June 2014

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

Σ 96

Version 1

IVD

Rx Only

For detection and quantitation of CMV DNA

For use with Rotor-Gene® Q MDx instruments

REF 4503245

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R1 MAT 1076957

QIAGEN®

Sample & Assay Technologies

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Contents

Intended Use

The *artus* CMV 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 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 RGQ MDx Kit must be interpreted within the context of all relevant clinical and laboratory findings.

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 and the Rotor-Gene Q MDx instrument for CMV DNA amplification and quantitation.

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

Summary and Explanation

The *artus* CMV RGQ MDx Kit constitutes a ready to use system for the detection and quantitation of CMV DNA using the polymerase chain reaction (PCR) on the Rotor-Gene Q MDx instrument. The CMV RG Master provided in the *artus* CMV RGQ MDx Kit contains reagents and enzymes for the specific amplification of a 105 bp region of the CMV Major Immediate Early Gene (MIE) DNA within the CMV genome. Oligonucleotide probes linked to fluorescent dyes specifically bind to the amplified product and permit the direct detection of CMV DNA in the “Test Channel” of the Rotor Gene Q MDx instrument.

In addition, the *artus* CMV RGQ MDx Kit contains a second heterologous amplification system (internal control) to identify possible inhibition in the PCR. The specific amplification of the internal control is detected in the “Control Channel” of the Rotor Gene Q MDx instrument.

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.

Principles 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 RGQ MDx Kit contains reagents and instructions for the detection and quantitation of CMV DNA in EDTA plasma.

The assay utilizes the EZ1 DSP Virus Kit (QIAGEN) and the EZ1 Advanced (XL)* instrument with the EZ1 Advanced (XL) DSP Virus Card for viral nucleic acid extraction. The EZ1 Advanced and the EZ1 Advanced XL instruments differ mainly in the number of samples which can be processed in one run. The EZ1 Advanced can process up to 6 samples, whereas the EZ1 Advanced XL can process up to 14 samples. The Rotor-Gene Q MDx instrument (QIAGEN), with the Rotor-Gene Q software 2.1.0 or higher, the *artus* CMV RGQ MDx Kit, and

* “EZ1 Advanced (XL)” is used to indicate “EZ1 Advanced or EZ1 Advanced XL”.

the Rotor-Gene Q *artus* CMV Assay Package1.2.7 or higher, is used 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. 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.

Two positive controls are provided with the *artus* CMV 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 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 RGQ MDx Kit are sufficient for 96 reactions on the Rotor-Gene Q MDx. The Rotor-Gene Q MDx rotor holds up to 72 reaction tubes.

Kit contents

artus CMV RGQ MDx Kit		
Catalog no.		**4503245**
Number of reactions		**96**
Blue	CMV RG Master	8 x 300 µl
Yellow	CMV Mg-Sol	600 µl
Red	CMV QS 1 1 x 10^4 copies/µl (1.19 x 10^6 IU/ml)	200 µl
Red	CMV QS 2 1 x 10^3 copies/µl (1.19 x 10^5 IU/ml)	200 µl
Red	CMV QS 3 1 x 10^2 copies/µl (1.19 x 10^4 IU/ml)	200 µl
Red	CMV QS 4 1 x 10^1 copies/µl (1.19 x 10^3 IU/ml)	200 µl
Violet	CMV Low Positive Control	200 µl
Black	CMV High Positive Control	200 µl
Green	CMV RG IC	2 x 1000 µl
White	H_2O	1 ml
artus *CMV RGQ MDx Kit Instructions for Use (Handbook)*		1

Materials Required but Not Provided

For DNA purification

Reagents

- EZ1 DSP Virus Kit (cat. no. 62724), version 4
- Water
- 70% ethanol

Equipment

- EZ1 Advanced instrument* (cat. no. 9001411) or EZ1 Advanced XL instrument* (cat. no. 9001492)
- EZ1 Advanced DSP Virus Card v1.0 or higher, with protocol "DSP Virus version 1.0" or higher (cat. no. 9018306) or EZ1 Advanced XL DSP Virus Card v1.0 or higher, with protocol "DSP Virus version 1.0" or higher (cat. no. 9018703)
- Heating block for 1.5 ml Tubes (e.g. Eppendorf® Thermomixer)
- Optional: Vortexer* (if frozen samples need to be mixed)

For sample tracking, one of the following is required:

- PC and TFT Monitor, minimum 17" (QIAGEN cat. no. 9016643), (or your own PC and monitor) with EZ1 Advanced Communicator Software (software supplied with EZ1 Advanced and EZ1 Advanced XL instruments)
- Printer (cat. no. 9018464) and accessory package for printer (cat. no. 9018465)

Consumables

- Pipets* and sterile, RNase-free pipet tips
- Soft paper tissue

* Ensure that instruments have been checked, maintained, and calibrated regularly according to the manufacturer's recommendations.

For PCR

Consumables

- Pipets* (adjustable)
- Sterile pipet tips with filters
- Strip Tubes and Caps, 0.1 ml (cat. no. 981103 or 981106), for use with 72-well rotor

Equipment

- Vortex mixer*
- Laboratory timer
- Benchtop centrifuge* with rotor for 2 ml reaction tubes
- Rotor-Gene Q MDx instrument* with 72-well rotor (cat. no. 9002035 or 9002036)
- Rotor-Gene Q Software version 2.1.0 or higher
- Rotor-Gene Q *artus* CMV Assay Package 1.2.7 or higher (cat. no. 9022860)
- Cooling block (Loading Block 72 x 0.1 ml Tubes, cat. no. 9018901)

Warnings and Precautions

For In Vitro Diagnostic Use

For prescription use only.

The *artus* CMV RGQ MDx Kit is not intended for use as a diagnostic test to diagnose CMV infection.

Due to inherent differences among technologies and patient populations, the user should perform method comparison with

* Ensure that instruments have been checked, maintained, and calibrated regularly according to the manufacturer's recommendations.

their own quantitative CMV test currently used in their clinical practice before switching to the *artus* CMV RGQ MDx Kit.

Due to inherent differences among technologies, it is recommended to assess the impact of any potential change in the method for quantification of CMV DNA during the clinical management of a patient.

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs). These are available online in convenient and compact 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 EZ1 DSP Virus System and the Rotor-Gene Q MDx instrument, see the kit handbooks and instrument user manuals supplied.

Discard sample and assay waste according to your local safety regulations.

24-hour emergency information

Chemical emergency or accident assistance is available 24 hours a day from:

CHEMTREC

USA ■ Tel: 1-800-424-9300

General precautions

The user should always pay attention to the following:

- Unless noted otherwise, strict compliance with this handbook, as well as with the *EZ1 DSP Virus Kit Handbook*, the *EZ1 Advanced (XL) User Manual*, and the *Rotor-Gene Q MDx (US) User Manual* is required for optimal results.
- Attention should be paid to the expiration date printed on the box. Do not use a kit after its expiration date.

- Do not mix components from different kit lots.
- Performance characteristics of this assay have been determined only with EDTA plasma specimens.
- Follow universal precautions. All patient specimens should be considered potentially infectious and handled accordingly.
- Follow preventive measures to avoid DNA contamination (see “Appendix: Prevention of contamination”, page 77).
- Use sterile pipet tips with filters (see Appendix: Prevention of contamination”, page 77).
- Use always new clean PCR reaction tubes and caps (see “Appendix: Prevention of contamination”, page 77).
- Dispose amplified samples without opening reaction tubes. Do not autoclave reaction tubes after the PCR.
- Thaw all components thoroughly at 18–25°C before starting an assay.
- When thawed, mix the components (by pipetting repeatedly up and down or by pulse vortexing) and centrifuge briefly.
- Work quickly and keep thawed components on ice or in the cooling block.

Reagent Storage and Handling

The components of the *artus* CMV RGQ MDx Kit should be stored at –15 to –30°C and are stable until the expiration date stated on the label. Repeated thawing and freezing (>2 x) should be avoided, as this may reduce assay sensitivity. If the reagents are to be used only intermittently, they should be frozen in aliquots. Storage at 2–8°C should not exceed a period of 5 hours. The *artus* CMV RGQ MDx Kit is shipped frozen on dry ice. If the reagents are thawed upon receipt, they should not be used.

Specimen Handling and Storage

The *artus* CMV RGQ MDx Kit is intended to be used with plasma samples obtained with EDTA as anticoagulant. 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.

If the plasma samples are not immediately analyzed, they can be stored at 20–25°C for 24 hours or up to 2 months at –15 to –30°C with no more than 2 freeze–thaw cycles.

Procedure

Overview

The procedure consists of 4 consecutive steps:

- Sample collection: Whole blood specimens are withdrawn with commercially available specimen collection tubes from Becton Dickinson, Sarstedt, or Greiner, following manufacturer's instructions. EDTA plasma is collected after blood centrifugation.
- Nucleic acid extraction: Extract viral DNA, using the EZ1 DSP Virus Kit in combination with the EZ1 Advanced (XL) instrument.
- Real-time PCR: Prepare the PCR reaction for the extracted DNA and positive and negative control material. Perform real-time PCR using the *artus* CMV RGQ MDx Kit in combination with the Rotor-Gene Q MDx instrument.
- Result interpretation: The Rotor-Gene Q *artus* CMV Assay Package evaluates the results of the CMV high and low positive controls, CMV quantitation standards, and the NTC to determine if the run is valid. If the run is valid, the internal control and target-specific results of each specimen are evaluated.

DNA isolation (Pre-amplification Area I)

Make sure to familiarize yourself with the EZ1 Advanced (XL) instrument before beginning the DNA extraction procedure. See the *EZ1 Advanced (XL) User Manual*.

Viral DNA is extracted from 400 µl of the patient sample using the EZ1 DSP Virus Kit on the EZ1 Advanced (XL) instrument, as described in the *EZ1 DSP Virus Kit Handbook*. Elution volume is 60 µl.

Note: Care should be taken to clearly label the EZ1 DNA eluates to avoid the possibility of mix-up.

Note: The use of carrier RNA is critical for the extraction efficiency and, consequently, for the DNA yield. Add the appropriate amount of carrier RNA to each extraction following the instructions in the *EZ1 DSP Virus Kit Handbook* and in "Preparation of AVE–IC–CARRIER mix", page 17.

If eluates have been frozen, they must be heated at 85°C for 15 minutes using a heating block. Before starting the PCR setup, eluates must be cooled down and briefly centrifuged.

Internal control

An internal control (CMV RG IC) is supplied with the *artus* CMV RGQ MDx Kit. This allows the user to both monitor the DNA isolation procedure and to check for possible PCR inhibition.

The internal control must not be added to the sample material directly. Internal control should be combined with elution buffer (AVE) and carrier RNA (CARRIER) stock solution in one mixture.

Preparation of AVE–IC–CARRIER mix

Determine the number (N) of reactions you are going to perform (this includes all clinical specimens, plus any process controls). Prepare the AVE–IC–CARRIER mix directly before use according to Table 1.

Table 1. Preparation of AVE–IC–CARRIER mix*

Number of reactions:	1	N + 1
Elution buffer (AVE)†	49.2 µl	(N + 1) x 49.2 µl
Internal control (CMV RG IC)	7.2 µl	(N + 1) x 7.2 µl
Carrier RNA (CARRIER) stock solution†	3.6 µl	(N + 1) x 3.6 µl
Total volume	**60 µl**	**(N + 1) x 60 µl**

* AVE–IC–CARRIER mix is added to each sample during the extraction process by the EZ1 Advanced (XL) instrument.

† From the EZ1 DSP Virus Kit.

Process controls

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.

Protocol: PCR

Important point before starting

- Take time to familiarize yourself with the Rotor-Gene Q MDx before starting the protocol. See the instrument user manual.

Things to do before starting

- Install the Rotor-Gene Q *artus* CMV Assay Package template before using the *artus* CMV RGQ MDx Kit for the first time (see "Appendix A: The Rotor-Gene Q *artus* CMV Assay Package", page 74).
- Make sure that the cooling block (accessory of the Rotor-Gene Q MDx instrument) is precooled to 2–8°C.
- Before each use, all reagents need to be thawed completely at 18–25°C, mixed (by repeated up and down pipetting or by quick vortexing), and centrifuged briefly.

Reagent preparation (Pre-amplification Area II)

1. **Place the desired number of PCR tubes into the adapters of the cooling block. Follow the order of the tubes shown in Table 2.**

Table 2. Order of the tubes in the PCR run

Tube position	Description
1	Low positive control
2	CMV QS 4
3	CMV QS 3
4	CMV QS 2
5	CMV QS 1
6	High positive control

7	NTC (H_2O)
8 and higher	Process control or samples

2. **Prepare a master mix according to Table 3 for the total number of samples (N), including the process controls.**

Table 3. Preparation of master mix

Number of samples:	1–33	34–48	49–65
CMV RG Master	(N+9)* x 25 µl	(N+10)* x 25 µl	(N+12)* x 25 µl
CMV Mg-Sol	(N+9)* x 5 µl	(N+10)* x 5 µl	(N+12)* x 5 µl
Total volume	(N+9)* x 30 µl	(N+10)* x 30 µl	(N+12)* x 30 µl

* The total number of reactions to prepare (N+9, N+10, N+12) is increased in order to avoid lack of master mix volume due to pipetting imprecision. If more runs are performed, fewer than 96 reactions might be obtained with the *artus* CMV RGQ MDx Kit.

3. **Prepare the master mix for the quantitation standards, low positive control, and high positive control according to Table 4.**

Table 4. Preparation of master mix

Number of reactions:	8[†]
CMV RG Master plus CMV Mg-Sol (prepared according to Table 3)	240 µl
CMV RG IC	16 µl[‡]
Total volume	256 µl

[†] The total number of reactions to prepare is set to 8 (7 + 1) to avoid a lack of master mix volume due to pipetting imprecision.

[‡] The volume increase caused by adding the internal control is negligible and does not affect the sensitivity of the detection system.

PCR setup (Pre-amplification Area III)

4. **For the samples and process controls, pipet 30 µl of the master mix, prepared according to Table 3, into each PCR tube (tube positions 8 and higher). For the quantitation standards, low positive control, high positive control, and NTC (tube positions 1–7), pipet 30 µl of the master mix prepared according to Table 4 (including CMV RG IC).**
5. **Add 20 µl of the eluted sample or process control DNA (Table 5) to the appropriate tubes. Mix well by pipetting up and down.**

Table 5. Preparation of PCR assay

Component	Volume
Master mix (mixture from Table 4 for tube positions 1–7; mixture from Table 3 for tube positions 8 and higher)	30 µl
Sample or control	20 µl
Total volume	50 µl

6. **Add 20 µl of low positive control for tube position 1, 20 µl of CMV QS 4 for tube position 2, 20 µl of CMV QS 3 for tube position 3, 20 µl of CMV QS 2 for tube position 4, 20 µl of CMV QS 1 for tube position 5, 20 µl of high positive control for tube position 6, and 20 µl of NTC (H_2O) for tube position 7. Mix well by pipetting up and down directly after adding the sample.**
7. **Close the PCR tubes carefully.**

Rotor-Gene Q MDx setup and run (Amplification/Detection Area)

8. **Transfer the PCR tubes from the cooling block into the appropriate positions in the 72-Well Rotor of the Rotor-Gene Q MDx (see Table 2, page 19). Transfer the Rotor-Gene Q MDx 4-strip tubes in the correct orientation, so that the position indices of the cooling adapter and the rotor match.**

   Note: Place the tubes into the rotor in consecutive order. If all of the tube positions have been occupied up to a specific number (n), the next tube must go into the subsequent numerical position (n + 1).

   Note: Fill any empty positions in the rotor with empty and capped PCR tubes.

9. **Place the rotor into the Rotor-Gene Q MDx instrument. Make sure that the locking ring (supplied with the Rotor-Gene Q MDx instrument) is placed on top of the rotor to prevent the tubes from opening during the run. Close the lid.**

10. Start the software by double-clicking on the "Rotor-Gene Q MDx Series Software" icon on the desktop. Select "New" from the "File" menu on the menu bar. Navigate to the "CMV Templates" folder in the dialog displayed (Figure 1).

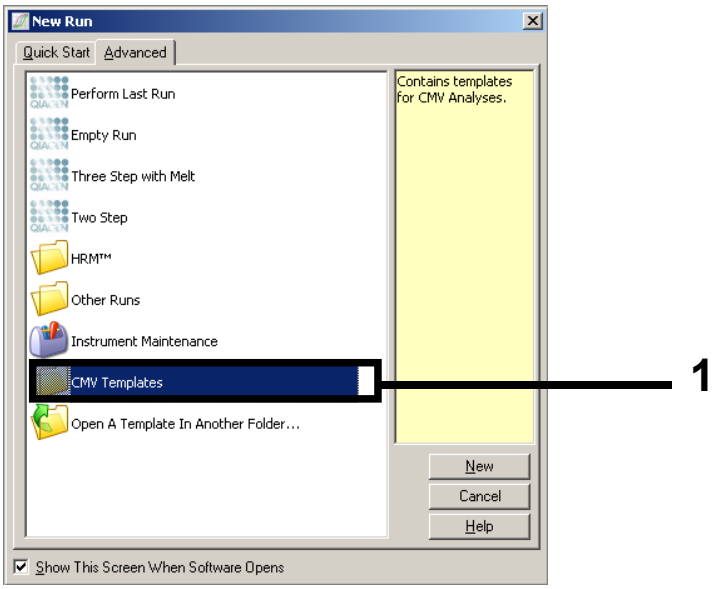

Figure 1. Opening the "CMV Templates" folder.

11. Select the "artus CMV Locked Template" (Figure 2) by double-clicking or with a single click, then click "New".

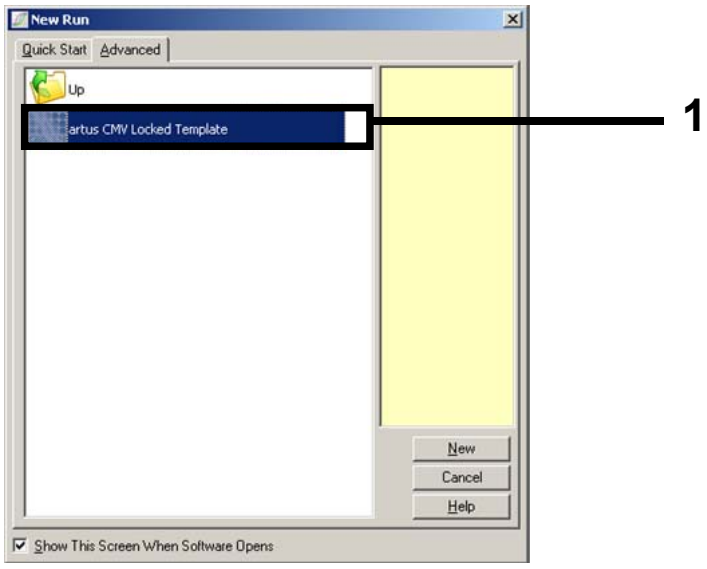

Figure 2. Selecting the "artus CMV Locked Template" in the "CMV Templates" folder.

12. The "Setup" screen appears (Figure 3). Enter the operator's name in the "Notes" dialog field, and enter the run ID in the "Run ID" dialog field. Check the "Locking Ring Attached" box. Enter the sample name of each

sample in the "Sample Name" dialog field. When all sample IDs have been entered, verify and click "Start Run".

Note: Entering the operator's name is optional and does not influence the functionality of the program.

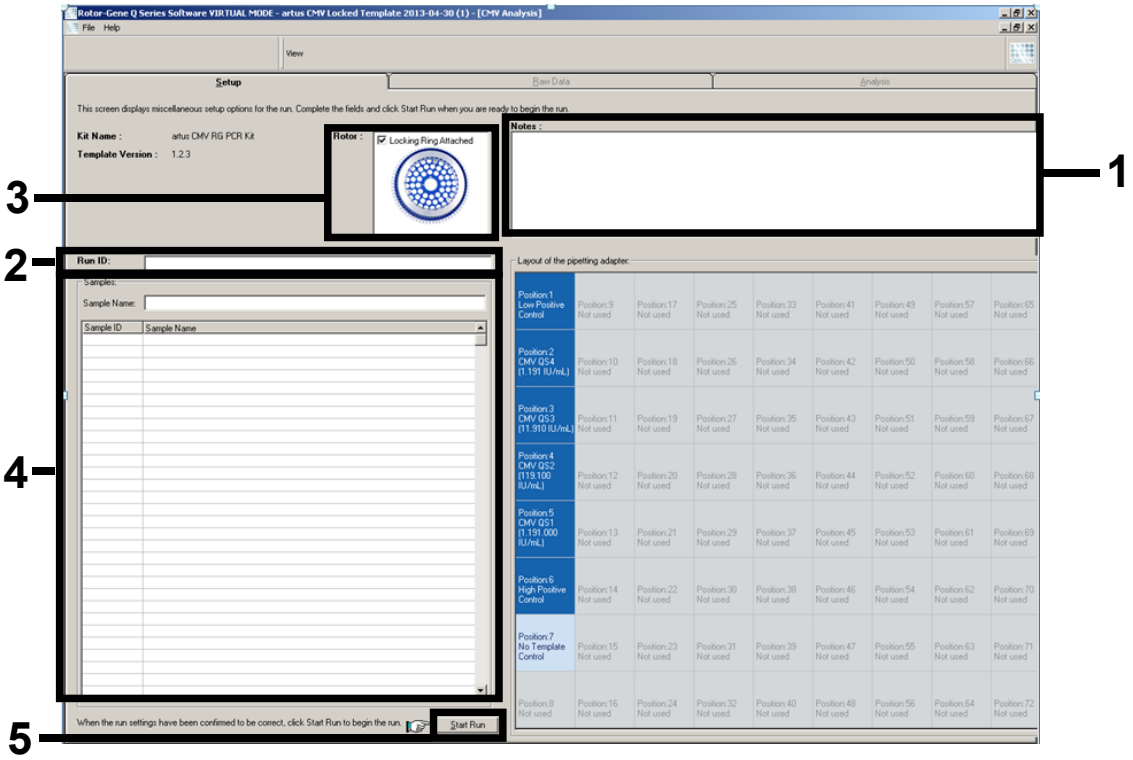

Figure 3. Entering the operator name, sample ID, run ID, and "Locking Ring Attached" box.

13. **If there are open positions, a warning will appear (Figure 4). Fill all open rotor positions with empty tubes and click "OK".**

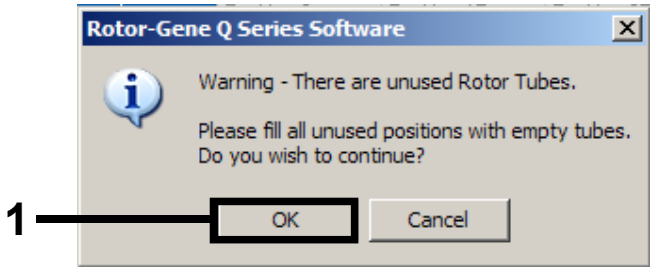

Figure 4. Warning of open rotor positions.

14. **Choose an appropriate file name and save as a .rex run file (Figure 5).**

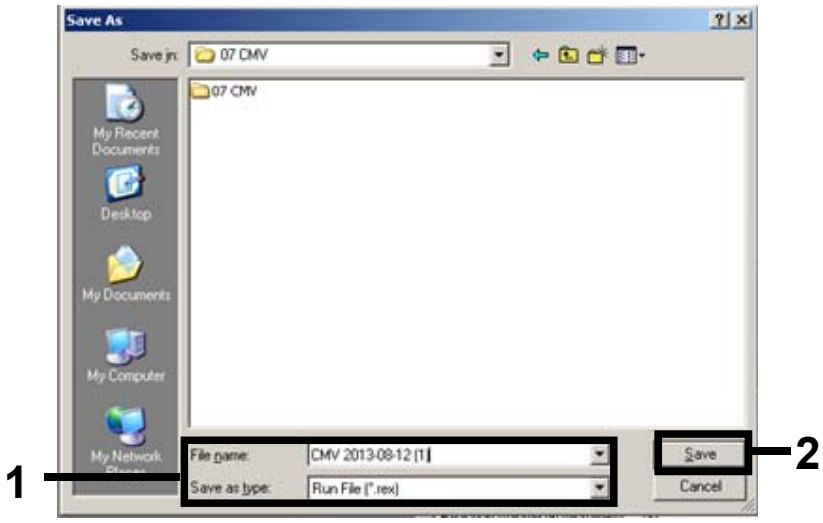

Figure 5. Saving the run file.

15. **The PCR run starts.**
    During the run, the "Raw Data" tab will open and real-time data for the "Test Channel" (CMV detection and quantitation) and the "Control Channel" will be displayed automatically.

Reports

16. **After the run is finished, the "Analysis" tab will open automatically.**
    The analysis for the "Test Channel" (CMV detection and quantitation) and the "Control Channel" will be displayed.

17. Click “Report”. Choose “CMV Analysis Report” and click “Show” (Figure 6).

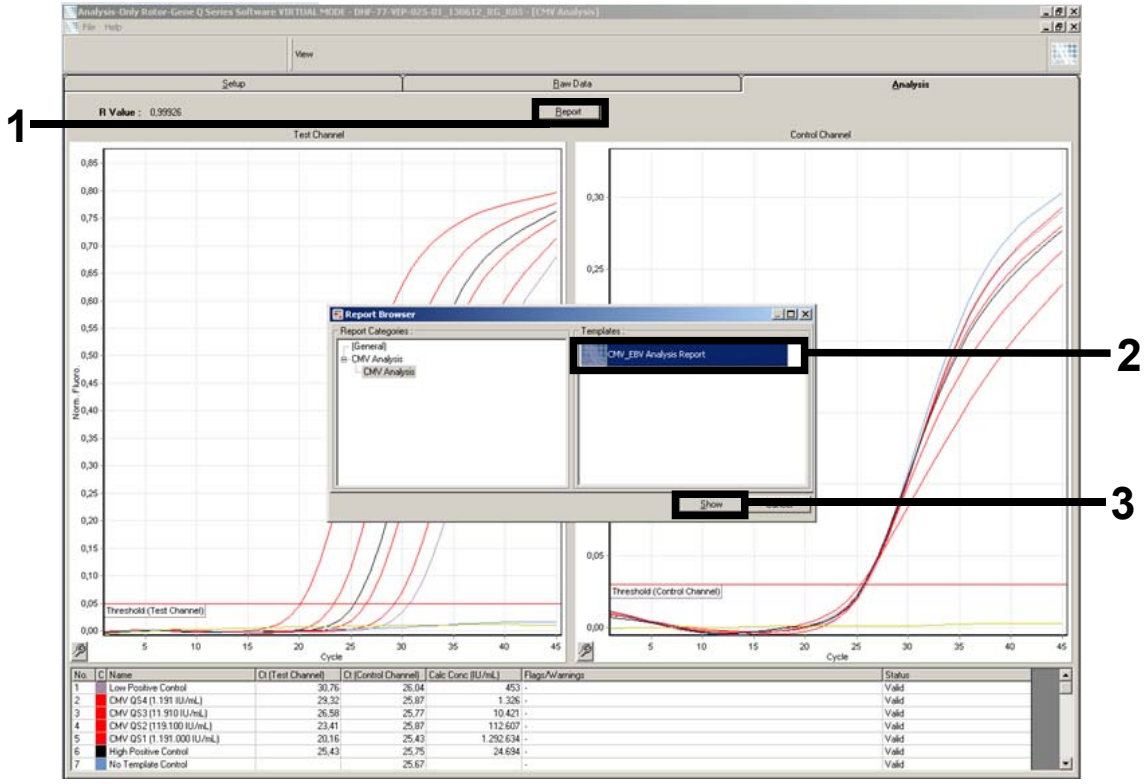

Figure 6. Choosing the “CMV Analysis Report”.

Interpretation of Control Results

The Rotor-Gene Q software determines whether control results are valid or invalid and therefore whether the run is valid or invalid.

Valid runs

The conditions in Table 6 must be met for a valid run.

Table 6. Conditions required for a valid run

Name	Test channel	Control channel	Status
CMV low positive control	Valid	–	Valid
CMV high positive control	Valid	–	Valid
CMV QS 1–4	Valid	–	Valid
NTC	Valid	Valid	Valid

Invalid runs

If the CMV low positive control, CMV high positive control, CMV QS 1–4, or NTC result is determined to be invalid, the software will provide a flag/warning message. An interpretation of the possible flag/warning messages is provided in Table 7.

Table 7. Description of flag/warning messages associated with invalid control results

Name	Flag/warning	CMV result	Status	Interpretation of result
QS 1–4	Control Sample [CMV QS (1–4)] failed rule [Minimum Fluorescence] on Test Channel. Detected Fluorescence: X. Min Fluorescence: X	Invalid	Invalid	Run is invalid: signal in the CMV Test Channel is out of specification.
QS 1–4	Control Sample [CMV QS (1–4)] failed rule [C_T Range] on Test Channel. Detected C_T: X. Min Ct: X Max C_T: X	Invalid	Invalid	Run is invalid: signal in the CMV Test Channel is out of specification.
QS 1–4	Failed rule [R Value] on Test Channel. Calculated R Value: X. Min R Value: X	Invalid	Invalid	Run is invalid: signal in the CMV Test Channel is out of specification.
LPC	Control Sample [Low Positive Control] failed rule [Concentration Range] on Test Channel. Detected Concentration: X. Min Concentration: X Max Concentration: X	Invalid	Invalid	Run is invalid: signal in the CMV Test Channel is out of specification.
QS 1–4	Control Sample [CMV QS (1–4)] failed rule [Minimum Fluorescence] on Test Channel. Detected Fluorescence: X. Min Fluorescence: X	Invalid	Invalid	Run is invalid: signal in the CMV Test Channel is out of specification.

Table continued on next page

Table 7. Continued

Name	Flag/warning	CMV result	Status	Interpretation of result
HPC	Control Sample [High Positive Control] failed rule [Concentration Range] on Test Channel. Detected Concentration: X. Min Concentration: X Max Concentration: X	Invalid	Invalid	Run is invalid: signal in the CMV Test Channel is out of specification.
NTC	Control Sample [No Template Control] failed rule [Minimum Fluorescence] on Control Channel. Detected Fluorescence: X. Min Fluorescence: X	–	Invalid	Run is invalid: signal in the Control Channel is out of specification.
NTC	Control Sample [No Template Control] failed rule [C_T Range] on Control Channel. Detected C_T: X. Min C_T: X Max C_T: X	–	Invalid	Run is invalid: double intersection in Control Channel.*
NTC	Control Sample [No Template Control] failed rule [Concentration Range] on Test Channel. Detected C_T value: X. Expected C_T value = "Not Detected"	Invalid	Invalid	Run is invalid: signal in the Test Channel is out of specification.

* Amplification curve crosses the threshold twice.

Retesting of invalid runs

Note: If the run is invalid due to failure of the low positive control, high positive control, QS 1–4, or NTC, all samples in that run must be retested using remaining purified nucleic acids.

Interpretation of Sample Results

For each sample in a valid run, the Rotor-Gene Q software indicates the status of the analysis for CMV (invalid, Detected, or Not Detected). The calculated CMV concentration is only provided for samples within the linear range. A description of the sample results provided by the Rotor-Gene Q software is provided in Table 8.

Table 8. Sample results determined by the Rotor-Gene Q software

Name	Flag/warning	CMV	Status	Interpretation of result
Sample ID	–	DNA detected	Detected	CMV DNA detected within linear range. Calculated concentration provided.
Sample ID	–	DNA not detected	Not Detected	CMV DNA not detected.
Sample ID	–	DNA detected	Detected, below LOQ	CMV DNA detected below LOQ (<159 IU/ml).
Sample ID	–	DNA detected	Detected, above linear range	CMV DNA detected above linear range (>7.94 x 10^7 IU/ml).
Sample ID	IC_LEFT_CT_ SHIFT	Invalid	Invalid	Not determined: Signal in the Control Channel is out of specification range.
Sample ID	IC_RIGHT_CT _SHIFT	Invalid	Invalid	Not determined: Signal in the Control Channel is out of specification range.

Table continued on next page

Table 8. Continued

Name	Flag/ warning	CMV	Status	Interpretation of result
Sample ID	IC_FLUORESCENCE	Invalid	Invalid	Not determined: Signal in the Control Channel is out of specification range.
Sample ID	IC_LEFT_CT_SHIFT IC_FLUORESCENCE	Invalid	Invalid	Not determined: Signal in the Control channel is out of specification range.
Sample ID	IC_RIGHT_CT_SHIFT IC_FLUORESCENCE	Invalid	Invalid	Not determined: Signal in the Control channel is out of specification range.
Sample ID (positive sample)	INVALID_DATA	Invalid	Invalid	Not determined: CMV test channel failed, caused by double intersection.*
Sample ID (negative sample)	EARLY_CT	Invalid	Invalid	Not determined: CMV test channel failed, caused by threshold intersection.

* Amplification curve crosses the threshold twice.

Table continued on next page

Table 8. Continued

Name	Flag/ warning	CMV	Status	Interpretation of result
Sample ID (IC of negative sample)	IC_INVALID_DATA	Invalid	Invalid	Not determined: Control channel failed, caused by double intersection.*
Sample ID	IC_FAIL IC_FLUORESCENCE	Invalid	Invalid	No result in CMV test channel; no result in control channel.

* Amplification curve crosses the threshold twice.

Retesting invalid samples

Samples with invalid results must be re-extracted and retested if no eluate is left. If you have remaining eluate, you may retest. If the retest fails, then resample and re-extract.

Quality Control

The CMV low positive control, high positive control, quantitation standards, and NTC (H_2O) are provided with the *artus* CMV RGQ MDx Kit and must be included in each run of the Rotor-Gene Q MDx instrument. Control results are evaluated to determine whether the run is valid. Acceptance criteria for the controls are automatically verified by the Rotor-Gene Q software. If the run is invalid, the eluates of the samples must be retested.

It is recommended to test a CMV negative process control, a CMV high positive process control, and a CMV low positive process control 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. Each laboratory should ensure compliance with applicable local,

state, and federal regulations, as well as the laboratory's quality control procedures.

Limitations

- A trained healthcare professional should interpret assay results in conjunction with the patient's medical history, clinical signs and symptoms, and the results of other diagnostic tests.
- Test performance characteristics have been evaluated only for individuals who have undergone kidney transplantation, have been diagnosed with CMV disease and are undergoing purine analogue (guanine) anti-CMV therapy. No information is available on test performance in patients undergoing other types of transplant procedures, neonates or pediatric patients, or AIDS or other immunocompromised patients; nor is information available on test performance in patients who have been diagnosed with CMV disease and are undergoing other therapies, or have life-threatening CMV disease.
- Testing for analytical reactivity with various currently known antiviral drug resistance CMV strains was limited. Although the targeted DNA sequence for this test is not known to be involved in some anti-CMV drug resistance pathways, the performance of the test may be affected when other resistance pathways are considered or new variants emerge.
- A specimen with a result of "CMV DNA Not Detected" cannot be presumed to be negative for CMV DNA.
- Analyte targets (viral nucleic acid) can persist in vivo, independent of virus viability. Detection of one or more analyte targets does not imply that the corresponding virus(es) are infectious or that they are the causative agents of clinical symptoms.
- The detection of viral DNA is dependent upon proper specimen collection, handling, transportation, storage, and preparation (including extraction). Failure to observe proper procedures in any of these steps can lead to

incorrect results. There is a risk of false negative results resulting from improperly collected, transported, or handled specimens.

- Although rare, mutations within the highly conserved regions of the viral genome covered by the kit's primers and/or probe may result in underquantitation or failure to detect the presence of the virus in these cases (false negative result).

Performance Characteristics

Traceability to the 1st WHO International Standard for Cytomegalovirus

Traceability of the *artus* CMV Quantitation Standards (QS) to the WHO International Standard (WHO Standard) NIBSC 09/162 was carried out by testing the Quantitation Standards against the WHO Standard. This was achieved through a Quality Control test to ensure that a 5,000 IU/ml concentration of the WHO Standard was accurately quantitated. No manufactured lot of Quantitation Standards was released for inclusion in the *artus* CMV RGQ MDx Kit unless they had successfully passed the acceptance criteria described in the test procedure. 5,000 IU/ml was chosen as a suitable concentration to be employed in the Quality Control test since this concentration is near the midpoint of the *artus* CMV RGQ MDx Kit calibration curve (logarithmic scale). In setting the QC release test acceptance criteria for the Quantitation Standards, a total of 113 replicates of the WHO Standard diluted to 5,000 IU/ml in negative plasma were employed. The acceptance criterion for the WHO Standard sample was set using the data obtained (logarithmic scale) from these test dilutions and defined as the mean value ± 4 standard deviations, corresponding to a range of ±0.30 $\log_{10}$ IU/ml.

Limit of blank and performance of negative samples

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 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 100 CMV-negative EDTA plasma samples were analyzed using 2 different EZ1 DSP Virus Kit manufacturing lots and 2 different Rotor-Gene Q instruments over a total of 4 runs. A total of 99 samples showed no result in the CMV Test Channel and fluorescence intensities below the given threshold (0.05). Therefore the performance of the negative samples for the *artus* CMV RGQ MDx Kit was 99% with a LOB less than 0.05 at cycle 45.

In addition, C_T values generated for each sample were analyzed. Ninety-nine of the 100 samples tested yielded a negative result using the *artus* CMV RGQ MDx Kit.

Limit of detection (LOD)

Limit of detection using the 1st WHO International Standard for Cytomegalovirus

The limit of detection (LOD) of the *artus* CMV RGQ MDx Kit was determined for the 1st WHO International Standard for Cytomegalovirus 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 10 different dilutions levels of the 1st WHO International Standard, starting with 892 IU/ml in EDTA plasma, was used. LOB was confirmed to be 0 IU/ml by analysis of blank samples.

Each dilution was determined in 6 replicates per run and day. All replicates of one dilution were tested in one PCR run. The test was performed with 3 different *artus* CMV RGQ MDx Kit lots and with each lot on 4 different days, by 3 different persons, on 4 different EZ1 Advanced XL and 3 different Rotor-Gene Q instruments, resulting in overall 72 data points per dilution.

A probit regression with SAS Software was performed and the 95% LOD value was determined. The results are shown in Table 9.

Table 9. Limit of detection using the 1st WHO International Standard for Cytomegalovirus

CMV target concentration (IU/ml)	Number of replicates tested (N)	Mean observed concentration (IU/ml)	Number of positives detected	Positive rate (%
892	72	574	72	100
282	72	173	72	100
141	72	83	72	100
89	72	50	72	100
56.3	72	30	72	100
28.1	72	17	60	83
8.9	72	9	34	47
2.8	72	7	14	19
0.9	72	7	4	6
0.3	72	6	3	4

The point estimate of the LOD for the *artus* CMV RGQ MDx Kit using the 1st WHO International Standard for Human Cytomegalovirus (NIBBSC 09/162, Merlin strain, genotype 1 based on glycoprotein B gene UL 55) is 54 IU/ml.

Limit of detection using glycoprotein B (gB) genotype 2

A dilution series consisting of 10 different dilution levels was used, starting with 794 IU/ml cultured CMV in CMV-negative EDTA plasma. Samples were analyzed using the *artus* CMV RGQ MDx Kit. The evaluation was performed with 3 different *artus* CMV RGQ MDx Kit lots, and testing was performed with each lot on 4 different days, by 4 different persons, on 3 different Rotor-Gene Q instruments. Each dilution level was tested in 6 replicates per lot and day. The LOD value of the *artus* CMV RGQ MDx Kit is 77 IU/ml CMV in EDTA plasma (Table 10).

Table 10. Limit of detection using glycoprotein B (gB) genotype 2

CMV target concentration (IU/ml)	Number of replicates tested (N)	Mean observed concentration (IU/ml)	Number of positives detected	Positive rate (%
794	72	603	72	100
251	72	151	72	100
125	72	90	72	100
79	72	46	72	100
50	72	32	69	95.8
25.1	71	18	45	63.4
7.92	72	11	27	37.5
2.50	72	10	14	19.4
0.79	72	10	5	6.9
0.25	71	8	1	1.4

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

The claimed LOD value obtained for genotype gB2 (77 IU/ml) was verified for both CMV gB3 and gB4 genotypes following the CLSI Guideline EP17-A2 (1).

A set of samples was prepared for each CMV gB genotype by diluting cultured virus (gB3) or clinical specimen (gB4) at the claimed LOD value concentration in 2 different EDTA plasma pools.

The test was performed on 5 different days. For each CMV gB genotype, samples were analyzed in 10 replicates each day, resulting in a total number of 50 analyzed samples. Two different *artus* CMV RGQ MDx Kit lots, two different EZ1 Advanced (XL) instruments, and two different Rotor-Gene Q instruments were used.

The *artus* CMV RGQ MDx Kit can detect the tested CMV gB genotypes, gB3 and gB4, at least with the same sensitivity as the genotype gB2, 77 IU/ml (Table 11).

Table 11. Limit of detection using glycoprotein B (gB) genotype 3 and 4

CMV gB genotype	CMV target concentration (IU/ml)	Number of replicates tested (N)	Number of positives detected	Positive rate (%)
gB3	77	50	49	98
gB4	77	50	47	94

Linear range and limit of quantitation

Linear range using glycoprotein B (gB) genotype 2

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

A dilution series of cultured CMV ranging from (1.19×10^2 to 7.94×10^7 IU/ml) in EDTA plasma was prepared to determine the linear range. Samples were analyzed using the *artus* CMV RGQ MDx Kit with a total of one EZ1 DSP Virus Kit lot and 3 *artus* CMV RGQ MDx Kit lots. Each dilution level was tested in 6 replicates.

The linear range of the *artus* CMV RGQ MDx Kit was determined to cover concentrations from 1.19 x 10^2 to 7.94 x 10^7 IU/ml or 2.08 $\log_{10}$ IU/ml to 7.90 $\log_{10}$ IU/ml CMV in EDTA plasma (Figure 7).

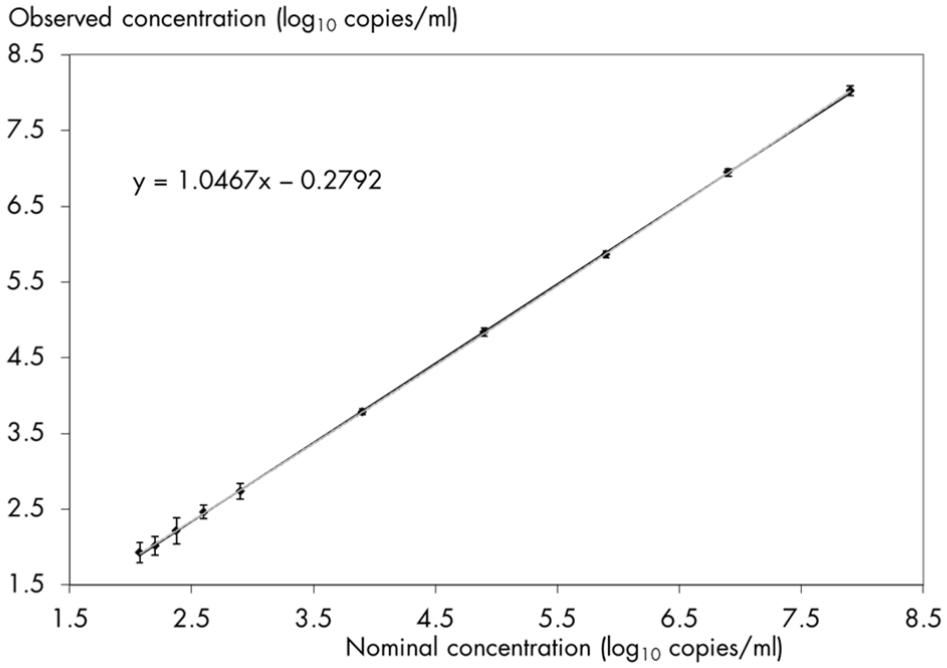

Figure 7. Linear range of the artus CMV RGQ MDx Kit for CMV gB2 genotype.

Linear range using glycoprotein B (gB) genotypes 1, 3, and 4

For the determination of the linear range for the other gB genotypes, a dilution series from the 1st WHO International Standard (NIBSC code 09/162, gB1), cultured virus (gB3), and a clinical specimen (gB4) were used. The concentrations analyzed for gB1 and gB3 ranged from 1.19 x 10^2 to 3.97 x 10^5 IU/ml. For gB4 a dilution series ranging from 1.19 x 10^2 to 7.94 x 10^4 IU/ml was used.

An assessment was performed using the polynomial evaluation of linearity to determine if the dataset was linear. None of the nonlinear coefficients in quadratic and cubic regressions were significant. Each dilution was analyzed in 8 replicates. All replicates of one dilution were tested in one Rotor-Gene Q run. The linear range was determined using one *artus* CMV RGQ MDx Kit lot.

Figure 8 and Table 12 summarize the results obtained for all CMV gB genotypes analyzed.

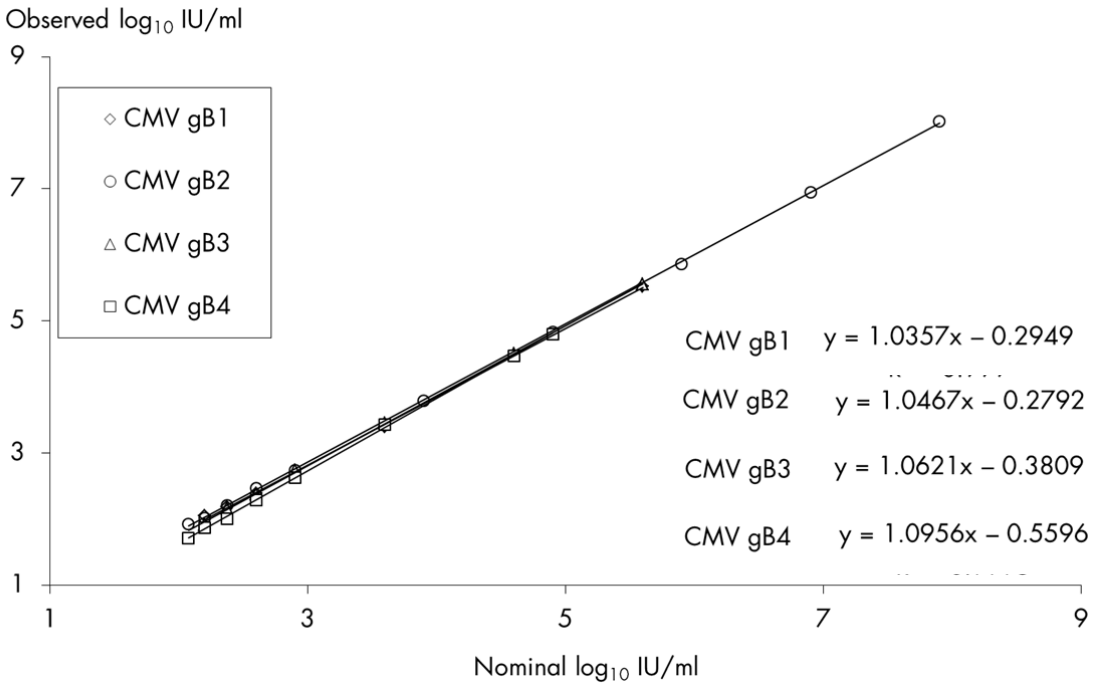

Figure 8. Linear range of the *artus* CMV RGQ MDx Kit for all CMV gB genotypes analyzed.

Table 12. Linear range of the *artus* CMV RG PCR Test for all CMV gB genotypes analyzed

gB genotype	Linear range
gB1	159 IU/ml to 3.97 x 10^5 IU/ml (2.20 $\log_{10}$ IU/ml to 5.60 $\log_{10}$ IU/ml)
gB2	119 IU/ml to 7.94 x 10^7 IU/ml (2.08 $\log_{10}$ IU/ml to 7.90 $\log_{10}$ IU/ml)
gB3	159 IU/ml to 3.97 x 10^5 IU/ml (2.20 $\log_{10}$ IU/ml to 5.60$\log_{10}$ IU/ml)
gB4	119 IU/ml to 7.94 x 10^4 IU/ml (2.08 $\log_{10}$ IU/ml to 4.90 $\log_{10}$ IU/ml)

The linear range of the *artus* CMV RGQ MDx Kit was determined to be 159 IU/ml to 7.94 x 10^7 IU/ml.

Table 13 contains the linear equation obtained for each genotype and the maximum difference between the gB1 (1st WHO International Standard) and the corresponding genotypes based on the linear fit.

Table 13. Linear equations obtained for all CMV gB genotypes analyzed

CMV gB genotype	Linear equation in gB genotype linearity study	Maximum difference between gB1 and corresponding gB genotype (log_{10} IU/ml)
1	y = 1.0357x – 0.2949	n.a.*
2	y = 1.0467x – 0.2792	0.08
3	y = 1.0621x – 0.3809	0.06
4	y = 1.0956x – 0.5596	0.13

* n.a.: not applicable.

Limit of quantitation (LOQ)

The limit of quantitation (LOQ) was determined by spiking the different CMV genotypes into 5 unique EDTA plasma pools at a concentration equal to the lower end of the linear range testing for each genotype. Data was generated on 5 different days with 12 replicates per genotype each day (e.g., day 1, pool 1, 12 replicates). A total of 60 data points for each genotype was obtained. Two different *artus* CMV RGQ MDx Kit lots were used.

Table 14 shows both criteria for the total analytical error (TAE = |bias| + 2 x standard deviation and TAE = $\sqrt{2}$ x 2 x standard deviation) for the log_{10} IU/ml following the recommendations of the CLSI Guideline EP17-A2 (1). The acceptable TAE was ≤1 log_{10} IU/ml.

Table 14. Limit of quantitation for the different CMV gB genotypes (log_{10} IU/ml)

CMV gB genotype	Nominal concentration (IU/ml)	Observed concentration (log_{10} IU/ml)	Average log_{10} IU/ml)	Bias (log_{10} IU/ml)	SD (log_{10} IU/ml)	TAE = |bias| + 2 x SD (log_{10} IU/ml)	TAE = $\sqrt{2}$ x 2 x SD (log_{10} IU/ml)
gB1	159	2.2	1.87	−0.33	0.25	0.84	0.72
gB2	159	2.2	2.03	−0.17	0.23	0.62	0.65
gB3	159	2.2	1.79	−0.41	0.26	0.93	0.73
gB4	159	2.2	1.89	−0.31	0.18	0.66	0.5

The claimed LOQ for all CMV gB genotypes is 159 IU/ml, with the geometric mean of the observed titer value of 107 IU/ml (2.03 log_{10} IU/ml).

Table 15 shows the linear range for the different gB genotypes according to the results obtained for the LOQ.

Table 15. Quantitation obtained for all CMV gB genotypes analyzed

gB genotype	LOQ
gB1	159 IU/ml to 3.97 x 10^5 IU/ml (2.20 $\log_{10}$ IU/ml to 5.60 $\log_{10}$ IU/ml)
gB2	159 IU/ml to 7.94 x 10^7 IU/ml (2.20 $\log_{10}$ IU/ml to 7.90 $\log_{10}$ IU/ml)
gB3	159 IU/ml to 3.97 x 10^5 IU/ml (2.20 $\log_{10}$ IU/ml to 5.60 $\log_{10}$ IU/ml)
gB4	159 IU/ml to 7.94 x 10^4 IU/ml (2.20 $\log_{10}$ IU/ml to 4.90 $\log_{10}$ IU/ml)

Analytical specificity (Cross-reactivity)

The analytical specificity of the *artus* CMV RGQ MDx Kit was evaluated by testing the cross-reactivity of a panel of different pathogens consisting of 21 viruses, 3 fungi, and 1 protozoan parasite. The pathogens were tested at the highest concentration available. Samples were prepared by diluting the organisms or DNA/RNA either in CMV negative EDTA plasma or in CMV-spiked EDTA at 2 concentrations (near the LOD value and within the linear range). Each sample was extracted and tested in 4 replicates. There were no false-positive or invalid results among the 25 pathogens tested (Table 16).

Table 16. Analytical specificity

Pathogen	Concentration*	CMV
Viruses		
Adenovirus type 2	1.26 x 10^9 $TCID_{50}$/ml	–
Adenovirus type 4	4.77 x 10^5 $TCID_{50}$/ml	–
Adenovirus type 5	2.75 x 10^{12} $TCID_{50}$/ml	–
BK polyomavirus deposited as BK virus	1.41 x 10^4 $TCID_{50}$/ml	–
EBV B95-8 strain (type 1) purified virus	1.50 x 10^8 copies/ml	–
Enterovirus type 71	3.62 x 10^4 $TCID_{50}$/ml	–
Hepatitis A virus RNA NAT assays	5.00 x 10^3 IU/ml	–
Hepatitis B virus DNA	5.00 x 10^4 IU/ml	–
Hepatitis C virus (HCV) RNA	7.75 x 10^3 IU/ml	–
HSV-1 MacIntyre strain purified virus	3.30 x 10^5 $TCID_{50}$/ml	–
Herpes simplex virus type 2 (HSV-2)	6.15 x 10^6 $TCID_{50}$/ml	–
Human herpesvirus 3 deposited as varicella-zoster	1.41 x 10^4 $TCID_{50}$/ml	–
HHV-6A GS strain purified viral lysate	2.50 x 10^9 VP/ml	–

* $TCID_{50}$: Tissue culture infective dose 50%; VP: Viral particles; CFU: Colony forming units; IU: International Unit.

Table continued on next page

Table 16. Continued

Pathogen	Concentration*	CMV
Viruses (continued)		
Human herpesvirus 6B strain Z-29	1.41 x 10^2 $TCID_{50}$/ml	–
HHV-7 H7-4 strain quantitated DNA control	6.00 x 10^5 copies/ml	–
KSHV/HHV-8 KS-1 strain quantitated viral DNA	6.00 x 10^5 copies/ml	–
HIV-1 RNA, 2nd International Standard	1.82 x 10^4 IU/ml	–
HTLV-I MT-2 strain purified virus	2.30 x 10^7 VP/ml	–
Human T lymphotropic virus type II (HTLV-II)	3.25 x 10^7 VP/ml	–
Parvo B19 DNA NAT assays.2nd I.S.	5.00 x 10^4 IU/ml	–
WNV	5.45 x 10^8 copies/ml	–
Fungi		
Aspergillus fumigatus Z014	1.09 x 10^7 CFU/ml	–
Candida albicans Z006	1.05 x 10^7 CFU/ml	–
Pneumocystis jirovecii	4.15 x 10^3 copies/ml	–
Parasite		
Plasmodium falciparum	5.00 x 10^7 IU/ml	–

* $TCID_{50}$: Tissue culture infective dose 50%; VP: Viral particles; CFU: Colony forming units; IU: International Unit.

Moreover, all samples with a CMV concentration near the LOD value were detected positive in the Test Channel in the presence of the tested organisms. Samples with a nominal concentration of 1.19 x 10^3 IU/ml CMV were quantified within ±0.25 $\log_{10}$ IU/ml.

In conclusion, there is no interference of the respective pathogens with the specific CMV detection in terms of sensitivity and quantitation.

Carryover/cross-contamination

The *artus* CMV RGQ MDx Kit showed no evidence of carryover or cross-contamination when 30 high positive CMV samples with 2.38 x 10^6 IU/ml were extracted and tested alternating positive samples with 30 CMV-negative samples. The CMV tested concentration represents the highest viral load observed within a diagnostic evaluation study analyzing 203 retrospectively and prospectively collected patient specimens.

Precision

The precision of the *artus* CMV RGQ MDx Kit was determined following the recommendations of the CLSI Guideline EP05-A2 (6) by testing a 4-member panel (a negative sample, a sample with a concentration near the LOD, and 2 concentrations in the linear range of the assay; all samples were in EDTA plasma). Each panel member was evaluated in duplicate in 2 runs per day for 20 days. A total of 2 different EZ1 Advanced and 2 different EZ1 Advanced XL instruments, as well as 3 different Rotor-Gene Q instruments were used for the testing. Three different EZ1 DSP Virus Kit lots and 3 different *artus* CMV RGQ MDx Kit lots were used for the study. A total of 3 different operators performed the test. The results are summarized in Tables 17–18.

Table 17: Precision of the artus CMV RGQ MDx Kit (in log10 IU/ml)

Nominal Values IU/ml, (log_{10} IU/ml)	Average Observed CMV DNA Titer (log_{10} IU/ml)	N of tests	Within-Run SD	Between-Run SD	Between-EZ1 Advanced Instrument SD*	Between-EZ1 DSP Virus Kit Lot SD*	Between RGQ Instrument SD*	Between *artus* CMV RGQ MDx Kit Lot SD*	Between-Operator SD*	Between Day SD*	Total SD
230 (2.362)	2.110	80	0.136	0.052	0.000	0.000	0.000	0.042	0.000	0.043	0.158
1,191 (3.076)	2.901	80	0.068	0.052	0.003	0.009	0.000	0.034	0.019	0.000	0.095
79,400 (4.900)	4.764	80	0.025	0.019	0.013	0.050	0.000	0.000	0.018	0.004	0.063
Negative		80	100% (80/80) "Not Detected" Results								

* Estimates of some components of variance have large uncertainty due to only 80 measurements.

Table 18: Precision of the artus CMV RGQ MDx Kit (in IU/ml)

Nominal Values (IU/ml)	Geometric Mean CMV DNA Titer (IU/ml)	N of tests	Within-Run %CV	Between Run %CV	Between EZ1 Advanced Instrument %CV*	Between EZ1 DSP Virus Kit Lot %CV*	Between RGQ Instrument %CV*	Between-*artus* CMV RGQ MDx Kit Lot %CV*	Between Operator %CV*	Between Day %CV*	Total %
230	129	80	32.1%	12.0%	0%	0%	0%	9.7%	0%	9.9%	37.6%
1,191	796	80	15.8%	12.0%	0.7%	2.1%	0%	7.8%	4.4%	0%	22.1%
79,400	58,076	80	5.8%	4.4%	3.0%	11.6%	0%	0%	4.2%	0.9%	14.6%
Negative		80	100% (80/80) "Not Detected" Results								

* Estimates of some components of variance have large uncertainty due to only 80 measurements.

Endogenous interfering substances

The potentially interfering substances were spiked into CMV-negative EDTA plasma in the presence of different concentrations of CMV. Samples were then tested using the *artus* CMV RGQ MDx Kit. Samples containing potentially interfering substances were compared to control EDTA plasma samples containing no spiked interfering substance. Each concentration level for each interfering substance was tested in 4 replicates.

The test concentrations for each interfering substance (Table 19) were selected based on available literature references and guidance provided by the CLSI Guideline EP07-A2 (3).

Table 19. Endogenous interfering substances

Potential interfering 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

All tested interfering substance concentrations showed no influence on the performance of the *artus* CMV RGQ MDx Kit with regards to specificity, sensitivity, and quantitation.

Exogenous interfering substances

The potentially interfering substances were spiked into EDTA plasma in the absence or presence of different concentrations of CMV. Samples were then tested with the *artus* CMV RGQ MDx Kit. Samples containing potentially interfering substances were compared to control EDTA plasma samples containing no spiked interfering substance. Each concentration level for each interfering substance was tested in 4 replicates.

The test concentrations for each interfering substance were selected based on available literature references and guidance provided by the CLSI Guideline EP07-A2 (3). The potentially interfering substances and the test concentrations are presented in Table 20.

Table 20. Exogenous interfering substances

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

All tested interfering substance concentrations showed no influence on the performance of the *artus* CMV RGQ MDx Kit at the CMV concentrations evaluated with regards to specificity, sensitivity, and quantitation.

Clinical performance

The clinical usefulness of the *artus* CMV RGQ MDx Kit 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. Kidney post-transplantation 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). 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 CMV DNAemia, as determined by the clinician, following antiviral treatment with ganciclovir or valganciclovir. Study endpoints for this evaluation were the 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 a comparator test results.

Prospective Study Results

Agreement at baseline threshold values

Agreement at baseline threshold values was established comparing the results from the *artus* CMV RGQ MDx Kit and the FDA approved test using the following arbitrary values: “Not Detected”, LOQ 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 21 and 22 below show the agreement data at baseline threshold values.

Table 21: Summary of *artus* CMV RGQ MDx Kit vs. FDA-approved Test by Threshold

Clinical Study Stratified by Threshold		FDA-approved Test		Total
		Not Detected	Detected	
artus CMV	Not Detected	1	0	1
	Detected	0	43	43
Total		1	43	44
		≤ LOQ IU/ml	> LOQ IU/ml	
artus CMV	≤ LOQ IU/ml	14	1	15
	> LOQ 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 22: Statistical Summary of *artus* CMV RGQ MDx Kit vs. FDA-approved Test by Viremia Threshold

Clinical Study 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)
LOQ 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% is at the LOQ threshold where some differences are expected given the two different LOQ's of the two tests. Percent agreement at 1,000 IU/ml threshold is 90.91% agreement. At 500 IU/ml the percent agreement was 95.45% and at Not Detected, it was 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 two consecutive CMV viral load measurements below the LOQ on different days, is presented in Tables 23 and 24 below. There were a total of 24 subjects with 229 specimens in this analysis that had baseline visit data greater than LOQ for both the *artus* CMV RGQ MDx Kit and the FDA-approved test.

Table 23: Summary of *artus* CMV RGQ MDx Kit vs. FDA-approved Test for Resolution of CMV Episode

Day	Clinical Study		FDA-approved Test		Total
			Not Resolved	Resolved	
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
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 24: Statistical Summary of *artus* CMV RGQ MDx Kit vs. FDA-approved Test for Resolution of CMV Episode

Clinical Study 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 Test and the FDA-Approved test is 83.33% and higher. For the resolution of CMV episode, the agreement ranges from 93.33% to 100.00%. For the no resolution of 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 LOQ 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 <LOQ, 500, 1000, and 10,000 IU/ml. The number of specimens falling into each time window is presented in Table 25. The number of specimens falling into the respective categories is presented in Table 26. Tables 27–32 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 25: 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 26: *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, <LOQ**	**≥LOQ and ≤500**	**>500 and ≤1,000**	**>1,000 and ≤10,000**	**>10,000**	**Total**
Not Detected	133	12	2	0	0	0	147
Detected, <LOQ	42	81	14	2	0	0	139
≥LOQ and ≤500	1	7	12	2	2	0	24
> 500 and ≤1,000	0	1	2	5	1	0	9
>1,000 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 and Negative Percent Agreement 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 1,000 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 27: *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, <LOQ**	**≥LOQ and ≤500**	**>500 and ≤1,000**	**>1,000 and ≤10,000**	**>10,000**	**Total**
Not detected	1	0	0	0	0	0	1
Detected, <LOQ	0	13	1	0	0	0	14
≥LOQ and ≤500	0	4	3	0	0	0	7
> 500 and ≤1,000	0	1	1	1	1	0	4
>1,000 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 28: *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, <LOQ**	**≥LOQ and ≤500**	**> 500 and ≤1,000**	**>1,000 and ≤10,000**	**>10,000**	**Total**
Not detected	15	2	2	0	0	0	19
Detected, <LOQ	17	37	4	2	0	0	60
≥LOQ and ≤500	0	2	5	0	1	0	8
> 500 and ≤1,000	0	0	1	1	0	0	2
>1,000 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 29: *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, <LOQ**	**≥LOQ and ≤500**	**> 500 and ≤1,000**	**>1,000 and ≤10,000**	**>10,000**	**Total**
Not detected	47	6	0	0	0	0	53
Detected, <LOQ	12	13	4	0	0	0	29
≥LOQ and ≤500	0	1	1	0	1	0	3
> 500 and ≤1,000	0	0	0	2	0	0	2
>1,000 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 30: *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, <LOQ**	**≥LOQ and ≤500**	**> 500 and ≤1,000**	**Total**
Not detected	33	1	0	0	34
Detected, <LOQ	4	6	3	0	13
≥LOQ and ≤500	1	0	1	2	4
> 500 and ≤1,000	0	0	0	1	1
>1,000 and ≤10,000	0	0	1	1	2
Total	38	7	5	4	54

Table 31: *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, <LOQ**	**≥LOQ and ≤500**	**Total**
Not detected	21	2	0	23
Detected, <LOQ	1	4	0	5
≥LOQ and ≤500	0	0	2	2
Total	22	6	2	30

Table 32: *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, <LOQ**	**≥LOQ and ≤500**	**Total**
Not detected	9	0	0	9
Detected, <LOQ	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 26 above. Of the 101 specimens, 43 were negative by the FDA-approved test while 42 were detected below the LOQ and 1 was ≥ LOQ and ≤500 IU/ml by the *artus* CMV RGQ MDx Kit; 20 were detected below the LOQ by the FDA-approved test while 12 were negative, 7 were ≥ LOQ and ≤500 IU/ml and 1 was > 500 and ≤1000 IU/ml by the *artus* CMV Test; 21 were ≥ LOQ and ≤500 IU/ml by the FDA-approved test while 2 were negative, 14 were detected below LOQ, 2 were > 500 and ≤1,000 IU/ml and 3 were > 1,000 and ≤10,000 IU/ml by the *artus* CMV RGQ MDx Kit; 9 were ≥500 and ≤1,000 IU/ml by the FDA-approved test while 2 were detected below LOQ, 2 were ≥ LOQ and ≤500 IU/ml and 5 were > 1,000 and ≤10,000 IU/ml by the *artus* CMV RGQ MDx

Kit; 6 were >1,000 and ≤10,000 IU/ml by the FDA-approved test while 2 were ≥LOQ 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; 2 were >10,000 IU/ml by the FDA-approved test and they were >1,000 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 < LOQ IU/ml by the FDA-approved test that were higher in CMV viral load (≥LOQ and <1,000 IU/ml) by the *artus* CMV RGQ MDx Kit; and there were 16 specimens that were ≥LOQ and ≤500 IU/ml by the FDA-approved test that were lower in CMV viral load (Not Detected or <LOQ) by the *artus* CMV RGQ MDx Kit.

Method Comparison Results

In addition to the prospective clinical study, a method comparison analysis was performed. The total of 73 specimens corresponding to 25 subjects out of the prospective clinical study that had a result above the LOQ 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 the *artus* CMV RGQ MDx Kit at three testing sites and by the FDA approved test at one site. Figure 9 shows a scatter plot with results from testing this panel and the concordant positive specimens from the clinical prospective study.

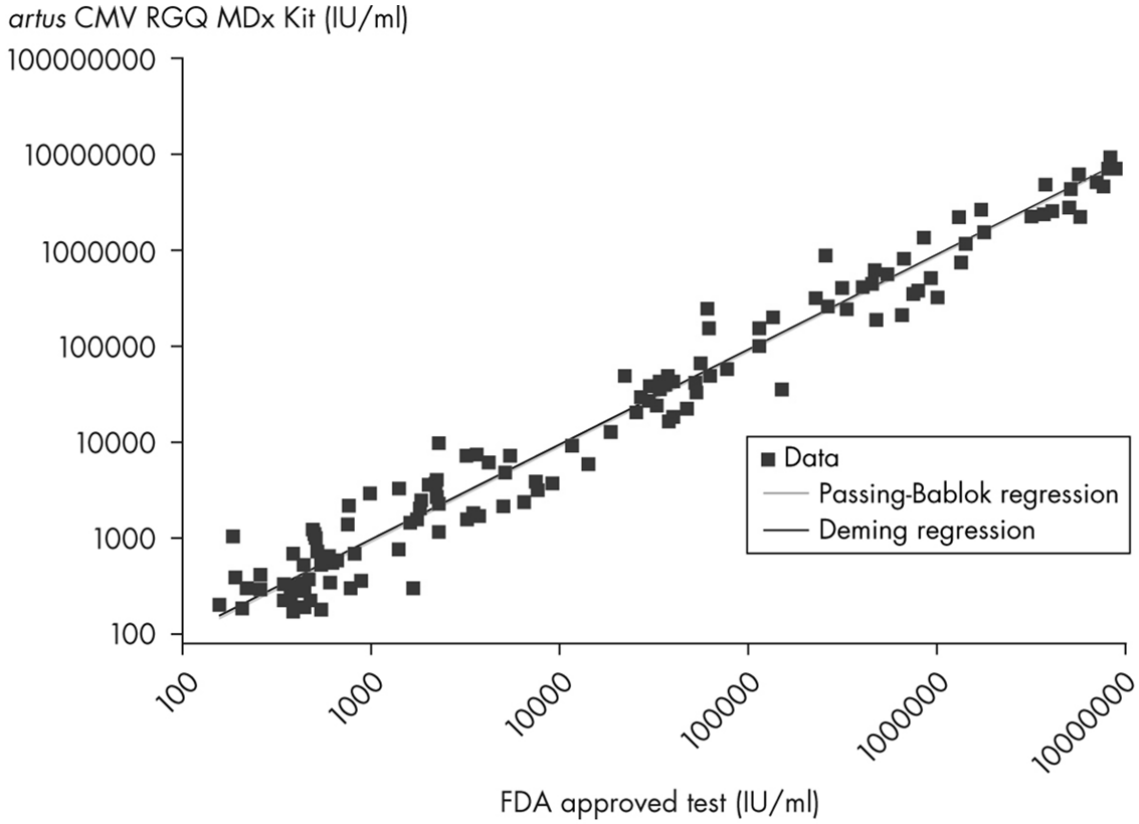

Figure 9. *artus* CMV RGQ MDx Kit versus FDA approved test for $\log_{10}$ IU/ml.

Tables 33 and 34 show the Deming regression estimates for the slope and intercept, and systematic difference between the *artus* CMV RGQ MDx Kit and the FDA-approved test.

Table 33. Deming regression estimates for *artus* CMV RGQ MDx Kit versus 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 34: Systematic Difference Between the *artus* CMV RGQ MDx Kit and the FDA-Approved Test

Value of the FDA- approved test	**Systematic Difference between the *artus* CMV RGQ MDx Kit and the FDA-approved test**
2.70 $\log_{10}$ IU/ml (500 IU/ml)	0.02 $\log_{10}$ IU/ml
3.00 $\log_{10}$ IU/ml (1,000 IU/ml)	0.02 $\log_{10}$ IU/ml
4.00 $\log_{10}$ IU/ml (10,000 IU/ml)	0.02 $\log_{10}$ 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.

Error! Reference source not found.35 and 36 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 35. Passing-Bablok regression estimates for *artus* CMV RGQ MDx Kit versus 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 36: Systematic Difference Between the *artus* CMV RGQ MDx Kit and the FDA-Approved Test

Value of the FDA- approved test	**Systematic Difference between the *artus* CMV RGQ MDx Kit and the FDA-approved test**
2.70 log_{10} IU/ml (500 IU/ml)	0.027 log_{10} IU/ml
3.00 log_{10} IU/ml (1,000 IU/ml)	0.030 log_{10} IU/ml
4.00 log_{10} IU/ml (10,000 IU/ml)	0.040 log_{10} 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 10 shows a difference plot presenting this difference between the *artus* CMV RGQ MDx Kit and the FDA-approved test (reporting log_{10} 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 37 and 38 show the analyses at the different subintervals.

Figure 10: Allowable Total Difference (ATD) Plot, All Sites Combined

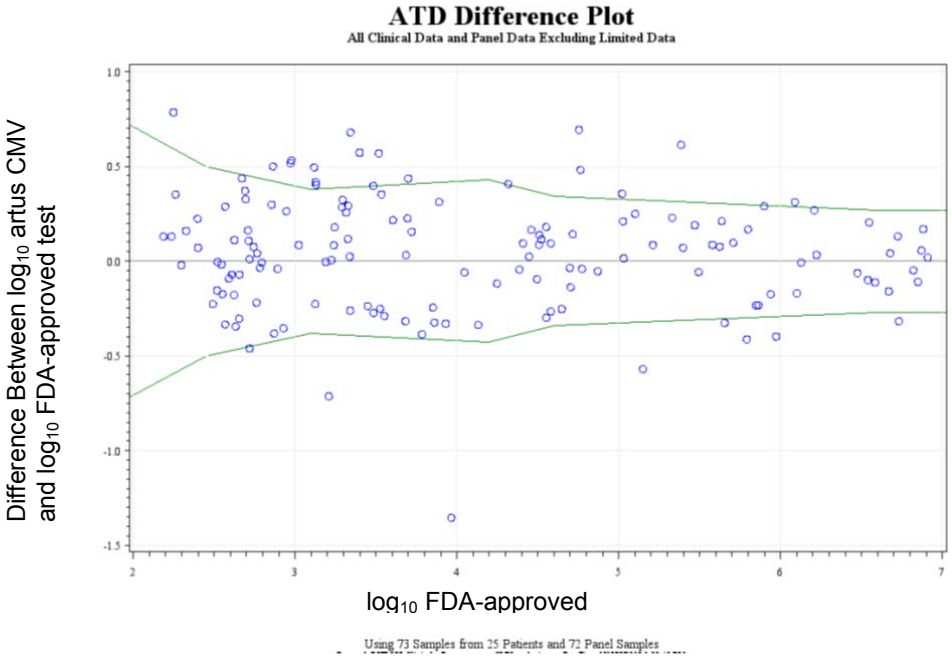

Table 37 below presents the total difference at 2.5th, 5.0th, 95.0th and 97.5th percentiles for the following three FDA-approved test measuring subintervals: less than 10,000 IU/ml (4.00 on the $\log_{10}$ scale); between 10,000 and 1,000,000 IU/ml (4.00 and 6.00 on the $\log_{10}$ scale respectively) and greater than 1,000,000 IU/ml (6.00 on the $\log_{10}$ scale).

Table 37: Percentile of the Difference between Log *artus* CMV and Log FDA-approved test Clinical and Panel Data – All Sites Combined

Range of FDA-approved test IU/ml		Difference between log *artus* CMV and log FDA-approved test Percentiles			
	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 38 shows the percentages of the samples that fall within the following three FDA-approved test measuring subintervals: between 137 IU/ml and 10,000 IU/ml (2.14 and 4.00 on the $\log_{10}$ scale); between 10,000 and

1,000,000 IU/ml (4.00 and 6.00 on the $\log_{10}$ scale respectively) and greater than 1,000,000 (6.00 on the $\log_{10}$ scale).

Table 38: 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 percent 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 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 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 and a 2.04 fold difference in IU/ml).

Negative (CMV IgG Neg) 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 3x3 matrices (Table 39).

Table 39: 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, < LOQ	Greater Than LOQ
Not Detected	41	0	0
Detected, < LOQ	1	0	0
Greater Than LOQ	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, < LOQ" by the *artus* CMV RGQ MDx Kit.

Reproducibility

The reproducibility of the *artus* CMV RGQ MDx Kit was evaluated at 3 investigational sites using a 10-member panel with 5 simulated specimens (2 of each in the panel), including negatives, high negatives, low positive, moderate positives, and high positives. The 10-member panel was tested in duplicate by 2 different technologists each day for 6 days at each site with 3 reagent kit lots. A total of 144 measurements was analyzed for each pair of simulated specimens.

The percentage of variance due to each component and SD of the $\log_{10}$ transformed CMV DNA concentration were calculated. The detectable difference in viral load between two test results for each expected $\log_{10}$ CMV DNA concentration was estimated by using the total variance and was calculated as the antilog of the 95% confidence limit for the standard deviation of the difference between two measurements.

The reproducibility of the test was also evaluated by calculating the negative percent agreement across the aforementioned factors in the negative panel member.

Tables 40 and 41, provide the overall summary of the percentage variance and standard deviation for the $\log_{10}$ IU/ml values, and %CV for IU/ml values, for each of the 10 panels across lot, site, operator, day, between run, and within run.

Table 40: Percentage of Total Variance and Standard Deviation for *artus* CMV RGQ MDx Kit (log_{10} IU/ml)

				Variance Components						
Sample type	Observed Mean (log_{10} IU/ml)	N of tests	Measure	Within-Run	Between-Run	Between-Day	Between-Operator	Between-Lot	Between-Site	Total
Low Positive	1.91	144	Percent Variance	87%	4.4%	8.6%	0%	0%	0%	100%
			SD	0.212	0.048	0.067	0	0	0	0.227
Moderate Positive	2.96	144	Percent Variance	63.4%	10.9%	6.0%	18.8%	0%	0.9%	100%
			SD	0.136	0.057	0.042	0.074	0	0.016	0.171
High Positive	5.03	144	Percent Variance	25.6%	7.9%	48.1%	15.4%	3.0%	0%	100%
			SD	0.048	0.026	0.065	0.037	0.016	0	0.094
Negative		144	98.6% (142/144) "Not Detected", Median="Not Detected", Maximum =1.47 log_{10}IU/ml							
High Negative		144	41.0% (59/144) "Not Detected", Median=0.83 IU/ml, 95th percentile=1.62 IU/ml, Maximum =1.90 IU/ml							

Table 41: Total variance, %CV for *artus* CMV RGQ MDx Kit (IU/ml)

Sample type	Observed Geometric Mean (IU/ml)	N of tests	Within-Run %CV	Between-Run %CV	Between-Day %CV	Between-Operator %CV	Between-Lot %CV	Between-Site %CV	Total %CV
Low Positive	82 IU/ml	144	51.8%	11.0%	15.4%	0%	0%	0%	56.0%
Moderate Positive	909 IU/ml	144	32.2%	13.1%	9.7%	17.2%	0%	3.6%	41.0%
High Positive	107,597 IU/ml	144	11.0%	6.1%	15.1%	8.5%	3.7%	0%	21.9%
Negative		144	98.6% (142/144) "Not Detected", Median="Not Detected", Maximum =29 IU/ml						
High Negative		144	41.0% (59/144) "Not Detected", Median=7 IU/ml, 95th percentile=41 IU/ml, Maximum =79 IU/ml						

The detectable fold difference can be used to serially assess a patient's viral load for statistically significant changes. Variations between measurements that are within the detectable fold difference could be due to variability in the test's reproducibility. Table 42 shows the estimated maximum total variation and 95% confidence limits that could be expected for a change between two consecutive CMV DNA determinations in a single patient at different nominal log_{10} CMV DNA concentrations.

Table 42: Detectable Viral Load Difference by log_{10} IU/ml

Observed Mean (log_{10} IU/ml)	N of Tests	Total precision SD (log_{10} IU/ml)	SD of Difference Between Two Measurements (log_{10} IU/ml)	95% Confidence Limit[1] (± log_{10} IU/ml)	Fold Detectable Difference[2]
2.96	144	0.171	0.242	0.474	2.98
5.03	144	0.094	0.133	0.261	1.82

[1] The 95% confidence limit for the difference between two measurements in the same subject. These measurements do not include within-subject biologic variation and they could be from the same sample tested at different times with different lots, testing sites, and operators.

[2] The 95% confidence limit for the fold difference of the ratio of two measurements in IU/ml (e.g., 10**0.474=2.98)

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Symbols

The following symbols may appear on the packaging and labeling:

	Contains sufficient for 96 tests
	Use by
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 Instructions for Use (Handbook) and n is the revision number
	Temperature limitation
	Manufacturer
	Consult instructions for use

Contact Information

For technical assistance and more information, please see our Technical Support Center at www.qiagen.com/Support or call the QIAGEN Technical Service Department in the USA at 800-DNA-PREP (800-362-7737) or visit www.qiagen.com.

QIAGEN GmbH, QIAGEN Strasse 1, 40724 Hilden, GERMANY

Appendix A: The Rotor-Gene Q *artus* CMV Assay Package

Assay packages contain the required files to run and analyze individual types of assays. For each assay package a separate software installation is required. The installation copies the required files to the system and creates one or more shortcuts on the desktop.

The Rotor-Gene Q *artus* CMV Assay Package is designed for use with the QIAGEN Rotor-Gene Q MDx instrument with a 72-well rotor. The package includes a template that can be printed to record the run information and a run template that defines and executes the PCR protocol.

Instructions for installation of the Rotor-Gene Q *artus* CMV Assay Package

Note: The Rotor-Gene Q *artus* CMV Assay Package is for Rotor-Gene Q Series Software version 2.1.0 or higher. Make sure the correct version of Rotor-Gene Q software is installed before proceeding with the installation.

Procedure

1. **Order the Rotor-Gene Q *artus* CMV Assay Package CD (cat. no. 9022860), available separately from QIAGEN.**
2. **Insert the CD into the CD drive of the PC connected to the Rotor-Gene Q MDx instrument.**
3. **Start the installation by double-clicking the file artus_CMV_Assay_Package_1.2.7.exe.**
   Version 1.2.7 is shown in the following screenshots. However, the version number may be higher for more recent versions of the assay package.

4. The setup wizard will appear. Click “Next” (Figure 11).

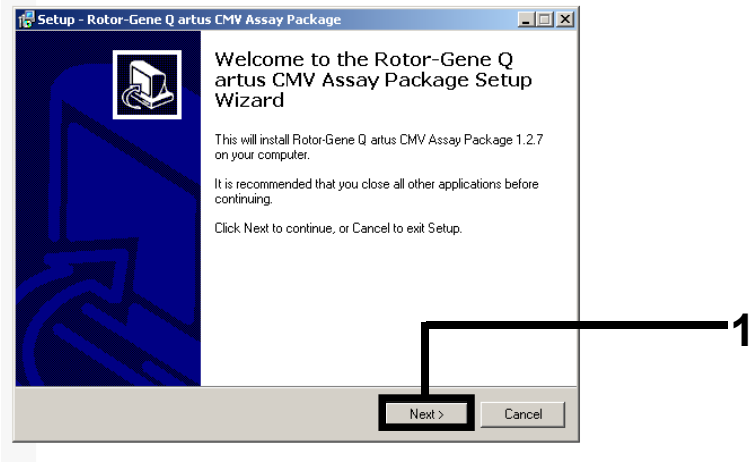

Figure 11.The “Setup” dialog box.

5. Read the License Agreement in the “License Agreement” dialog box and accept the agreement by checking “I accept the agreement”. Click “Next” (Figure 12).

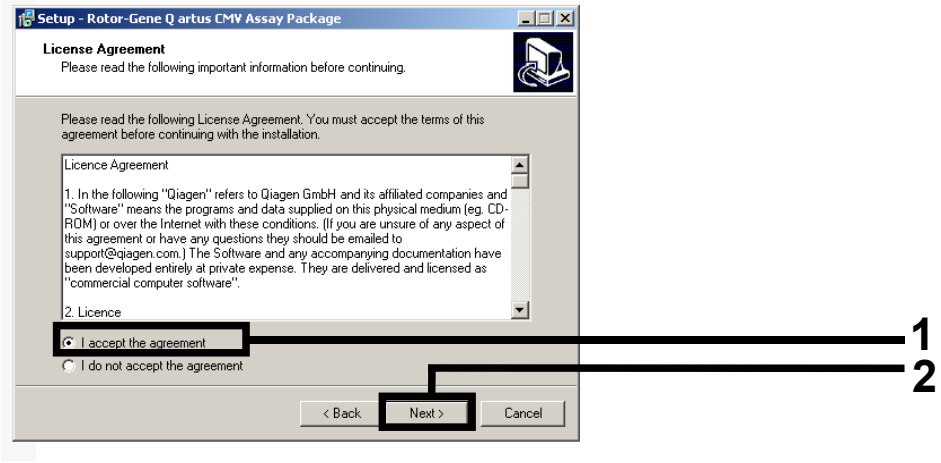

Figure 12. The “License Agreement” dialog box.

6. **The template setup will start automatically and a "Setup" dialog box will appear. Click "Finish" to exit the setup wizard (Figure 13).**

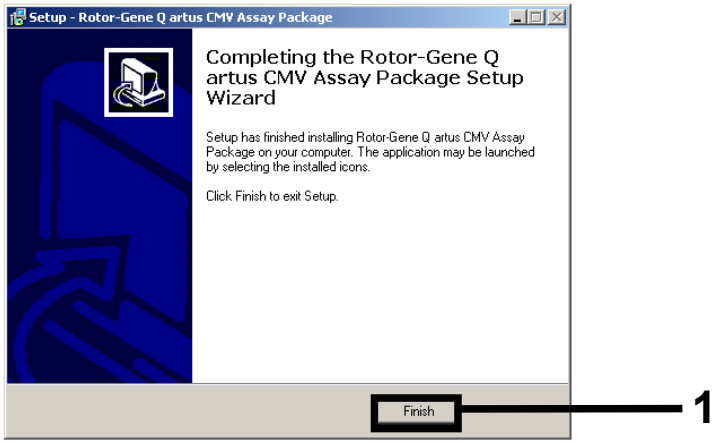

Figure 13. Completing the setup wizard.

7. **Restart the computer. A shortcut to the "artus CMV Locked Template" will be generated automatically and appear on the desktop.**

Appendix B: 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 (4). 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) (5).

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 80). 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, floorcloth, etc.).

Personnel and clothing

All personnel are key to the prevention of contamination.

* 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.

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.

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.
- Discard all waste in closed containers.
- Decontamination may be done 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.

Ordering Information

Product	Contents	Cat. no.
artus CMV RGQ MDx Kit (96)	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
Rotor-Gene Q *artus* CMV Assay Package CD	Software protocol package for use with the *artus* CMV RGQ PCR Kit and the QIAGEN Rotor-Gene Q MDx instrument with a 72-well rotor	9022860
EZ1 DSP Virus Kit — for automated purification of viral DNA using the EZ1 Advanced or the EZ1 Advanced XL		
EZ1 DSP Virus Kit (48)	For 48 viral nucleic acid preps: Prefilled Reagent Cartridges, Disposable Tip Holders, Disposable Filter-Tips, Sample Tubes, Elution Tubes, Buffers, Carrier RNA	62724
EZ1 Advanced DSP Virus Card	Preprogrammed card for EZ1 DSP Virus protocol	9018306
EZ1 Advanced XL DSP Virus Card	Preprogrammed card for EZ1 DSP Virus protocol	9018703
EZ1 Advanced instruments — for automated purification of nucleic acids from 1–6 or 1–14 human samples		
EZ1 Advanced	Robotic workstation for automated purification of nucleic acids from up to 6 samples using EZ1 Kits, 1-year warranty on parts and labor	9001411

Product	Contents	Cat. no.
EZ1 Advanced XL	Robotic workstation for automated purification of nucleic acids from up to 14 samples using EZ1 Kits, 1-year warranty on parts and labor	9001492
Rotor-Gene Q MDx and accessories		
Rotor-Gene Q MDx (US) Platform	Real-time PCR cycler with 6 channels,* laptop computer, software, accessories, 1-year warranty on parts and labor	9002035
Rotor-Gene Q MDx (US) System	Real-time PCR cycler with 6 channels,* laptop computer, software, accessories, 1-year warranty on parts and labor, installation and training	9002036
Loading Block 72 x 0.1 ml Tubes	Aluminum block for manual reaction setup with a single-channel pipet in 72 x 0.1 ml tubes	9018901
Strip Tubes and Caps, 0.1 ml (250)	250 strips of 4 tubes and caps for 1000 reactions	981103
Strip Tubes and Caps, 0.1 ml (2500)	10 x 250 strips of 4 tubes and caps for 10,000 reactions	981106

* Red and HRM channels are not intended for use with FDA cleared or approved nucleic acid tests.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at www.qiagen.com or can be requested from QIAGEN Technical Services or your local distributor.

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