LightCycler 480
DNA R-gene® Kits
Programming

- **The use of these guideline is dedicated for Argene kits that mentionned the instrument LightCycler 480 in their package insert.**

- **Some products have their own amplification program following the same principle. In this case, please refer to their Outlined procedure.**

Products to be amplified relate to the extracted DNA obtained with the extraction methods recommended in the corresponding datasheet.

Plan the experiment as described in the datasheet.

- **AMPLIFICATION PROGRAM: "60°C R-gene" Program**

Steps		Time	Temperature	Cycles	Wavelength for signal reading
Taq Polymerase Activation		15 min.	95°C	1	-
Amplification	Denaturation	10 sec.	95°C	45	-
	Annealing Elongation	40 sec.	60°C		FAM (465-510) and/or VIC/HEX/Yellow555 (533-580) end of the annealing
Cooling (*Optional*)		30 sec.	40°C	1	-

1. Throughout the patient follow-up, it is imperative to use the same protocol and to use the same extraction and amplification instrument.

2. This guideline is based on the LightCycler 480 Version 1.5.0 Software.

LightCycler 480 Programming

In the room reserved for amplification

For multiple wavelengths detection, the use of a colour compensation file is imperative to interpret the results on the LightCycler 480.

Make sure that LightCycler 480 used is a LightCycler 480 System II (Fig1).
The optical system of the LightCycler 480 System I does not allow an automatic colour compensation.

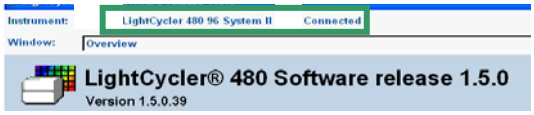

FIG.1

STARTING THE LIGHTCYCLER SOFTWARE VERSION 1.5.0

- Switch on the computer, its screen and the LightCycler 480.
- Start the LightCycler Software by clicking its icon LIGHTCYCLER®480 SW 1.5 (Fig.2).
- Enter the user name and the software password.
- In EXPERIMENT CREATION field :
  Select WHITE PLATES or CLEAR PLATES (Fig.3).
- Click on NEW EXPERIMENT to open the programming and run module.

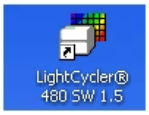

FIG.2

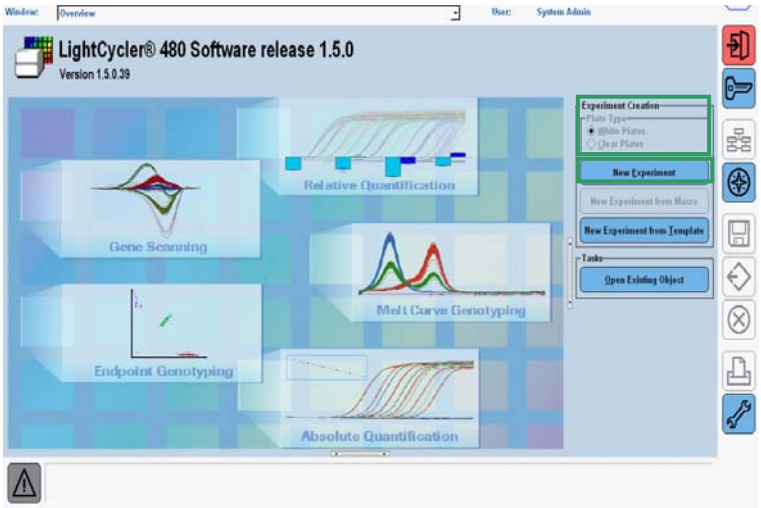

FIG.3

ENTRY OF THE PROGRAM DATA

- In the DETECTION FORMAT field select DUAL COLOR HYDROLYSIS PROBE/UPL PROBE (Fig.4)
- Set the REACTION VOLUME to 25 µL.

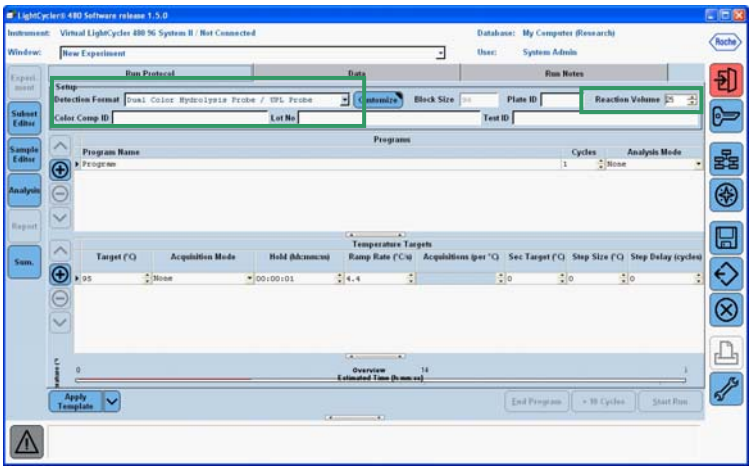

FIG.4

ENTRY OF THE AMPLIFICATION PARAMETERS:

- In PROGRAM NAME column (Fig.5):
- Enter ACTIVATION in the 1st row.
- Click the «+».
- Enter AMPLIFICATION in the 2nd row.
- Click the «+».
- Enter COOLING in the 3rd row.

- In the CYCLES column:
- Enter 1 in the 1st row.
- Enter 45 in the 2nd row.
- Enter 1 in the 3rd row.

- In the ANALYSIS MODE column:
- Select NONE in the 1st row.
- Select QUANTIFICATION in the 2nd row.
- Select NONE in the 3rd row.

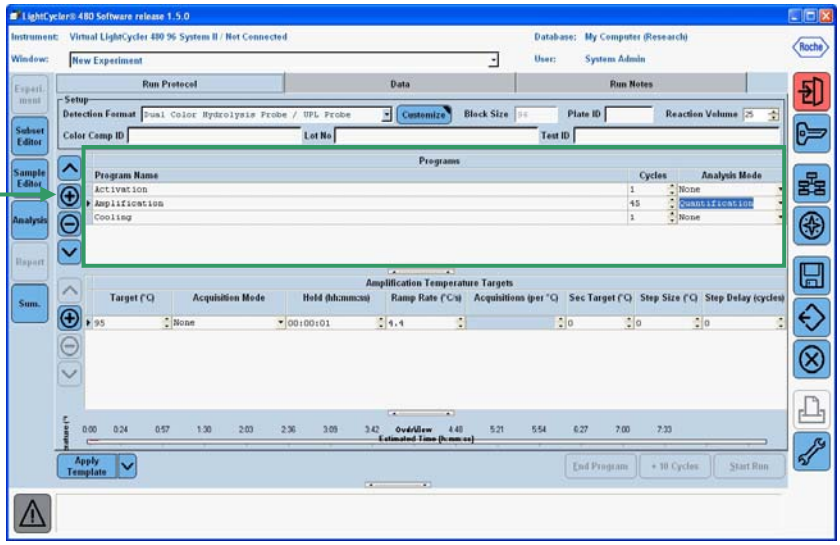

FIG.5

Activation step

- Click on the ACTIVATION program:
  - Set 95 in the TARGET column
  - Set 00:15:00 in the HOLD column (Fig.6).

Amplification step

- Click on the AMPLIFICATION program:
  - Set 95 in the 1st row of the TARGET column.
  - Set 00:00:10 in the 1st row of the HOLD column.
  - Click the «+» 1 time.
  - Set 60 in the 2nd row of the TARGET column.
  - Select SINGLE in the ACQUISITION MODE column.
  - Set 00:00:40 in the 2nd row of the HOLD column.

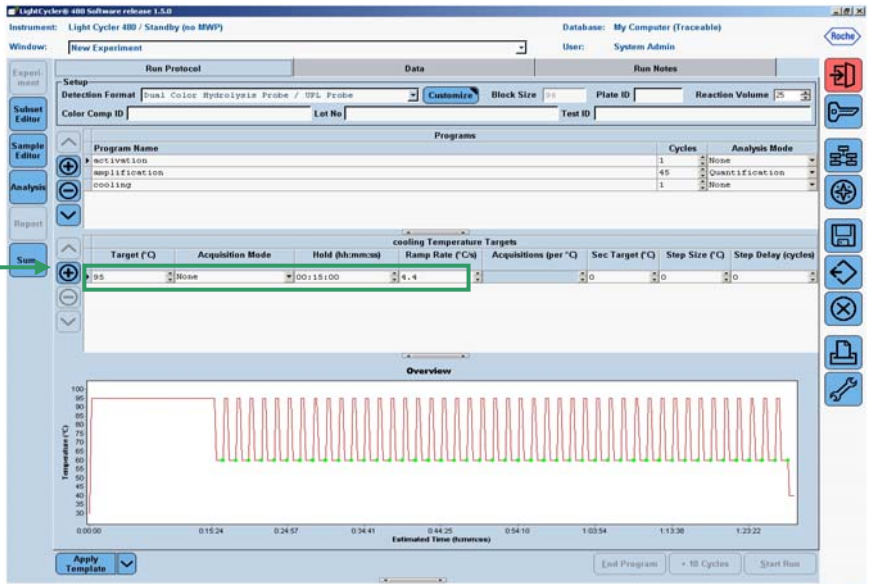

FIG.6

Cooling step

- Click on the COOLING program.
  - Set 40 in the TARGET column
  - Set 00:00:30 in the HOLD column.

ENTRY of PARAMETERS FOR RESULTS INTERPRETATION

- Click on **Sample Editor**
  - In the **Step 1: Select Workflow** field Select **Abs Quant** (Fig.7).
  - In the **Step 2: Select Samples** field Select **All samples**.
  - In the **Select Filter Combinations** field Select the check box **465-510** and/or **533-580**.

  ⚠ **Use the 533-580 nm channel only for multiple wavelengths detection.**

  - In the **Units** field enter **Copies/mL**.

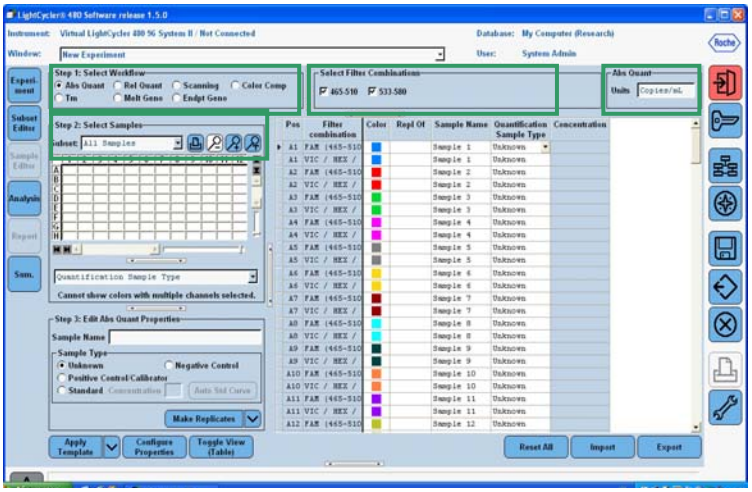

Fig.7

SAVE THE "60°C Rgene" PROGRAM

- Click the tick button near **Apply Template** to store this program for subsequent experiments and select **Save as Template** (Fig.8).

Fig.8

A new window appears:

- Click on the **"+"** next to **Templates**
- Click on **Run Templates**
- Enter the name of the program: **60°C Rgene.**
- Click on **Run Templates** again and validate by clicking on the tick button.

Note: **If this program has already been saved, it can be launched directly by clicking on New Experiment from Template in the main menu and selecting the appropriate program (60°C Rgene).**

Running the Program

- Run the amplification program (stored according to the instructions described in section “LightCycler 480 Programming”) by clicking in **EXPERIMENT** section on **START RUN** (Fig 9).
- To store the file, click on the record image.
- Type the name of your file *(ex: **EBV RGENE YYYY-MM-DD**)* and validate.

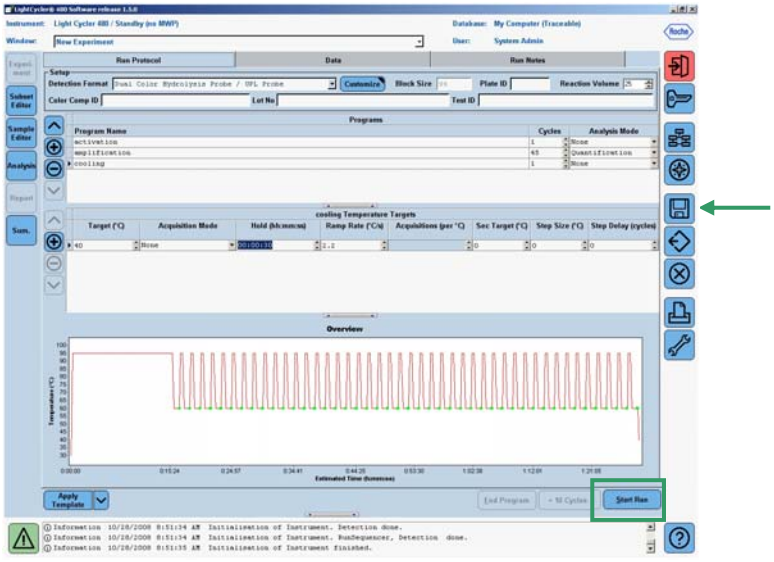

FIG.9

DEFINE THE SAMPLES

- Click on **SAMPLE EDITOR** to specify the samples, standards and controls to be amplified in each position of the block.
- Fill the **SAMPLE NAME** column in the tab.
- Define the samples in the **QUANTIFICATION SAMPLE TYPE** column in the tab as described in the table below.

	Quantitative kits		Qualitative kits	
Channels	FAM (465-510)	VIC/HEX/YELLOW555 (533-580)	FAM (465-510)	VIC/HEX/YELLOW555 (533-580)
Patient samples	UNKNOWN	UNKNOWN	UNKNOWN	UNKNOWN
Quantification standard (**QS**)	STANDARD	UNKNOWN	Not applicable	
Sensitivity control (**SC**)	UNKNOWN	UNKNOWN	Not applicable	
Extraction+inhibition control (**IC2W0**)	NEGATIVE CONTROL	POSITIVE CONTROL/CALIBRATOR	NEGATIVE CONTROL	POSITIVE CONTROL/CALIBRATOR
Positive control (**PC**)	Not applicable		POSITIVE CONTROL/CALIBRATOR	UNKNOWN
Negative Amplification Control (**R0**)	NEGATIVE CONTROL	UNKNOWN	NEGATIVE CONTROL	UNKNOWN

If several parameters are detected in the same experiment, each quantification standard range has to be identified one by one. Not applicable for qualitative detection kits.

ONLY FOR QUANTIFICATION:

- Enter the concentration of the standard(s) (cp/mL) in the **CONCENTRATION** column.

Conditions					Standard concentration to be entered			
R-gene® Kit	**Extraction method**	**Specimen**	**Sample volume to be extracted**	**Elution volume**	**QS1**	**QS2**	**QS3**	**QS4**
69-002 EBV R-gene®	QIAamp DNA Blood Mini kit	Whole blood	200 µL	100 µL	5 000 000	500 000	50 000	5 000
		Plasma / CSF		50 µL	2 500 000	250 000	25 000	2 500
	QIAcube	Whole blood		100 µL	5 000 000	500 000	50 000	5 000
		Plasma / CSF		50 µL	2 500 000	250 000	25 000	2 500
	MagNA Pure Compact MagNA Pure LC System	Whole blood		100 µL	5 000 000	500 000	50 000	5 000
		Plasma / CSF		50 µL	2 500 000	250 000	25 000	2 500
	NucliSENS® easyMAG®	Whole blood / Plasma / CSF		50 µL	2 500 000	250 000	25 000	2 500
	BioRobot EZ1 Workstation	Whole blood	350 µL	200 µL	6 000 000	600 000	60 000	6 000
	m2000sp Abbott	Whole blood / plasma / CSF / BAL / biopsies	800 µL (extract 300 µL)	250 µL (eluate 150 µL)	8 000 000	800 000	80 000	8 000
	Versant kPCR Molecular System SP	Plasma	400 µL (extract 250 µL)	65 µL (eluate 50 µL)	2 500 000	250 000	25 000	2 500
69-003 CMV R-gene® **69-100** CMV HHV6, 7,8 R-gene®	QIAamp DNA Blood Mini kit	Whole blood Amniotic Fluid	200 µL	100 µL	2 500 000	250 000	25 000	2 500
		Serum / Plasma / CSF		50 µL	1 250 000	125 000	12 500	1 250
	QIAcube	Whole blood Amniotic Fluid		100 µL	2 500 000	250 000	25 000	2 500
		Serum / Plasma / CSF		50 µL	1 250 000	125 000	12 500	1 250
	MagNA Pure Compact MagNA Pure LC System	Whole blood Amniotic Fluid		100 µL	2 500 000	250 000	25 000	2 500
		Serum / Plasma / CSF		50 µL	1 250 000	125 000	12 500	1 250
	NucliSENS® easyMAG®	Whole blood						
		Serum / Plasma / CSF						
	m2000sp Abbott	Whole blood / plasma / BAL / urine / biopsies / amniotic fluid	800 µL (extract 300 µL)	250 µL (eluate 150 µL)	4 000 000	400 000	40 000	4 000
	Versant kPCR Molecular System SP	Plasma	400 µL (extract 250 µL)	65 µL (eluate 50 µL)	1 250 000	125 000	12 500	1 250

Conditions					Standard concentration to be entered			
R-gene® Kit	Extraction method	Specimen	Sample volume to be extracted	Elution volume	QS1	QS2	QS3	QS4
69-004 HSV1 HSV2 VZV R-gene® **71-015** HSV1 r-gene® **71-016** HSV2 r-gene® **71-017** VZV r-gene®		QIAamp DNA Blood Mini kit	CSF / BAL / Ophthalmologic specimens / Gynaecological, smears / ENT cutaneous / plasma	200 µL	50 µL	500 000	50 000	5 000	500
		QIAamp MinElute Virus Spin Kit	CSF					
	QIAcube	QIAamp DNA Blood Mini kit	CSF / BAL / Ophthalmologic specimens / Gynaecological, smears / ENT cutaneous / plasma					
		QIAamp MinElute Virus Spin Kit	CSF					
	MagNA Pure Compact MagNA Pure LC System		CSF					
	NucliSENS® easy MAG®		CSF					
	Versant kPCR Molecular System SP		CSF	400 µL (extract 250 µL)	65 µL (eluate 50µL)			
69-010 ADENOVIRUS R-gene®		QIAamp DNA Blood Mini kit	Whole blood / Respiratory samples* / Stool* / biopsies	200 µL	100 µL	2 500 000	250 000	25 000	2 500
			Plasma / CSF		50 µL	1 250 000	125 000	12 500	1 250
		QIAamp DNA Stool Mini kit	Stool*		100 µL	2 500 000	250 000	25 000	2 500
	QIAcube	QIAamp DNA Blood Mini kit	Whole blood / Respiratory samples* / Stool* / biopsies		100 µL	2 500 000	250 000	25 000	2 500
			Plasma / CSF		50 µL	1 250 000	125 000	12 500	1 250
		QIAamp DNA Stool Mini kit	Stool*		100 µL	2 500 000	250 000	25 000	2 500
	MagNA Pure Compact MagNA Pure LC System		Plasma / Respiratory samples*		50 µL	1 250 000	125 000	12 500	1 250
			Whole blood / Stool*		100 µL	2 500 000	250 000	25 000	2 500
	NucliSENS® easyMAG®		Respiratory samples* / Whole blood / Stool*		50 µL	1 250 000	125 000	12 500	1 250
	BioRobot M48 QIAGEN		Respiratory samples*		100 µL	2 500 000	250 000	25 000	2 500
	m2000sp Abbott		Whole blood / biopsies / Respiratory samples*	800 µL (extract 300 µL)	250 µL (eluate 150µL)	4 000 000	400 000	40 000	4 000
	Versant kPCR Molecular System SP		Plasma / CSF	400 µL (extract 250 µL)	65 µL (eluate 50µL)	1 250 000	125 000	12 500	1 250

***** *For a quantitative detection in cp/PCR, see 69-010 datasheet, section 11.3.*

Conditions					Standard concentration to be entered			
R-gene® Kit	**Extraction method**	**Specimen**	**Sample volume to be extracted**	**Elution volume**	**QS1**	**QS2**	**QS3**	**QS4**
69-013 **BK Virus R-gene®**	QIAamp DNA Blood Mini kit	Whole blood / Urine	200 µL	100 µL	2 500 000	250 000	25 000	2 500
		Plasma		50 µL	1 250 000	125 000	12 500	1 250
	QIAcube	Whole blood / Urine		100 µL	2 500 000	250 000	25 000	2 500
		Plasma		50 µL	1 250 000	125 000	12 500	1 250
	MagNA Pure Compact	Whole blood / Urine		100 µL	2 500 000	250 000	25 000	2 500
		Plasma		50 µL	1 250 000	125 000	12 500	1 250
	MagNA Pure LC System	Whole blood		100 µL	2 500 000	250 000	25 000	2 500
		Plasma / Urine		50 µL	1 250 000	125 000	12 500	1 250
	NucliSENS® easyMAG®	Whole blood / Plasma		50 µL	1 250 000	125 000	12 500	1 250
		Urine		100 µL	2 500 000	250 000	25 000	2 500
	Versant kPCR Molecular System SP	Plasma / Urine	400 µL (extract 250 µL)	65 µL (eluate 50µL)	1 250 000	125 000	12 500	1 250

Data Analysis

GENERATE ANALYSIS FILE(S)

- At the end of the run, in **Analysis** section, in **Create New Analysis** field double click on **Abs Quant / 2nd derivative Max** (Fig.10). Then, validate (Fig.11).

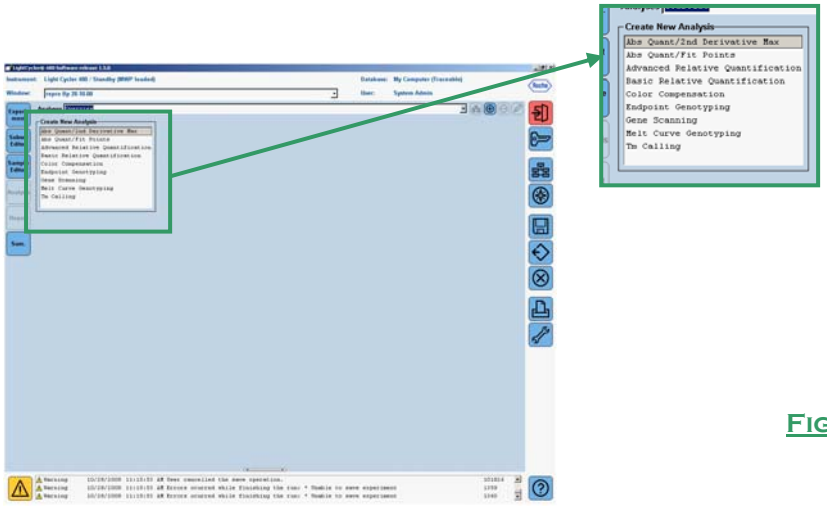

Fig.10

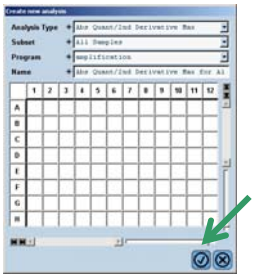

Fig.11

ACTIVATION OF COLOUR COMPENSATION FILE/OBJECT

- Click on the tick button near to **Color comp** (Fig.12) then select **In Database** and choose the file : **Universal CC FAM (510)-VIC (580)** and validate.

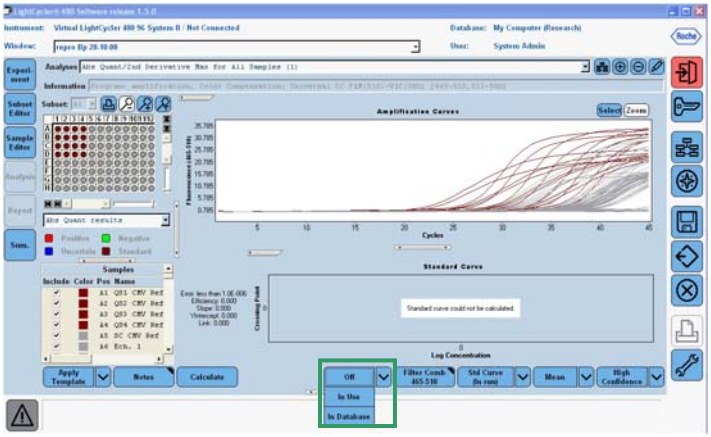

Fig.12

- A new window appears:

- Select the check box **FAM (465-510)** and **VIC/HEX/Yellow555 (533-580)** and validate (Fig13).

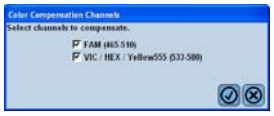

Fig.13

ANALYSIS OF SAMPLES AND STANDARDS

- Click on **FILTER COMB** and choose **FAM (465-510)** (Fig.14)

- Click on **CALCULATE** .

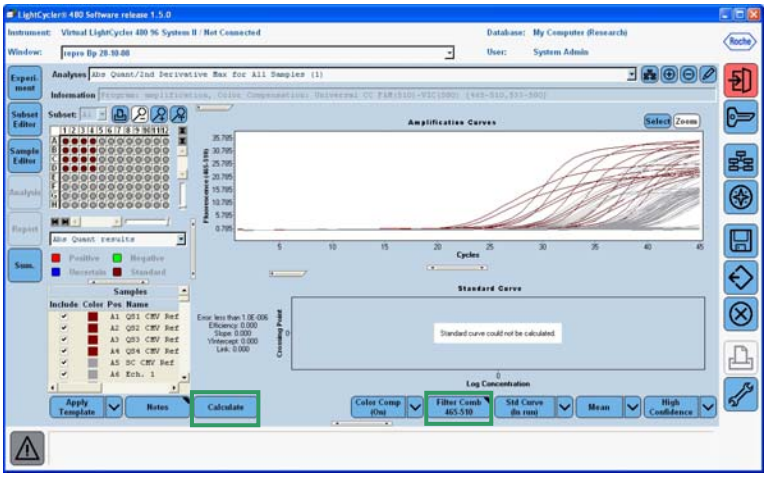

FIG.14

ONLY FOR QUANTIFICATION:

Standard curve: Viral Load Quantification

- The quantification measure must be performed with the **ABS QUANT/2ND DERIVATIVE MAX** method.
- **If the standard curve is created with all 4 quantification standards (QS1, QS2, QS3, QS4):**
  - A calculated concentration is displayed for each well from the column **CONC (COPIES /ML)**.
  - Read the calculated concentration for each positive sample.
- **If the standard curve is imported from the QS3:**
  - Click on the tick button next to **USE EFFICIENCY** then select **STD CURVE (EXTERNAL)**.
  - Choose a standard curve previously saved.
    Example: "HSV1 R-gene or HSV2 R-gene or VZV R-gene – Lot number-date".
  - Read the calculated concentration for each positive in the column **CONC (COPIES /ML)**.

CREATION OF THE EXTERNAL STANDARD CURVE (Optional)

ONLY FOR QUANTIFICATION

- An external standard curve can be created:
  - When the **R-gene®** kit is used for the first time.
  - With every new batch number of the kit.
  - When the standard curve has been created more than 3 months ago.
- The external standard curve is created with the 4 quantification standards (**QS1, QS2, QS3, QS4**).
- This curve can be stored and imported in subsequent runs when using products of the same batch number. However, the quantification standard **QS3** ALWAYS has to be created with every new run.
- The value of the **EFFICIENCY** and the **CP** of **QS3** must be between:

	69-002	69-003	69-004 71-015 71-016 71-017	69-100	69-010	69-013
Efficiency	1.900 2.050	1.900 2.050	1.700 2.100	1.900 2.050	1.900 2.050	1.800 2.100
CP	28 - 32	30 - 35	31 - 35	**CMV:** 30 - 35 **HHV6:** 30 - 34	29 - 33	28 - 32

- If the value of the **EFFICIENCY** or the **CP** value of **QS3** does not correspond to these parameters, the curve cannot be validated as an external standard curve. A new curve has to be created with all four quantification standards (**QS1, QS2, QS3, QS4**).
- Store the external standard curve by clicking on the tick button near to **STANDARD CURVE (IN RUN)** then choose **SAVE AS EXTERNAL**.
- Type the name of standard curve (*for example: "HSV1 R-gene or HSV2 R-gene or VZV R-gene – Lot number-date"*) and store it in the file **STD CURVE**.
- Repeat for each parameter to be analysed.

ANALYSIS OF CONTROLS (IC2sample, IC2W0)

- Click on the **Filter Comb** and choose **VIC/HEX/Yellow555 (533-580)**

71-101 *(DICO Extra r-gene®, Argene) has to be read at FAM (465-510)*

- Check that the validated colour compensation file was activated (**Color Comp (on)**)

- Click on **Calculate.**

- Read the calculated CP in the **CP** column for each sample inhibition control (**IC2Sample**) and compare this value to the CP value of the reference extraction+ inhibition control (**IC2W0**).

NOTE : **Do not read the CP of the quantification standards (QS), sensitivity control (SC), negative control (R0) and positive control (PC) at VIC/HEX/Yellow555 (533-580).**

The interpretation results are only based on the comparison of the CP obtained for each sample inhibition control (IC2sample) with the CP obtained with reference extraction + inhibition control (IC2W0).

Interpretation of Results

- Detailed interpretation is described in each corresponding datasheet.

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