HSV1 HSV2 VZV R-gene™ - ref.: 69-004

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EXTRACTION

- IN EXTRACTION ROOM -

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

CSF, cutaneous, mucous and gynaecological smears, ENT and ophtalmological specimens, BAL, plasma.

- 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
	QIAamp® DNA Blood Mini Kit	200 µL of sample + 10 µL IC2	CSF, BAL, ophtalmological specimens, gynaecological smears, ENT cutaneous, plasma.		50 µL
	QIAamp® MinElute® Virus Spin Kit		CSF		50 µL
QIAcube	QIAamp® DNA Blood Mini Kit		CSF, BAL, ophtalmological specimens, gynaecological smears, ENT cutaneous, plasma.	Blood and body fluid spin protocol V3	50 µL
	QIAamp® MinElute® Virus Spin Kit		CSF		
MagNAPure Instrument	MagNA Pure Compact Nucleic Acid Isolation Kit I		CSF	Total_NA_Plasma _100_400	50 µL
NucliSENS® easyMAG™	NucliSens® EasyMag reagents		CSF	Generic	50 µL
Versant® kPCR Molecular System SP - SIEMENS	Sample Preparation 1.0 Reagents	400 µL of sample + 10 µL IC2 *(extract 250 µL)*	CSF	Sample Preparation Protocol 5	65 µL *(eluate 50 µL)*

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

ANCILLARY REAGENTS:

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

GLOSSARY:

IC2	Internal Control 2
W0	Water for extraction
R1, R2, R3	HHV Amplification premix
QS	Quantification Standard
SC	Sensitivity Control
CT	Crossing Threshold
IC2sample	Sample Extraction + Inhibition Control
IC2W0	Reference Extraction + Inhibition Control

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AMPLIFICATION

- IN AMPLIFICATION ROOM -

Validated on:

LightCycler® 530 & 560	SmartCycler® FCTC / FAM & Cy3	Applied Biosystems FAM & VIC	Rotor-Gene™ GREEN & YELLOW	Stratagene® Versant® kPCR AD FAM & HEX

HSV1 HSV2 VZV R-gene™ AMPLIFICATION PROGRAM

- Enter the following program:

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

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**.

HSV1 HSV2 VZV 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).
- Distribute 15 µL of the R1 and/or R2 and/or R3 in amplification tubes.
- Add 10 µL sample, **QS**, **SC**, extracted **W0**.
- Centrifuge for 15 sec / gently move the microplate if applicable.

LAUNCHING HSV1 HSV2 VZV R-gene™ PROGRAM

- Enter the following concentrations of the standards in copies / mL.

	QUANTIFICATION
QS1	500 000
QS2	50 000
QS3	5 000
QS4	500

- 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;
  - setting **30** as default parameter in "**MANUAL THRESH FLUOR UNITS**" for SmartCycler®.
  - 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 between 31 and 35 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	-4.339 < Slope < -3.103	
LightCycler® 2.0 / LightCycler® 480	1.7 < Efficiency < 2.1	
SmartCycler® 2.0	-0.322 < Slope < -0.230	
Rotor-Gene™	0.7 < Efficiency < 1.1	
Applied Biosystems	-4.339 < Slope < -3.103	*NOT APPLICABLE*
Stratagene®, Versant® kPCR AD	0.7 < Efficiency < 1.1	

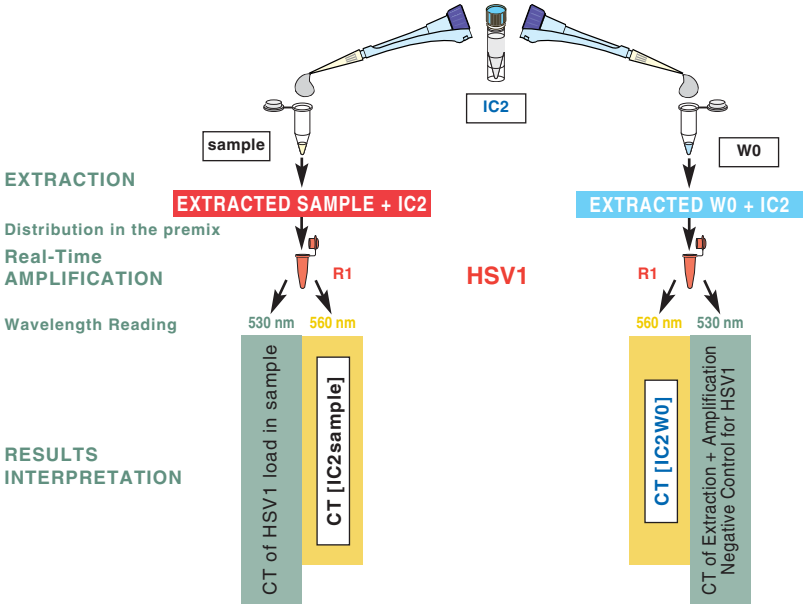
- **Quantification**

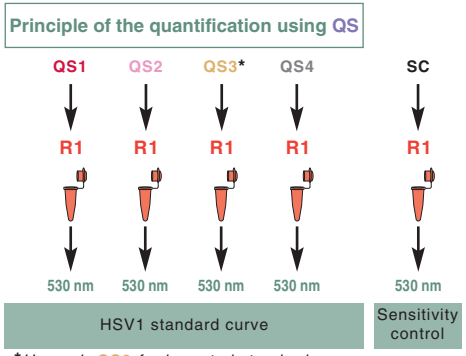
EXTRACTION and INHIBITION CONTROL	CT [IC2sample] ≤ CT [IC2W0] + 3 cycles		CT [IC2sample] > CT [IC2W0] + 3 cycles	
	NON INHIBITED and correctly extracted sample		INHIBITED or badly extracted sample	
SAMPLE	Calculated CT	Non calculated CT	Calculated CT	Non calculated CT
HSV-1, HSV-2,VZV quantification	Validated quantification	Sample validated as negative	Perform quantification again. Sample validated as positive.	NOT VALID

This outline procedure does not replace the original protocol.

O.P.69-004_VD_230710

PRINCIPLE for HSV1 QUANTIFICATION





*Use only QS3 for imported standard curve.

Sample interpretation

CT [IC2sample] ≤ CT [IC2W0] + 3 cycles

The sample is correctly extracted and doesn't contain inhibitory agents of amplification.