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artus® CMV QS-RGQ MDx Kit Instructions for Use (Handbook)

Σ 72

Version 1

For in vitro diagnostic use

For detection and quantitation of CMV DNA

For use with the QIAsymphony® RGQ MDx system

IVD

REF 4503346

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Sample to Insight

QIAGEN

Contents

Intended Use

The *artus*® CMV QS-RGQ MDx Kit is an in vitro nucleic acid amplification test for the quantitation of human cytomegalovirus (CMV) DNA in human EDTA plasma. The *artus* CMV QS-RGQ MDx Kit is intended for use as an aid in the management of solid organ transplant patients who are undergoing anti-CMV therapy.

The test measures CMV DNA levels in EDTA plasma and can be used to assess CMV viral load response to antiviral drug therapy. The results from the *artus* CMV QS-RGQ MDx Kit must be interpreted within the context of all relevant clinical and laboratory findings.

The *artus* CMV QS-RGQ MDx Kit is configured for use with the QIAsymphony® Rotor-Gene® Q MDx system (QS-RGQ MDx) in conjunction with the QIAsymphony DSP Virus/Pathogen Midi Kit for DNA extraction, Rotor-Gene AssayManager® and the Rotor-Gene Q MDx instrument for CMV DNA amplification and quantitation.

The *artus* CMV QS-RGQ MDx Kit is not intended for use as a screening test for blood or blood products.

Summary and Explanation

The *artus* CMV QS-RGQ MDx Kit constitutes a ready to use system for the detection and quantitation of CMV DNA using polymerase chain reaction (PCR) on the Rotor-Gene Q MDx instrument, analysis via Rotor-Gene AssayManager, with sample preparation and assay setup using the QIAsymphony SP and AS instruments.

Note: It is recommended you do not interchange testing systems between the EZ1® DSP Virus System (EZ1 DSP Virus Kit and EZ1 Advanced instruments) using the *artus* CMV RGQ MDx Kit and the QIAsymphony Rotor-Gene Q MDx System (QS-RGQ MDx) using the *artus* CMV QS-RGQ MDx Kit when monitoring a patient.

Pathogen Information

CMV, also known as herpesvirus-5 (HHV-5), belongs to the *Herpesviridae* family. Viruses in this family have double-stranded DNA and their main characteristic is the capacity to remain latent within the body. Although primary infection with CMV is mostly asymptomatic in healthy people, immunocompromised patients develop a mononucleosis-like syndrome with prolonged fever, mild hepatitis, sore throat and inflammation of the lymph nodes.

Solid organ transplant patients represent a risk group for CMV infection. In these patients, a primary infection can result in bone marrow suppression, pneumonia, myocarditis, encephalitis, hepatitis, cystitis, retinitis, enteritis and pancreatitis.

Principle of the Procedure

Pathogen detection by PCR is based on the amplification of specific regions of the pathogen genome. In real-time PCR, the amplified product is detected via fluorescent dyes. These are linked to oligonucleotide probes that bind specifically to the amplified product. Monitoring the fluorescence intensities during the PCR run (i.e., in real time) allows the detection of the accumulating product without having to reopen the reaction tubes after the PCR run.

The *artus* CMV QS-RGQ MDx Kit contains reagents and instructions for the detection and quantitation of CMV DNA in EDTA plasma. The assay utilizes the QIAsymphony SP instrument and QIAsymphony DSP Virus/Pathogen Midi Kit for viral DNA extraction and the QIAsymphony AS instrument for assay setup. The eluate transfer between QIAsymphony SP and QIAsymphony AS is done automatically with no manual intervention. The Rotor-Gene Q MDx instrument is used in conjunction with Rotor-Gene AssayManager for amplification and detection.

The CMV RG Master contains primers/probes, enzymes and other reaction components (except magnesium solution) needed for the specific amplification of a 105 bp region of the major immediate-early (MIE) gene and for the direct detection of the specific amplicons in the

Test Channel of the Rotor-Gene Q MDx instrument. Quantitation Standards (CMV QS 1–4) are supplied, which allow the determination of the amount of viral DNA.

In addition, the CMV RG Master contains a second heterologous primer/probe set to detect the internal control (CMV RG IC). The internal control result identifies a loss of DNA during the extraction or a possible inhibition in the PCR. The specific amplification of CMV RG IC is detected in the Control Channel of the Rotor-Gene Q MDx instrument. The quantitative results of the CMV RG IC must fall within a specified range for the assay to be valid. The C_T value is the number of cycles required to reach a defined threshold. The ΔC_T value corresponds to the difference between the C_T of the IC in a sample and the C_T of the IC in the NTC ($\Delta C_T = C_T\ IC_{Sample} - C_T\ IC_{NTC}$). A valid indicator of a CMV negative sample is the ΔC_T value within a defined range.

Two positive controls are provided with the *artus* CMV QS-RGQ MDx Kit. The low positive control contains non-infectious CMV nucleic acid fragments at a concentration near the limit of quantitation. The low positive control is used to monitor for substantial reagent failure. The high positive control contains non-infectious CMV nucleic acid fragments at a concentration that is in the middle of the linear range of the *artus* CMV QS-RGQ MDx Kit. The high positive control is used to verify that the calibration status of the assay is maintained within acceptable limits. The quantitative results of the low and high positive controls must fall within a specified range for the assay to be valid. PCR-grade water (H_2O) is provided as a negative control (no template control, NTC). The NTC is used to check a possible contamination with target nucleic acid during the PCR setup.

Materials Provided

The contents of the *artus* CMV QS-RGQ MDx Kit are sufficient for 3 x 24 reactions on the QIAsymphony-RGQ MDx. The Rotor-Gene Q MDx rotor holds up to 72 reaction tubes.

Kit contents

***artus* CMV QS-RGQ MDx Kit**			**(72)**
Catalog number			**4503346**
Number of reactions			**72**
Cap color	**Component name**	**Symbol**	**Amount**
Blue	CMV RG Master	MASTER ‡	3 x 950 µl
Yellow	CMV Mg-Sol*	MG-SOL ‡	3 x 600 µl
Green	CMV Internal Control	IC ‡	1 x 1000 µl
Red	CMV QS 1†	QS	1 x 200 µl
Red	CMV QS 2†	QS	1 x 200 µl
Red	CMV QS 3†	QS	1 x 200 µl
Red	CMV QS 4†	QS	1 x 200 µl
Violet	CMV Low Positive Control	CONTROL + ‡	1 x 200 µl
Black	CMV High Positive Control	CONTROL + ‡	1 x 200 µl
White	PCR-grade H_2O	CONTROL − ‡	1 x 1000 µl
artus *CMV QS-RGQ MDx Kit Instructions for Use (Handbook)*			1

* Magnesium solution.
† Quantitation standard.
‡ See page 93 for a list of symbols and definitions.

Materials Required but Not Provided

Important: Make sure that instruments used in this procedure have been checked and calibrated according to the manufacturer's recommendations.

General laboratory equipment

- Adjustable pipets and sterile pipet tips with filters
- Vortex mixer
- Benchtop centrifuge with rotor for 2 ml reaction tubes
- Water bath at 37°C and 70°C

Additional equipment and materials for sample preparation on QIAsymphony SP

- QIAsymphony SP (module of QIAsymphony RGQ MDx)
- QIAsymphony software version 4.0.3, or higher
- QIAsymphony DSP Virus/Pathogen Midi Kit (cat. no. 937055)
- Elution Microtube Rack (QS, Cooling Adapter, EMT, v2, Qsym, cat. no. 9020730) in combination with the QIAsymphony SP/AS Transfer Frame
- Tube Insert 3B, Insert, 2.0 ml v2, samplecarr. (24), (Qsym, cat. no. 9242083)
- 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 (EMTR) (cat. no. 19588)
- Tip disposal bags (cat. no. 9013395)
- Micro tubes 2.0 ml Type H, without skirted base (Sarstedt®, cat. no. 72.693) or Micro tubes 2.0 ml Type I, with skirted base (Sarstedt, cat. no. 72.694), for use with samples
- Tubes, 14 ml, 17 x 100 mm round-bottom polystyrene test tube (Corning®, cat. no. 352051), for use with internal control
- Recommended: Materials for external specimen process controls

Additional equipment and materials for assay setup on QIAsymphony AS

- QIAsymphony AS (module of QIAsymphony RGQ MDx)
- Cooling Adapter, Reagent Holder 1 (cat. no. 9018090)
- Cooling Adapter, RG Strip Tubes 72 (cat. no. 9018092)
- Strip Tubes and Caps, 0.1 ml (cat. no. 981103)
- Tubes, conical, 2 ml, Qsym AS (cat. no. 997102)
- Tubes, conical, 5 ml, Qsym AS (cat. no. 997104)
- 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)

Additional equipment and materials for PCR on Rotor-Gene Q MDx

- Rotor-Gene Q MDx (module of QIAsymphony RGQ MDx)
- Rotor-Gene AssayManager version 1.0.x (where x = 4 or higher) (module of QIAsymphony RGQ MDx)
- 72-Well Rotor (cat. no. 9018903)

Warnings and Precautions

For in vitro diagnostic use

The *artus* CMV QS-RGQ MDx Kit is to be used by laboratory professionals trained in the use of QIAsymphony SP/AS, Rotor-Gene Q MDx and Rotor-Gene AssayManager.

Safety information

When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles. For more information, consult the appropriate safety data sheets (SDSs). These are available online in PDF format at **www.qiagen.com/safety** where you can find, view and print the SDS for each QIAGEN kit and kit component.

For safety information for the QIAsymphony DSP Virus/Pathogen Midi Kit, refer to the applicable kit handbook. For safety information regarding instrument modules, refer to the applicable instrument user manual. Discard sample, liquid and assay waste according to your national and local safety and environmental regulations.

CAUTION **Risk of personal injury**

Treat all specimens as potentially infectious material.

General precautions

Always pay attention to the following:

- Follow preventive measures to avoid DNA contamination (see "Appendix: Prevention of Contamination", page 88.
- Use sterile pipet tips with filters.
- During manual steps, keep tubes closed when possible and avoid contamination.
- Do not mix components from kits with different lot numbers.
- Make sure that the required adapters are precooled to 2–8°C.
- Work quickly and keep PCR reagents on ice or in the cooling block before loading.
- Proceed continuously from one part of the workflow to the next. Do not exceed 30 minutes of transfer time between QIAsymphony AS and Rotor-Gene Q MDx.
- Do not refill or reuse reagents already used in the assay preparation as this may lead to a reduced performance and incorrect results.
- Thaw all assay components thoroughly at room temperature (15–25°C) before starting an assay.
- When thawed, mix the components (by pipetting repeatedly up and down or by pulse vortexing) and centrifuge briefly. Ensure that no foam or bubbles are present in the reagent tubes.

- Follow universal safety precautions. All patient specimens should be considered potentially infectious and handled accordingly.
- Make sure that maintenance has been performed and replaceable parts (e.g., tip guards) are reinstalled.
- Make sure that the Application Process files and required Rotor-Gene AssayManager plug-in are installed.

Reagent Storage and Handling

The components of the *artus* CMV QS-RGQ MDx Kit are shipped on dry ice and should be stored at –15°C to –30°C upon arrival, separately from patient material in order to eliminate possible contamination. If the *artus* CMV QS-RGQ MDx Kit is not frozen on arrival, do not use the reagents and contact QIAGEN Technical Services or local distributors. Reagents are stable until the expiration date stated on the label.

Repeated thawing and freezing (>2x) should be avoided, as this may reduce assay performance. If the reagents are to be used only intermittently, they should be frozen in aliquots and the aliquots labeled clearly with the information from the original reagent tube. Storage at 2–8°C should not exceed a period of 5 hours.

All reagents from the assay kit that are loaded on the QIAsymphony are for use in that run only. Do not remove the residual components to use them for a second PCR.

Specimen Handling, Storage and Preparation

Whole blood specimens are withdrawn following manufacturer's instructions, and plasma is collected after centrifugation. Plasma is obtained within 24 hours of the whole blood being drawn. Blood samples can be stored at 20–25°C for up to 24 hours before collecting the plasma, but they cannot be frozen. Information about specimen handling and storage for EDTA plasma is given in Table 1.

Table 1. Specimen handling, storage, and preparation for human EDTA plasma

Specimen collection	Human EDTA plasma
Specimen transport	Shatterproof transport Shipment within 24 hours of collection Samples should be shipped cool (2–8°C)
Specimen storage (including time needed for transport)	EDTA whole blood: 20–25°C for up to 48 hours 2–8°C for up to 72 hours EDTA plasma: 20–25°C for up to 48 hours 2–8°C for up to 5 days –15°C to –30°C for up to 8 weeks
Specimen preparation	Place 600 µl of EDTA plasma into a Sarstedt 2.0 ml Micro tube Type H, without skirted base (cat. no. 72.693) or Sarstedt Micro tube 2.0 ml Type I, with skirted base (cat. no. 72.694) and load onto the QIAsymphony SP (avoid generating foam)

Table 2. General information on setup conditions for running the *artus* CMV assay on the QIAsymphony RGQ MDx system

Kit	*artus* CMV QS-RGQ MDx Kit (cat. no. 4503346)
Sample material	EDTA plasma
Front-end purification	QIAsymphony DSP Virus/Pathogen Midi Kit (cat. no. 937055)
Sample volume (including excess volume)	600 µl of EDTA plasma

Table continued next page

Table continued

Assay Parameter Set	artus_CMV_QS-RGQ MDx_V1
Default Assay Control Set	Cellfree500_V5_DSP artus CMV RG
Elution volume	85 µl
IC volume per sample	6 µl
Required QIAsymphony software version	Version 4.0.3, or higher
Required QIAsymphony SP/AS configuration profile	Default profile 1
Master mix volume	30 µl
Template volume	20 µl
Number of reactions	24–72* (including all controls to be loaded onto QIAsymphony SP and QIAsymphony AS; this corresponds to 17–65 clinical samples)
Runtime on QIAsymphony SP/AS module	For 24 reactions: approximately 95 minutes For 72 reactions: approximately 260 minutes
Required Rotor-Gene AssayManager software version	Version 1.0.x, where x = 4 or higher
Rotor-Gene AssayManager Assay Profile	AP_artus_CMV_QS-RGQ_MDx_V1
Rotor-Gene AssayManager plug-in	Epsilon (US) Version 1.0.x, where x = 1 or higher
Runtime on Rotor-Gene Q MDx module	Approximately 105 minutes

* Ensure that the limit of 72 reactions and 1 assay rack adapter is not exceeded. Avoid extended incubation time (>30 minutes) between completion of the assay set-up and transfer to the Rotor-Gene Q MDx module.

Procedure

Controls

Internal control

The CMV internal control (CMV RG IC) supplied with the *artus* CMV QS-RGQ MDx Kit contains an exogenous amplification system to identify possible PCR inhibition and monitor reagent integrity. The internal control is added to each sample and is detected in the Control Channel of the Rotor-Gene Q MDx instrument.

Positive controls

The CMV positive controls (CMV Low Positive Control and CMV High Positive Control) supplied with the *artus* CMV QS-RGQ MDx Kit monitor the efficiency of the downstream assay. These positive controls are loaded onto QIAsymphony AS. (See page 35 for further details on loading positive controls.)

Negative control

The CMV negative control (PCR-grade water supplied with the *artus* CMV QS-RGQ Kit) is loaded onto QIAsymphony AS before amplification in place of an extracted sample. The negative control monitors the PCR for contamination and is referred to as **NTC** (no template control) in the QIAsymphony software.

External specimen process controls

External specimen process controls (SPC) are not required to perform the *artus* CMV QS-RGQ MDx Kit test; however, positive and negative controls should be routinely tested in each laboratory according to the guidelines or requirements of local, state and/or federal regulations or accrediting organizations.

The high positive specimen process control (H-SPC) and the low positive specimen process control (L-SPC) are intended to monitor the entire process. The negative specimen process control (N-SPC) detects reagent or environmental contamination by CMV.

It is recommended to test negative and positive process controls for CMV in each PCR run. The process controls should be treated as samples and subjected to the same DNA isolation procedure. Previously characterized samples may be used for this purpose. (See page 28 for further details on loading specimen process controls.)

Quantitation standards

The quantitation standards (QS) CMV QS 1–4 supplied with the *artus* CMV QS-RGQ MDx Kit are used for creating a standard curve and are loaded onto the QIAsymphony AS for the assay setup. The standard curve is valid for the run it was created for and cannot be applied to subsequent runs.

Assay Control Sets and Assay Parameter Sets

Assay Control Sets are the combination of an extraction protocol plus additional parameters, such as internal control, for sample purification on the QIAsymphony SP. An Assay Control Set is preinstalled for each protocol.

Assay Parameter Sets are the combination of an assay definition with additional parameters defined, such as replicate count and number of assay standards, for assay setup on the QIAsymphony AS.

For the integrated run on the QIAsymphony SP/AS, the Assay Parameter Set, **artus_CMV_QS-RGQ MDx_V1**, is directly linked to the upfront Assay Control Set, **Cellfree500_V5_DSP artus CMV RG**, specifying the associated sample purification process.

Preparation of carrier RNA and internal control

The internal control (IC) must be prepared before loading onto the QIAsymphony AS. The internal control (CMV RG IC) supplied with the *artus* CMV QS-RGQ MDx Kit must be added to carrier RNA (CARRIER)-Buffer AVE (AVE) mixture supplied with the QIAsymphony DSP Virus/Pathogen Midi Kit. The total volume of the internal control-carrier RNA (CARRIER)-Buffer AVE (AVE) mixture is 120 µl per sample.

1. Add 1350 µl Buffer AVE to the Carrier RNA tube.
2. Invert the Carrier RNA–Buffer AVE mixture thoroughly to mix.
3. Divide the Carrier RNA–Buffer AVE mixture into labeled aliquots based on usage.
   Note: The concentration of the Carrier RNA–Buffer AVE mixture is 1 µg/µl.
4. Store the Carrier RNA–Buffer AVE mixture at 2–8°C for up to 2 weeks.

We recommend using the IC Calculator within the QIAsymphony Management Console (QMC) to calculate the volumes for the mixture of Internal Control (IC), Carrier RNA and Buffer AVE. Alternatively, the calculation can be done according to Table 3.

1. Open the QMC.
2. Select the **IC Calculator** icon.
3. Select **Cellfree500_V5_DSP artus CMV RG** from the **ACS** drop-down list.
4. Enter the required number of samples including specimen process controls.
5. Select the labware used for the internal control.
6. Select an elution volume of **85 µl**.
7. Select **Internal Control/Sample** and **6 µl**.
8. Press **Calculate** to calculate the reagent volumes required to prepare the internal control. On the right side of the screen, the IC calculator displays the reagent volumes and the tube type required to prepare the internal control.
9. Label the applicable tube for the internal control.

10. Add the specified volume of Buffer AVE to the tube.
11. Add the specified volume of CMV IC to the tube.
12. Add the specified volume of Carrier RNA–Buffer AVE mixture to the tube.
13. Invert the internal control thoroughly to mix.

Table 3 represents the addition of 6 µl internal control to the sample. We recommend preparing fresh mixtures for each run just before use.

Table 3. Preparation of carrier RNA and internal control (CMV RG IC)

	n = number of samples and controls
Component	**n ≥ 24 Volume (µl) 14 ml Corning tubes***
Carrier RNA–Buffer AVE mixture	(n + 5) x 5
CMV Internal Control	(n + 5) x 6
Buffer AVE	(n + 5) x 109
Final volume per sample (excluding dead volume)	120
Total volume for n samples	**(n + 5) x 120**

* Tubes 14 ml, 17 x 100 mm polystyrene round-bottom (Corning, cat. no. 352051). Internal control mixture corresponding to 5 additional samples (n+5) (i.e., 600 µl) is required. Do not fill more than 13.92 ml total volume.

Reagent preparation

Reagent cartridge

Reagents for sample preparation are contained in the reagent cartridge of the QIAsymphony DSP Virus/Pathogen Midi Kit. Each trough of the reagent cartridge contains a particular reagent and is labeled with the applicable reagent name.

Each time before using the reagent cartridge, make sure that Buffers QSL2 and QSB1 in the reagent cartridge do not contain a precipitate.

If a precipitate has formed, perform the following procedure to dissolve the precipitate.

1. If the reagent cartridge has not been pierced, remove the troughs containing Buffers QSL2 and QSB1 from the reagent cartridge. If the reagent cartridge has been pierced, make sure that the troughs are sealed with Reuse Seal Strips.
2. Place the unopened troughs or entire reagent cartridge sealed with Reuse Seal Strips in a water bath for 30 minutes at 37°C with occasional shaking to dissolve precipitate.
3. Remove the unopened troughs or reagent cartridge from the water bath.
4. As applicable, replace the troughs in the correct positions of the reagent cartridge.
5. Allow the reagent cartridge to cool to room temperature (15–25°C).

Magnetic particles

Resuspend the magnetic particles in the reagent cartridge before each use.

1. Remove the magnetic-particle trough from the reagent cartridge.
   If the reagent cartridge is already opened, make sure the magnetic-particle trough is sealed with a Reuse Seal Strip.
2. Vortex the magnetic-particle trough for 3 minutes.
3. Replace the magnetic-particle trough in the reagent cartridge.

Protocol: Viral DNA purification and assay setup on the QIAsymphony SP/AS

Important points before starting

- Training is mandatory to operate QIAsymphony SP, QIAsymphony AS and Rotor-Gene Q MDx. The training can be provided either by QIAGEN Field Service or trained persons in the laboratory. If you have not received training, please contact QIAGEN Technical Services. Refer to the applicable user manuals for additional instructions on operating the instruments.
- Avoid vigorous shaking of the reagent cartridge. Otherwise foam may be generated which can lead to liquid-level detection problems.

- Work quickly and keep PCR reagents on ice or in the cooling block before loading.
- The *artus* CMV QS-RGQ MDx Kit reagent volumes are optimized for a maximum of 3 batches of 24 reactions or 1 batch of 72 reactions per kit per run.
- Proceed continuously from one part of the workflow to the next.
  Eluates from the sample preparation and all components of the *artus* CMV QS-RGQ MDx Kit are stable onboard the instrument for the time required for sample preparation and assay setup of 72 assay reactions. The time includes 30 minutes for the transfer from QIAsymphony AS to Rotor Gene Q MDx.
- Do not use an Elution Microtubes CL rack that has already been used on a different QIAsymphony SP instrument. Do not enter a rack ID manually.

Things to do before starting

- Before each run, thaw the required components of the *artus* CMV QS-RGQ MDx Kit required for that run thoroughly at room temperature (15–25°C). Mix all components by repeated up and down pipetting or by pulse vortexing followed by centrifuging for at least 3 seconds. Make sure that no foam or bubbles are present in the reagent tubes.

  Note: The operating conditions of the QIAsymphony SP/AS are 18–26°C.
  The operating conditions of the Rotor-Gene Q MDx are 18–30°C.

  Refer to the applicable user manual for more information on instrument operating conditions.
- Make sure that the required adapters are precooled to 2–8°C.
- Prepare all required reagents. Prepare mixtures containing carrier RNA (CARRIER) and internal controls just before starting. For more information, see “Preparation of carrier RNA and internal control”, page 17.
- Before starting an integrated run, make sure that all instruments are clean and that the replaceable parts are loaded (e.g., tip guards) as described in the maintenance instructions in the applicable user manuals.
- Make sure to perform maintenance regularly to minimize the risk of cross-contamination.

- Make sure that QIAsymphony SP/AS process profile **Default Profile 1** is active. The selected profile is shown at the bottom-right corner of the QIAsymphony touchscreen. The profile may be changed in the **Configuration** menu of the **Tools** tab by a user logged in as "Supervisor".
- Make sure that all necessary protocol files are installed on QIAsymphony SP/AS:
  - Assay Parameter Set: artus_CMV_QS-RGQ MDx_V1
  - Assay Control Set: Cellfree500_V5_DSP artus CMV RG
  - Assay Definition: AD_artus_CMV_QS-RGQ_MDx_V1
  - BioScript: Cellfree500_V5_DSP

Load the "Waste" drawer on QIAsymphony SP

Sample Prep Cartridges and 8-Rod Covers used during a protocol run are racked in empty unit boxes in the "Waste" drawer. A waste container collects the liquid waste generated during the protocol run. The "Waste" drawer can only be closed if the waste container is in place.

Important: Do not autoclave the filled waste bottle. Make sure to empty the waste bottle to prevent waste overflow.

The following table provides an overview of the "Waste" drawer requirements on the QIAsymphony SP.

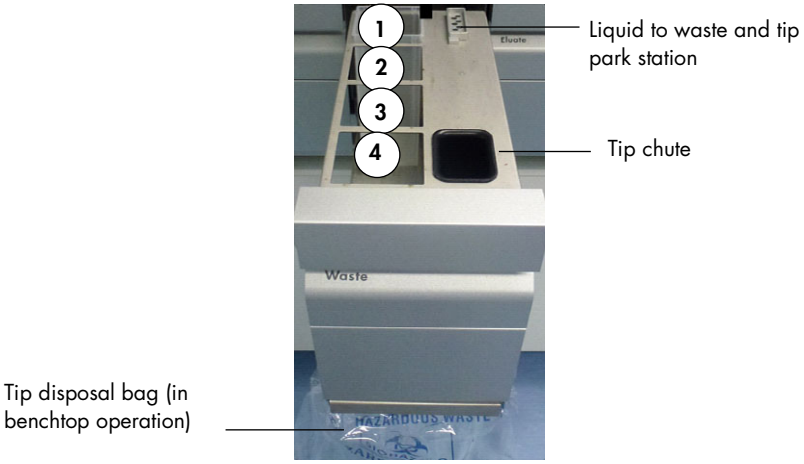
Location	Requirement
Unit box holder 1–4	Empty unit boxes
Waste bag holder	Waste bag (replace the waste bag if necessary)
Liquid waste bottle holder	Empty liquid waste container
Tip chute holder	Tip chute
Tip park station	Empty tip park station

1. Close all drawers as well as the QIAsymphony SP and QIAsymphony AS hoods.
2. Power ON the QIAsymphony SP/AS and wait until the initialization procedure has finished and the **Integrated Run** screen appears.

Note: The power switch is located at the bottom-left corner of QIAsymphony SP.

3. Log in to the QIAsymphony SP/AS.
4. Open the "Waste" drawer.
5. Empty and install the liquid waste bottle.
   Important: Make sure the lid is removed from the liquid waste container.
6. Insert the tip chute.

   Note: Different tip chutes must be used depending on if QIAsymphony SP and QIAsymphony AS are on a benchtop or a QIAsymphony Cabinet SP/AS.
7. Insert the tip park station.
8. Insert empty unit boxes into the "Waste" drawer for the plastic waste generated during the protocol run.
   Important: Make sure that there is at least 1 empty unit box in slot 4 (slot closest to you).
   Important: Make sure that the covers of the unit boxes are removed before loading the unit boxes into the "Waste" drawer. If using 8-Rod Cover boxes for collecting plastic waste, make sure that the box spacer has been removed.



Position of unit boxes (1–4) in "Waste" drawer.

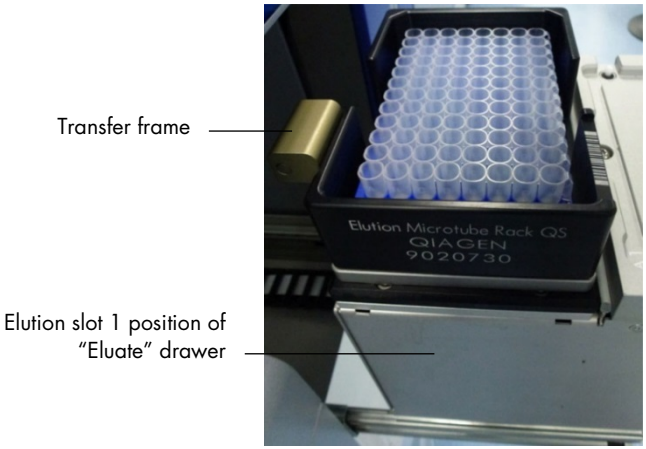
The following table details the required empty unit boxes required based on the number of batches.

	One batch 24 samples	Two batches 48 samples	Three batches 72 samples
Empty unit boxes	2	3	4

9. Install an empty tip disposal bag for used filter-tips.
   Note: The presence of a tip disposal bag is not checked by the instrument. Make sure that the tip disposal bag is properly attached before starting a run. For more information on installing a tip disposal bag, refer to the applicable user manuals.
   Note: The installation location of the tip disposal bag will vary depending on whether QIAsymphony SP and QIAsymphony AS are on a benchtop or a QIAsymphony Cabinet SP/AS.
10. Close the "Waste" drawer and perform an inventory scan of the "Waste" drawer.

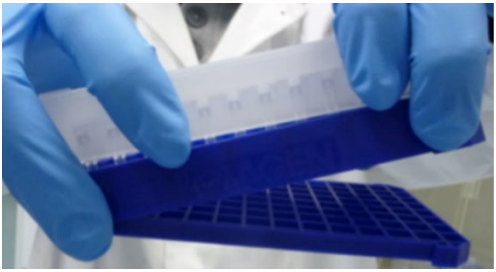
Load the "Eluate" drawer on QIAsymphony SP

1. Place the Elution Microtubes Rack QS cooling adapter onto the transfer frame.



Elution Mictrotubes CL rack on Elution Microtubes Rack QS adapter and transfer frame.

2. Open the "Eluate" drawer.
3. Place the assembly of the cooling adapter and transfer frame onto elution slot 1 of the "Eluate" drawer.
4. Remove the bottom of the Elution Microtubes CL rack by twisting the rack until the bottom comes out. We recommend leaving the lid of the Elution Microtubes CL rack on the rack until it is placed on the rack adapter.



Removing the bottom of the Elution Microtubes CL rack.

5. Using the handheld bar code scanner, scan elution slot 1. Alternatively, press the **Elution Slot 1** button on the touchscreen. The **Eluate Drawer/Elution Slot/Change Rack 1** screen appears.
6. Using the handheld bar code scanner, scan the bar code on the Elution Microtubes CL rack.
   Important: Do not use an Elution Microtubes CL rack that has already been used on a different QIAsymphony instrument. Do not manually enter a rack ID.
7. Place the Elution Microtubes CL rack into the assembly of the adapter and transfer frame on slot 1 in the "Eluate" drawer. Make sure that the orientation of the rack in the adapter is correct (i.e., skewed edge of the rack points to the right side of the adapter placed on elution slot 1).
8. Remove the lid of the Elution Microtubes CL rack.
9. Close the "Eluate" drawer.
10. Press **OK**. QIAsymphony SP performs an inventory scan of the "Eluate" drawer. Wait until the inventory scan is complete.

Load the "Reagents and Consumables" drawer on QIAsymphony SP

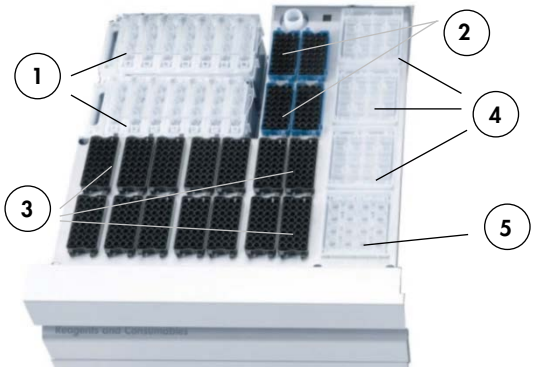
Sample Prep Cartridges and 8-Rod Covers are provided in unit boxes that are loaded directly on QIAsymphony SP. There are 28 Sample Prep Cartridges or twelve 8-Rod Covers per unit box.

Disposable filter-tips are provided in color-coded racks containing 32 filter-tips. Filter-Tips, 200 µl are provided in blue racks, and Filter-Tips, 1500 µl are provided in gray racks.

- Avoid vigorous shaking of the reagent cartridge as foam may be generated resulting in liquid-level detection problems.
- Make sure that the magnetic particles are resuspended before proceeding. See "Magnetic particles," page 19, for additional instructions.
- Make sure that Buffers QSL2 and QSB1 in the reagent cartridge do not contain a precipitate. If a precipitate has formed, see "Reagent cartridge," page 18.
- The piercing lid is sharp. Take care when placing it onto the reagent cartridge.
- Tips have filters to help prevent cross-contamination. Make sure not to touch the filter-tips to the instrument drawer to prevent contamination.
- Do not refill tip racks or unit boxes for Sample Prep Cartridges or 8-Rod Covers before starting the protocol run. QIAsymphony SP can use partially used tip racks and unit boxes.

The following table provides an overview of the recommended "Reagents and Consumables" drawer requirements on QIAsymphony SP.

Location	Requirement
Position A1	One reagent cartridge in position A1 for up to 72 samples
Tip rack holder 1–4	Filter-Tips, 200 µl
Tip rack holder 5–18	Filter-Tips, 1500 µl
Unit box holder 1–3	Unit boxes containing Sample Prep Cartridges
Unit box holder 4	Unit boxes containing 8-Rod Covers



1 Reagent cartridges 1–2 **3** Tip racks 5–18 **5** Unit box 4

2 Tip racks 1–4 **4** Unit boxes 1–3

Positions of reagent and consumables on QIAsymphony SP

The following table describes the plasticware loaded on QIAsymphony SP for an integrated QIAsymphony RGQ MDx run with the *artus* CMV QS-RGQ MDx Kit.

	One batch 24 samples*	**Two batches 48 samples***	**Three batches 72 samples***
Disposable filter-tips, 200 µl†	32 2 racks	56 2 racks	80 3 racks
Disposable filter-tips, 1500 µl†	89 3 racks	162 6 racks	235 8 racks
Sample prep cartridges	21	42	63
8-Rod Covers	3	6	9

* Performing more than one inventory scan requires additional disposable filter-tips. Use of fewer than 24 samples per batch decreases the number of disposable filter-tips required per run.

† Number of required filter-tips includes filter-tips for one inventory scan per reagent cartridge.

The numbers of filter-tips given may differ from the numbers displayed on the touchscreen depending on the settings.

1. Open the "Reagents and Consumables" drawer.
2. Place the reagent cartridge into the gray reagent cartridge holder. Make sure that all reagent troughs are completely inserted; if not, press reagent troughs into position.
3. Resuspend the magnetic particles by vortexing the magnetic-particle trough for 3 minutes before proceeding. See "Magnetic particles," page 19, for additional instructions.
4. Remove the lid or Reuse Seal Strips from the magnetic-particle trough.
5. Open the enzyme tubes in the enzyme rack and place the enzyme tube lids onto the cap holders on the gray reagent cartridge holder.
6. Make sure the enzyme tubes do not contain air bubbles
   If bubbles are present, aspirate bubbles from the surface.
7. Mount the enzyme rack on the reagent cartridge.
8. If using the reagent cartridge for the first time, mount the piercing lid on top of the reagent cartridge and gently click it into place (noticeable click on left and right sides). If using a partially used reagent cartridge, remove the reuse seal strips from the trough.
   Important: Make sure to place the piercing lid onto the reagent cartridge in the correct orientation.

Reagent cartridge in reagent cartridge holder with enzyme rack and piercing lid.

9. Load the required reagent cartridges into the "Reagents and Consumables" drawer.
   Note: One reagent cartridge is sufficient for up to 72 samples.
10. Press the **R+C** button on the touchscreen.

11. Determine the number of consumables required for the run. See the table of plasticware on page 26 to determine the number of consumables required.
12. Remove the covers of the unit boxes to be loaded.
13. Load the required Sample Prep Cartridges and 8-Rod Covers into the "Reagents and Consumables" drawer.

    Note: Check that all plastic consumables are aligned properly inside the unit boxes before loading on QIAsymphony SP as the plastic consumables may shift during transit or storage. If necessary, twist the plastic box slightly to align the stacks of plastic consumables appropriately.
14. Load the required 200 µl filter-tips in tip rack holders 1–4.

    Make sure the filter-tip racks click into place.
15. Load the required 1500 µl filter-tips in tip rack holders 5–18.

    Make sure the filter-tip racks click into place.

    Recommendation: Load more than the required number of filter-tips of each size so that sufficient filter-tips are available for automated error handling.
16. Press **OK** on the touchscreen.
17. Close the "Reagents and Consumables" drawer and perform an inventory scan of the "Reagents and Consumables" drawer.

Load the specimen process controls in tube carrier

External specimen process controls (high positive specimen process control [H-SPC], low positive specimen process control [L-SPC], negative specimen process control [N-SPC]) are not required to perform the *artus* CMV QS-RGQ MDx Kit test; however, they are recommended. The optional specimen process controls will be analyzed as samples. Rotor-Gene AssayManager will not automatically invalidate a run with invalid results for the specimen process controls.

Important: When loading tubes, make sure the tube is inserted completely in the tube carrier before unscrewing the lid and any label is fully visible to allow the bar code to be read automatically. Orient samples in the tube carrier so that the bar codes are completely visible.

1. Suspend 600 µl of a known H-SPC in a Sarstedt Micro tube 2.0 ml.
2. Place the tube in a Tube Insert 3B.
3. Place the tube containing the H-SPC into position 1 of the first tube carrier.
4. Suspend 600 µl of a known L-SPC in a Sarstedt Micro tube 2.0 ml.
5. Place the tube in a Tube Insert 3B.
6. Place the tube containing the L-SPC into position 2 of the first tube carrier.
7. Suspend 600 µl of a known N-SPC in a Sarstedt Micro tube 2.0 ml.
8. Place the tube in a Tube Insert 3B.
9. Place the tube containing the N-SPC in position 3 of the first tube carrier.

Load samples on QIAsymphony SP

Do not load more than 65 samples, including CMV SPCs, in one run in the tube carriers on the QIAsymphony SP so that the capacity of the Rotor-Gene Q MDx rotor is not exceeded. The CMV High Positive Control, CMV Low Positive Control, the negative control (NTC) as well as the four quantitation standards (CMV QS 1–4) will be loaded on the QIAsymphony AS. A run of 72 reactions will require the preparation of 3 tube carriers with only the first tube carrier containing the optional specimen process controls.

Prepare samples according to the information in Table 1, page 13.

Important: Consider all specimens as potentially infectious. See "Warnings and Precautions," page 10, for additional instructions.

Note: Check for clots in the primary sample when transferring to the Sarstedt Micro tube 2.0 ml for the QIAsymphony SP sample preparation. Do not use specimens that show any accumulation of fibrinogen or other signs of clots that cause potential obstructions for the pipetting process.

1. Carefully open the primary sample tube and place 600 µl of EDTA plasma in a Sarstedt Micro tube 2.0 ml.
2. Place the sample tube in a Tube Insert 3B.
3. Place the tube into the tube carrier containing the specimen process controls in the order in which the sample will be placed onto the Elution Microtubes CL rack.
4. Repeat for each plasma sample, continuing to fill each tube carrier to a maximum of 65 samples.
   Note: Do not load additional specimen process controls after the first tube carrier is prepared; these controls in the first tube carrier are for the entire run.
5. If the samples are bar-coded, orient the samples in the tube carrier so that the bar codes are visible.
6. Make sure that sample tubes are correctly loaded, clicked into the tube carrier and the caps are removed from the tubes.
7. Insert all tube carriers in the "Sample" drawer in slots 1–3.
   Place the sample carrier on its corresponding loading slot on the drawer lid and wait until the LED light is blinking in green then insert the whole sample carrier at a constant speed. The LED light changes to orange when the tube carriers are loaded correctly.

Define the QIAsymphony SP batch

Refer to the applicable user manuals for more information about integrated runs on QIAsymphony SP and QIAsymphony AS.

1. Press the **Integrated Run** tab on the touchscreen.
2. Press **Define run**.
3. Select **SP Batch 1** or, if performing continuous loading, the applicable batch number of the tube carrier with the specimen process controls.
4. Press **Edit samples**.
   If any samples have been provided without bar codes, select the samples without ID and manually assign IDs to these samples.

Important: Make sure to assign the sample type of **Sample** to any specimen process controls in the run.

Note: If there are any bar code reading errors, remove the sample carrier containing the samples and any CMV specimen process controls, and reinsert the sample carrier rather than entering the bar codes manually. Otherwise, press **Sample ID** and enter the sample ID.

5. Press **OK**.
6. Repeat the procedure for all batches and samples for the integrated QIAsymphony RGQ MDx run.

Define the assay

1. Press the corresponding SP Batch button.
2. Press **Define assays**.
3. Select the samples to be processed with the assay.
4. Select the **artus_CMV_QS-RGQ MDx_V1** Assay Parameter Set under the **artus QS-RGQ** category.
5. Press **OK**.
6. Repeat procedure for all batches and samples to be run.

Define the QIAsymphony AS batch

1. Select all QIAsymphony SP batches that should be processed in one integrated QIAsymphony RGQ MDx run.
2. Press **Create AS batch**.
   Note: All QIAsymphony SP batches assigned to the same QIAsymphony AS batch will be processed in the same assay setup procedure for the integrated QIAsymphony RGQ MDx run.
3. Press **OK** to queue the run.

Load and define the internal control

1. Prepare the internal control.
   See "Preparation of carrier RNA and internal control," page 17, for instructions.
2. Attach a blank label to the 14 ml tube(s), or mark the tube area facing the bar code scanner with a permanent marker.
   Note: For certain liquid levels in unlabeled 14 ml tubes, scan errors can occur due to the clear liquid in the tube.
3. Place the tube(s) in a tube carrier.
4. Insert the tube carrier in slot "A" of the "Sample" drawer.
   The LED light turns orange when the tube carriers are loaded correctly.
5. Press the **Define ICs** button.
6. Select the positions of the internal control.
7. Select the corresponding internal control **Cellfree500_V5_DSP artus CMV RG** from the **Required** folder.
8. Make sure that the correct labware is assigned. If not, assign the correct labware by pressing **IC Tubes**. Refer to the table on page 26 for the correct labware.
9. Press **OK**.

Start the QIAsymphony SP run

The position of samples on the assay rack can be displayed before the start of the run. After creation of the AS batch, press the Assays drawer button (**A**) on the touchscreen and select the respective **Assay** slot. The sample type of each position will be displayed (**Type**) if the toggle button **Sample** is pressed.

1. Press the Run button to start the run. A message appears.
2. Read and confirm the message. All processing steps are fully automated.
   Important: Do not pause or stop the run during processing unless an emergency occurs. If the run is paused or stopped, the samples are flagged as "unclear". Rotor-Gene AssayManager will invalidate "unclear" flagged samples.

Recommendation: Wait beside the QIAsymphony SP until the liquid level detection of the internal control tubes is complete. When complete, the QIAsymphony SP carrier status changes to **running**.

Note: Until reagents are loaded, it is possible to continuously load samples and add the samples to this run or a new integrated QIAsymphony RGQ MDx run.

Load consumables on QIAsymphony AS

1. Install an empty tip disposal bag below the "Assays" drawer for benchtop operation or in the waste bin for QIAsymphony Cabinet SP/AS operation.

Tip disposal bag below the "Assays" drawer in benchtop operation

2. Open the hood and insert the tip chute inside the instrument.

   Note: The type of tip chute installed depends on if QIAsymphony SP and QIAsymphony AS are on a benchtop or a QIAsymphony Cabinet SP/AS.

3. Close the hood. A message appears.
4. Read and confirm the message.
5. Open the "Eluate and Reagents" drawer and the "Assays" drawer of QIAsymphony AS.
6. Press the yellow **5 Assay** panel on the touchscreen.
7. Fill the required number of 0.1 ml Strip Tubes (4 tubes equals 1 segment) in a precooled RG Strip Tubes 72 QS cooling adapter.

   Important: Load complete strip tubes. Do not break strip tubes.

   Note: The touchscreen displays **QIA#981103 *StripTubes 0.1** for 0.1 ml Strip Tubes. The * (asterisk) indicates the strip tubes can be cooled using a cooling adapter with bar code.

8. Load RG Strip Tubes 72 QS cooling adapter filled with the strip tubes in slot 5 of the "Assays" drawer.
9. Press **Rack ID** on the touchscreen, enter a user-defined rack ID and press **OK**.

   Note: It is also possible to use the automatic ID function.
10. Press **Load**.
11. Load at least the number of filter-tips provided in the **Assay Setup | Loading Information** screen.

    Important: Start loading tip racks from the positions at the back near the cooling adapters. In rare cases, the pipetting head may not be able to reach some positions toward the hood and this may cause the instrument to automatically pause.

    Recommendation: Load more than the required number of filter-tips of each size so that sufficient filter-tips are available for automated error handling.

Load reagents on QIAsymphony AS

Important: Before each run, thaw the components of the *artus* CMV QS-RGQ MDx Kit required for that run thoroughly at room temperature (15–25°C). Mix all components by repeated up and down pipetting or by pulse vortexing followed by centrifuging for at least 3 seconds. Make sure that no foam or bubbles are present in the reagent tubes.

Take care when combining previously aliquoted kit components and make sure not to mix different reagents or reagent lots.

Viscous reagents can be difficult to handle with manual pipets. Make sure to transfer the entire volume of the CMV Master into the respective tube.

If the required volume for a reagent (CMV Master or CMV Mg-Sol) exceeds the volume of the reagent, combine the reagent into one tube. One tube of CMV Master and CMV Mg-Sol is sufficient for 24 reactions plus a negative control. For a run of less than 24 reactions, we recommend loading 100 µl of CMV Mg-Sol. If the volume of internal control (IC) required is less than 100 µl, load 100 µl.

1. Press the slot 3 Reagent (yellow) panel on the touchscreen.

2. Prepare the applicable precooled cooling adapter reagent holder as displayed on the touchscreen.

   Important: Do not include lids on tubes.

Reagent holder.

3. Select the tube positions on the touchscreen; load an empty tube for the master mix.
4. Fill at least the required volume of the applicable reagents and CMV Low Positive Control, CMV High Positive Control, NTC and CMV QS 1–4 in the required tubes in the corresponding positions as indicated on the touchscreen.

   Alternatively, select **List View** on the touchscreen and prepare the reagent adapter accordingly. A **Loading Information File** can also be downloaded and printed via the QMC or USB port after the QIAsymphony AS batch is defined and queued.
5. Press the **Scan Kit Barcode** button on the touchscreen and press the light-blue kit bar code line.

   Important: If the kit bar code is not scanned at this step, Rotor-Gene AssayManager will reject the QIAsymphony AS result file during import.
6. Press the text field and, using the handheld bar code scanner, scan the kit bar code on the upper side of the *artus* CMV QS-RGQ MDx Kit.
7. Load the prepared cooling adapter reagent holder onto slot 3 of the "Eluate and Reagents" drawer.
8. Press the **Load** button.
9. Close the "Eluate and Reagents" and "Assays" drawers.

Start the QIAsymphony AS run

1. Press **Scan** to enter the scan dialog.
2. Press **Scan** to perform an inventory scan of all components on QIAsymphony AS.
   Recommendation: Wait beside the instrument until the scan is complete.

   The inventory scan checks the slots, adapters, filter-tips and the tip chute as well as the correct loading of specific reagent volumes. If required, correct any errors.
   After the scan is finished, the calculated integrated run time is shown on the **Integrated Run Overview** screen.

   After sample preparation on QIAsymphony SP is complete, assay setup starts automatically and the eluate racks are transferred to QIAsymphony AS.
3. Check the end time of the QIAsymphony AS batch to remove the assay rack.

   Important: Make sure to transfer the assay rack to Rotor-Gene Q MDx within 30 minutes of the run finishing on QIAsymphony AS. The maximum time permitted from the end of the run on QIAsymphony AS until the start of the run on Rotor-Gene Q MDx is 30 minutes.

Remove the assay rack from QIAsymphony AS

1. After the QIAsymphony AS run is finished, open the "Assays" and the "Eluate and Reagents" drawers.
2. Remove the adapter with the strip tubes and close the tubes with the strip tube caps.
   Recommendation: Mark the strip tube caps to ensure correct positioning. Use a cooled transport frame to avoid contamination.
   Note: Check the tubes with CMV High Positive Control, CMV Low Positive Control, the negative control (NTC) and the four quantitation standards (CMV QS 1–4) and visually confirm similar liquid levels in the tubes to show that the transfers were performed correctly.
3. Press slot 5 **Assay**.

4. Press the **Remove** button.
   Note: Keep the strip tubes with PCR reactions in the cooled adapter on ice and load the Rotor-Gene Q MDx instrument within the shown remaining timeframe.
5. Remove the cooling adapter reagent holder and discard the reagents according to your local safety regulations.
6. Press slot 3 **Reagent**.
7. Press the **Remove** button.
8. Close the "Assays" and the "Eluate and Reagents" drawers.
9. Press **Scan** to enter the scan dialog.
10. Press **Scan** to perform an inventory scan for cooling adapters on the left and right drawers. This option is typically preselected.
11. Press the green **Integrated Batch** button to remove the run.
    A message appears.
12. Read and confirm the message.
    The final result file is created. and can be transferred to either a USB stick or to a defined folder (**\log\Results\AS**) via the QMC

Transfer result file from QIAsymphony AS

The result files must be downloaded and transferred to Rotor-Gene Q MDx using either QIAsymphony Management Console (QMC) or a USB flash drive. Use either "Procedure to transfer result file using the QMC," below, or "Procedure to transfer result file using the USB stick," below, to transfer the result files.

Procedure to transfer result file using the QMC

1. Log in to the instrument.
2. Select the **File Transfer** icon.
3. Choose file format **Result File AS**.

4. Select the result file with the correct time stamp and batch ID from the list of **Remote Site** files (right column).
5. Transfer result file to the **Local Site**.
   Note: The file is saved under the path defined in **Tools**, **Options**, **File Transfer**, in the folder **\log\Results\AS**.
6. If multiple batches for QIAsymphony AS are configured in an integrated run, proceed to "Load consumables on QIAsymphony AS," page 33, to reload QIAsymphony AS for a second assay setup.
7. Once the second assay is set up or if a second assay is not required, proceed to "Protocol: PCR on Rotor-Gene Q MDx," page 39.

Procedure to transfer result file using the USB stick

1. Insert the USB stick.
2. Select **Tools**.
3. Select **File Transfer**.
4. Select **Result Files** in the **Save to USB Stick** column.
5. Press the **Transfer** button.
   A message appears.
6. Read and confirm the message.
7. After successful transfer, press **OK** and remove the USB stick.
8. If multiple batches for QIAsymphony AS are configured in an integrated run, proceed to "Load consumables on QIAsymphony AS," page 33, to reload QIAsymphony AS for a second assay setup.
9. Proceed to "Protocol: PCR on Rotor-Gene Q MDx," page 39.

Protocol: PCR on Rotor-Gene Q MDx

Note: To avoid exceeding the recommended standing time of the PCR reaction, proceed with PCR on the Rotor-Gene Q MDx before starting QIAsymphony SP post-run maintenance activities such as removing the eluate rack and sample carrier. For a description of maintenance activities, see "Maintenance," on page 43.

Familiarize yourself with Rotor-Gene Q MDx before starting the protocol. Refer to the applicable instrument user manual.

The *artus* CMV QS-RGQ MDx Kit must be run on Rotor-Gene Q MDx using automated interpretation of results with Rotor-Gene AssayManager. The cycling parameters are locked for the run.

Things to do before starting

- Make sure the *artus* CMV QS-RGQ MDx Kit Application Package has been downloaded, unzipped and the applicable files uploaded to QIAsymphony SP/AS and imported to Rotor-Gene Assay Manager. If necessary, download the application package from the *artus* CMV QS-RGQ MDx Kit catalog page on the **Product Resources** tab under **Protocol Files**. Refer to the applicable user manual for additional instructions on installing the application files.
- For system-wide process safety, a user with an "Administrator" user role must activate the following settings in the Rotor-Gene AssayManager software for the closed mode of Rotor-Gene Q MDx: **Material number required**, **Valid expiry date required** and **Lot number required**. The applicable settings are accessed under **Configuration**, **Settings**, **Global Settings**, **Work List**.
- For automatic interpretation of results using the *artus* CMV QS-RGQ MDx Kit with Rotor-Gene AssayManager, the latest Epsilon (US) Plug-in, V1.0.x or higher, must be installed to your Rotor-Gene AssayManager. Refer to the Rotor-Gene AssayManager product page to access the latest version of the plug-in. Start the installation process by double-clicking on **RGAM_Epsilon_US_Plug-in.Installation.msi**, and follow the installation

instructions. For a detailed description refer to the section "Installing Plug-ins" in the *QIAsymphony RGQ MDx (US) User Manual*.

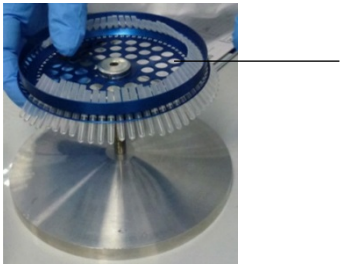
- To use the corresponding assay profile of the *artus* CMV QS-RGQ MDx Kit, the file **AP_artus_CMV_QS-RGQ_MDx_V1.iap** must be imported to Rotor-Gene AssayManager. To import the assay profile into Rotor-Gene AssayManager, navigate to the **Configuration** environment and click the **General Information** tab. Click **Open assay profile** and select the **AP_artus_CMV_QS-RGQ_MDx_V1.iap** file in the open file dialog. Click **Open**. The assay profile is loaded and added to the list of available assay profiles.

  Note: The same version of an assay profile cannot be imported twice.

After installing the applicable plug-in and importing the applicable assay profile, Rotor-Gene AssayManager can use the information provided in the QIAsymphony AS result file to set up a run for real-time PCR amplification and subsequent automated interpretation of results.

Set up Rotor-Gene Q MDx

1. Place a 72-Well Rotor on the Rotor Holder and press so that a click is heard.



2. Place the strip tubes from the QIAsymphony AS in the 72-Well Rotor.
   Important: Make sure to transfer the strip tubes to Rotor-Gene Q MDx in the correct orientation, starting at position 1 so that the position indices of the cooling adapter and the 72-Well Rotor match.
3. Visually confirm that the negative control was transferred correctly by checking that the volume of negative control is the same as the volume of the sample tubes.
   Note: The negative control is in the last strip tube position of the Rotor-Gene Q MDx run.

4. Use empty, capped strip tubes to fill all unused positions.
   Note: Using empty strip tubes will guarantee an optimal temperature distribution in Rotor-Gene Q MDx.
5. Attach the locking ring.
6. Load Rotor-Gene Q MDx with the rotor and locking ring (there will be a noticeable click).

Set up the Rotor-Gene Q MDx run

1. Start Rotor-Gene AssayManager.
2. Select **Closed mode** and log in.
3. If not already preselected, select the **Setup** environment.
4. Import the QIAsymphony AS result file at the bottom of the screen. Select the source **QIAsymphony** as **Import type**.
5. Press **Import.**
6. If using a USB stick, select the USB stick in the **Select file** dialog, select **log/results/AS**, open the corresponding QIAsymphony AS result file, and click **Open**.
   If using a USB stick for data transfer directly from QIAsymphony SP/AS, unzip the result file from QIAsymphony AS. On most computers, unzip files by right-clicking the file and then clicking **Extract** in the menu that opens. Files must be unzipped in order to be imported into Rotor Gene AssayManager.
7. A message appears. Read and confirm the message.
8. After successful import, make sure that the check box **Show only work lists never applied before**, located in the lower-left corner of the screen, is checked.
9. Select the corresponding work list from the work list manager list and click the **Apply** button.
10. Enter an experiment name.
11. Select the cycler to be used in the **Cycler selection** dialog.
12. Make sure that the locking ring is correctly attached and press the button to confirm on the touchscreen.

13. Close the Rotor-Gene Q MDx lid.

14. Click the **Start run** button.

    Note: If using multiple cycler runs, change to the corresponding cycler environment to see the progress of the run.

15. When the run is finished, click **Finish run…**.

16. For users logged in with the "Operator" role: Click **Release**.
    For users logged in with the "Approver" role: Click **Release and go to approval**.

Release and report PCR results

The general functionality of the **Approval** environment is described in *Rotor-Gene AssayManager Plug-in Epsilon (US) Online User Manual*.

After a run has finished and the cycler has been released, the experiment will be stored in the internal database. The analysis of the acquired data is performed automatically depending on the plug-in corresponding to the assay profile and the rules and parameter values defined by the assay profile.

Note: The user role "Approver" is required to approve a run.

If the run was released with the **Release and go to approval** option, the run is automatically open in the **Approval** environment and the approval can be performed.

The first step in the approval process is to filter the assay to be approved. This is done by using filter criteria in the **Approval** environment.

1. Press **Apply filter** (or choose your own filter options beforehand).
2. Select the experiment.
3. Click **Start approval**.

   In the Epsilon (US) Plug-in, the amplification curves of all controls (excluding CMV specimen process controls) are automatically analyzed, and a specific result for each target is determined. The results for controls in this plug-in do not have to be approved because tailored analysis parameters and rules are applied to the raw data of the controls. This ensures the detection of any abnormal or invalid amplification curve

behavior by Rotor-Gene AssayManager. Each lot of controls is tested against predetermined specifications to ensure consistent product quality. Therefore only the approval buttons for test samples are activated.

Note: A result automatically set to "INVALID" by Rotor-Gene AssayManager cannot be converted to a valid result anymore, even if the result is rejected.

4. Optional: Enter comments.
5. Check results.
6. Click **Release / report data….**
7. Click **OK**.
   The report will be generated and stored automatically.
8. Unload the Rotor-Gene Q MDx and discard the tubes according to your local safety regulations.

Maintenance

Remove the eluate rack

Upon completion of a QIAsymphony SP/AS run, the eluate rack automatically returns to the QIAsymphony SP module. To continue any operations with QIAsymphony SP, the eluate rack must be removed in the software as well as physically from the module.

Note: The removal of the eluate rack can be done during the Rotor-Gene Q MDx PCR run.

1. Open the "Eluate" drawer.
2. Select **Elution Slot 1** on the touchscreen.
3. Press **Remove** and **Yes**.
4. Close the Elution Microtubes CL rack with the lid and unload the rack from the adapter.
5. Close the "Eluate" drawer.
6. Press **OK**. Wait until the scan has finished.

Remove the sample carrier.

Note: The removal of the samples can be done during the Rotor-Gene Q MDx PCR run.

1. Open the "Sample" drawer.
2. Remove the sample carriers and samples from QIAsymphony SP. Discard samples according to your local safety regulations.
3. When all QIAsymphony AS batches have finished, perform the QIAsymphony maintenance as described in the applicable user manual.
   Note: This can be done during the Rotor-Gene Q MDx PCR run. If the QIAsymphony run is not complete, proceed to the next step and perform maintenance at a later time.

Unload Rotor-Gene Q MDx and perform the maintenance as described in the applicable user manual.

Discard all reagent aliquots and prepared reagents according to applicable local safety regulations.

Interpretation of Results

This section describes interpretation of results on Rotor-Gene Q MDx. Review the sample status information from the QIAsymphony SP/AS result files for analysis of the complete sample-to-result workflow. Only report samples with a valid status. The *artus* CMV QS-RGQ MDx Kit Assay Profile for EDTA plasma samples contains rules for interpreting the assay results automatically.

Target results of the assay are as follows:

- Every sample and control displays a result for the CMV target
- A result for the CMV IC is displayed for all samples and the NTC
- C_T values are shown for both the CMV target and the CMV IC
- A CMV concentration based on the generated standard curve is shown

Positive and negative control results

The results of the positive and negative controls confirm that the performance of the assay was successful and the test results may be reported. The following results are required for a valid assay:

- The sample result for the CMV High Positive Control must be **Valid (Signal detected)**
- The sample result for the CMV Low Positive Control must be **Valid (Signal detected)**
- The sample result for the CMV Negative Control (NTC) must be **Valid (CMV: No signal; CMV IC: Signal detected)**

If the sample result for a control is displayed as **INVALID**, results for every sample in the run will display **INVALID**. Do not report test results, and test all samples in a new integrated QIAsymphony RGQ MDx run.

Specimen process control results

External specimen process controls (SPC) are optional but recommended. The *artus* CMV QS-RGQ MDx Kit Assay Profile does not contain rules for the automatic analysis of the specimen process controls as the specimen process controls are classified as samples. You must check the results of the specimen process controls to confirm the following results:

- The H-SPC must report a **CMV positive** sample result
- The L-SPC must report a **CMV positive** sample result
- The N-SPC must report a **CMV negative** sample result

If results for the H-SPC, L-SPC or N-SPC fall outside the specifications predefined by the laboratory, follow established standard procedures for root-cause analysis and appropriate evaluation of the sample validity status. Do not report test results, and test all samples in a new integrated QIAsymphony RGQ MDx run.

Quantitation standards

All quantitation standards (CMV QS 1–4) must be **Valid (Signal detected)** for sample results to be valid. If the generated standard curve does not match the defined quality criteria all samples of this run will be INVALID. Test all samples in a new integrated QIAsymphony RGQ MDx run.

Sample results

For each sample in a valid run, Rotor-Gene AssayManager indicates the status of the analysis for CMV: INVALID, Signal Detected or NOT DETECTED. The internal control (CMV IC) signal for positive test samples is not evaluated. The following table describes the possible sample result and interpretation of the result.

Status	CMV concentration (IU/ml)	Interpretation
Valid	>159 – <7.94 x 10^7	CMV DNA detected within linear range; calculated concentration provided
Valid	0.0	CMV DNA NOT_DETECTED
Valid	≤159	CMV_DNA_DETECTED_BELOW_LLOQ*†
Valid	≥7.94 x 10^7	CMV_DNA_DETECTED_ABOVE_ULOQ*
INVALID	Not applicable	Invalid‡: No interpretation can be made; retest the sample

* Results outside the linear range of the assay may not be accurate.

† In alignment with the *artus* CMV RGQ MDx workflow using the *artus* CMV RGQ MDx Kit (cat. no. 4503245), the Rotor-Gene AssayManager assay profile will flag samples ≤159 IU/ml with CMV_DNA_DETECTED_BELOW_LLOQ.

‡ If the whole run is invalid, repeat the QIAsymphony RGQ MDx run with new specimens or from specimens already collected and handled according to the instructions in Table 1 (see page 13).

Samples reported as INVALID will have one or more flags associated to explain the cause. Flags will be shown for both targets (CMV and IC), explaining why the targets were invalidated by the software. For universal flags included in the Epsilon (US) Plug-in, also refer to the *Rotor-Gene AssayManager Plug-in Epsilon (US) User Manual*.

The automated analysis of the *artus* CMV QS-RGQ MDx assay may provide the following assay-specific flags:

Flag	Description
ABOVE_ACCEPTED_RANGE	The target value is higher than the defined range. This can be a C_T, endpoint-fluorescence, concentration or calculated value, e.g., mean C_T or delta C_T.
ANALYSIS_FAILED	Assay is set to invalid because the analysis has failed due to various reasons. Contact QIAGEN Technical Services.
ASSAY_INVALID	The assay is invalid because at least one external control is invalid.
AUDAS_CONFLICT	Results from the automatic data scan (AUDAS) are in conflict with results from the core analysis. An unambiguous automatic assessment of data validity is not possible.
BELOW_ACCEPTED_RANGE	The target value is lower than the defined range. This can be a C_T, endpoint-fluorescence, concentration or calculated value, e.g., mean C_T or delta C_T.
CMV_DNA_DETECTED _BELOW_LLOQ	CMV DNA was detected but reproducibility of quantitation is not assured since the quantitative result is below the linear range of the assay.
CMV_DNA_DETECTED _ABOVE_ULOQ	CMV DNA was detected but reproducibility of quantitation is not assured since the quantitative result is above the linear range of the assay.
CONSECUTIVE_FAULT	Target that was used for calculation of this target is invalid.
CURVE_SHAPE_ANOMALY	The raw data amplification curve shows a shape that deviates from the established behavior for this assay. There is a high likelihood of incorrect results or misinterpretation of results.

Flag	Description
FLAT_BUMP	The raw data amplification curve shows a shape like a flat bump deviating from the established behavior for this assay. There is a high likelihood of incorrect results or misinterpretation of results (e.g., wrong C_T value determination).
IC_INVALID	The internal control is invalid. Target and internal control share the same tube.
IC_LEFT_CT_SHIFT	A negative sample is invalid due to the C_T value of the sample IC < –3 compared to the C_T value of the NTC IC (Delta C_T rule).
IC_RIGHT_CT_SHIFT	A negative sample is invalid due to the C_T value of the sample IC > 2 when compared to the C_T of the NTC IC (Delta C_T rule).
IC_NO_SIGNAL	No internal control signal detected. Target and internal control share the same tube.
INVALID_CALCULATION	Calculation for this target failed.
MULTIPLE_THRESHOLD _CROSSING	The amplification curve crosses the threshold more than once. An unambiguous C_T cannot be determined.
NO_BASELINE	No initial baseline has been found. The subsequent analysis cannot be performed.
NO_VALUE	The target has no value but it is expected to have one. This value does not have to be in certain range. This can be a C_T endpoint-fluorescence, concentration or calculated value, e.g., mean C_T or delta C_T.
RUN_FAILED	Assay is set to invalid due to a problem with the cycler or the cycler connection.
RUN_STOPPED	Assay is set to invalid because the run has been stopped manually.

Flag	Description
SATURATION	The raw data fluorescence is saturating strongly before the inflection point of the amplification curve.
SPIKE	A spike in the raw data fluorescence is detected in the amplification curve but outside the region where the C_T is determined.
SPIKE_CLOSE_TO_CT	A spike is detected in the amplification curve close to the C_T.
STEEP_BASELINE	A steeply rising baseline for the raw data fluorescence is detected in the amplification curve.
STRONG_BASELINE_DIP	A strong drop in the baseline for the raw data fluorescence is detected in the amplification curve.
STRONG_NOISE	Strong noise is detected outside the growth phase of the amplification curve.
STRONG_NOISE_IN _GROWTH_PHASE	Strong noise is detected in the growth (exponential) phase of the amplification curve.
TOO_LESS_CORRELATION _IN_STANDARD_CURVE	A lower limit for the correlation coefficient (R^2 or R value) is not reached.
UNEXPECTED_VALUE	The target has a value, but it is not expected to have one. This can be a C_T, endpoint-fluorescence, concentration or calculated value, e.g., mean C_T or delta C_T.
UPSTREAM	Sample status was set to “Invalid” or “Unclear” by an upstream process (e.g., QIAsymphony).
WAVY_BASE _FLUORESCENCE	Wavy baseline for the raw data fluorescence detected in the amplification curve.

Troubleshooting guide

This troubleshooting guide may be helpful in solving any problems that may arise. For more information, consult your clinical coordinator, or visit **www.qiagen.com**.

		Comments and suggestions
General handling		
	Error message displayed on the touchscreen	If an error message is displayed during an integrated run, refer to the user manuals supplied with your instruments.
Precipitate in reagent trough of opened cartridge of the QIAsymphony DSP Virus/Pathogen Midi Kit		
a)	Buffer evaporation	Excessive evaporation may lead to increased salt concentration or decreased alcohol concentrations in buffers. Discard reagent cartridge. Make sure to seal buffer troughs of a partially used reagent cartridge with Reuse Seal Strips when not being used for purification.
b)	Storage of reagent cartridge	Storage of reagent cartridge at less than 15°C may lead to formation of precipitates. To dissolve the precipitates, see "Reagent cartridge," page 18, for additional instructions.
Low yield of nucleic acids		
a)	Magnetic particles were not completely resuspended	Before starting the procedure, make sure that the magnetic particles are fully resuspended. See "Magnetic particles," page 19, for additional instructions.
b)	Frozen samples were not mixed properly after thawing	Thaw frozen samples with mild agitation to ensure thorough mixing.

		Comments and suggestions
c)	Carrier RNA not added	Make sure to add the Carrier RNA. See "Preparation of carrier RNA and internal control," page 17, for additional instructions. Repeat the sample preparation procedure with new samples.
d)	Degraded nucleic acids	Samples were stored incorrectly or subjected to too many freeze–thaw cycles. Repeat the purification procedure with new samples.
e)	Incomplete sample lysis	Storage of reagent cartridge at less than 15°C may lead to formation of precipitates. To dissolve the precipitates, see "Reagent cartridge," page 18, for additional instructions.
f)	Clogging of pipet tip due to insoluble material	Insoluble material was not removed from the sample prior to starting the QIAsymphony purification procedure. To remove insoluble material, centrifuge the sample at 3000 x *g* for 1 minute, and transfer the supernatant to a new sample tube.
QIAsymphony AS detects insufficient Master		
	Insufficient Master transferred to tube	Combine the contents of an appropriate number of CMV Master tubes into one tube before use. Combine the contents of an appropriate number of CMV Mg-Sol tubes into one tube before use. Viscous reagents can be difficult to handle with manual pipets. Make sure to transfer the entire volume of the Master in the tube. For viscous reagents, we recommend aspirating an extra volume of 5% when using manual pipets (e.g., adjust the pipet to 840 µl for an 800 µl volume). Alternatively, after slowly dispensing the liquid and performing a blowout at the target tube's wall, remove the tip from the liquid, release the pipet plunger, and wait for an additional 10 seconds. Residual liquid will flow down the tip and can be blown out by pressing the pipet plunger a second time. The use of PCR grade filter-tips labeled as "low retention" can improve the recovery of liquid.

		Comments and suggestions
No signal with positive controls (CMV High Positive Control, CMV Low Positive Control)		
a)	Incorrect configuration of the PCR	Make sure that assay setup was performed correctly and that the correct Assay Parameter Set was used. Repeat the PCR, if necessary. See "Assay Control Sets and Assay Parameter Sets," page 16.
b)	The storage conditions for one or more kit components did not comply with the instructions	Check the storage conditions and the expiration date on the kit label of the reagents and use a new kit, if necessary.
c)	The *artus* CMV QS-RGQ MDx Kit has expired	Check the storage conditions and the expiration date on the kit label of the reagents and use a new kit, if necessary.
Weak or no signal of the internal control of a negative sample subjected to purification using the QIAsymphony DSP Virus/Pathogen Midi Kit, and simultaneous absence of a signal in the CMV target channel		
a)	The PCR was inhibited	Make sure that you use the validated procedure and closely following the instructions.
b)	DNA was lost during extraction	An absent signal of the internal control can indicate the loss of DNA during the sample preparation. Make sure that you use the validated procedure and closely following the instructions. See "Low yield of nucleic acids," above.
c)	The storage conditions for one or more kit components did not comply with the instructions	Check the storage conditions and the expiration date on the kit label of the reagents and use a new kit, if necessary.
d)	The *artus* CMV QS-RGQ MDx Kit has expired	Check the storage conditions and the expiration date on the kit label of the reagents and use a new kit, if necessary.

		Comments and suggestions
Signals with the negative controls in the CMV target channel of the analytical PCR		
a)	Contamination occurred during preparation of the PCR	Repeat the integrated QIAsymphony RGQ MDx run with new reagents. If possible, close the PCR tubes directly after addition of the sample to be tested. Make sure that work space and instruments are decontaminated at regular intervals.
b)	Contamination occurred during extraction	Repeat the integrated QIAsymphony RGQ MDx run with new reagents. Make sure that work space and instruments are decontaminated at regular intervals.

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of *artus* CMV QS-RGQ MDx Kit is tested against predetermined specifications to ensure consistent product quality.

Limitations

- The *artus* CMV QS-RGQ MDx Kit is to be used by laboratory professionals trained in the use of the QIAsymphony RGQ MDx system.
- The product is to be used by personnel specially instructed and trained in the in vitro diagnostics procedures only.
- Any diagnostic results that are generated must be interpreted in conjunction with other clinical or laboratory findings.
- Strict compliance with the user manual(s) is required for optimal PCR results.
- Attention should be paid to expiration dates printed on the box and labels of all components. Do not use expired components.
- Improper collection, storage or transport may lead to false negative results
- Inhibitors present in the sample and/or errors in following the assay procedure may lead to false negative result.
- Fibrinous samples or samples that show other signs of clot accumulation may obstruct the pipette tips and result in false results due to insufficient volume transfer during the sample preparation process.
- Although rare, mutations within the highly conserved regions of the viral genome covered by the kit's primers and/or probe may result in failure to detect the presence of the virus in these cases. Validity and performance of the assay design are revised at regular intervals.

Performance Characteristics

Limit of blank

The limit of blank (LOB) is defined as the highest measurement result that is likely to be observed for a blank sample. In the case of the *artus* CMV QS-RGQ MDx Kit, an appropriate parameter to analyze for the LOB is the end-point fluorescence intensity in the Test Channel. The fluorescence levels of negative samples should remain below a given threshold value (0.05) to generate a result "CMV DNA NOT_DETECTED".

The performance of the test using negative samples determines the probability of potential false positive results.

A total of 120 CMV-negative EDTA plasma samples were analyzed. The test was performed using a single kit lot and single QIAsymphony RGQ MDx system over 3 runs. A total of 119 samples showed no result in the CMV Test Channel and fluorescence intensities were below the given threshold (0.05). Therefore, the performance of the negative samples for the *artus* CMV QS-RGQ MDx Kit was 99.16% with a LOB less than 0.05 at cycle 45.

Limit of detection

Limit of detection using the 1st WHO International Standard for human cytomegalovirus

The limit of detection (LOD) of the *artus* CMV QS-RGQ MDx Kit was determined for the 1st WHO International Standard for human cytomegalovirus (HCMV), NIBSC code 09/162 [Merlin strain, genotype 1 based on glycoprotein B gene *UL55*],* and following the Clinical and Laboratory Standards Institute (CLSI) Guideline EP17-A2 (1). The LOD is defined as the lowest amount of analyte in a sample that is detected with a 95% probability, and it was determined by probit analysis. For this purpose a dilution series consisting of 6 different dilutions levels of the 1st WHO International Standard for HCMV, starting with 320 IU/ml in EDTA plasma, was used.

* National Institute for Biological Standards and Control, Potters Bar, Hertfordshire, EN6 3QG.

Each dilution was determined in 10 replicates per day for 7 days, and 2 replicates on one day resulting in a total of 72 data points per dilution. All replicates of each dilution were tested in one PCR run. The test was performed with 3 different *artus* CMV QS-RGQ MDx Kit lots and with each lot on at least 2 different days, by 2 different persons, on a single QIAsymphony RGQ MDx system. The hit rates are shown in Table 4.

Table 4. Hit rates for determining the limit of detection using the 1st WHO International Standard for human cytomegalovirus on the QIAsymphony RGQ MDx platform

Concentration (IU/ml)	Total number of samples	Total number of samples amplified	Hit rate
10	72	26	36%
20	72	49	68%
40	72	65	90%
80	72	71	99%
160	72	72	100%
320	72	72	100%

A probit regression with SAS® Software was performed and the 95% LOD value was determined. The concentration estimate of the LOD for the *artus* CMV QS-RGQ MDx Kit using the 1st WHO International Standard for HCMV is 51 IU/ml. A summary of the predicted probability with the 95% confidence interval over all kits for the QIAsymphony RGQ MDx is shown in Table 5. The concentration estimates of the LOD for each kit lot are shown in Table 6.

Table 5. Predicted probability (C95) summary and concentration estimate of the LOD for the QIAsymphony RGQ MDx platform over all kits

Platform	Probability	Concentration estimate (IU/ml)	Lower 95% confidence limit (IU/ml)	Upper 95% confidence limit (IU/ml)
QIAsymphony RGQ MDx	0.95	51.881	41.433	72.183

Table 6. Concentration estimates of the LOD for each of 3 kit lots

Platform	Kit lot	Probability	Concentration estimate (IU/ml)	Lower 95% confidence limit (IU/ml)	Upper 95% confidence limit (IU/ml)
QIAsymphony RGQ MDx	1	0.95	45.981	33.903	81.547
	2	0.95	50.855	36.068	99.855
	3	0.95	62.897	40.683	167.299

Limit of detection using glycoprotein B (gB) CMV genotypes 2, 3 and 4

A common method of CMV genotyping is based on variations in the *UL55* gene that encodes CMV glycoprotein B (gB). The claimed LOD value obtained for CMV genotypes gB2, gB3 and gB4 using the *artus* CMV QS-RGQ MDx Kit (77 IU/ml) was verified by testing 72 replicates on the QIAsymphony RGQ MDx system.

A set of samples was prepared for each CMV gB genotype by diluting an Acrometrix® standard (gB2) or cultured CMV virus (gB3 and gB4) at the claimed LOD value concentration. The test was performed on 2 different days for each CMV gB genotype. On each day, 36 replicates were tested resulting in a total number of 72 replicates per genotype. The results showed that the *artus* CMV QS-RGQ MDx Kit can detect the 3 tested CMV gB genotypes — gB2, gB3 and gB4 — at 77 IU/ml (Table 7).

Table 7. Proportion of CMV positive results with exact two-sided 95% confidence limits at 77 IU/ml for CMV glycoprotein B (gB) genotypes 2, 3 and 4

CMV genotype	Proportion CMV positive	Exact lower two-sided 95% confidence limit	Exact upper two-sided 95% confidence limit
gB2	92.96% (62/71)	84.33%	97.67%
gB3	100.0% (72/72)	95.01%	100.0%
gB4	100.0% (72/72)	95.01%	100.0%

Linear range and limit of quantitation (LOQ)

Linear range using glycoprotein B (gB) genotype 1

The linear range of the *artus* CMV QS-RGQ MDx Kit using CMV genotype gB 1 was determined following recommendations of the CLSI Guideline EP06-A (2).

A series of 10 dilutions of the 1st WHO International Standard for HCMV (NIBSC code 09/162, genotype gB1) ranging from 39.7 IU/ml to 3.97 x 10^5 IU/ml in EDTA plasma was prepared to determine the linear range. Samples were analyzed using the *artus* CMV QS-RGQ MDx Kit and each dilution level was tested in 6 replicates.

The linear range of the *artus* CMV QS-RGQ MDx Kit was determined to cover concentrations from 39.7 IU/ml to 3.97 x 10^5 IU/ml CMV in EDTA plasma (Figure 1).

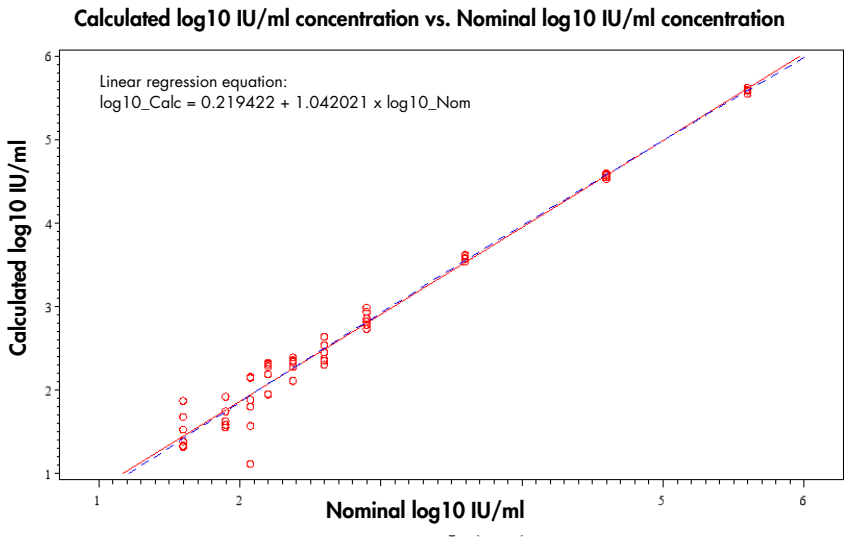


Figure 1. Linear range of the *artus* CMV QS-RGQ MDx Kit for the 1st WHO International Standard for HCMV. The red line represents the linear regression line; the blue line represents the quadratic regression line.

Linear range using glycoprotein B (gB) genotype 2

The linear range of the *artus* CMV QS-RGQ MDx Kit using CMV genotype gB2 was determined following recommendations of the CLSI Guideline EP06-A (2).

A series of 10 dilutions of CMV genotype gB2 ranging from 119.1 IU/ml to 7.94 x 10^7 IU/ml in EDTA plasma was prepared to determine the linear range. Samples were analyzed using the *artus* CMV QS-RGQ MDx Kit and each dilution level was tested in 6 replicates. The linear range of the *artus* CMV QS-RGQ MDx Kit was determined to cover concentrations from 119.1 IU/ml to 7.94x 10^7 IU/ml CMV in EDTA plasma (Figure 2).

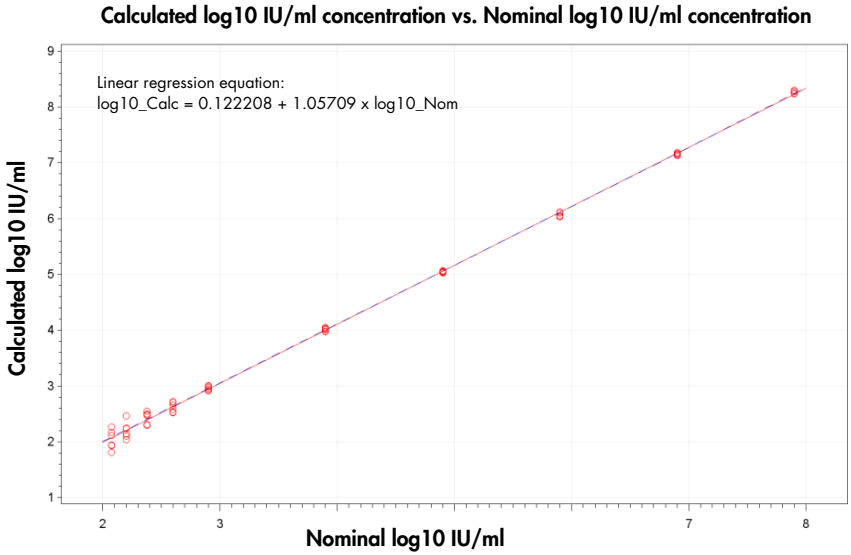


Figure 2. Linear range of the *artus* QS-CMV RGQ MDx Kit for HCMV gB genotype 2. The red line represents the linear regression line; the blue line represents the quadratic regression line.

Determination of LLOQ

The lower limit of quantitation (LLOQ) was determined by spiking the 1st WHO International Standard for HCMV (NIBSC code 09/162, gB1) into an EDTA plasma pool to equal the lower end of the linear range. The data were verified with 72 replicates, and 3 *artus* CMV QS-RGQ

MDx Kit lots were used. These data were generated as part of the LOD study on 8 different days on a single QIAsymphony RGQ MDx system.

The LLOQ was defined as the lowest concentration within the linear range with a total analytical error (TAE; standard deviation of the difference between two log10 measurements) that is ≤1.0 log10 IU/ml.

The fold detectable difference (FDD) was calculated as follows:

FDD = 10^((Total SD)*sqrt(2)*2) and results are included in Table 8.

The LLOQ of the *artus* CMV QS-RGQ MDx Kit system was verified at 80 IU/ml, with a TAE of 0.73 and FDD 5.76 (Table 8).

In alignment with the *artus* CMV RGQ MDx workflow using the *artus* CMV RGQ MDx Kit (cat. no. 4503245), the Rotor Gene AssayManager assay profile will flag samples ≤159 IU/ml with CMV_DNA_DETECTED_BELOW_LLOQ.

Table 8. Results for determination of the lower limit of quantitation (LLOQ) including values for FDD and TAE (number of valid tests = 71)

Given (IU/ml)	Nominal (log10 IU/ml)	Mean (calculated) (log10 IU/ml)	Bias	Total precision SD (in logs)	SD of difference between two measurements	95% Confidence limit (±log10)	Fold detectable difference	Total analytical error
80	1.903	1.714	–0.189	0.27	0.28	0.76	5.76	0.73

Cross-reactivity and microbial interference

The analytical specificity of the *artus* CMV QS-RGQ MDx Kit was evaluated by testing the cross-reactivity of a panel of different pathogens consisting of 21 viruses, 2 fungi, and 1 protozoan parasite. The pathogens were tested at the highest concentration available. Samples were prepared by diluting the organisms into EDTA spiked with CMV (AD-169 strain, gB2) at 3x LOD. Each sample was extracted and tested in 6 replicates. There were no false-negative or invalid results among the 24 pathogens tested (Table 9).

Table 9. Pathogens tested for cross-reactivity and microbial interference

Pathogen	Test concentration	Unit
Adenovirus Type 2	1.26×10^9	TCID50/ml
Adenovirus Type 4	4.77×10^5	TCID50/ml
Adenovirus Type 5	3.40×10^5	TCID50/ml
Herpes simplex virus 1	3.30×10^5	TCID50/ml
Herpes simplex virus 2	1.45×10^6	TCID50/ml
Varicella zoster virus (human herpesvirus 3; HHV-3)	6.00×10^3	TCID50/ml
BK virus	2.01×10^4	copies/ml
Epstein Barr virus	9.40×10^7	copies/ml
Human herpesvirus 7 (HHV-7)	4.20×10^5	TCID50/ml
Kaposi's sarcoma-associated herpesvirus (HHV-8)	6.00×10^5	copies/ml
HHV-6A	1.00×10^9	VP/ml
HHV-6B	1.00×10^5	copies/ml
Human T-lymphotropic virus 1	2.30×10^7	VP/ml
Human T-lymphotropic virus 2	3.25×10^7	VP/ml
West Nile virus	3.50×10^8	TCID50/ml
Enterovirus 71	3.62×10^4	TCID50/ml
Human immunodeficiency virus 1	1.82×10^4	IU/ml
Hepatitis A virus	5.00×10^3	IU/ml
Hepatitis B virus	5.00×10^4	IU/ml
Hepatitis C virus	7.75×10^3	IU/ml
Parvovirus B19	5.00×10^4	IU/ml
Aspergillus fumigatus	1.09×10^7	CFU/ml
Candida albicans	1.05×10^7	CFU/ml
Plasmodium falciparum	0.96%	% parasitemia

Carryover/cross-contamination

The *artus* CMV QS-RGQ MDx Kit showed no evidence of carryover or cross-contamination when 150 high-positive CMV samples (gB3) with a concentration of 3.0 x 10^6 IU/ml were extracted and tested in a checker-board format alternating positive samples with CMV-negative samples. The tested concentration of CMV represents the highest viral load observed within a diagnostic evaluation study analyzing 203 patient specimens collected retrospectively and prospectively. The percentage of correct calls was 100% (215/215) with a 95% confidence interval of 98.6%–100% (Table 10).

Table 10. Percentage carryover rate with one-sided exact 95% confidence limit

Sample type	Percentage correct calls	Exact lower one-sided 95% confidence limit	Exact upper one-sided 95% confidence limit
Negative	100.0% (215/215)	98.6%	100.0%

Precision and lot-to-lot variation

The precision of the *artus* CMV QS-RGQ MDx Kit was determined following the recommendations of the CLSI Guideline EP05-A2 (3) by testing a 4-member panel:

- 1 negative sample
- 1 sample with a 3x LOD concentration
- 2 samples with concentrations in the linear range of the assay

All samples were prepared by spiking EDTA plasma with the appropriate amount of 1st WHO International Standard for HCMV (NIBSC code 09/162, gB1).

Each panel member was evaluated in triplicate in 2 runs per day for 20 days. Three different QIAsymphony RGQ MDx systems were used for the study, with 3 different *artus* DSP Virus/Pathogen Midi Kit lots and 3 different *artus* CMV QS-RGQ MDx Kit lots. The tests were performed by 3 different operators. The results are summarized in Table 11, Table 12 and Table 13. The total standard deviation (SD) for the samples across the 3 kit lots is shown in Table 14.

Table 11. Sample 3x LOD (n = 120): Summary of precision and lot-to-lot variation data for 3 lots of the *artus* CMV QS-RGQ MDx Kit

Variance component	Variance	SD	SD lower two-sided 95% confidence limit	SD upper two-sided 95% confidence limit	Log normal %CV	Proportion of total variance
Day	0.000	0.018	0.008	3.371E76	4.21%	0.64%
Residual	0.051	0.226	0.198	0.263	55.82%	97.36%
Operator	0.000	0.000	–	–	0.00%	0.00%
Kit lot (extraction and PCR)	0.000	0.000	–	–	0.00%	0.00%
Instrument	0.001	0.032	0.012	146.217	7.48%	2.00%
Run within day	0.000	0.000	–	–	0.00%	0.00%

Table 12. Sample low-positive (n = 120): Summary of precision and lot-to-lot variation data for 3 lots of the *artus* CMV QS-RGQ MDx Kit

Variance component	Variance	SD	SD lower two-sided 95% confidence limit	SD upper two-sided 95% confidence limit	Log normal %CV	Proportion of total variance
Day	0.000	0.000	–	–	0.00%	0.00%
Residual	0.006	0.077	0.067	0.092	17.97%	63.81%
Operator	0.000	0.000	–	–	0.00%	0.00%
Kit lot (extraction and PCR)	0.000	0.000	–	–	0.00%	0.00%
Instrument	0.000	0.011	0.003	1.447E14	2.46%	1.22%
Run within day	0.003	0.057	0.042	0.092	13.26%	34.97%

Table 13. Sample high-positive (n = 120): Summary of precision and lot-to-lot variation data for 3 lots of the *artus* CMV QS-RGQ MDx Kit

Variance component	Variance	SD	SD lower two-sided 95% confidence limit	SD upper two-sided 95% confidence limit	Log normal %CV	Proportion of total variance
Day	0.000	0.000	–	–	0.00%	0.00%
Residual	0.001	0.032	0.028	0.038	7.35%	40.84%
Operator	0.000	0.000	–	–	0.00%	0.00%
Kit lot (extraction and PCR)	0.000	0.004	–	–	0.85%	0.55%
Instrument	0.000	0.013	0.005	4.811	2.95%	6.59%
Run within day	0.001	0.036	0.028	0.051	8.30%	52.02%

Table 14. Total standard deviation across 3 kit lots with the 95% confidence interval by sample concentration in log10 calculated concentration (IU/ml)

Sample type	n	SD	SD lower two-sided 95% confidence limit	SD upper two-sided 95% confidence limit	Log normal %CV
3x LOD	120	0.228	0.203	0.262	56.46%
Low-positive	120	0.096	0.086	0.110	22.49%
High-positive	120	0.049	0.044	0.056	11.33%

Endogenous interfering substances

Endogenous substances with a potential to interfere with the *artus* CMV QS-RGQ MDx Kit assay were spiked into (a) CMV-negative EDTA plasma and (b) in EDTA plasma containing a 3x LOD concentration of CMV (AD-169 strain, gB2). Samples were tested with the *artus* CMV QS-RGQ MDx Kit. Samples containing potentially interfering substances were compared to

control EDTA plasma samples containing no spiked interfering substance. The concentration level for each interfering substance was tested in 6 replicates.

The test concentrations for each interfering substance (Table 15) were selected based on current claims for the *artus* CMV RGQ PCR Kit (cat. no. 4503245; EZ1-RGQ) workflow and guidance provided by the CLSI Guideline EP07-A2 (4).

Table 15. Test concentrations of potentially interfering endogenous substances

Substance	Test concentration
Bilirubin (conjugated)	30.3 mg/dl
Bilirubin (unconjugated)	20.3 mg/dl
Hemoglobin	2 g/dl
Human genomic DNA	10 µg/dl
Total protein (albumin)	11 g/dl
Triglyceride (intralipid)	1.1 g/dl

At the tested concentrations, none of the interfering substances influenced the performance of the *artus* CMV QS-RGQ MDx Kit with regards to specificity, sensitivity and quantitation.

Exogenous interfering substances

Exogenous substances with a potential to interfere with the *artus* CMV QS-RGQ MDx Kit assay were spiked into (a) CMV-negative EDTA plasma and (b) in EDTA plasma containing a 3x LOD concentration of CMV (AD-169 strain, gB2). Samples were then tested with the *artus* CMV QS-RGQ MDx Kit. Samples containing potentially interfering substances were compared to control EDTA plasma samples containing no spiked interfering substance. The concentration level for each interfering substance was tested in 6 replicates.

The test concentrations for each interfering substance (Table 16) were selected based on current claims for the *artus* CMV RGQ PCR Kit (cat. no. 4503245; EZ1-RGQ) workflow and guidance provided by the CLSI Guideline EP07-A2 (4).

Table 16. Test concentrations of potentially interfering exogenous substances

Substance	Test concentration
Augmentin® (Amoxicillin+Clavulanic acid)	Amoxicillin:125 mg/l Clavulanic acid: 25 mg/l
Azathioprine-sodium	4 mg/l
Cefotaxim	1 g/l
Cidofovir	81 mg/l
Cyclosporine	1.125 g/l
Di-sodium EDTA	1.5 mg/l
Fluconazole	1 mg/l
Foscarnet (phosphonoformic acid trisodium hexahydrate)	700 mg/l
Ganciclovir	32 mg/l
Heparin-sodium	3000 U/l
Mycophenolate sodium	80 mg/l
Tazobac® (Piperacillin+Tazobactam)	Piperacillin: 1 g/l Tazobactam:125 mg/l
Prednisolone-21-hydrogensuccinate, sodium salt	4 mg/l
Prednisone	0.5 mg/l
Rapamycin	100 mg/l
Sulfamethoxazole	200 mg/l
Ticarcillin	1 g/l
Trimethoprim	5.2 mg/l
Valganciclovir hydrochloride	22 mg/l
Vancomycin	125 mg/l

At the tested concentrations, none of the interfering substances influenced the performance of the *artus* CMV QS-RGQ MDx Kit with regards to specificity, sensitivity and quantitation.

On-board stability

Samples were tested at 3 concentrations of CMV (AD-169 strain, gB2) in pooled negative EDTA plasma. The CMV concentrations were (a) 3x LOD (231 IU/ml), (b) a low-positive concentration within the linear range of the assay (1000 IU/ml) and (c) CMV negative. Samples were tested in replicates of 20 using a single lot of reagents on a single QIAsymphony RGQ MDx instrument.

Test samples and reagents were incubated on-board the instrument for 65 minutes at room temperature before the QIAsymphony SP/AS run was initiated. Following setup on the QIAsymphony AS instrument, the PCR reaction mix was left for a further 65 minutes on the QIAsymphony AS instrument before the PCR reaction was initiated on the Rotor-Gene Q MDx instrument. These results were compared to the results of a separate run set up using the same reagents under the same conditions but without the two prolonged delays.

Negative samples showed 100% negative calls (n = 40) and the 3x LOD (231 IU/ml) samples demonstrated a hit rate of 100% for positive calls (n = 40).

The differences in mean log10 calculated concentration (IU/ml) between the stability time points for the 3x LOD sample (231 IU/ml) and the low-positive sample (1000 IU/ml) are reported in Table 17.

Table 17. Difference in mean log10 calculated concentration (IU/ml) between stability conditions for samples (Method: Satterthwaite)

Sample type (concentration)	n	Difference in mean	Lower 95% confidence limit	Upper 95% confidence limit	p-value
3x LOD	40	0.010	–0.112	0.132	0.869
Low-positive	40	0.048	–0.024	0.120	0.183

The difference in mean log10 IU/ml between the incubated results and the non-incubated results for the 3x LOD and the 1000 IU/ml samples was 0.01 and 0.048, respectively.

Comparison of blood collection tubes

Blood collection tubes from BD™, Sarstedt® and Greiner were used to collect samples from patients. EDTA-plasma was isolated from each of the whole blood samples following manufacturer's instructions. CMV-negative samples were used to create 5 blood donor pools per sample tube type. The pools were split into 3 equal aliquots. One aliquot was used for CMV-negative samples; two aliquots were spiked with CMV-positive material (AD-169 strain, gB2) to generate samples at 3x LOD CMV and 1000 IU/ml CMV. Testing was carried out in duplicate using one *artus* CMV QS-RGQ MDx kit lot on two QIAsymphony RGQ MDx systems.

All negative samples tested were negative in the CMV Test Channel. All 3x LOD samples were positive in the CMV Test Channel. For the 1000 IU/ml samples, the 95% confidence limits for the differences in log10 concentration (IU/ml) between each pairing of the collection tube types fell within ±2x intermediate precision of the assay (Table 18).

Table 18. Differences in mean log10 concentration (IU/ml) between pairs of blood collection tubes with 95% confidence limits (sample: 1000 IU/ml; n = 30)

Tube 1	Tube 2	Difference in mean	Standard error	P value	Lower 95% confidence limit	Upper 95% confidence limit
Greiner	BD	0.030	0.038	0.445	–0.049	0.109
Sarstedt	BD	0.027	0.038	0.480	–0.052	0.106
BD	Sarstedt	–0.027	0.038	0.480	–0.106	0.052
Greiner	Sarstedt	0.002	0.038	0.954	–0.077	0.081
BD	Greiner	–0.030	0.038	0.445	–0.109	0.049
Sarstedt	Greiner	–0.002	0.038	0.954	–0.081	0.077

The data in Table 18 demonstrate that the use of different blood collection tubes has no impact upon the *artus* CMV QS-RGQ PCR MDx assay when run using the QIAsymphony RGQ MDx workflow.

Clinical Performance

The *artus* CMV QS-RGQ MDx Kit and *artus* CMV RGQ MDx Kit are in vitro nucleic acid amplification tests for the quantitation of human cytomegalovirus (CMV) DNA in human EDTA plasma.

The *artus* CMV QS-RGQ MDx Kit is configured for use with the QIAsymphony Rotor-Gene Q MDx system in conjunction with the QIAsymphony DSP Virus/Pathogen Midi Kit for DNA extraction. The Rotor-Gene Q MDx instrument in combination with Rotor-Gene AssayManager is used for CMV DNA amplification and quantitation.

The *artus* CMV RGQ MDx Kit is configured for use with the EZ1 DSP Virus System (EZ1 DSP Virus Kit and EZ1 Advanced instruments) for DNA extraction. The Rotor-Gene Q MDx instrument with Rotor-Gene Q software 2.1 or 2.3 and the respective Rotor-Gene Q *artus* CMV Assay Package (1.2.7 or 3.1.1) are used for CMV DNA amplification and detection.

Clinical comparison between EZ1-RGQ and QS-RGQ workflows

Two workflows were compared: *artus* CMV RGQ MDx Kit (EZ1-RGQ) and *artus* CMV QS-RGQ MDx Kit (QS-RGQ). A total of 180 paired positive and negative clinical samples as well as 124 contrived positive CMV samples were tested on both workflows and the results for each compared. The results for the *artus* CMV RGQ MDx workflow were generated at one test site (test site 3). The results for the *artus* CMV QS-RGQ MDx workflow were generated at 3 test sites (test sites 1, 2 and 3).

Samples that fell within the LLOQ for both systems were analyzed by Deming and Passing-Bablok regressions. The parameter estimates, along with their standard errors and corresponding 95% confidence intervals, are reported in Table 19.

Table 19. Results of regression analyses of combined samples QS-RGQ log10 concentration (IU/ml) vs. EZ1-RGQ log10 concentration (IU/ml) over all sites (number of observations = 455)

Regression	Response variable	Explanatory variable	Intercept	Intercept lower two-sided 95% confidence limit	Intercept upper two-sided 95% confidence limit	Slope	Slope lower two-sided 95% confidence limit	Slope upper two-sided 95% confidence limit
Deming	QS-RGQ log10 IU/ml	EZ1-RGQ log10 IU/ml	0.227	0.135	0.319	0.986	0.969	1.004
Passing-Bablok	QS-RGQ log10 IU/ml	EZ1-RGQ log10 IU/ml	0.263	0.174	0.339	0.985	0.969	1.004

A plot of the QS-RGQ assay results vs. EZ1-RGQ assay results with the fitted Deming and Passing-Bablok regression lines overlaid is shown in Figure 3.

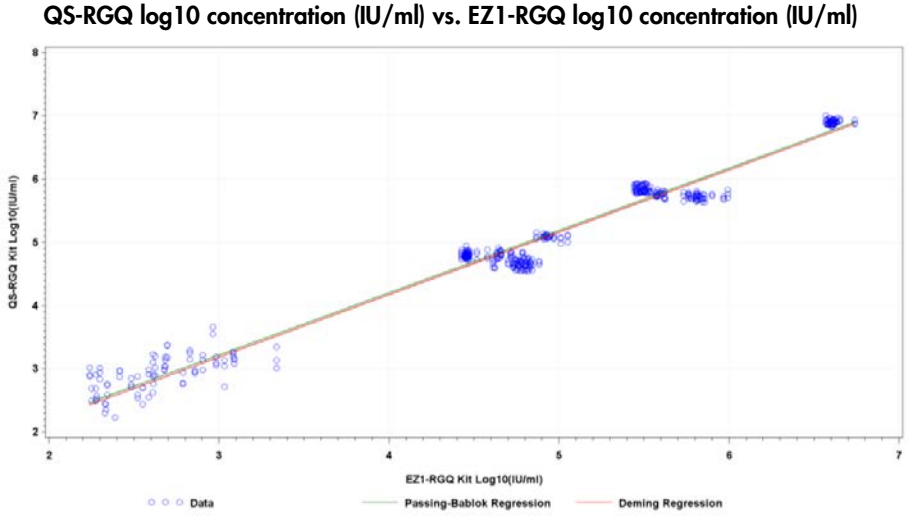


Figure 3: Regression plot QS-RGQ log10 concentration (IU/ml) vs. EZ1-RGQ log10 concentration (IU/ml) of combined samples over all sites showing Passing-Bablok and Deming lines. Samples that are between LLOQ and ULOQ for both kits are included for analysis.

A Bland-Altman plot of difference in calculated log10 concentration (IU/ml) against mean log10 concentration (IU/ml) between the QS-RGQ assay results and the EZ1-RGQ assay results (Figure 4) shows that the mean difference between the workflows was 0.16 log10 concentration (IU/ml).

Difference in calculated log10 IU/ml against mean log10 IU/ml: QS-RGQ and EZ1-RGQ

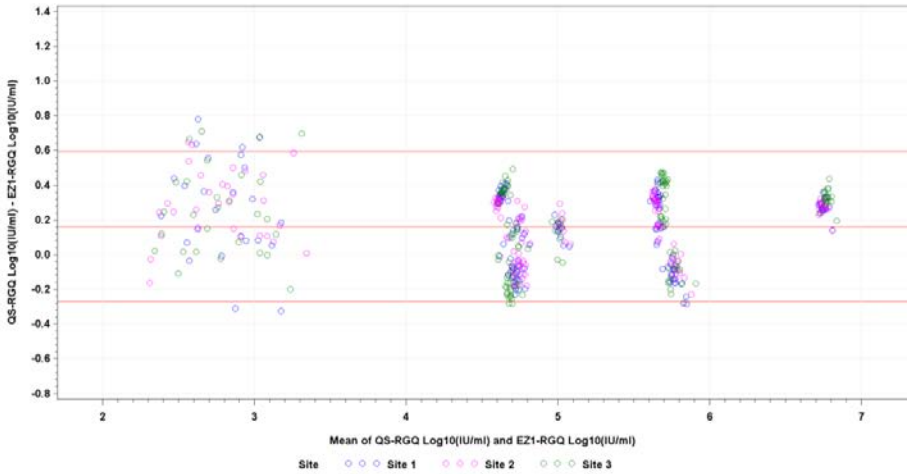


Figure 4. Combined samples Bland-Altman plot of difference in calculated log10 concentration (IU/ml) against mean log10 concentration (IU/ml) between QS-RGQ and EZ1-RGQ assay results. Horizontal reference lines at –0.27, 0.16 and 0.59 denote mean of difference [QS-RGQ log10 IU/ml – EZ1-RGQ log10 IU/ml] and its corresponding 95% prediction interval. Samples that are between LLOQ and ULOQ for both kits are included for analysis. Sites are identified by color.

The estimated difference in log10 concentration (IU/mL) at 1000 IU/ml between the QS-RGQ kit and EZ1-RGQ kit was calculated from the Deming regression (Table 20).

Table 20. Deming regression with estimated difference at 1000 IU/ml

Regression	EZ1-RGQ Kit log10 concentration (IU/ml)	QS-RGQ Kit log10 concentration (IU/ml)	Difference (log10 IU/ml)
Deming	3.00	3.187	0.187

The difference in log10 concentration (IU/ml) at the medical decision point of 1000 IU/ml viral load (5) was validated as 0.187 log10 IU/ml.

Allowable total difference (ATD)

Allowable total difference (ATD) zones for the difference between two measurements based on the reproducibility of the EZ1-RGQ system were computed for the following intervals:

- Between LLOQ and 1000 IU/ml (approximately 2.18 and 3 on the log10 scale)
- Between 1000 and 100,000 IU/ml (3 and 5 on the log10 scale respectively)
- Greater than 100,000 (5 on the log10 scale) up to ULOQ

The number of samples within the ATD for each site and over all sites and samples is shown in Table 21.

Table 21. Number of samples that fall within the ATD zones for concentration intervals at all sites (combined samples)

Concentration interval (IU/ml)	ATD lower limit	ATD upper limit	Number of samples within ATD zone (%)			
			Site 1	Site 2	Site 3	Over all sites
LLOQ to 1000	–0.699	0.699	20/21 (95.24%)	26/26 (100.0%)	23/24 (95.83%)	69/71 (97.18%)
1000 to 100,000	–0.526	0.526	65/65 (100.0%)	65/65 (100.0%)	65/65 (100.0%)	195/195 (100.0%)
100,000 to ULOQ	–0.289	0.289	44/63 (69.84%)	44/63 (69.84%)	30/63 (47.62%)	118/189 (62.43%)

Samples at concentrations in the low to mid-range (up to 100,000 IU/ml) are well within the ATD limits at 97.18% and 100%, respectively. Samples at concentrations of ≥100,000 IU/ml fall within the ATD in 62.43% of the cases (Table 21).

The clinical decision point for the management of CMV infections in transplant patients is generally accepted to be around 1000 IU/ml. A lower limit of detection of less than 1000 IU/ml (using either whole blood or plasma) may be inadequate to detect disease (5). At 1000 IU/ml, 97–100% of the results of the QS-RGQ and EZ1-RGQ workflows fall within the ATD. The level at which differences were observed is 2.0 log10 higher than the medical decision point. As such, patient treatment should not be impacted by the potential differences observed at much higher viral load concentrations. During treatment, the trend in viral load over time is of relevance rather than the absolute viral load (5).

Clinical performance of the *artus* CMV RGQ MDx Kit (EZ1-RGQ)

The clinical usefulness of the *artus* CMV RGQ MDx Kit (EZ1-RGQ workflow) was evaluated during a prospective study at 5 clinical laboratories in the United States. A method comparison analysis and a study with a truly negative (IgG-negative) cohort were also performed. Post-kidney transplant patients with CMV DNAemia were enrolled.

Specimens were collected at different time points (baseline, day 7, day 14, day 21 and day 28 post-treatment initiation and/or day 49 post-treatment/end of treatment) and during the course of anti-viral treatment (ganciclovir or valganciclovir; 6). The specimens collected across multiple time points were tested with the *artus* CMV RGQ MDx Kit and an FDA-approved test. A total of 368 specimens were evaluated from 44 evaluable subjects.

The primary endpoint for this evaluation was the resolution of clinically significant DNAemia, as determined by the clinician, following antiviral treatment with ganciclovir or valganciclovir. Study endpoints for this evaluation were positive percent agreement (PPA) and negative percent agreement (NPA) between the *artus* CMV RGQ MDx Kit viral load levels and the resolution of CMV DNAemia at the termination of antiviral therapy treatment in kidney transplant patients as defined by results from a comparator test.

Prospective study results

Agreement at baseline threshold values

Agreement at baseline threshold values was established by comparing the results from the *artus* CMV RGQ MDx Kit and the FDA-approved test using the following arbitrary values: "Not Detected", LLOQ of the respective assays (*artus* CMV RGQ MDx Kit at 159 IU/ml and FDA-approved test at 137 IU/ml), 500 IU/ml and 1000 IU/ml. Tables 22 and 23 below show the agreement data at baseline threshold values.

Table 22. Summary of the *artus* CMV RGQ MDx Kit vs. FDA-approved test by threshold

Clinical study stratified by threshold		FDA-approved test (N)		Total (N)
		Not detected	Detected	
artus CMV	Not detected	1	0	1
	Detected	0	43	43
Total		1	43	44
		≤LLOQ IU/ml	>LLOQ IU/ml	
artus CMV	≤LLOQ IU/ml	14	1	15
	>LLOQ IU/ml	5	24	29
Total		19	25	44
		≤500 IU/ml	>500 IU/ml	
artus CMV	≤500 IU/ml	22	0	22
	>500 IU/ml	2	20	22
Total		24	20	44
		≤1000 IU/ml	>1000 IU/ml	
artus CMV	≤1000 IU/ml	25	1	26
	>1000 IU/ml	3	15	18
Total		28	16	44

Table 23. Statistical summary of *artus* CMV RGQ MDx Kit vs. FDA-approved test by viremia threshold

Threshold	% ≤Threshold agreement (n/N)	% >Threshold agreement (n/N)	% Overall agreement (n/N)
Not Detected	100.00 (1/1)	100.00 (43/43)	100.00 (44/44)
LLOQ IU/ml	73.68 (14/19)	96.00 (24/25)	86.36 (38/44)
500 IU/ml	91.67 (22/24)	100.00 (20/20)	95.45 (42/44)
1000 IU/ml	89.29 (25/28)	93.75 (15/16)	90.91 (40/44)

Overall agreement analysis ranges from 86.36% to 100.00%. The 86.36% agreement is at the LLOQ threshold where some differences are expected given the different LLOQs of the *artus* CMV RGQ MDx Kit and FDA-approved tests. Percent agreement at the 1000 IU/ml threshold is 90.91%. At 500 IU/ml the percent agreement is 95.45% and at "Not Detected", it is 100.00%.

Agreement at resolution of CMV episode

Agreement analysis between the *artus* CMV RGQ MDx Kit and the FDA-approved test for resolution of CMV episode (as defined by 2 consecutive CMV viral load measurements below the LLOQ on different days) is presented in Tables 24 and 25 below. There were a total of 24 subjects with 229 specimens in this analysis that had baseline visit data greater than LLOQ for both the *artus* CMV RGQ MDx Kit and the FDA-approved test.

Table 24. Summary of *artus* CMV RGQ MDx Kit vs. FDA-approved test for resolution of CMV episode

			FDA-approved test		
Day	**Clinical study**		**Not resolved**	**Resolved**	**Total**
7	*artus* CMV	Not resolved	17	0	17
		Resolved	1	6	7
	Total		18	6	24
14	*artus* CMV	Not resolved	6	1	7
		Resolved	3	14	17
	Total		9	15	24
21	*artus* CMV	Not resolved	6	0	6
		Resolved	2	16	18
	Total		8	16	24
28	*artus* CMV	Not resolved	4	1	5
		Resolved	1	18	19
	Total		5	19	24

Table continued next page

Table continued

Day	Clinical study		FDA-approved test: Not resolved	Resolved	Total
35	*artus* CMV	Not resolved	4	1	5
		Resolved	1	18	19
	Total		5	19	24
42	*artus* CMV	Not resolved	3	1	4
		Resolved	0	20	20
	Total		3	21	24
133	*artus* CMV	Not resolved	1	1	2
		Resolved	1	21	22
	Total		2	22	24

Table 25. Statistical summary of *artus* CMV RGQ MDx Kit vs. FDA-approved test for resolution of CMV episode

Day	% Not resolved agreement (n/N)	% Resolved agreement (n/N)	% Overall agreement (n/N)
7	94.44 (17/18)	100.00 (6/6)	95.83 (23/24)
14	66.67 (6/9)	93.33 (14/15)	83.33 (20/24)
21	75.00 (6/8)	100.00 (16/16)	91.67 (22/24)
28	80.00 (4/5)	94.74 (18/19)	91.67 (22/24)
35	80.00 (4/5)	94.74 (18/19)	91.67 (22/24)
42	100.00 (3/3)	95.24 (20/21)	95.83 (23/24)
133*	50.00 (1/2)	95.45 (21/22)	91.67 (22/24)

* Includes day 84 from pivotal study.

The overall agreement between the *artus* CMV RGQ MDx Kit and FDA-approved test is 83.33% and higher. For instances where there was resolution of the CMV episode, the agreement ranges from 93.33% to 100.00%. For instances where there was no resolution of the CMV episode, the agreement ranges from 50.00% to 100.00%.

Overall agreement at different viral load levels and time windows

The overall agreement analysis included all evaluable subjects (44) and specimens (368). The viral load levels from the *artus* CMV RGQ MDx Kit and the FDA-approved test were stratified using the LLOQ values of the respective test (*artus* CMV RGQ MDx Kit at 159 IU/ml and FDA-approved test at 137 IU/ml) and arbitrarily determined viral load values of <LLOQ, 500, 1000 and 10,000 IU/ml. The number of specimens falling into each time window is presented in Table 26. The number of specimens falling into the respective categories is presented in Table 27. Tables 28–33 show the comparison of the *artus* CMV RGQ MDx Kit to the FDA-approved test in tracking the CMV viral load across different time points.

Table 26. Number of specimens within each time window

Time window	Number of samples within window
Baseline	44
Between day 1 and day 14	111
Between day 15 and day 28	94
Between day 29 and day 42	54
Between day 43 and day 56	30
Between day 57 and day 70	22
Between day 71 and day 84	9
Between day 85 and day 98	1
Between day 99 and day 112	1
Between day 113 and day 126	1
Between day 127 and day 140	1
Total	**368**

Table 27. *artus* CMV RGQ MDx Kit vs. FDA-approved test — all specimens

	FDA-approved test response (IU/ml)						
***artus* CMV RGQ MDx Kit response (IU/ml)**	**Not detected**	**Detected, <LLOQ**	**≥LLOQ and ≤500**	**>500 and ≤1000**	**>1000 and ≤10,000**	**>10,000**	**Total**
Not detected	133	12	2	0	0	0	147
Detected, <LLOQ	42	81	14	2	0	0	139
≥LLOQ and ≤500	1	7	12	2	2	0	24
>500 and ≤1000	0	1	2	5	1	0	9
>1000 and ≤10,000	0	0	3	5	17	2	27
>10,000	0	0	0	0	3	19	22
Total	176	101	33	14	23	21	368

Positive percent agreement (PPA) and negative percent agreement (NPA) results:

- For threshold LLOQ: PPA = 80.2% (73/91) and NPA = 96.8% (268/277)
- For threshold 500 IU/ml: PPA = 89.7% (52/58) and NPA = 98.1% (304/310)
- For threshold 1000 IU/ml: PPA = 93.2% (41/44) and NPA = 97.5% (316/324)
- For threshold 10,000 IU/ml: PPA = 90.5% (19/21) and NPA = 99.1% (344/347)

Table 28. *artus* CMV RGQ MDx Kit vs. FDA-approved test — all specimens at baseline

	FDA-approved test response (IU/ml)						
***artus* CMV RGQ MDx Kit response (IU/ml)**	**Not detected**	**Detected, <LLOQ**	**≥LLOQ and ≤500**	**>500 and ≤1000**	**>1000 and ≤10,000**	**>10,000**	**Total**
Not detected	1	0	0	0	0	0	1
Detected, <LLOQ	0	13	1	0	0	0	14
≥LLOQ and ≤500	0	4	3	0	0	0	7
>500 and ≤1000	0	1	1	1	1	0	4
>1000 and ≤10,000	0	0	0	3	7	0	10
>10,000	0	0	0	0	2	6	8
Total	1	18	5	4	10	6	44

Table 29. *artus* CMV RGQ MDx Kit vs. FDA-approved test — all specimens between days 1 and 14 from baseline

	FDA-approved test response (IU/ml)						
***artus* CMV RGQ MDx Kit response (IU/ml)**	**Not detected**	**Detected, <LLOQ**	**≥LLOQ and ≤500**	**>500 and ≤1000**	**>1000 and ≤10,000**	**>10,000**	**Total**
Not detected	15	2	2	0	0	0	19
Detected, <LLOQ	17	37	4	2	0	0	60
≥LLOQ and ≤500	0	2	5	0	1	0	8
>500 and ≤1000	0	0	1	1	0	0	2
>1000 and ≤10,000	0	0	1	1	7	1	10
>10,000	0	0	0	0	1	11	12
Total	32	41	13	4	9	12	111

Table 30. *artus* CMV RGQ MDx Kit vs. FDA-approved test — all specimens between days 15 and 28 from baseline

	FDA-approved test response (IU/ml)						
***artus* CMV RGQ MDx Kit response (IU/ml)**	**Not detected**	**Detected, <LLOQ**	**≥LLOQ and ≤500**	**>500 and ≤1000**	**>1000 and ≤10,000**	**>10,000**	**Total**
Not detected	47	6	0	0	0	0	53
Detected, <LLOQ	12	13	4	0	0	0	29
≥LLOQ and ≤500	0	1	1	0	1	0	3
>500 and ≤1000	0	0	0	2	0	0	2
>1000 and ≤10,000	0	0	1	0	3	1	5
>10,000	0	0	0	0	0	2	2
Total	59	20	6	2	4	3	94

Table 31. *artus* CMV RGQ MDx Kit vs. FDA-approved test — all specimens between days 29 and 42 from baseline

	FDA-approved test response (IU/ml)				
***artus* CMV RGQ MDx Kit response (IU/ml)**	**Not detected**	**Detected, <LLOQ**	**≥LLOQ and ≤500**	**>500 and ≤1000**	**Total**
Not detected	33	1	0	0	34
Detected, <LLOQ	4	6	3	0	13
≥LLOQ and ≤500	1	0	1	2	4
>500 and ≤1000	0	0	0	1	1
>1000 and ≤10,000	0	0	1	1	2
Total	38	7	5	4	54

Table 32. *artus* CMV RGQ MDx Kit vs. FDA-approved test — all specimens between days 43 and 56 from baseline

	FDA-approved test response (IU/ml)			
***artus* CMV RGQ MDx Kit response (IU/ml)**	**Not detected**	**Detected, <LLOQ**	**≥LLOQ and ≤500**	**Total**
Not detected	21	2	0	23
Detected, <LLOQ	1	4	0	5
≥LLOQ and ≤500	0	0	2	2
Total	22	6	2	30

Table 33. *artus* CMV RGQ MDx Kit vs. FDA-approved test — all specimens between days 57 and 70 from baseline

	FDA-approved test response (IU/ml)			
***artus* CMV RGQ MDx Kit response (IU/ml)**	**Not detected**	**Detected, <LLOQ**	**≥LLOQ and ≤500**	**Total**
Not Detected	9	0	0	9
Detected, <LLOQ	7	5	1	13
Total	16	5	1	22

Of the 368 specimens, there were 101 specimens that were not in the same category with regards to the quantification result as stratified in Table 27 above.

The distribution of the quantification results for these 101 specimens is as follows:

- Of the 43 that were negative by the FDA-approved test, 42 were detected below the LLOQ and 1 was ≥LLOQ and ≤500 IU/ml by the *artus* CMV RGQ MDx Kit
- Of the 20 that were detected below the LLOQ by the FDA-approved test, 12 were negative, 7 were ≥LLOQ and ≤500 IU/ml, and 1 was >500 and ≤1000 IU/ml by the *artus* CMV RGQ MDx Kit

- Of the 21 that were ≥LLOQ and ≤500 IU/ml by the FDA-approved test, 2 were negative, 14 were detected below LLOQ, 2 were >500 and ≤1000 IU/ml, and 3 were >1000 and ≤10,000 IU/ml by the *artus* CMV RGQ MDx Kit
- Of the 9 that were ≥500 and ≤1000 IU/ml by the FDA-approved test, 2 were detected below LLOQ, 2 were ≥LLOQ and ≤500 IU/ml, and 5 were >1000 and ≤10,000 IU/ml by the *artus* CMV RGQ MDx Kit
- Of the 6 that were >1000 and ≤10,000 IU/ml by the FDA-approved test, 2 were ≥LLOQ and ≤500 IU/ml, 1 was >500 and ≤1000 IU/ml, and 3 were ≥10,000 IU/ml by the *artus* CMV RGQ MDx Kit
- Of the 2 that were >10,000 IU/ml by the FDA-approved test, both were >1000 and ≤10,000 IU/ml by the *artus* CMV RGQ MDx Kit.

Overall, the *artus* CMV RGQ MDx Kit results and the FDA-approved test results were comparable as far as clinical relevance of the result. In the clinical study, out of the 368 specimens, 343 (93.2%) specimens had results that were comparable to the FDA-approved test with regards to clinical relevance of the result, in terms of having an impact on initiating and stopping treatment with antivirals and clinical management of the patient. Of the remaining 25 specimens, there were 9 specimens that were "Not Detected" or <LLOQ IU/ml by the FDA-approved test that were higher in CMV viral load (≥LLOQ and <1000 IU/ml) by the *artus* CMV RGQ MDx Kit, and there were 16 specimens that were ≥LLOQ and ≤500 IU/ml by the FDA-approved test that were lower in CMV viral load ("Not Detected" or <LLOQ) by the *artus* CMV RGQ MDx Kit.

Method comparison results

In addition to the prospective clinical study, a method comparison analysis was performed. A total of 73 specimens, corresponding to 25 subjects out of the prospective clinical study that had a result above the LLOQ for both the *artus* CMV RGQ MDx Kit and the FDA-approved test, was supplemented with a panel of 72 samples made up of cultured CMV diluted in human plasma across the linear range of the *artus* CMV RGQ MDx Kit, and equally distributed for testing by the *artus* CMV RGQ MDx Kit at 3 testing sites and by the FDA-approved test at

1 site. Figure 5 shows a scatter plot with results from testing this panel and the concordant positive specimens from the prospective clinical study.

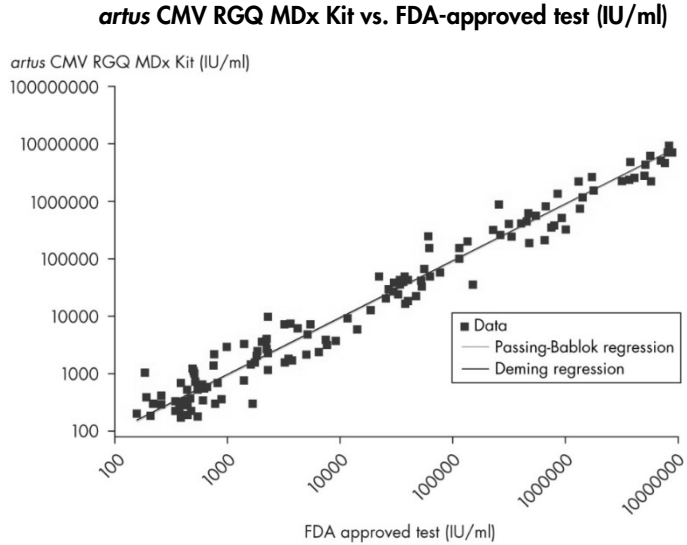


Figure 5. *artus* CMV RGQ MDx Kit vs. FDA-approved test in IU/ml (log scale).

Tables 34 and 35 show the Deming regression estimates for the slope and intercept, and the systematic difference between the *artus* CMV RGQ MDx Kit and the FDA-approved test.

Table 34. Deming regression estimates for *artus* CMV RGQ MDx Kit vs. FDA-approved test

Intercept	Intercept lower two-sided 95% confidence limit	Intercept upper two-sided 95% confidence limit	Slope	Slope lower two-sided 95% confidence limit	Slope upper two-sided 95% confidence limit
0.02	–0.13	0.17	1.00	0.97	1.03
0.02*	–0.14*	0.22*	1.00*	0.97*	1.04*

* Values re-estimated using bootstrap sampling at the subject level.

Table 35. Systematic difference between the *artus* CMV RGQ MDx Kit and the FDA-approved test (Deming regression estimates)

Value of the FDA-approved test	Systematic difference between the *artus* CMV RGQ Kit and the FDA-approved test
2.70 log10 IU/ml (500 IU/ml)	0.02 log10 IU/ml
3.00 log10 IU/ml (1000 IU/ml)	0.02 log10 IU/ml
4.00 log10 IU/ml (10,000 IU/ml)	0.02 log10 IU/ml

The Deming regression estimates show high concordance between the quantitative results of the *artus* CMV RGQ MDx Kit and the FDA-approved test across the measurement range.

Tables 36 and 37 show the Passing-Bablok regression estimates for the slope and intercept, and systematic difference between the artus CMV RGQ MDx Kit and the FDA-approved test.

Table 36. Passing-Bablok regression estimates for *artus* CMV RGQ MDx Kit vs. FDA-approved test

Intercept	Intercept lower two-sided 95% confidence limit	Intercept upper two-sided 95% confidence limit	Slope	Slope lower two-sided 95% confidence limit	Slope upper two-sided 95% confidence limit
0.00	–0.14	0.16	1.01	0.97	1.04
0.00*	–0.16*	0.21*	1.01*	0.97*	1.05*

* Values re-estimated using bootstrap sampling at the subject level.

Table 37. Systematic difference between the *artus* CMV RGQ MDx Kit and the FDA-approved test (Passing-Bablok regression estimates)

Value of the FDA-approved test	Systematic difference between the *artus* CMV RGQ Kit and the FDA-approved test
2.70 log10 IU/ml (500 IU/ml)	0.027 log10 IU/ml
3.00 log10 IU/ml (1000 IU/ml)	0.030 log10 IU/ml
4.00 log10 IU/ml (10,000 IU/ml)	0.040 log10 IU/ml

The Passing-Bablok regression results are similar to the Deming regression results. The estimates show high concordance between the two assays across the measurement range. Bootstrap sampling at the subject level showed that there was no dependency or correlation based on the multiple time points from the same subject.

In addition to the bias scatter plots, the method comparison was analyzed assessing the Allowable Total Difference (ATD) zone for two measurements of the *artus* CMV RGQ MDx Kit based on the reproducibility of the FDA-approved comparator test, calculating the percentages of the samples at low, medium and high subintervals that fall within the ATD zone. Similarly, the percentiles of the total difference between the *artus* CMV RGQ MDx Kit and the FDA-approved test were reported for each subinterval. Figure 6 shows a difference plot presenting this difference between the *artus* CMV RGQ MDx Kit and the FDA-approved test (reporting log10 IU/ml values), and an overlay with the ATD zone limits based on the mean observed values and 95% confidence limit of the FDA-approved test. Tables 38 and 39 show the analyses at the different subintervals.

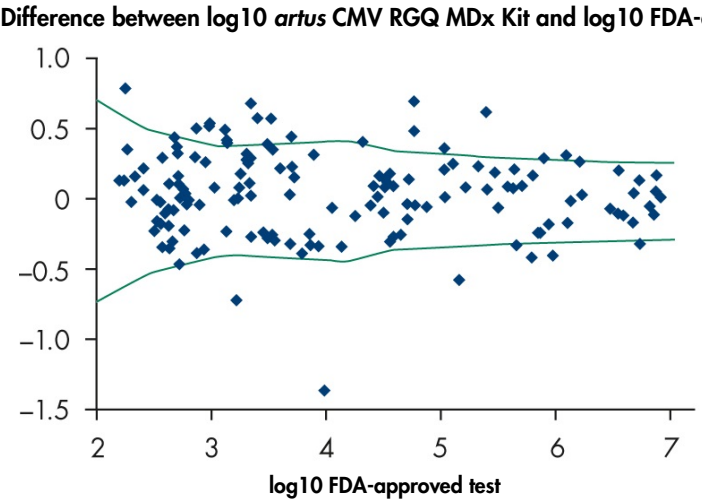


Figure 6. Allowable Total Difference (ATD) plot — all sites combined.

Table 38 below presents the total difference at 2.5th, 5.0th, 95.0th and 97.5th percentiles for the following 3 FDA-approved test measuring subintervals: less than 10,000 IU/ml (4.00 on the log10 scale); between 10,000 and 1,000,000 IU/ml (4.00 and 6.00 on the log10 scale respectively) and greater than 1,000,000 IU/ml (6.00 on the log10 scale).

Table 38. Percentile of the difference between log10 *artus* CMV and log10 FDA-approved test clinical and panel data — all sites combined

		Difference between log10 *artus* CMV and log10 FDA-approved test percentiles			
Result of FDA-approved test IU/ml	**N**	**2.5%**	**5.0%**	**95.0%**	**97.5%**
All	145	–0.46	–0.38	0.52	0.61
Less than 10,000	80	–0.59	–0.38	0.55	0.63
Between 10,000 and 1,000,000	47	–0.41	–0.40	0.48	0.61
Greater than 1,000,000	18	–0.32	–0.32	0.31	0.31

Table 39 shows the percentages of the samples that fall within the following 3 FDA-approved test measuring subintervals: between 137 IU/ml and 10,000 IU/ml (2.14 and 4.00 on the log10 scale); between 10,000 and 1,000,000 IU/ml (4.00 and 6.00 on the log10 scale respectively) and greater than 1,000,000 (6.00 on the log10 scale).

Table 39. Specimens/samples within the ATD clinical and panel data — all sites combined

Samples within ATD	**Samples within ATD for the FDA-approved test between 137 IU/ml and 10,000 IU/ml**	**Samples within ATD for the FDA-approved test between 10,000 IU/ml and 1,000,000 IU/ml**	**Samples within ATD for the FDA-approved test greater than 1,000,000 IU/ml**
82.1% (119/145)	81.3% (65/80)	80.9% (38/47)	88.9% (16/18)

The percentage of samples in the ATD was 82.1%.

The data show that across the entire range of FDA-approved test values, the 2.5th and 97.5th percentiles of the differences between methods are –0.46 and 0.61 respectively (representing a 0.35-fold and a 4.07-fold difference in IU/ml) and that the percentiles and corresponding fold differences become tighter at higher concentrations, as expected. For the lower subinterval (between 137 IU/ml and 10,000 IU/ml), the 2.5th and 97.5th percentiles of the differences between methods were –0.59 and 0.63 respectively (representing a 0.26-fold and a 4.79-fold difference in IU/ml). For the middle subinterval (between 10,000 and 1,000,000), the 2.5th and 97.5th percentiles of the difference between methods are –0.46 and 0.61, almost the same as across the entire range. For the higher subinterval (greater than 1,000,000), the 2.5th and 97.5th percentiles of the differences between methods are –0.32 and 0.31 respectively (representing a 0.48-fold and a 2.04-fold difference in IU/ml).

Negative (CMV IgG-negative) arm results

For the negative arm of the study, a total of 42 evaluable subjects out of the 58 enrolled were analyzed. The *artus* CMV RGQ MDx Kit was compared to the FDA-approved test and data was presented in 3 x 3 matrices (Table 40).

Table 40. CMV IgG-negative arm comparison of the *artus* CMV RGQ MDx Kit vs. the FDA-approved test

Negative specimens	**FDA-approved test**		
artus CMV RGQ MDx Kit	Not detected	Detected, <LLOQ	>LLOQ
Not detected	41	0	0
Detected, <LLOQ	1	0	0
>LLOQ	0	0	0

The agreement between the *artus* CMV RGQ MDx Kit and the FDA-approved test in the CMV IgG-negative specimens showed that of the 42 specimens, 41 (97.6%) had no CMV detected by both tests. One specimen was not detected by the FDA-approved test and was "Detected, <LLOQ" by the *artus* CMV RGQ MDx Kit.

Appendix: Prevention of Contamination

The detection of pathogens using polymerase chain reaction (PCR) and the subsequent detection of the PCR product by means of fluorescence-labeled, single-stranded oligonucleotides (probes) has become an important pillar in human diagnostics. The special advantages of this technology are above all its rapidness, its large linear quantification range as well as its very high specificity and sensitivity. Due to the high detection sensitivity, less than one copy of a DNA sequence per microliter of sample material may already be detected (7). This high sensitivity at the same time bears the risk that through inappropriate handling of samples even minute DNA quantities of the pathogen from another sample may result in a positive signal in real-time PCR (cross-contamination). These signals lead to false positive results in molecular diagnostics. Preventive measures concern the facilities, personnel and laboratory equipment, as well as the materials used. In addition, the identification of DNA contaminations, the search for their origins, and their elimination can be a very time-consuming and cost-intensive procedure. Therefore, preventive measures to protect against a contamination are of significant importance.

It is, therefore, of utmost importance to identify possible contaminations, to protect oneself from contaminations (prevention), and to eliminate already existing DNA contaminations (decontamination) (8).

Facilities

Local facilities permitting, one particular laboratory should be established for every working step in the execution of a PCR analysis. The focus ought to be on the spatial separation of sample preparation from assay setup.

The workflow in the laboratory should proceed in a unidirectional manner, beginning in the pre-amplification areas (I, II and III) and moving to the amplification/detection area. If this is not possible in exceptional cases, the relevant materials and objects should be thoroughly decontaminated (see “Cleaning of contaminated working areas and materials”, page 91).

The following assay steps should be performed in separate areas with dedicated supplies and equipment:

- DNA extraction — pre-amplification (area I)
- Reagent preparation — pre-amplification (area II)
- PCR setup — pre-amplification (area III)
- Rotor-Gene Q MDx setup/run — amplification/detection area

Color labeling may be useful to raise awareness for the particular sensitization of the personnel and for the demarcation of the working areas and corresponding equipment, e.g., pipets.

It is recommended to prepare the master mix on a designated work bench, separated from the potentially positive material. The use of a work bench with an integrated UV lamp* is another option for preventing contamination. This is especially useful where spatial separation of the working areas is not possible.

Air circulation from the work bench into the room should be avoided. If possible, the working area should be decontaminated before and after an activity (e.g., using UV light and/or commercially available decontaminating agents). Separate cleaning equipment should be available for every laboratory (bucket, floor cloth, etc.).

Personnel and clothing

All personnel are key to the prevention of contamination. The wearing and regular changing of laboratory clothing (laboratory coats, disposable sleeve protectors) and gloves should be standard. Door handles and other objects that are not directly linked to the working activities should not be touched with gloves. If there is uncertainty during the handling of sample material or equipment (e.g., touching the rim of a tube with the glove), gloves should always be changed. Separate laboratory wear should be available for every area.

* For the decontamination of the working areas, UV lamps should be switched on at least for 30 minutes. Materials and equipment which may react sensitively to UV light (e.g., centrifuge plastic lids), should be protected against UV light and be decontaminated separately. In addition, "dead spaces" within the laboratory that are not reached by the UV light should be cleaned to prevent possible contamination. Be aware of UV bulb shelf life.

Color differentiation of the laboratory wear for the individual working areas is advantageous. Such clothing must always be changed when entering or leaving a room. Laboratory clothing should regularly be cleaned (once per week) or one-way clothing for subsequent disposal may be used (e.g., overalls, disposable sleeve protectors, etc.). Care should be taken that the laboratory coats are stored inside the room and not removed from it.

Materials

All materials and devices should only be transported unidirectionally between work areas. If this is not possible, the relevant materials and devices (e.g., plastic holders for reaction tubes, cooling blocks) are to be carefully and preventively decontaminated. Each working place should, thus, have its own equipment (e.g., pipets, pipet tips, centrifuges, vortexers).

The placement of tips by hand onto the pipet is to be avoided. The pipet piston may be protected against contaminations by the use of direct displacement pipets and corresponding tips.

Pipets and consumables, as well as waste containers, should always remain in the work area and be decontaminated. Waste should be disposed of in lockable containers and in a plastic bag.

Wooden racks for storage of reaction tubes should not be used. Potentially contaminated material may attach to wood, due to its surface characteristics. For the same reason, wood is more difficult to decontaminate. Therefore, racks made of plastic should always be used.

Elimination of contamination (decontamination)

DNA contamination is usually not obvious. After working with DNA, all objects and materials should be regarded as potentially contaminated and be thoroughly cleaned.

Only relatively small DNA molecules (100-500 bp) are generally necessary for real-time PCR. Therefore, the appropriate solutions for the decontamination of nucleic acid contaminations must guarantee that the DNA will be reduced to sufficiently small fragments or nucleotides in order to prevent subsequent amplification during the PCR. The cleaning of the surfaces with alcoholic solutions is not suitable for DNA contamination since alcohols do not destroy the

structure of nucleic acids but merely serve for disinfection. Autoclaving (heat sterilization, 121°C at 1 bar excess pressure) of materials is also inappropriate for the effective destruction of nucleic acids since it also only guarantees the killing of microorganisms. Additionally, contamination may arise due to aerosol formation during the opening of the autoclave.

Cleaning of contaminated working areas and materials

Working areas may be decontaminated by irradiation with appropriate UV light (<280 nm). The use of a suitable reflector may increase the efficiency of the UV light by up to 90%. UV light with a low wavelength (approximately 220 nm) leads to the formation of ozone gas (O_3). Ozone, however, is injurious to health. Therefore, an adequate exhaust system for the gas must be guaranteed.

Cleaning of the equipment and surfaces of working area should include:

- Pipets
- Tip boxes
- Tube racks
- Cooling blocks
- Centrifuges
- Vortexers
- Surfaces of cab, hood, bench, etc.
- Discarding of all waste in closed containers
- Decontamination with commercially available decontamination agents

Decontamination of real-time device

In case of contamination of a real-time PCR device, the recommendations given in the relevant user manual should be observed or the manufacturer should be contacted. In general, decontamination should be done without aggressive cleaning agents, acids, or bases. Instead, the use of commercial decontamination solutions and low-lint cloth (e.g., KIMTECH®) is recommended in order to avoid a damage of the optical system of the instrument. The same applies to the instrument's accessories.

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Symbols

The following table describes the symbols that may appear on the labeling or in this document.

 <N>	Contains reagents sufficient for <N> reactions
	Use by
IVD	In vitro diagnostic medical device
REF	Catalog number
LOT	Lot number
MAT	Material number (i.e., component labeling)
COMP	Components (i.e., a list of what is included)
CONT	Contains (contents)
NUM	Number (i.e., vials, bottles)
Rn	R is for revision of the Handbook and n is the revision number
	Temperature limitation
	Manufacturer
	Consult instructions for use
	Caution
MASTER	Master mix
MG-SOL	Magnesium solution
IC	Internal control
CONTROL +	Positive control
CONTROL –	Negative control
GTIN	Global Trade Item Number

Ordering Information

Product	Contents	Cat. no.
artus CMV QS-RGQ MDx Kit	For 72 reactions: 3 Masters, Mg-Sol, Internal Control, Water (PCR grade), 6 Positive Controls, 4 Quantitation Standards	4503346
artus CMV RGQ MDx Kit	For 96 reactions: Master, Mg Solution, 4 Quantitation Standards, 2 Positive Controls (CMV High Positive Control and Low Positive Control), Internal Control, Water (PCR grade)	4503245
QIAsymphony DSP Virus/Pathogen Midi Kit	For 96 preps (1000 µl each): includes 2 Reagent Cartridges and enzyme racks and accessories	937055
QIAsymphony RGQ MDx System, Rotor-Gene AssayManager	QIAsymphony SP module; QIAsymphony AS module; Real-time PCR cycler 6 channels (green, yellow, orange, red, crimson and HRM); software for routine testing in combination with the QIAsymphony RGQ MDx system; laptop computer, software, accessories	9002341

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