

February 2018

QlAsymphony[®] RGQ Application Sheet

artus[®] CMV QS-RGQ Kit (sample type: plasma)

R3

IVD

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0197

REF

4503363 *artus* CMV QS-RGQ Kit, version 1



Check availability of new electronic labeling revisions at www.qiagen.com/products/artuscmvprkitce.aspx before test execution.

General Information

Kit	<i>artus</i> CMV QS-RGQ Kit, Version 1 (cat.no. 4503363)
Validated sample material	Human EDTA plasma
Front-end purification	QIAasymphony DSP Virus/Pathogen Midi Kit (cat. no. 937055)
Sample volume (including excess volume)	1200 µl
Assay Parameter Set	<i>artus</i> _CMV_plasma1000_V5
Default Assay Control Set	Cellfree1000_V7_DSP_artus_CMV
Elution volume	60 µl
Required software version	Version 4.0 or higher
Master mix volume	30 µl
Template volume	20 µl
Number of reactions	6–24
Runtime on AS module	For 6 reactions: approximately 9 minutes For 72 reactions: approximately 35 minutes

Materials Required but Not Provided

Purification kit

- QIAasymphony DSP Virus/Pathogen Midi Kit (cat. no. 937055)

Adapters for the QIAasymphony SP

- Elution Microtube Rack QS (Cooling Adapter, EMT, v2, Qsym, cat. no. 9020730)
- Transfer frame
- Tube Insert 3B (Insert, 2.0ml v2, samplecarr. (24), Qsym, cat. no. 9242083)

Consumables for the QIAasymphony SP

- Sample Prep Cartridges, 8-well (cat. no. 997002)
- 8-Rod Covers (cat. no. 997004)
- Filter-Tips, 1500 µl (cat. no. 997024)
- Filter-Tips, 200 µl (cat. no. 990332)
- Elution Microtubes CL (cat. no. 19588)
- Tip disposal bags (cat. no. 9013395)
- Micro tubes 2.0 ml Type H or Micro tubes 2.0 ml Type I (Sarstedt®, cat. nos. 72.693 and 72.694, www.sarstedt.com) for use with samples and internal controls

Adapters and reagent holders for the QIAasymphony AS

- Reagent holder 1 QS (Cooling Adapter, Reagent Holder 1, Qsym, cat. no. 9018090)
- RG Strip Tubes 72 QS (Cooling Adapter, RG Strip Tubes 72, Qsym, cat. no. 9018092)

Consumables for the QIAasymphony AS

- Strip Tubes and Caps, 0.1 ml (cat. no. 981103)
- Tubes, conical, 2 ml, Qsym AS (cat. no. 997102) or Micro tubes 2.0 ml Type I (Sarstedt, cat. no. 72.694.005)
- Alternatively: Tubes, conical, 5 ml, Qsym AS (cat. no. 997104) or Tubes with flat base from PP (Sarstedt, cat. no. 60.558.001)
- Filter-Tips, 1500 µl (cat. no. 997024)
- Filter-Tips, 200 µl (cat. no. 990332)
- Filter-Tips, 50 µl (cat. no. 997120)
- Tip disposal bags (cat. no. 9013395)

Specimen Handling and Storage

Sample collection	Blood sample 5–10 ml EDTA blood 8x overhead mix — no agitation! Heparinized human samples must not be used.
Sample storage	Separation: 20 minutes centrifugation, 800–1600 x g within 24 hours post-collection Transfer the isolated plasma into a sterile polypropylene tube The sensitivity of the assay can be reduced if samples are frozen as a matter of routine or stored for a longer period of time.
Sample transport	Shatterproof transport Shipment within 24 hours Mail shipment according to legal instructions for the transport of pathogen material* Blood samples should be shipped cool (2 to 8°C)
Interfering substances	Heparin (≥ 10 IU/ml) affects the PCR. Samples collected in tubes containing heparin as an anticoagulant or samples from heparinized patients must not be used.
Sample preparation	Prevent formation of foam in or on the samples Samples should be equilibrated to room temperature (15–25°C) before starting the run.

* International Air Transport Association (IATA). Dangerous Goods Regulations.

Procedure

Preparation of carrier RNA and addition of the internal control to the samples

Using the QIAasymphony DSP Virus/Pathogen Midi Kit in combination with the *artus* CMV QS-RGQ Kit requires introduction of the internal control (CMV RG IC) into the purification procedure to monitor the efficiency of sample preparation and downstream assay.

For a multi-assay run where both CMV and EBV will be assayed in the same PCR, make sure that the CMV RG IC, from the *artus* CMV QS-RGQ Kit, is used in the purification process. Use a CMV RG IC from the same lot for both sample preparation and for assay setup of the PCR controls. Do not use a CMV RG IC with a different lot number.

Internal controls must be added with carrier RNA (CARRIER)–Buffer AVE (AVE) mixture, and the total volume of the internal control–carrier RNA (CARRIER)–Buffer AVE (AVE) mixture remains 120 µl.

The table represents the addition of internal control to the isolation at a ratio of 0.1 µl per 1 µl elution volume. We recommend preparing fresh mixtures for each run just before use. Alternatively, the “IC Calculator” Tool in the QIAasymphony Management Console may be used.

Component	Volume (µl) (Sarstedt tubes)*	Volume (µl) (Corning tubes)†
Stock carrier RNA (CARRIER)	5	5
Internal control‡	9	9
Buffer AVE	106	106
Final volume per sample (excluding dead volume)	120	120
Total volume for n samples	$(n \times 120) + 360^{\S}$	$(n \times 120) + 600^{\P}$

* Micro tubes 2.0 ml Type H and Micro tubes 2.0 ml Type I, Sarstedt cat. nos. 72.693 and 72.694.

† Tubes 14 ml, 17 x 100 mm polystyrene round-bottom (Corning® Inc., cat. no. 352051; Becton Dickinson was the previous supplier of this tube and Corning Inc. is now the new supplier).

‡ The calculation of the amount of internal control is based on the initial elution volumes (90 µl). Additional void volume depends on the type of sample tube used.

§ Internal control mixture corresponding to 3 additional samples (i.e., 360 µl) is required. Do not fill more than 1.92 ml total volume (corresponding to a maximum of 13 samples. These volumes are specific for Micro tubes 2.0 ml Type H and Micro tubes 2.0 ml Type I, Sarstedt cat. nos. 72.693 and 72.694).

¶ Internal control mixture corresponding to 5 additional samples (i.e., 600 µl) is required. Do not fill more than 13.92 ml total volume (corresponding to a maximum of 111 samples. These volumes are specific for Tubes 14 ml, 17 x 100 mm polystyrene round-bottom, Corning Inc., cat. no. 352051; Becton Dickinson was the previous supplier of this tube and Corning Inc. is now the new supplier).

QIAsymphony SP setup

“Waste” drawer

Unit box holder 1–4	Empty unit boxes
Waste bag holder	Waste bag
Liquid waste bottle holder	Empty and install liquid waste bottle

“Eluate” drawer

Elution rack	Elution Microtubes CL in Elution Microtube Rack QS and Transfer frame Use slot 1, cooling position
Elution volume*	Preselected elution volume: 60 µl Initial elution volume: 90 µl

* The elution volume is preselected for the protocol. This is the minimum accessible volume of eluate in the final elution tube. The initial volume of elution solution is required to ensure that the actual volume of eluate is the same as the preselected volume.

“Reagents and Consumables” drawer

RC Position 1 and 2	Load 1 reagent cartridge (RC) for up to 48 samples or 2 new reagent cartridges (RC) for up to 96 samples
Tip rack holder positions 1–18	Load sufficient racks of disposable filter-tips, 200 µl and 1500 µl (see “Required plasticware for 1–4 sample batches”, page 7)
Unit box holder positions 1–4	Load unit boxes containing sample prep cartridges and 8-Rod Covers (see “Required plasticware for 1–4 sample batches”, page 7)

“Sample” drawer

Sample type	Human EDTA Plasma
Sample volume (including excess volume)	1200 µl
Sample tubes	Micro tubes 2.0 ml Type H or Micro tubes 2.0 ml Type I (Sarstedt, cat. nos. 72.693 and 72.694)
Insert	Tube Insert 3B (cat. no. 9242083)

Required plasticware for 1–4 sample batches

Component	One batch, 24 samples*	Two batches, 48 samples*	Three batches, 72 samples*	Four batches, 96 samples*
Disposable filter-tips, 200 µl†‡	28	52	76	100
Disposable filter-tips, 1500 µl†‡	113	206	309	402
Sample prep cartridges§	21	42	54	72
8-Rod Covers¶	3	6	9	12

* Use of more than one internal control tube per batch and performing more than one inventory scan requires additional disposable filter tips.

† There are 32 filter-tips/tip rack.

‡ Number of required filter-tips includes filter-tips for 1 inventory scan per reagent cartridge.

§ There are 28 sample prep cartridges/unit box.

¶ There are twelve 8-Rod Covers/unit box.

QIAsymphony AS setup

Consumables

During the setup, the appropriate positions for each consumable on the QIAsymphony AS module are indicated on the touchscreen of the instrument.

Consumable	Name on touchscreen	For use with adapter/ reagent holder
Strip Tubes and Caps, 0.1 ml (250)	QIA#981103 *StripTubes 0.1	RG Strip Tubes 72 QS
Tubes, conical, 2 ml, Qsym AS (500) ^{†‡}	QIA#997102 *T2.0 ScrewSkirt [§]	Reagent holder 1 QS
Tubes, conical, 5 ml, Qsym AS (500) ^{†‡}	QIA#997104 *T5.0 ScrewSkirt [§]	Reagent holder 1 QS

* Indicates labware that can be cooled using a cooling adapter with bar code.

[†] For master mix components, system-prepared master mix, assay standards, and assay controls.

[‡] Alternatively, the Sarstedt tubes described in "Material Required but Not Provided", page 3, can be used.

[§] The suffix "(m)" in the touchscreen indicates that liquid level calculations for the respective tube have been optimized for reagents forming a concave meniscus.

Adapters and reagent holders

Rack/reagent holder	Name	Number required [¶]
Reagent holders	Reagent holder 1 QS	1
Sample racks	RG Strip Tubes 72 QS	1

[¶] Calculated for an assay run with 72 reactions.

Filter-tips

Load tip racks starting with tip slots 1, 2 and 3 in the "Eluate and Reagents" drawer, and then load tip racks into tip slots 7, 8 and 9 in the "Assays" drawer.

Consumable	Name on touchscreen	Minimum number for 24 reactions	Minimum number for 72 reactions
Filter-Tips, 1500 µl (1024)	1500 µl	4	5
Filter-Tips, 200 µl (1024)	200 µl	10	8
Filter-Tips, 50 µl (1024)	50 µl	25	73
Tip Disposal Bags	–	1	1

PCR on the Rotor-Gene Q*

Please refer to the software-specific protocol sheet *Settings to run artus QS-RGQ Kits* at www.qiagen.com/products/artuscmvpcrkkitce.aspx for protocol details.

Specific settings for the *artus* CMV QS-RGQ Kit

With Rotor-Gene® Q software 2.1 or higher, the specific settings are shown below.

Reaction Volume (µl)	50
Hold	Hold Temperature: 95 degrees Hold Time: 10 minutes
Cycling	45 times 95 degrees for 15 seconds 65 degrees for 30 seconds (Acquire on Green, Yellow and activate touchdown function for 10 cycles) 72 degrees for 20 seconds
Auto-Gain Optimisation Setup	65 degrees (Samples: Green; IC: Yellow)

Multi-assay run

The detection range of the fluorescence channels has to be determined according to the fluorescence intensities in the PCR tubes. Click **Gain Optimisation** in the **New Run Wizard** dialog box to open the **Auto-Gain Optimisation Setup** dialog box (see Step 6 and Figure 7 in the protocol sheet *Settings to run artus QS-RGQ Kits*).

For a single assay run, set the calibration temperature to **65** to match the annealing temperature of the amplification program. For a multi-assay run where both CMV and EBV will be assayed in the same PCR, adjust the fluorescence channel intensities manually.

* If applicable, Rotor-Gene Q 5plex HRM instrument with a production date of January 2010 or later. The production date can be obtained from the serial number on the back of the instrument. The serial number is in the format "mmyynnn" where "mm" indicates the production month in digits, "yy" indicates the last two digits of the production year, and "nnn" indicates the unique instrument identifier.

1. Click **Edit** (Figure 1) to edit the fluorescence channels.

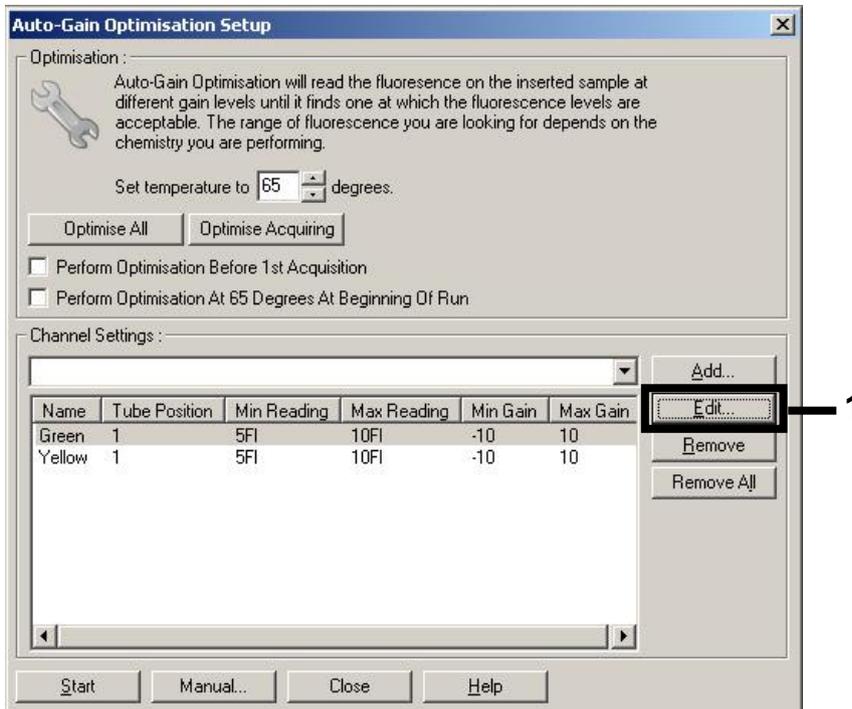


Figure 1. Adjusting the fluorescence channel intensity manually. Adjust the intensity for every fluorescence channel at different tube positions for different assays (CMV and EBV).

2. Set the tube position for a tube for the first *artus* assay (e.g., CMV). Set the tube position for all fluorescence channels, and click **OK** (Figure 2).

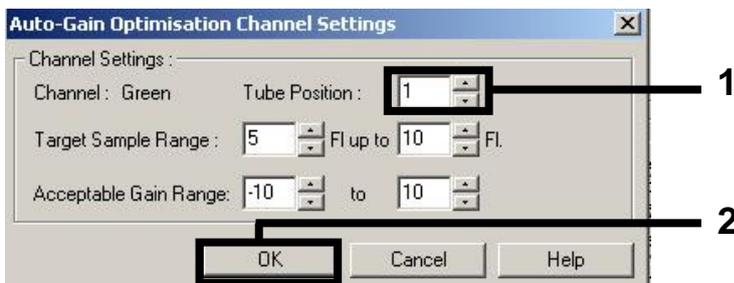


Figure 2. Setting the tube position.

- Click **Start** to begin the gain optimization for the first *artus* assay (Figure 3).

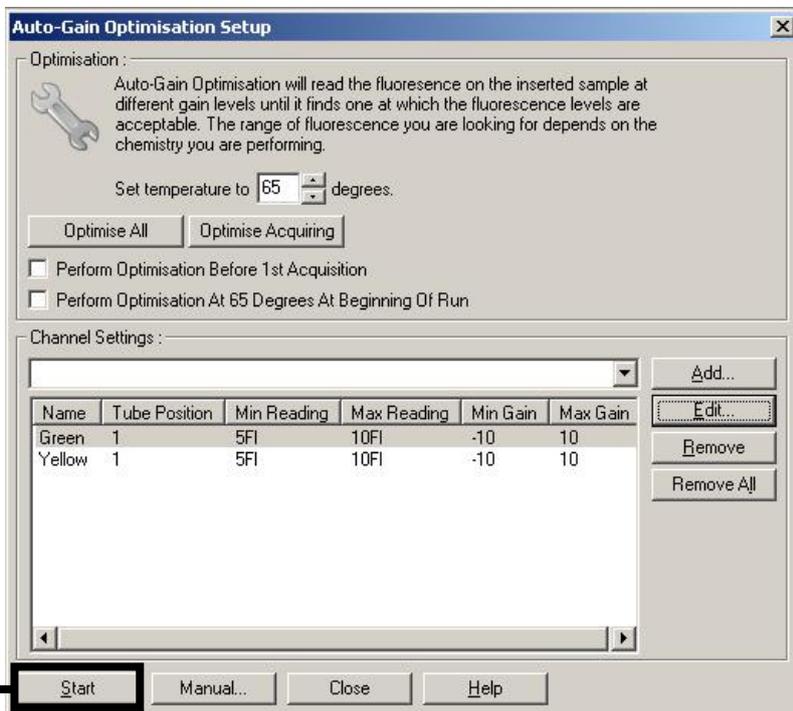


Figure 3. Starting the gain optimization.

- A new **Running Auto-Gain Optimisation** window opens. Wait until **Completed** appears in this window (Figure 4). Write down the selected gain values for both channels, and then click **Close** (Figure 4).

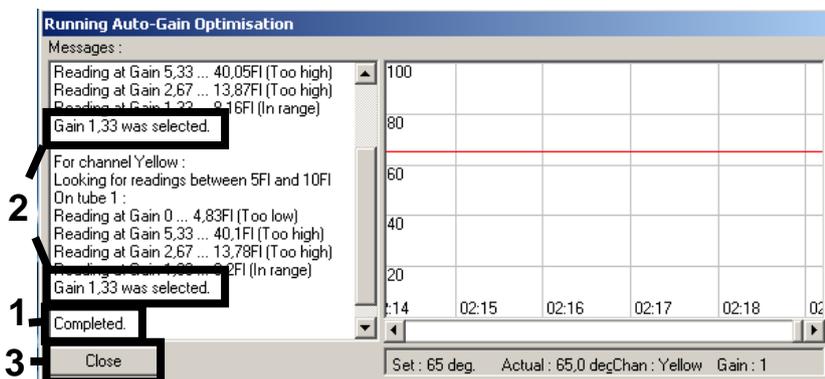


Figure 4. Gain optimization completed. Note gain values (in this case, 1.33 for both fluorescence channels).

- Repeat steps 1–4 for a tube position for the second *artus* assay (e.g., EBV).

6. Click **Edit Gain** to edit the gain values manually (Figure 5).

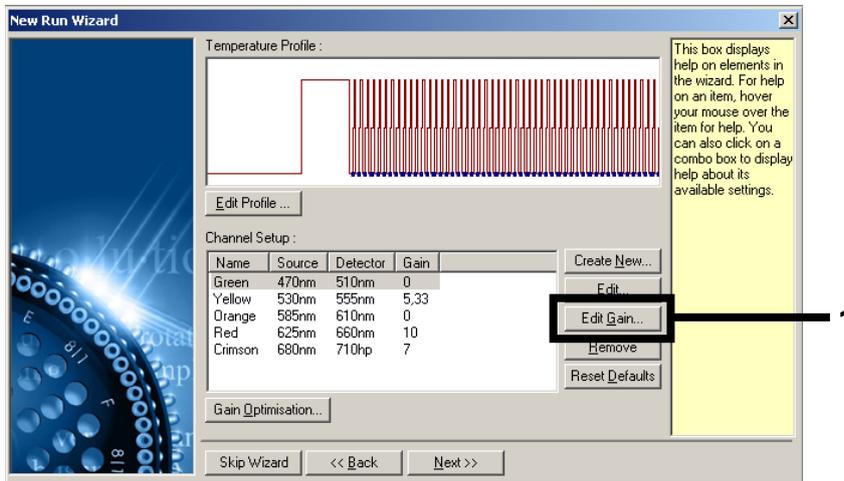


Figure 5. Editing the gain values manually.

7. Select the lowest gain value for Cycling Green noted in step 4, and enter this value manually in the **Gain for Green** window (Figure 6). Select the lowest gain value for Cycling Yellow noted in step 4, and enter this value manually in the **Gain for Yellow** window (Figure 6).

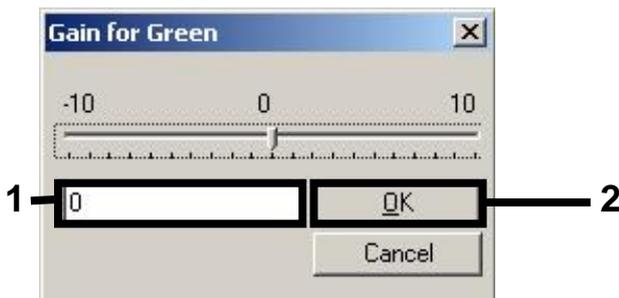


Figure 6. Manually entering the lowest gain values.

- The gain values determined by the channel calibration (or manually assigned) are saved automatically and are listed in the last menu window of the programming procedure (Figure 7). Click **Start Run**.

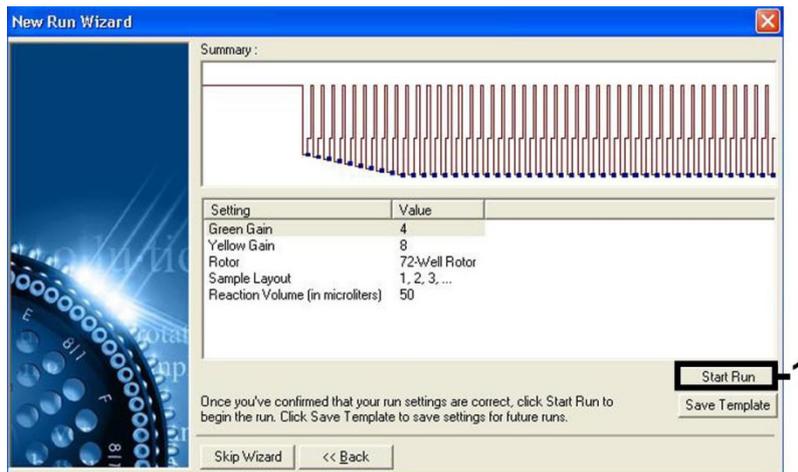


Figure 7. Starting the run.

Interpretation of results

This section describes interpretation of results on the Rotor-Gene Q. Review also the sample status information from the QIAasymphony SP/AS result files for analysis of the complete sample-to-result workflow. Only samples with a valid status should be used.

The *artus* CMV QS-RGQ Kit can be run on the Rotor-Gene Q using manual analysis with Rotor-Gene Q software 2.1 or higher. The following sections describe interpretation of results using Rotor-Gene Q software 2.1 or higher.

Signal detection and conclusions

Signal in channel Cycling Green	Signal in channel Cycling Yellow	Quantitative result (copies/ml)	Interpretation
Yes	Yes	<42.5	Valid result: CMV DNA detected, <79.4 copies/ml. Quantitation not possible since the quantitative result is below limit of detection. Reproducibility of the positive result is not assured.
Yes	Yes	≥42.5 and <79.4	Valid result: CMV DNA detected, <79.4 copies/ml. Quantitation not possible since the quantitative result is below the linear range of the assay.
Yes	Yes/No*	≥79.4 and ≤1 x 10 ⁸	Valid result: CMV DNA detected at the calculated concentration. Quantitative result is within the linear range of the assay.
Yes	Yes/No*	>1 x 10 ⁸	Valid result: CMV DNA detected, >1 x 10 ⁸ copies/ml. Quantitation not possible since the quantitative result is above the linear range of the assay.†
No	Yes	–	Valid result: No CMV DNA is detectable. ‡
No	No	–	Invalid result: No result can be concluded. §

* In this case, the detection of a signal in the Cycling Yellow channel is dispensable, since high initial concentrations of CMV DNA (positive signal in the Cycling Green channel) can lead to a reduced or absent fluorescence signal of the internal control in the Cycling Yellow channel (competition).

† If quantitation is desired, dilute the sample with CMV-free plasma and reprocess. Multiply the quantitative result from the reprocessed sample by the dilution factor.

‡ If the C_T value for the internal control of a negative sample is more than 3 cycles higher than the C_T value for the internal control of the no template control in the run (C_T IC Sample – C_T IC NTC >3), then the sample should be treated as invalid. No result can be concluded.

§ Information regarding error sources and their solution can be found in “Troubleshooting Guide” of the artus CMV QS-RGQ Kit Handbook.

Threshold setup for the PCR analysis

The optimal threshold settings for a given combination of Rotor-Gene Q instrument and *artus* QS-RGQ Kit should be set empirically by testing each individual combination since it is a relative value depending on the overall diagnostic workflow. The threshold can be set at a preliminary value of 0.04 for the analysis of the first PCR run, but this value should be fine-tuned in a comparative analysis of the next runs of the workflow. The threshold should be set manually just above the background signal of the negative controls and negative samples. The mean threshold value calculated from these experiments will most likely work for the majority of future runs, but the user should nevertheless review the generated threshold value at regular intervals. The threshold value will usually be in the range of 0.03–0.05 and should be rounded to no more than three decimal places.

Quantitation

The quantitation standards (CMV QS 1–4) in the *artus* CMV QS-RGQ Kit are treated as previously purified samples and the same volume is used (20 µl). To generate a standard curve on Rotor-Gene Q Instruments, all 4 quantitation standards should be used and defined in the **Edit Samples** dialog box on the Rotor-Gene Q instrument as standards with the specified concentrations (see the instrument user manual).

Note: The quantitation standards are defined as copies/µl in the eluate. The following equation has to be applied to convert the values determined using the standard curve into copies/ml of sample material.

$$\text{Result in sample material (copies/ml)} = \frac{\text{Result in eluate (copies/}\mu\text{l)} \times \text{Initial Elution Volume (90 }\mu\text{l)}^*}{\text{Sample Volume (ml)}}$$

As a matter of principle, the initial sample volume should be entered in the equation above. This has to be considered when the sample volume has been changed prior to the nucleic acid extraction (e.g., reducing the volume by centrifugation or increasing the volume by adding to the volume required for the isolation).

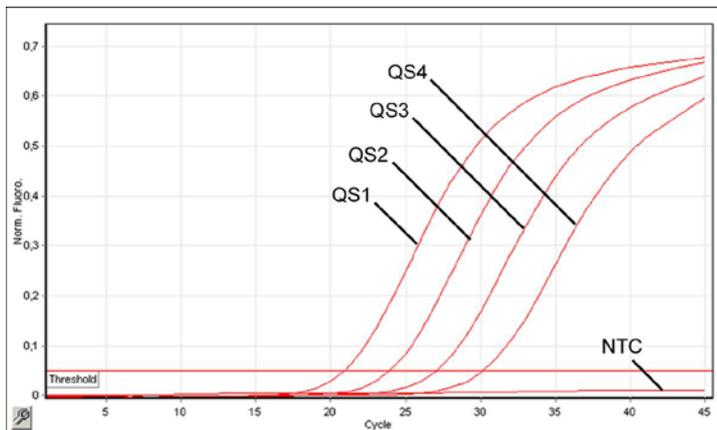
For a multi-assay run where both CMV and EBV were assayed in the same PCR, make sure that samples are analyzed separately for CMV and EBV, with the corresponding quantitation standards.

* The calculation is based on the initial elution volumes (90 µl).

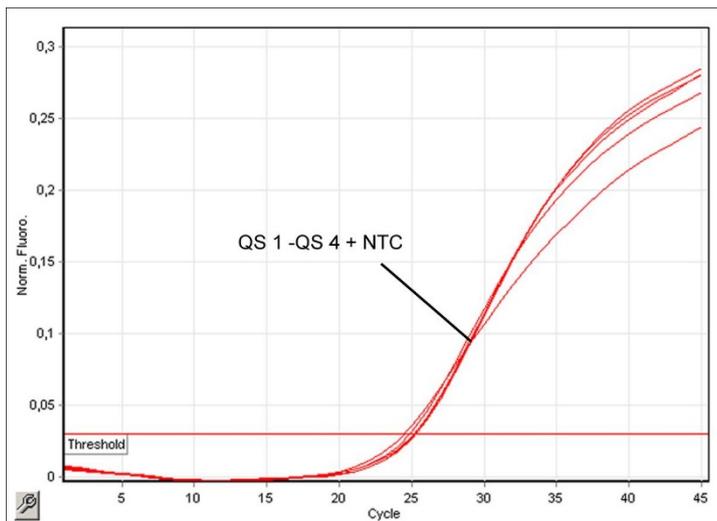
Conversion factor

1.00 copies/ml corresponds to 1.64 IU/ml for detection of CMV DNA derived from human EDTA plasma on the Rotor Gene Q. This conversion factor applies when adhering to the validated workflow as stated on this Application Sheet. The conversion factor is an approximation based on an average factor across the assay's dynamic range. The conversion factor was established by a regression analysis of multiple dilution series of the 1st WHO International Standard compared against a reference method reporting in IU/ml.

Examples of positive and negative PCR reactions



Detection of the quantitation standards (CMV QS 1-4) in fluorescence channel Cycling Green. NTC: No template control (negative control).



Detection of the internal control (IC) in fluorescence channel Cycling Yellow with simultaneous amplification of the quantitation standards (CMV QS 1-4). NTC: No template control (negative control).

Document revision history

R3, February 2018	Removed footnote regarding setup of 216 assays. Changed to new versions of QIASymphony protocols. Updated required materials for setup of maximum 72 reactions. Added information for multi-assay run with EBV. Added information on use of QMC "IC Calculator" tool. Updated naming of Corning labware (previously Becton Dickinson). Added specific Rotor-Gene Q run settings (use of touchdown function, acquisitions). Added information to interpretation of results to include case "pathogen positive and IC negative". Removed instructions regarding use of Rotor-Gene AssayManager®. Added Conversion factor information.
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