

## EXTRACTION

- IN EXTRACTION ROOM -

- Homogenize **IC2** and samples to be analysed:

**CSF, whole blood, amniotic fluid, plasma, serum, urine, BAL, biopsies.**

- Add 10 µL **IC2** to the appropriate volume of each sample to be extracted in 1 extraction tube.
- Add 10 µL **IC2** to the appropriate volume of **W0** in 1 extraction tube.
- Perform extraction as below:

INSTRUMENT	KIT	Sample + IC2 Volumes	SAMPLE TYPE	PROTOCOL	ELUTION VOLUME		
QIAcube	QIAamp® DNA Blood Mini Kit	200 µL of sample + 10 µL IC2	Whole blood, amniotic fluid.	Blood and body fluid spin protocol V3	100 µL		
			Plasma, serum, CSF.		50 µL		
			Whole blood, amniotic fluid.		100 µL		
MagNAPure Compact® Instrument	MagNA Pure Compact Nucleic Acid Isolation Kit I		Whole blood.	DNA_Blood_100_400	100 µL		
			Plasma, serum, CSF.		50 µL		
			Amniotic fluid.		100 µL		
MagNA Pure LC System® Instrument	MagNA Pure DNA Isolation Kit I	Whole blood.	Total_NA_Plasma_100_400	100 µL			
		Plasma, serum, CSF.		50 µL			
		Amniotic fluid.		100 µL			
NucliSENS® easyMAG™	NucliSENS® easyMAG reagents	Whole blood.	DNA I Blood_Cell High Performance	100 µL			
		Amniotic fluid.		100 µL			
		Plasma, serum, CSF.		50 µL			
m2000sp™ Abbott	Sample Preparation System DNA	800 µL of sample + 10 µL IC2 (extract 300 µL)	Whole blood, plasma, BAL, urine, biopsies, amniotic fluid.	DNA-Blood-LL-300-150 v081507	250 µL (eluate 150 µL)		
		400 µL of sample + 10 µL IC2 (extract 250 µL)			Plasma.	Sample Preparation Protocol 5	65 µL (eluate 50 µL)

Store extracted samples at -18°C/-22°C

### ANCILLARY REAGENTS:

- Quanti HHV8 QS r-gene™ - ref.: 68-008**
- CELL Control r-gene™ - ref.: 71-106**
- Colour Compensation r-gene™ - ref.: 71-103 for LightCycler® 2.0 Instruments use only.**

### GLOSSARY:

<b>IC2</b>	Internal Control 2
<b>W0</b>	Water for extraction
<b>R5</b>	CMV and IC2 Amplification premix
<b>R6, R7, R8</b>	HHV Amplification premix
<b>QS</b>	Quantification Standard
<b>SC</b>	Sensitivity Control
<b>CT</b>	Crossing Threshold
<b>IC2sample</b>	Sample Extraction + Inhibition Control
<b>IC2W0</b>	Reference Extraction + Inhibition Control

## AMPLIFICATION

- IN AMPLIFICATION ROOM -

Validated on:

LightCycler® 530 & 560	Applied Biosystems® FAM & VIC	Rotor-Gene™ GREEN & YELLOW	Stratagene® Versant® kPCR AD FAM & HEX	Opticon™ i.Cycler®
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### CMV HHV6,7,8 R-gene™ AMPLIFICATION PROGRAM

- Enter the following program:

STEPS	TIME	TEMPERATURE	CYCLES	FLUORESCENCE ACQUISITION
Taq Polymerase Activation	15 min.	95°C	1	-
Amplification	10 sec.	95°C	45	530 + 560 nm end of the elongation
	20 sec. for Stratagene			
	40 sec.	60°C		

Note 1: for LightCycler® instruments, set **SEEK TEMPERATURE** to the temperature corresponding to the fluorescence acquisition.

Note 2: for LightCycler® instruments, add a cooling step: 30 sec. / 40°C / 1 cycle at the end of the PCR.

Note 3: for Applied Biosystems® instruments, select **NONE** in **PASSIVE REFERENCE**.

Note 4: for Rotor-Gene™, calibrate the signal by clicking on **GAIN OPTIMISATION**.

Note 5: for Stratagene® and Versant® kPCR Molecular System AD instruments, select **NONE** in **REFERENCE DYE**.

### CMV HHV6,7,8 R-gene™ AMPLIFICATION PREPARATION

- Plan number of amplification tubes taking into account:
  - The virus(es) to be detected;
  - The necessity to create standard curve or not;
  - The negative/inhibition control(s), sensitivity control(s) (**SC**).
- Distribute 15 µL of the **R5** and/or **R6** and/or **R7** and/or **R8** in amplification tubes.
- Add 10 µL sample, **QS**, **SC**, extracted **W0**.
- Centrifuge for 15 sec / gently move the microplate if applicable.

### LAUNCHING CMV HHV6,7,8 R-gene™ PROGRAM

- Enter the concentrations of the standards according to extraction method used.

	Extraction 200 µL Elution in 50 µL (copies/mL)	Extraction 200 µL Elution in 100 µL (copies/mL)	m2000sp™ Extraction 300 µL Elution in 250 µL (copies/mL)	Versant® kPCR Molecular System SP Extraction 250 µL Elution in 65 µL (copies/mL)
<b>QS1</b>	1 250 000	2 500 000	4 000 000	1 250 000
<b>QS2</b>	125 000	250 000	400 000	125 000
<b>QS3</b>	12 500	25 000	40 000	12 500
<b>QS4</b>	1 250	2 500	4 000	1 250

- Select the type of each amplification tube as: **SAMPLE** or **UNKNOWN** or **STANDARD** or **NEGATIVE CONTROL** or **POSITIVE CONTROL**.

## RESULTS

### 1. DATA ANALYSIS

- Import the external standard curve (if applicable).
- Identify and quantify the positive samples by:
  - using "fit point" method with LightCycler®1.0;
  - using "second derivative maximum" method for LC2.0 and LC480;
  - adjusting threshold line for Applied Biosystems®, Rotor-Gene™, Stratagene® and Versant® kPCR Molecular System AD.

### 2. INTERPRETATION

- Conditions for test validation:
  - Negative Control: NO SIGNAL.
  - IC2W0 (560 nm): CT ≤ 32 CYCLES.
  - QS3:
    - CT **CMV** between 30 and 35 CYCLES.
    - CT **HHV-6** between 30 and 34 CYCLES.
    - CT **HHV-8** between 27 and 32 CYCLES

- Slope / Efficiency:

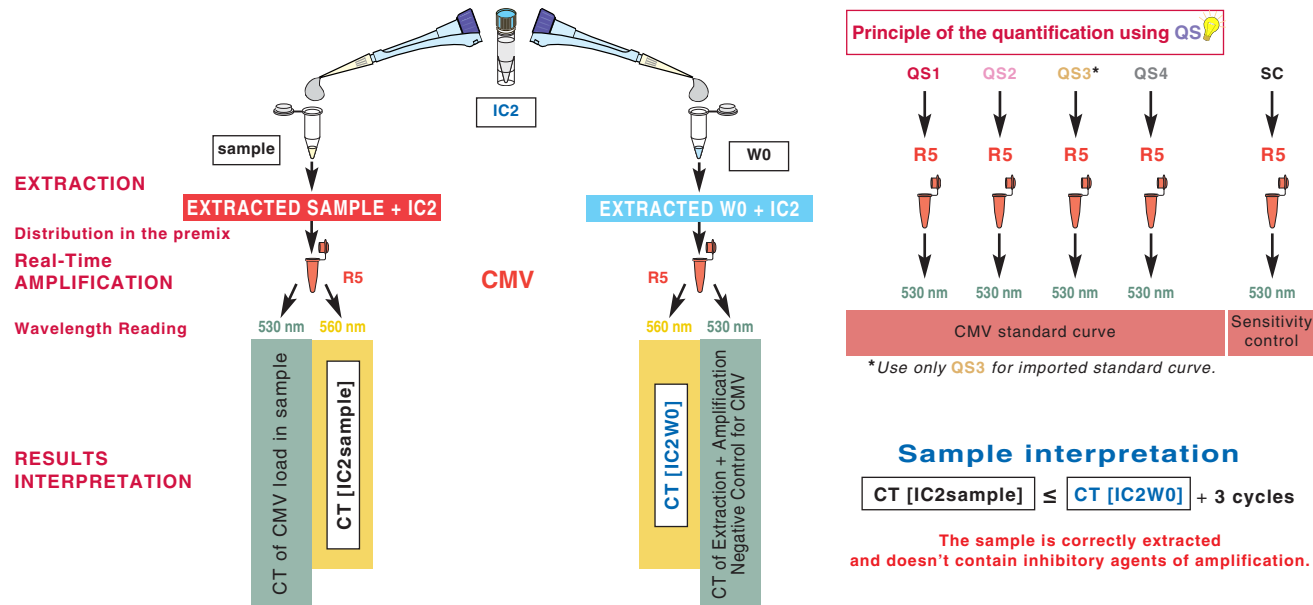
Real-Time PCR Platform	VALUABLE SLOPE / EFFICIENCY	
	If all QS are tested.	If all QS are tested to be stored for subsequent importation.
LightCycler® 1.0	-3.917 < Slope < -3.103	-3.587 < Slope < -3.208
LightCycler® 2.0 / LightCycler® 480	1.8 < Efficiency < 2.1	1.9 < Efficiency < 2.05
Rotor-Gene™	0.8 < Efficiency < 1.1	0.9 < Efficiency < 1.05
Applied Biosystems®	-3.917 < Slope < -3.103	NOT APPLICABLE
Stratagene®MX3000P, Versant® kPCR AD	0.8 < Efficiency < 1.1	
Opticon™	-3.917 < Efficiency < -3.103	

### Quantification

EXTRACTION and INHIBITION CONTROL	CT [IC2sample] ≤ CT [IC2W0] + 3 cycles		CT [IC2sample] > CT [IC2W0] + 3 cycles	
	NON INHIBITED and correctly extracted sample		INHIBITED or badly extracted sample	
SAMPLE	Calculated CT	Non calculated CT	Calculated CT	Non calculated CT
CMV or HHV6 or HHV8 quantification	Validated quantification	Sample validated as negative	Perform quantification again. Sample validated as positive.	NOT VALID
HHV7 or HHV8 qualitative	POSITIVE	NEGATIVE	POSITIVE	NOT VALID

HHV8 can be quantified by using HHV8 QS provided in Argene, ref.: 68-008. This outline procedure does not replace the original protocol.

## PRINCIPLE for CMV QUANTIFICATION



💡 If HHV8 quantification is required, add HHV8 QS from Argene Quanti HHV8 QS r-gene™, ref.: 68-008 in R8 premix to create HHV8 Standard Curve.