: year-month)
LOT	Lot (xxxx)
REF	Code
	Manufacturer
Σ	Contains sufficient for <n> tests

EDMA code: 15040240
CND: W0105040205

The ***quanty CMV*** kit is CE marked diagnostic kit according to the European *in vitro* diagnostic directive 98/79/CE.

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Revised: ST. RT12-ENG.10
Revised: 13th October 2016