SUMMARY OF SAFETY AND EFFECTIVENESS DATA (SSED)

I. GENERAL INFORMATION

Device Generic Name:	*In vitro* real-time polymerase chain reaction (PCR) based assay for CMV viral load measurement in human plasma
Device Trade Name:	*artus*® CMV RGQ MDx Kit
Device Procode:	PAB
Applicant's Name and Address:	QIAGEN, Inc. 1201 Clopper Road Gaithersburg, MD 20878
Date(s) of Panel Recommendation:	None
Premarket Approval Application (PMA) Number:	P130027
Date of FDA Notice of Approval:	June 2, 2014
Expedited:	Not applicable

II. INDICATIONS FOR 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.

III. CONTRAINDICATIONS

None

IV. WARNINGS AND PRECAUTIONS

The warnings and precautions can be found in the *artus* CMV RGQ MDx Kit labeling.

V. DEVICE DESCRIPTION

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. Automated DNA extraction is performed using the EZ1 DSP Virus System, which consists of the EZ1 DSP Virus Kit and either the EZ1 Advanced instrument or the EZ1 Advanced XL instrument. Pathogen detection is based on the specific amplification of a fragment of the CMV genome using the *artus* CMV RGQ MDx Kit on the Rotor-Gene Q MDx (RGQ MDx) Instrument.

CMV DNA is isolated from human plasma using silica-based nucleic acid purification and magnetic beads on the EZ1 DSP Virus System. After DNA purification, eluates are ready to use with the *artus* CMV RGQ MDx Kit. The CMV RG Master provided in the kit contains reagents and enzymes for the specific amplification of a 105 bp region of the CMV *Major Immediate Early* (MIE) gene DNA, and for the direct detection of the amplicon on the RGQ MDx Instrument. The *artus* CMV RGQ MDx Kit provides four quantitation standards (CMV QS 1-4) which allow the quantitation of CMV DNA in the sample. Additionally, the *artus* CMV RGQ MDx Kit contains a second heterologous amplification system (Internal Control) to control the DNA extraction and to identify possible inhibition of the PCR. Oligonucleotide probes linked to fluorescence dyes specifically bind to the amplified product. The specific amplification of CMV target and the signal of the Internal Control are detected as fluorescence in different channels on the RGQ MDx Instrument allowing the detection and quantitation of CMV DNA.

Target Selection

The CMV *Major Immediate Early* (MIE) gene was chosen as a target for the detection of CMV DNA. The target sequence is located specifically in the gene for the IE-1 protein, corresponding to the same region of strain AD-169 (accession number X17403). The MIE gene plays a key role in affecting activation and repression of viral and cellular genes; the region selected is specific for CMV and is highly conserved between the different CMV strains.

Alignment of sequences for three of the major gB genotypes (gB1 from Merlin strain, gB2 from AD-169 strain, and gB3 from Toledo strain) showed 100% identity for the target sequence of the *artus* CMV RGQ MDx Kit (105 of 105 base pairs). No sequence information was available for another gB genotype, gB4.

Specimen Preparation

The *artus* CMV RGQ MDx Kit is intended to be used with plasma samples obtained with EDTA as anticoagulant. The viral DNA extraction is performed with the EZ1 DSP Virus System, which consists of the EZ1 DSP Virus Kit and an instrument from the EZ1 Advanced Instrument family (either the EZ1 Advanced or the EZ1 Advanced XL). A volume of 400 µl of EDTA plasma is used as a sample input volume. The extraction

procedure using the EZ1 DSP Virus System comprises four steps: lyse, bind, wash and elute.

The proteolysis of viruses in plasma is performed with proteinase K and lysis buffer under highly denaturing conditions at elevated temperatures. This step ensures the digestion of virus coat proteins and the inactivation of RNases. During the binding step, binding buffer is added to the lysed samples to adjust binding conditions. Lysates are then thoroughly mixed with magnetic beads to allow the absorption of the viral DNA to the silica surface. Salt and pH conditions ensure that proteins and other contaminants, which can inhibit downstream enzymatic reactions, are not bound to the magnetic beads. Contaminants are efficiently washed away during a sequence of wash steps using first wash buffer 1, then wash buffer 2 and then ethanol. Finally during the elution step, the viral DNA is eluted in 60 µl of Elution Buffer (AVE). Extracted DNA can be stored at 4°C for up to 5 days or at -15 to -30°C for up to 6 months.

The extraction procedure is programmed on the EZ1 Advanced (or EZ1 Advanced XL) DSP Virus Card. These protocols provide both on-screen instructions for the user and operating commands for the EZ1 Advanced (or EZ1 Advanced XL) instrument.

PCR Amplification and Detection

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) on the thermocycler RGQ MDx Instrument. The initial high temperature that denaturizes the double stranded DNA of CMV permits the subsequent specific binding of the primers. DNA polymerase from *Thermus aquaticus* (*Taq* pol) catalyzes the amplification reaction of each cycle at the selected elongation temperature and the amount of amplicon increases exponentially.

The *artus* CMV RGQ MDx Kit also contains a second heterologous amplification system which allows the user both to control the DNA extraction and to identify a possible inhibition of the PCR. This Internal Control system is designed as a competitive PCR; a positive signal in the Internal Control channel in the absence of CMV DNA indicates that the PCR has not been inhibited.

Two different probes are used, one for the specific detection of the CMV amplicon and one for the Internal Control. The probes are flanked by a reporter fluorescence dye at one end (FAM for the CMV amplicon and HEX for the Internal Control) and a quencher at the opposite end.

In the RGQ MDx Instrument, fluorescent dyes are excited from the bottom of the sample chamber by a light-emitting diode. In the absence of a specific target sequence, the physical proximity of both dyes greatly reduces the emission of fluorescence by the reporter upon excitation. After binding of the probes to the amplicons, the exonuclease activity of the *Taq* polymerase permits the cleavage of the probes, liberating the fluorescence dyes and permitting the emission of fluorescence by the reporter once excited. Emitted fluorescence passes through the thin walls at the bottom of each PCR tube, through the emission filters on the side of the chamber and is detected by a

photomultiplier tube. Detection is performed as each tube aligns with the detection optics; tubes spin pass the excitation/emission optics every 150 milliseconds.

The RGQ MDx Instrument measures the fluorescence at each amplification cycle allowing the detection of both amplicons in real time. The fluorescence signals indicate the progress of the PCR reactions. Specific CMV signal is detected in the Green Channel and the signal of the Internal Control in the Yellow Channel. The RGQ MDx Instrument plots the level of fluorescence at each cycle as a curve and reports the cycle at which the fluorescence crosses a pre-defined background level (threshold). This cycle is known as the threshold cycle (C_T) and is inversely proportional to the initial amount of CMV DNA.

CMV Quantitation

The *artus* CMV RGQ MDx Kit provides four quantitation standards (CMV QS 1-4) at four different concentrations, which allow the quantitation of CMV DNA in the sample. These standards are produced using primers homologous to the CMV reference strain AD 169 to amplify a 504 bp DNA fragment in the region of the *MIE* gene. The fragment is isolated and the concentration measured spectrophotometrically. The stock solution is used to produce QS1 (10,000 copies/µL; QS2 (1,000 copies/µL), QS3 (100 copies/µL) and QS4 (10 copies/µL) by serial dilutions. These standards are treated as purified samples, and are amplified and detected together with the samples on the RGQ MDx Instrument. A linear regression is calculated by the RGQ software at the end of the procedure with the C_T values obtained from each standard and their respective concentration.

The equation for the linear regression is then used to calculate the concentration of CMV DNA in copies/mL, using the C_T value obtained for the positive detected sample. Low C_T values indicate higher CMV DNA concentrations and high C_T values indicate lower CMV DNA concentrations. The test software provides results converted from copies/mL to International Units per mL (IU/mL) and all subsequent results are reported in IU/mL.

Controls

- No Template Control: PCR grade nuclease free water is supplied for use with the No Template Control (NTC). The No Template Control contains water and all of the assay components except nucleic acid, and is used to detect reagent contamination with target nucleic acid or increased background in the amplification reaction. This includes potential contamination of kit components due to handling errors or contamination of the Master Mix with positive samples during PCR set-up. It also indicates contamination of the tubes or caps due to improper handling.
- High and Low Positive Control: The Low Positive Control (LPC) provided with the *artus* CMV RGQ MDx Kit contains a linear CMV DNA 504 bp sequence from the *MIE* gene at a concentration near the limit of quantitation. The Low Positive Control is used to monitor for substantial reagent failure. The quantitative result must fall within a specified range for the assay to be valid (between 315 and 1785 copies/mL). The High Positive Control (HPC) contains a linear CMV DNA 504 bp sequence from the *MIE* at a concentration which is in the middle of the linear range. The High Positive Control is used to verify that the calibration status of the assay is maintained

within acceptable limits. The quantitative result must fall within a specified range for the assay to be valid (16,800-53,400 copies/mL).

- Internal Control: The Internal Control is a non-CMV, 282 bp synthetic nucleic acid sequence provided with the *artus* CMV RGQ MDx Kit that is co-extracted and co-amplified with the target nucleic acid. The Internal Control is added to samples prior to extraction and monitors the integrity of the reagents, equipment function, and the presence of inhibitors in the samples. A specific response must be observed with negative specimens to be accepted. Inherent in every PCR reaction is the risk of false negative results because of inhibitory substances that impair amplification. To minimize the consequences of this risk, an Internal Control was implemented into the design of the assay to detect potential inhibition. The Internal Control of the *artus* CMV RGQ MDx Kit contains non-CMV DNA which is amplified simultaneously with the target specific DNA in a single PCR reaction. The emitted light of the specific target is measured in the Green Channel, while the light of the Internal Control is measured in the Yellow Channel. The Master reagent of the *artus* CMV RGQ MDx Kit contains primers and probes for the amplification and detection of both the target specific sequence and the Internal Control sequence. Both detection systems share essential components such as enzymes and dNTPs which drive the PCR reaction. The concentrations of the Internal Control components were optimized so that the amplification of the Internal Control DNA does not impair the sensitivity of the target specific system but still creates a stable fluorescence signal for the Internal Control. If a signal is detected in the Green Channel, the result is positive for the presence of cytomegalovirus DNA. Although high amounts of target specific DNA in a sample can decrease the fluorescence signal of the Internal Control, such an outcome is irrelevant with respect to interpretation due to the positive signal in the target specific channel. If no signal is detected in the target specific Green Channel for a sample but simultaneously a signal in the Internal Control Yellow Channel is detected with an allowed CT shift of -1 up to +4 in comparison to the No Template Control (ΔC_T), the result is negative for the presence of cytomegalovirus DNA in the sample. For a negative result to be considered valid, the C_T value should be between the allowed shift (-1.0 and +0.4) compared to the NTC in the Internal Control Yellow Channel. The signal in the Internal Control channel confirms that the PCR reaction was not impaired and thereby the result is valid. If no signal is detected in the Green or Yellow Channels, the result is invalid. The PCR could be inhibited by unknown substances in the sample or the reagents could have been handled differently than prescribed

- 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 1 must be met for a valid run.

Table 1: 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 2.

Table 2: 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 [CT Range] on Test Channel. Detected CT: X. Min Ct: X Max CT: 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.
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 [CT Range] on Control Channel. Detected CT: X. Min CT: X Max CT: X	–	Invalid	Run is invalid: double intersection in Control Channel.*
NTC	Control Sample [No Template Control] failed rule [Concentration Range] on Test Channel. Detected CT value: X. Expected CT value = "Not Detected"	Invalid	Invalid	Run is invalid: signal in the Test Channel is out of specification.

Retesting of invalid runs:

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 acid.

Instrument and Software

- EZ1 Advanced and EZ1 Advanced XL: The EZ1 Advanced and EZ1 Advanced XL instruments perform fully automated nucleic acid purification from up to 6 or 14 samples respectively using magnetic particles in combination with the EZ1 DSP Virus Kit. The automated steps include:
  - Reading reagent and sample information with a handheld bar code scanner connected to the instrument.
  - Lysis of samples.
  - Binding of nucleic acids to magnetic particles.
  - Washing and elution of nucleic acid.
  - Generating a report file that either will be transmitted to a PC or printed on an external printer after the protocol run is finished.
  - Using UV radiation for decontamination.

  The user inserts a card containing protocol(s) into the instrument. After starting worktable setup using the control panel and bar code reader, the user loads samples, reagent cartridges, filter-tips in tip holders, and elution tubes onto the instrument worktable. The user then closes the instrument door and starts the protocol. The door locks automatically at the start of the protocol. The protocol provides the necessary instructions for the instrument to carry out automated nucleic acid purification.

  The aspiration and dispensing of samples and reagents and the separation of magnetic particles are performed by the pipettor head. The temperature of samples is regulated by a heating system.

- Rotor-Gene Q MDx: The Rotor-Gene Q MDx (RGQ MDx) is a real-time PCR analyzer designed for rapid thermocycling and real-time detection of amplified DNA.

  The RGQ MDx Instrument incorporates a centrifugal rotary design for thermal cycling where the tubes spin in a chamber of moving air, keeping all samples at a

uniform temperature. Samples are heated and cooled in a low-mass-air oven according to a software controlled cycle that initiates the different phases of the PCR cycle.

The RGQ MDx Instrument is capable of supporting up to six optical channels (six excitation sources and six detection filters). Only two of these channels (for FAM and HEX fluorophores) are used with the *artus* CMV RGQ MDx Kit.

The *artus* CMV Assay Package contains a template to set the PCR run parameters, assess run validity and calculate the results. The RGQ MDx instrument software supports real-time analysis procedures. The software determines C_T values and calculates the CMV concentration. A system of Flags/Warnings is embedded within the software in order to inform the user of potential problems with the assay and to indicate non-valid test runs or non-valid samples within a valid test run (inappropriate level of DNA or Internal Control failure). No results are reported for invalid runs or for non-valid samples.

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). Status is reported as follows:

i. “Not Detected”: No CMV DNA detected in Target Specific Channel.

ii. “Detected, Below LOQ (<159 IU/mL)”: CMV DNA detected in Target Specific Channel, but at a level below the Limit of Quantitation (below 159 IU/mL).

iii. “Detected”: CMV DNA was detected within the linear range of the assay and the quantitated value expressed in IU/mL is provided.

iv. “Detected, Above Linear Range (>7.97 x10^7 IU/mL)”: CMV DNA detected in Target Specific Channel, but at a level above the linear range (greater than 7.97 x10^7 IU/mL).

v. “Invalid”: The sample result is invalid and the sample should be retested.

Negative control samples that do not meet the acceptance criteria in the Internal Control channel are flagged with a warning and no results are reported.

The calculated CMV concentration (in IU/mL) is only provided for samples within the measuring interval. A description of the sample results provided by the Rotor-Gene Q software is provided in Table 3.

Table 3: 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 (IU/mL).
Sample ID	–	DNA not detected	**Not Detected**	CMV DNA not detected.

Name	Flag/warning	CMV	Status	Interpretation of Result
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 x10^7 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.
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.
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 there is remaining eluate, it is appropriate to retest. If the retest fails, then it is necessary to resample and re-extract.

Quality Control

The CMV Low Positive Control, High Positive Control, Quantitation Standards, and NTC (H2O) 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.

Kit Configuration and Components

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 (Table 4).

Table 4: *artus* CMV RGQ MDx Kit content.

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, version 4
- Water
- 70% ethanol

Equipment

- EZ1 Advanced instrument or EZ1 Advanced XL instrument
- EZ1 Advanced DSP Virus Card v1.0 or higher, with firmware 1.0.1 and protocol "DSP Virus version 1.0" or higher or EZ1 Advanced XL DSP Virus Card v1.0 or higher, with firmware 1.0.1 and protocol "DSP Virus version 1.0" or higher

- 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, 17" (or user's PC and monitor) with EZ1 Advanced Communicator Software (software supplied with EZ1 Advanced and EZ1 Advanced XL instruments)
- Printer and accessory package for printer

Consumables

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

For PCR

Consumables

- Pipets (adjustable)
- Sterile pipet tips with filters
- Strip Tubes and Caps, 0.1 mL, 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
- Rotor-Gene Q Software version 2.1.0 or higher
- Rotor-Gene Q *artus* CMV Assay Package 1.2.7 or higher
- Cooling block (Loading Block 72 x 0.1 mL Tubes)

VI. ALTERNATIVE PRACTICES AND PROCEDURES

Currently, one alternative *in vitro* nucleic acid amplification test for the quantitative measurement of cytomegalovirus (CMV) DNA in human plasma has been FDA-approved in the United States. The pp65 antigenemia assay is another alternative to measurement of CMV viral load that has been used in transplant centers. The test is a fluorescent assay based on detection of infected cells in peripheral blood. The test is comparable in sensitivity to laboratory developed CMV amplification-based assays but has largely been supplanted by CMV PCR assays due to greater reliability and technical ease of the latter assays.

Each alternative has its own advantages and disadvantages. A patient should fully discuss these alternatives with his/her physician to select the method that best meets expectations and lifestyle.

VII. MARKETING HISTORY

The *artus* CMV RGQ MDx Kit has not been marketed in the United States or any other country.

VIII. POTENTIAL ADVERSE EFFECTS OF THE DEVICE ON HEALTH

Below is a list of the potential adverse effects (e.g., complications) associated with the use of the device.

The primary adverse events from use of the device are caused by an inaccurate measurement result of CMV viral load. Failure of the *artus* CMV RGQ MDx Kit to perform as indicated, or human error in the use of the test or the interpretation of the test result, may result in an incorrect test result that is too low or too high.

An erroneous low test result or a false negative result may lead to inappropriate patient management decisions, a premature discontinuation of antiviral therapy, or may instill a false sense of security in a patient or clinician. Similar assays that differ in LOD or other analytic characteristics may lead to different durations of treatment; theoretically a less sensitive assay could lead to earlier discontinuation of treatment relative to an alternative assay, with perhaps greater risk of relapse. Although clinical practice has evolved to recommend two consecutive "negative" responses as a treatment endpoint, this is based on different tests across different institutions. At the very low viral levels where "differences" between negative and positive assays appear, there may not be clinical repercussions from a patient 'discontinued early' from treatment.

An erroneous high test result, or a false positive result, may contribute to a change in therapy, unnecessary treatment, prolonged duration of therapy, or create anxiety in the patient. This may be mitigated by local practice; as experience with these assays evolves at local institutions, it is likely that "low level" positive results in the context of otherwise negative results will be recognized and that therapy would be unlikely to be unnecessarily prolonged.

The risk of these adverse events is readily mitigated in clinical practice by serial and/or repeat measurement as well as clinical evaluation, which would likely show symptomatic improvement.

To aid in the management of solid-organ transplant patients who are undergoing anti-CMV drug therapy, the results from the *artus* CMV RGQ MDx Kit must be interpreted in the context of all relevant clinical and laboratory findings.

IX. SUMMARY OF PRECLINICAL STUDIES

A. Laboratory Studies

Traceability to the 1st WHO International Standard for Human Cytomegalovirus (HCMV)

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 (3.70 $\log_{10}$ 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 it corresponded to a range of ± 0.30 $\log_{10}$ IU/ml. Preparation of the WHO Standard for use in the Quality Control test of the Quantitation Standards consisted of the following:

1. The WHO Standard was diluted to 5,000 IU/ml in human plasma confirmed to be negative for CMV. At least 14 separate dilutions were prepared.
2. Each of the 14 dilutions was extracted using the EZ1 DSP Virus Kit on the EZ1 Advanced Instrument.
3. The extracted eluates were pooled and dispensed into several aliquots.

The Quality Control test of the Quantitation Standards consisted of the following:

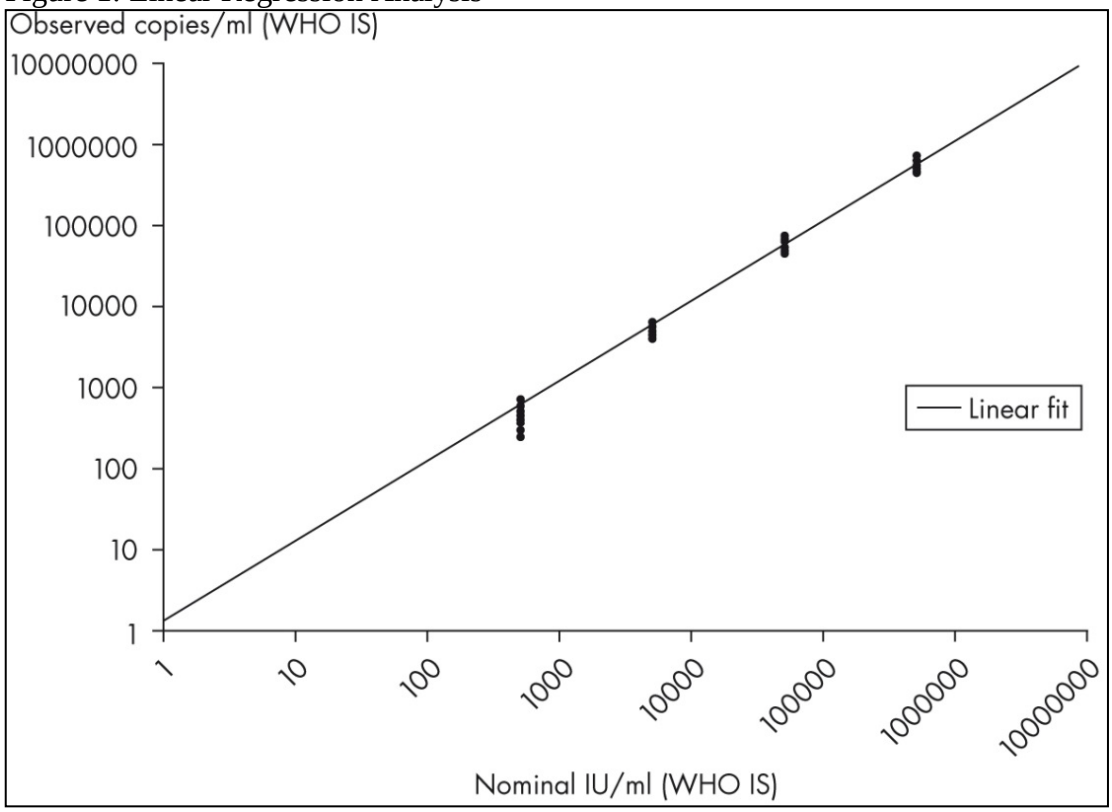
1. Quantitation Standards and one aliquot of the extracted WHO Standard were tested using a previously released *artus* CMV RGQ MDx Kit.
2. Both single test results must meet the respective QC release specifications. For the WHO Standard, the quantified value must meet the defined acceptance criteria.
3. In addition, the CT values of the Quantitation Standards were assessed against the CT values obtained with a previously released lot of Quantitation Standards to ensure consistency over time.

Correspondence between IU/mL and copies/mL

In order to establish correspondence between U/mL to copies/mL, a serial dilution of the 1st WHO International Standard for Cytomegalovirus (WHO IS) for NAT based assays (NIBSC code 09/162; Merlin strain) in EDTA plasma ranging from 5.00×10^2 to 5.00×10^5 IU/mL was extracted with the EZ1 DSP Virus System and analyzed with the *artus* CMV RGQ MDx Kit. The 1st WHO International Standard for Cytomegalovirus [1] represents the glycoprotein B (gB) genotype 1. Six rounds of CMV DNA extraction were conducted on 3 different days. In each run, 3 replicates of each dilution level were processed (CMV WHO Standard in EDTA plasma). All samples were subjected to single extraction using 3 different EZ1 DSP Virus Kit lots. The analysis of all eluates was performed in duplicates with 3 *artus* CMV RGQ MDx Kit lots on 3 Rotor-Gene Q MDx instruments.

Figure 1 shows the linear regression analysis of IU/mL versus copies/mL.

Figure 1: Linear Regression Analysis



The equation obtained was:
$Y_{copies/mL} = 0 + 1.2594212 \times X_{IU/mL}$

Which is equivalent to:

$X_{IU/mL} = Y_{copies/mL} / 1.2594212 = 0.794 \times Y_{copies/mL}$

Therefore, the equation for converting copies to international units is:

$X_{IU/mL} = 0.794 \times Y_{copies/mL}$
(i.e.; 100 copies/mL = 79.4 IU/mL; 100 IU/mL = 125.94 copies/mL.)

Characterization of the Different Genotypes used in the Studies

Throughout the different analytical studies, genotype gB 1 was represented by the 1st WHO International Standard for Human Cytomegalovirus for NAT Techniques (NIBSC code: 09/162); gB2 was represented by strain AD-169; gB3 by a strain Toledo provided by the Universitätsklinikum Ulm, Germany; and gB4 was represented by an EDTA-whole blood clinical isolate provided by the Groupe Hospitalier Pitié-Salpêtrière Paris, France.

Limit of Detection (LOD)

The Limit of Detection (LOD) value of the *artus* CMV RGQ MDx Kit was determined to be 77 IU/mL (1.89 log_{10} IU/mL) CMV in EDTA plasma.

Limit of Detection using the 1st WHO International Standard for Human 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 [2]. 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 three different *artus* CMV RGQ MDx Kit lots and with each lot on four different days, by three different operators, on four different EZ1 Advanced XL and three different Rotor-Gene Q instruments, resulting in 72 overall data points per dilution.

A probit regression with SAS Software was performed and the LOD value was determined. The LODs for three different lots were all close to 54 IU/mL. The results for the combined data are shown in Table 5.

Table 5: 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

CMV target concentration (IU/mL)	Number of replicates tested (N)	Mean observed concentration (IU/mL)	Number of positives detected	Positive rate (%)
0.3	72	6	3	4

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 determined to be 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. 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 values of the three lots were the same, and the final LOD for the *artus* CMV RGQ MDx Kit for the combined data is 77 IU/mL (using probit analysis) (Table 6).

Table 6: 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 [2].

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 LoD value of the *artus* CMV RGQ MDx Kit for all lots combined was confirmed to be at least 77 IU/mL (Table 7).

Table 7: 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

Conclusion: The data were consistent with the claimed Limit of Detection (LOD) value of the *artus* CMV RGQ MDx Kit of 77 IU/mL (1.89 $\log_{10}$ IU/mL) CMV in EDTA plasma.

Limit of Blank Confirmation 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 negative samples determines the probability of potential false positive results. A total of 100 characterized CMV-negative EDTA plasma samples from individual donors were analyzed using two different EZ1 DSP Virus Kit manufacturing lots and two different Rotor-Gene Q instruments, over a total of four runs.

The fluorescence values at cycle 45 were measured for all samples, and the percentage of samples with fluorescence less than 0.05 was calculated. A 99% (99/100) of samples showed no result in the CMV Test Channel and fluorescence intensities below the given threshold (0.05) (95% CI: 94.6% -99.8%).

In addition, C_T values generated for each sample were analyzed. The results showed that 99 of the 100 negative samples tested yielded a negative detection result using the *artus* CMV RGQ MDx Kit.

To assess the missed sample, the residual eluate obtained from the discordant sample was re-tested in duplicate, obtaining negative results in both cases. Re-amplification of the sample was tested again for each duplicate, obtaining again negative results for both

cases. These results suggest that the sample may have been contaminated with CMV during the initial PCR reaction set up. Note that the same initial PCR reaction and set of samples was used to assess performance with DNA negative samples and the same result was obtained.

Linear Range and Limit of Quantitation

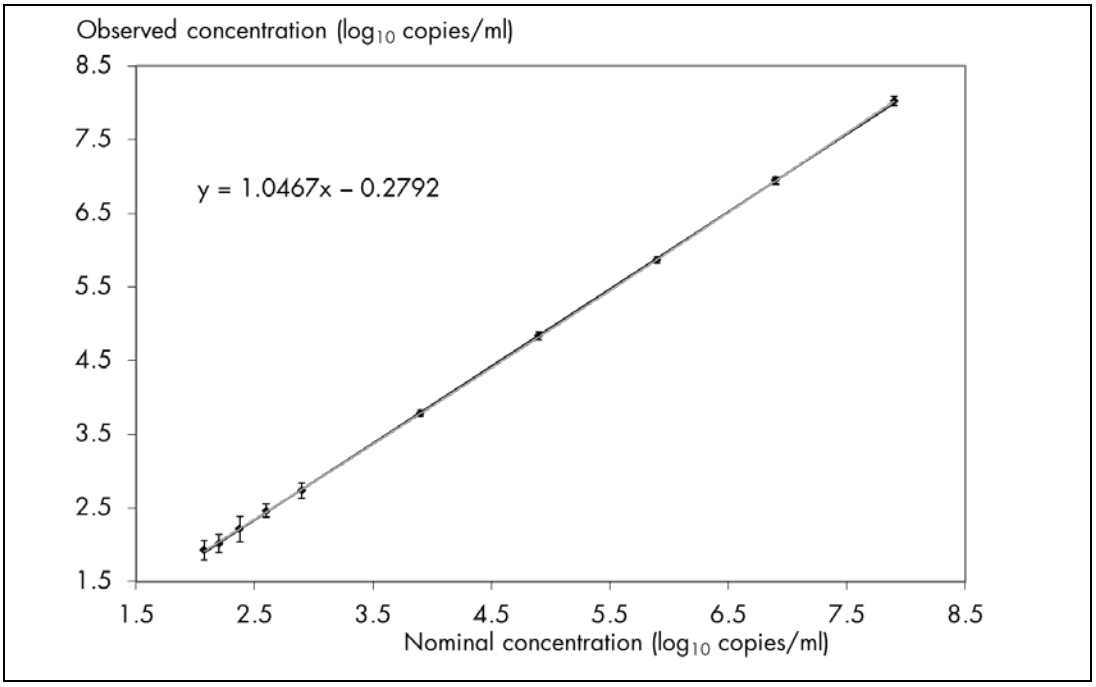
Linear Range Using Glycoprotein B (gB) Genotype 2

The linear range of the *artus* CMV RGQ MDx Kit was determined following recommendations of the CLSI Guideline EP06-A [3].

A dilution series of cultured CMV ranging from 2.08 $\log_{10}$ IU/mL to 7.90 $\log_{10}$ IU/mL (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 results were analyzed to assess if the replicates at each concentration demonstrated a predefined SD (in $\log_{10}$ IU/mL) of ≤0.20; otherwise the concentrations were considered to be outside the linear range. In addition, 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.

Figure 2: 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, 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 2.08 to 5.60 $\log_{10}$ IU/mL (1.19 x 10^2 to 3.97 x 10^5 IU/mL). For gB4, a dilution series ranging from 2.08 to 4.90 $\log_{10}$ IU/mL (1.19 x 10^2 to 7.94 x 10^4 IU/mL) was used.

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.

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.

Linear regression analyses of the mean $\log_{10}$ observed titer vs. the nominal $\log_{10}$ titer for each of the gB genotype linearity panels is shown in Figure 3. Table 8 summarizes the results obtained for all CMV gB genotypes analyzed.

Figure 1: Linear range of the *artus* CMV RGQ MDx Kit for all CMV gB genotypes analyzed.

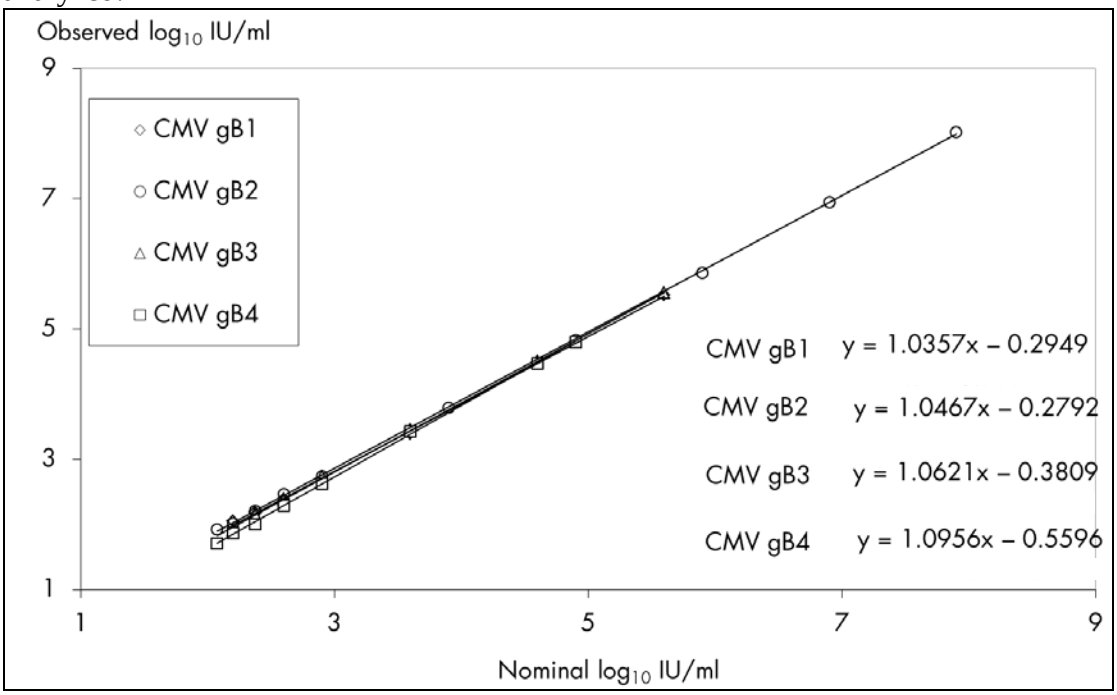


Table 8: Linear range of the *artus* CMV RG PCR Test for all CMV gB genotypes analyzed and maximum difference between regression equations

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)

Table 9: CMV gB Genotypes Linearity Study — Observed Titer Summary for CMV Glycoprotein B Genotype 1. Best fitted 1st-order line is y = 1.0357x - 0.2949

Nominal concentration ($\log_{10}$ IU/mL)	N	Mean Observed ($\log_{10}$ IU/mL)	SD $\log_{10}$ IU/mL	Bias	Predicted 1st- order	Deviation from linearity
5.60	8	5.52	0.02	-0.08	5.51	0.01
4.60	8	4.49	0.01	-0.11	4.47	0.02
3.60	8	3.39	0.04	-0.21	3.43	-0.04
2.90	8	2.65	0.08	-0.25	2.71	-0.06
2.60	8	2.38	0.10	-0.31	2.40	-0.02
2.38	8	2.19	0.15	-0.22	2.17	0.02
2.20	8	2.05	0.15	-0.15	1.98	0.07
2.08	8	1.83	0.24	-0.25	1.86	-0.03

For gB1, the assay was linear at interval [2.10 $\log_{10}$ IU/mL – 5.60 $\log_{10}$ IU/mL], with deviation from linearity ≤0.07 $\log_{10}$ IU/mL; bias was ≤0.31 $\log_{10}$ IU/mL.

Table 10: CMV gB Genotypes Linearity Study — Observed Titer Summary for CMV Glycoprotein B Genotype 2. Best fitted 1st-order line is y = 1.0467x - 0.2792

Nominal concentration ($\log_{10}$ IU/mL)	N	Mean Observed ($\log_{10}$ IU/mL)	SD $\log_{10}$ IU/mL	Bias	Predicted 1st- order	Deviation from linearity
7.90	18	8.02	0.06	0.12	7.99	0.03
6.90	18	6.95	0.05	0.05	6.94	0.01
5.90	18	5.86	0.05	-0.04	5.90	-0.04
4.90	18	4.83	0.05	-0.07	4.85	-0.02
3.90	18	3.79	0.04	-0.11	3.80	-0.01
2.90	18	2.73	0.11	-0.17	2.76	-0.03
2.60	18	2.46	0.09	-0.14	2.44	0.02
2.38	18	2.21	0.17	-0.17	2.21	0.00
2.20	18	2.02	0.13	-0.18	2.02	0.00
2.08	18	1.93	0.13	-0.15	1.89	0.04

For gB2, the assay was linear at interval [2.10 $\log_{10}$ IU/mL – 7.90 $\log_{10}$ IU/mL], with deviation from linearity ≤ 0.04 $\log_{10}$ IU/mL; bias was ≤0.18 $\log_{10}$ IU/mL.

Table 11: CMV gB Genotypes Linearity Study — Observed Titer Summary for CMV Glycoprotein B Genotype 3. Best fitted 1st-order line is y = 1.0621x - 0.3809

Nominal concentration ($\log_{10}$ IU/mL)	N	Mean Observed ($\log_{10}$ IU/mL)	SD $\log_{10}$ IU/mL	Bias	Predicted 1st- order	Deviation from linearity
5.60	8	5.56	0.02	-0.04	5.57	-0.01
4.60	8	4.50	0.01	-0.10	4.50	0.00
3.60	8	3.45	0.03	-0.15	3.44	0.01
2.90	8	2.73	0.06	-0.17	2.70	0.03
2.60	8	2.37	0.06	-0.22	2.38	-0.01
2.38	8	2.16	0.18	-0.22	2.15	0.01

2.20	8*	1.91	0.17	-0.29	1.96	-0.05
2.08	8	1.68	0.18	-0.40	1.83	-0.15

*1 outlier value of 1.28 $\log_{10}$ IU/mL was excluded

For gB3, the assay was linear at interval [2.10 $\log_{10}$ IU/mL – 5.60 $\log_{10}$ IU/mL], with deviation from linearity ≤ 0.15 $\log_{10}$ IU/mL; bias was ≤0.40 $\log_{10}$ IU/mL.

Table 12: CMV gB Genotypes Linearity Study — Observed Titer Summary for CMV Glycoprotein B Genotype 4. Best fitted 1st-order line is y = 1.0956x -0.5596

Nominal concentration ($\log_{10}$ IU/mL)	N	Mean Observed ($\log_{10}$ IU/mL)	SD $\log_{10}$ IU/mL	Bias	Predicted 1st- order	Deviation from linearity
4.90	8*	4.80	0.03	-0.10	4.81	-0.01
4.60	8	4.46	0.04	-0.14	4.48	-0.02
3.60	8	3.43	0.04	-0.17	3.38	0.05
2.90	8	2.63	0.09	-0.27	2.62	0.01
2.60	8	2.29	0.08	-0.31	2.29	0.00
2.38	8	2.00	0.17	-0.38	2.05	-0.05
2.20	8	1.86	0.12	-0.34	1.85	0.01
2.08	8	1.71	0.14	-0.37	1.79	-0.01

*1 outlier value of 5.09 $\log_{10}$ IU/mL was excluded

For gB4, the assay was linear at interval [2.10 $\log_{10}$ IU/mL – 4.90 $\log_{10}$ IU/mL], with deviation from linearity ≤0.05 $\log_{10}$ IU/mL; bias was ≤0.38 $\log_{10}$ 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 other 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.

The claimed linear range of the assay, based on the linearity results, the homology of sequences in the target region among the genotypes, the single primers/probe used, and the tight overlap in the curves, was considered to be between 159 and 7.94 x 10^7 IU/mL, or 2.20 $\log_{10}$ IU/mL to 7.90 $\log_{10}$ IU/mL CMV in EDTA plasma, with maximum deviation from linearity of less or equal to 0.15 $\log_{10}$ IU/mL.

Lower Limit of Quantitation (LOQ)

The lower 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 limit of the linear range 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.

The LOQ was defined as the lowest level of CMV that can be reliably detected and at which the total error is ≤1.0 $\log_{10}$ IU/mL, where total error is calculated as |Bias| + 2SD. Meeting the |Bias| + 2SD≤ 1.0 $\log_{10}$ IU/mL criterion ensures that, for samples with assay values equal to the LOQ, there is 95% or greater probability that the measured value will be within 1.0 $\log_{10}$ IU/mL of the true value. In addition, total error is such that difference between two measurements >1.0 $\log_{10}$ IU/mL is statistically significant (a true change is detected). The standard deviation for the difference between two measurements is $\sqrt{2}$ x SD, and meeting the $\sqrt{2}$ x 2 x SD ≤ 1.0 $\log_{10}$ IU/mL criterion ensures that, for samples with assay values equal to the LOQ, the difference between two measurements of more than 1.0 log10 IU/mL is statistically significant.

Table 14 shows both criteria for the total analytical error (TAE = |Bias| + 2 x SD ≤1.0 log10 IU/mL and $\sqrt{2}$ x 2 x SD ≤1.0 $\log_{10}$ IU/mL) for the $\log_{10}$ IU/mL (Tables 14 and 15) following the recommendations of the CLSI Guideline EP17-A2 [2].

The LoQs for two individual lots were similar. The LoQ results for the combined lots are presented in Table 14.

Table 14: Lower limit of quantitation for the different CMV gB genotypes ($\log_{10}$ IU/mL)

CMV gB genotype	Nominal Concentration (IU/mL)	Nominal Concentration ($\log_{10}$ IU/mL)	Average Measured Concentration ($\log_{10}$ IU/mL)	Bias ($\log_{10}$ IU/mL)	SD ($\log_{10}$ IU/mL)	TAE = |Bias| + 2 x SD ($\log_{10}$ IU/mL)	$\sqrt{2}$ x 2 x SD ($\log_{10}$ IU/mL)
gB1	159	2.20	1.87	–0.33	0.25	0.84	0.72
gB2	159	2.20	2.03	–0.17	0.23	0.62	0.65
gB3	159	2.20	1.79	–0.41	0.26	0.93	0.73
gB4	159	2.20	1.89	–0.31	0.18	0.66	0.50

The claimed LOQ for the *artus* CMV RGQ MDx Kit considering all CMV gB genotypes is 159 IU/mL (2.20 $\log_{10}$ 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: LOQ 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)

gB genotype	LOQ
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 plasma at 2 concentrations (near the LOD value, and within the linear range). Each sample was extracted and tested in four 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	–
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	–

Pathogen	Concentration*	CMV
Parvo B19 DNA	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	–
Protozoan 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.

All negative CMV EDTA plasma samples tested negative. 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 around the middle of the linear range (3.08 $\log_{10}$ IU/mL, 1.19 x 10^3 IU/mL CMV) were quantified within ±0.25 $\log_{10}$ IU/mL.

The results showed no cross-reactivity of the respective pathogens with the specific CMV detection in terms of sensitivity and quantitation.

Interference - Endogenous Interfering Substances

Potentially interfering endogenous substances that may be found in patient specimens were spiked into CMV-negative EDTA plasma in the presence of different concentrations of CMV (3 x LOD, and 1,191 IU/mL). 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 CMV concentration level for each interfering substance was tested in four replicates.

Qualitatively, samples were considered positive if a C_T value was detected in the target specific channel. Samples were considered negative if no C_T value was detected in the target specific channel and the sample validity criteria for the internal control were met.

The samples were also analyzed for potential inhibition by assessing the validity criteria of the Internal Control for negative specimens which is an allowed C_T shift of -1 up to +4 in comparison to the No Template Control (ΔC_T).

For all tested samples, the IU/mL values were transformed into $\log_{10}$ IU/mL. On the basis of the logarithmic values, the average for the control (CMV in EDTA-plasma without interferent) was calculated. Subsequently, the difference between a single value and the average control value was calculated for each sample to evaluate whether all samples were quantified within the pre-specified acceptance criteria (based on International

Consensus Guidelines on the Management of Cytomegalovirus in Solid Organ Transplantation [7]).

The test concentrations for each interfering substance (Table 17) were selected based on available literature references and guidance provided by the CLSI Guideline EP07-A2 [3].

Table 17: Endogenous Interfering Substances

Potential interfering substance	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 analytical specificity, sensitivity, and quantitation.

Interference - Exogenous Interfering Substances

Potentially interfering exogenous 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 four replicates.

Qualitatively, samples were considered positive if a C_T value was detected in the target specific channel. Samples were considered negative if no C_T value was detected in the target specific channel and the sample validity criteria for the internal control was met. The samples were also analyzed for potential inhibition by assessing the validity criteria of the Internal Control for negative specimens which is an allowed C_T shift of -1 up to +4 in comparison to the No Template Control (ΔC_T).

For all tested samples the copies/mL values were transformed into $\log_{10}$ copies/mL. On the basis of the logarithmic values, the average for the control (CMV in EDTA-plasma without interferent) was calculated. Subsequently, the difference between a single value and the average control value was calculated for each sample to evaluate whether all samples were quantified within the specified acceptance criteria.

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 18.

Table 28: Exogenous Interfering Substances

Potential interfering substance	**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 analytical specificity, 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 by alternating positive samples with 30 CMV-negative samples in five extraction runs and one PCR run. The CMV tested concentration represents the highest viral load observed

within a diagnostic evaluation study analyzing 203 retrospectively and prospectively collected patient specimens.

All CMV negative samples were analyzed regarding the presence of a C_T value in the target specific channel to check for a possible carryover event. The samples were also analyzed regarding the validity criteria for the Internal Control for negative specimens (C_T shift of -1 up to +4 in comparison to the No Template Control). On the basis of these results the percentage of valid negative results for all negative samples tested was calculated.

No C_T was observed in the target specific channel for any negative sample. Since the Internal Control of all samples showed a C_T value within the predefined acceptance range, 100% of the negative samples were considered to be truly negative. No cross-contamination occurred throughout the test system, including nucleic acid purification and real-time PCR detection.

Precision

The precision of the *artus* CMV RGQ MDx Kit was determined following the recommendations of the CLSI Guideline EP05-A2 [4] 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 for 20 days, with two runs per day, and two replicates per run. A total of two different EZ1 Advanced and two different EZ1 Advanced XL instruments, as well as three different Rotor-Gene Q instruments were used for the testing. Three different EZ1 DSP Virus Kit lots and three different *artus* CMV RGQ MDx Kit lots were used for the study. A total of three different operators performed the test. The results are summarized in Table 19 and Table 20.

Table 19: Precision of the *artus* CMV RGQ MDx Kit (in $\log_{10}$ 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 20: 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 %	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 %CV
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.

Specimen Stability

The stability of CMV DNA in EDTA Whole Blood, in EDTA Plasma, and after extraction under various storage conditions when using the *artus* CMV RGQ MDx Kit was evaluated using CMV from cultured virus stocks.

The stability testing was conducted in four separate parts:

- Stability of CMV in EDTA Whole Blood
- Stability of CMV in EDTA Plasma
- Stability of purified CMV DNA
- Stability of CMV covering the complete storage process.

A contrived sample stability study was performed for all parts, in which CMV whole virus was added to either CMV-negative whole blood or plasma samples from five randomly selected individuals pre-screened to ensure they did not contain endogenous CMV. Samples were tested with the *artus* CMV RGQ MDx Kit after storage at defined conditions. Samples subjected to the various storage conditions were compared to a baseline value (T_0) obtained at the start of the incubation period. Whole blood or plasma samples not spiked with CMV DNA were also subjected to the various storage conditions and served as a negative control.

Negative samples should show no detectable signal in the target specific channel. Samples prepared near the LOD (3x LOD) should be detected positive throughout the course of the study. The acceptance criteria for the sample with 3 x LOD and the sample with concentration 3.08 $\log_{10}$ IU/mL (1,191 IU/mL), within the linear range, were based on information derived from the International Consensus Guidelines on the Management of Cytomegalovirus in Solid Organ Transplantation (Kotton et al, Transplantation 2010; 89: 779-795) [7].

The contrived sample specimen stability is defined in Table 21 for the different specimen types.

Table 21: Contrived Sample Specimen Stability

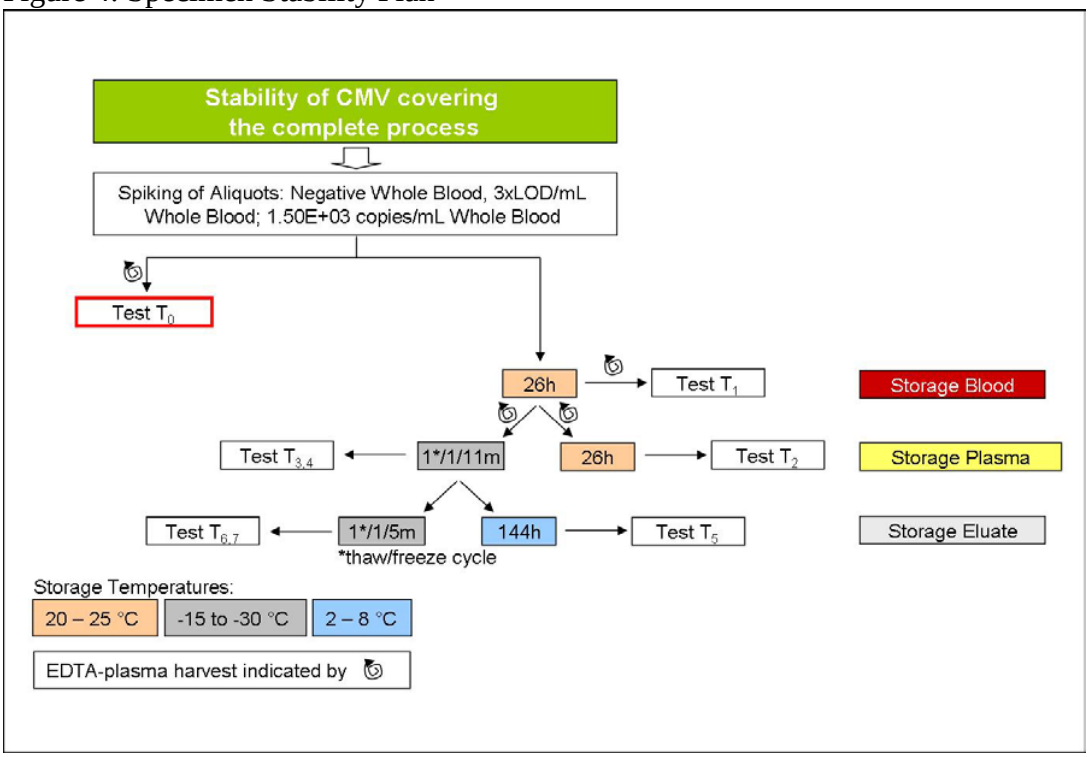
Specimen Type	Storage Temperature	Duration
EDTA Whole Blood	20-25°C	48 hours
	2-8°C	72 hours
EDTA Plasma	20-25°C	48 hours
	2-8°C	120 hours
	-15 to -30°C	12 months
Eluted DNA	2-8°C	120 hours
	-15 to -30°C	6 months

In addition to the specimen stability study using CMV from viral culture, a limited specimen stability study using actual clinical specimens was started to demonstrate the stability of CMV DNA in EDTA Whole Blood, in EDTA Plasma, and after extraction under various storage conditions when using the *artus* CMV RGQ MDx Kit. The results generated to date are presented here.

Whole blood from a clinical sample containing CMV DNA was added to CMV negative whole blood from 5 randomly selected donors pre-screened to ensure they did not contain endogenous CMV. Samples subjected to various storage conditions were compared to a baseline value (T_0) obtained at the start of the incubation period. The testing schedules for each storage condition were extended beyond the desired storage claim so that stability was supported by test data and not based on an extrapolation. Whole blood not spiked with a clinical sample containing CMV DNA was also subjected to the various storage conditions and served as a negative control.

Whole blood samples containing 3x LOD and 3.08 $\log_{10}$ IU/mL (1,191 IU/mL) of CMV DNA were maintained at 20 to 25°C for 26 hours. EDTA plasma was then prepared from the whole blood and the resulting plasma maintained at -15 to -30°C for a total of 13 months or 20 to 25°C for 26 hours. After the plasma was stored at -15 to -30°C for 13 months, nucleic acid was extracted from EDTA plasma samples using the EZ1 DSP Virus System. The resulting eluates were maintained at -15 to -30°C for 7 months or at 2 to 8°C for 144 hours (6 days). After designated storage conditions, the samples were tested with the *artus* CMV RGQ MDx Kit. Samples stored at -15 to -30°C included two freeze thaw cycles one month apart during the incubation period. This is represented in Figure 4 as "1/1/x m", where "x" is the number of months the samples were stored after the two freeze-thaw cycles. Time points that have been completed are indicated with a checkmark, and time points yet to be tested are indicated with a grey arrow. Time points T_3 and T_6 are tested after two months of the 13 month incubation at -15 to -30°C. The last of the samples are scheduled to be tested in January 2015.

Figure 4: Specimen Stability Plan



	Donor #2	Negative				3x LOD		1,191 IU/mL			
	Storage Condition	Sample ID	Target Specific Channel C_T	Internal Control Channel C_T	Internal Control Channel ΔC_T	Sample ID	Result	Sample ID	CMV IU/mL	CMV $\log_{10}$ IU/mL	$\Delta\log_{10}$ IU/mL compared to baseline
T0	Baseline (0 hr)	0240004 0240005	0.00 0.00 0.00	26.26 26.48 26.32	1.07 1.29 1.13	0240025 0240026 0240027	Detected Detected Detected	0240046 0240047 0240048	1,232 1,011 1,052	3.09 3.01 3.02	3.04 (Avg)**
T1	Whole Blood RT/26 hr*	0240064	0.00	27.50	2.01	0240069	Detected	0240074	823	2.92	-0.12
T2	Whole Blood RT/26 hr + Plasma RT/26 hr	0240079	0.00	28.03	2.36	0240084	Detected	0240089	504	2.70	-0.34
T3	Whole Blood RT/26 hr + Plasma - 20°C*/2 mo	0240094	0.00	27.32	1.86	0240099	Detected	0240104	600	2.78	-0.26
	Donor #3	Negative				3x LOD		1,191 IU/mL			
	Storage Condition	Sample ID	Target Specific Channel C_T	Internal Control Channel C_T	Internal Control Channel ΔC_T	Sample ID	Result	Sample ID	CMV IU/mL	CMV $\log_{10}$ IU/mL	$\Delta\log_{10}$ IU/mL compared to baseline

T0	Baseline (0 hr)	0240007 0240008 0240009	0.00 0.00 0.00	25.92 25.93 25.98	0.73 0.74 0.79	0240028 0240029 0240030	Detected Detected Detected	0240049 0240050 0240051	1,404 1,550 1,864	3.15 3.19 3.27	3.20 (Avg)
T1	Whole Blood RT/26 hr	0240065	0.00	26.51	1.02	0240070	Detected	0240075	1,279	3.11	-0.10
T2	Whole Blood RT/26 hr + Plasma RT/26 hr	0240080	0.00	26.87	1.20	0240085	Detected	0240090	1,253	3.10	-0.10
T3	Whole Blood RT/26 hr + Plasma - 20°C/2 mo	0240095	0.00	26.52	1.06	0240100	Detected	0240105	1,181	3.07	-0.13
	Donor #4	**Negative**				**3x LOD**		**1,191 IU/mL**			
	Storage Condition	**Sample ID**	**Target Specific Channel**	**Internal Control Channel C_T**	**Internal Control Channel ΔC_T**	**Sample ID**	**Result**	**Sample ID**	**CMV IU/mL**	**CMV $\log_{10}$ IU/mL**	**$\Delta\log_{10}$ IU/mL compared to baseline**
T0	Baseline (0 hr)	0240010 0240011	0.00 0.00 0.00	26.28 26.43 26.37	1.09 1.24 1.18	0240031 0240032 0240033	Detected Detected Detected	0240052 0240053 0240054	1,067 1,038 1,349	3.03 3.02 3.13	3.06 (Avg)
T1	Whole Blood RT/26 hr	0240066	0.00	27.57	2.08	0240071	Detected	0240076	1,850	2.93	-0.13
T2	Whole Blood RT/26 hr + Plasma RT/26 hr	0240081	0.00	27.85	2.18	0240086	Detected	0240091	590	2.77	-0.29
T3	Whole Blood RT/26 hr + Plasma - 20°C/2 mo	0240096	0.00	27.28	1.82	0240101	Detected	0240106	687	2.84	-0.22
	Donor #5	**Negative**				**3x LOD**		**1,191 IU/mL**			
	Storage Condition	**Sample ID**	**Target Specific Channel**	**Internal Control Channel C_T**	**Internal Control Channel ΔC_T**	**Sample ID**	**Result**	**Sample ID**	**CMV IU/mL**	**CMV $\log_{10}$ IU/mL**	**$\Delta\log_{10}$ IU/mL compared to baseline**
T0	Baseline (0 hr)	0240013 0240014	0.00 0.00 0.00	25.96 25.97 25.91	0.77 0.78 0.72	0240034 0240035 0240036	Detected Detected Detected	0240055 0240056 0240057	1,026 1,173 924	3.01 3.07 2.97	3.02 (Avg)
T1	Whole Blood RT/26 hr	0240067	0.00	26.24	0.75	0240072	Detected	0240077	977	2.99	-0.03
T2	Whole Blood RT/26 hr + Plasma RT/26 hr	0240082	0.00	26.59	0.92	0240087	Detected	0240092	619	2.79	-0.22
T3	Whole Blood RT/26 hr + Plasma - 20°C/2 mo	0240097	0.00	26.08	0.62	0240102	Detected	0240107	530	2.72	-0.29

	Donor #6	**Negative**				**3x LOD**		**1,191 IU/mL**			
	Storage Condition	**Sample ID**	**Target Specific Channel C_T**	**Internal Control Channel C_T**	**Internal Control Channel ΔC_T**	**Sample ID**	**Result**	**Sample ID**	**CMV IU/mL**	**CMV $\log_{10}$ IU/mL**	**$\Delta\log_{10}$ IU/mL compared to baseline**
T0	Baseline (0 hr)	0240016 0240017	0.00 0.00 0.00	26.10 26.85 26.73	0.91 1.66 1.54	0240037 0240038 0240039	Detected Detected Detected	0240058 0240059 0240060	2,095 1,970 1,671	3.32 3.29 3.22	3.28 (Avg)
T1	Whole Blood RT/26 hr	0240068	0.00	26.86	1.37	0240073	Detected	0240078	1,081	3.03	-0.25
T2	Whole Blood RT/26 hr + Plasma RT/26 hr	0240083	0.00	27.22	1.55	0240088	Detected	0240093	1,191	3.08	-0.20
T3	Whole Blood RT/26 hr + Plasma - 20°C/2 mo	0240098	0.00	26.90	1.44	0240103	Detected	0240108	785	2.90	-0.38

The data for all samples tested to date demonstrated the following:

- Negative Samples: No detectable signal observed in the target specific channel.
- 3x LOD: All samples were detected in the target specific channel.
- The 1,191 IU/mL sample values remained between ±0.38 log10 IU/mL of the baseline control average at time (T0).

The data collected supported a clinical specimen stability of whole blood at room temperature for 24 hr, and plasma at room temperature for 24 hr and at -20C for two months.

Kit Stability

The stability of the *artus* CMV RGQ MDx Kit and kit reagents under possible usage conditions was determined. Five different aspects of kit stability were examined:

- Closed Bottle Stability: Demonstrates stability of the kit under intended storage conditions.
- Component In Use Stability: Demonstrates stability of reagents under simulated usage conditions.
- Reaction Mix In Use Stability: Demonstrates stability of prepared Reaction Mix under simulated usage conditions.
- Open Bottle Stability: Demonstrates stability of reagents that have been opened and returned to storage conditions.
- Transport Simulation Stability: Demonstrates stability of reagents that have been handled under simulated shipping conditions.

For each part, reagents were stored under prescribed conditions, and then used to perform the *artus* CMV RGQ MDx Kit assay. To demonstrate stability, the assay acceptance criteria must have been met, as well as the criteria established for a panel of samples.

The samples used in the stability testing include kit controls, derivatives of kit controls, and samples intended to simulate clinical specimens at two different concentrations (Table 22). For the purposes of this study, the medical decision points were defined as those within the linear range of the assay and near the limit of detection. For the *artus* CMV RG PCR kits to be considered stable, these test samples should produce results that fall within predefined acceptance ranges throughout the course of the study. These ranges were the same at both baseline (i.e., time zero) and each subsequent time point.
For the assay run controls, functional testing at each time point should yield results that fall within the pre-defined acceptance criteria necessary to validate the run. The acceptance criteria for these run controls were established during the development of the assay, and are applicable both at baseline and at each subsequent time point.
The QS4 1:10 dilution sample was prepared to resemble a CMV DNA concentration near the limit of detection. A stable product should detect this sensitivity-challenging sample throughout its shelf life. The negative, and the 397 and 397,000 IU/mL samples reflect clinical samples, they consist of CMV particles spiked into negative EDTA-plasma. The 397 IU/mL sample (near LoD), should produce positive results throughout the course of the stability study. The acceptance criteria for the 397,000 IU/mL sample were based on an internal evaluation which accounted for the viral load variability of this particular sample under non-stressed test conditions. The viral load measurement of this sample should fall within this acceptable range, both at baseline and at each subsequent time point (Table 22).

Table 22: Samples Included in Kit Stability Testing

Sample	Acceptance Criteria	Description
NTC	Not detected	Assay Acceptance Criteria
QS4	C_T: 27-34	Assay Acceptance Criteria
QS3	C_T: 24-30	Assay Acceptance Criteria
QS2	C_T: 21-27	Assay Acceptance Criteria
QS1	C_T: 18-24	Assay Acceptance Criteria
Negative Sample	Not detected	CMV negative plasma sample
397 copies/mL Sample	Detected	CMV Virus spiked into CMV- negative plasma near LOD
QS 4 (1:10)- 4 replicates	Detected	CMV DNA sample near LOD (QS4 diluted 1:10)
CMV RG LPC	250-1,418 IU/mL	Assay Acceptance Criteria; CMV DNA sample in linear range
CMV RG HPC	13,353-42,400 IU/mL	Assay Acceptance Criteria; CMVDNA sample in linear range

397,000 IU/mL Sample	4.90-6.01 $\log_{10}$ IU/mL	CMV Virus spiked into CMV-negative plasma in linear range
NTC (IC Channel)	C_T: 22.65-28.65	Assay Acceptance Criteria

The kit stability of the *artus* CMV RGQ MDx Kit is defined in Table 23.

Table 23: Kit Stability

Stability Type	Storage Condition	Duration
Closed Bottle Stability	-15°C to -30°C	24 months
Component In Use Stability	2°C to 8°C	5hr
	Room Temperature	2 hr
Master Mix In Use Stability	2°C to 8°C	5 hr
	Room Temperature	2 hr
Open Bottle Stability	-15°C to -30°C after closed bottle	24 months
Transport Simulation Stability	-15°C to -30°C or dry ice	24 months

Reproducibility Study

The reproducibility of the *artus* CMV RGQ MDx Kit was evaluated using 3 sites. A 10 member panel with 5 simulated specimens (2 of each in the panel) including negatives, high negatives, low positives, moderate positives and high positives was provided for testing. The 10 member panel was tested in duplicate by two 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 24 and 25, below 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 ten panels across lot, site, operator, day, between run and within-run.

Table 24: 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

				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**
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 log_{10}IU/mL, 95th percentile=1.62 log_{10}IU/mL, Maximum =1.90 log_{10}IU/mL							

Table 25: 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	81 IU/mL	144	51.9%	11.1%	15.5%	0%	0%	0%	56.1%
Moderate Positive	912 IU/mL	144	32.1%	13.2%	9.7%	17.2%	0%	3.7%	41.0%
High Positive	107,152 IU/mL	144	11.1%	6.0%	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. The following table 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 26: 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)

Conclusions Drawn from the Non Clinical Studies

The *artus* CMV RGQ MDx Kit assay was evaluated to demonstrate performance claims for Traceability, LOD, LOQ, linearity, cross-reactivity, exogenous and endogenous

interference, carryover, precision/reproducibility, and sample/reagent kit stability. The results of the non-clinical studies, in conjunction with results of the clinical trial studies below, support the intended use statement of the *artus* CMV RGQ MDx Kit assay.

X. SUMMARY OF PRIMARY CLINICAL STUDIES

The clinical usefulness of the *artus* CMV RGQ MDx Kit for the quantitation of human cytomegalovirus (CMV) DNA in human EDTA plasma to assess virological response to antiviral therapy as an aid in the management of solid organ transplant patients who are undergoing anti-CMV therapy was determined by the clinical study described below. Data from this clinical study were the basis for the PMA approval decision. A summary of the study is presented below.

Clinical Usefulness Study

A. Study Design

The clinical performance of the *artus* CMV RGQ MDx Kit was evaluated during a prospective multi-center study at 5 clinical study collection and/or testing sites, separated geographically within 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), during the course of anti-viral treatment (ganciclovir or valganciclovir), and/or day 49 post-treatment/end of treatment. 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 out of 44 eligible subjects.

The statistical methodology used for the analyses is presented below, under "Statistical Methods and Data Analysis". The performance assessment and outcome is presented below, under "Effectiveness Results".

1. Clinical Inclusion and Exclusion Criteria

Enrollment in the study was limited to subjects meeting the following inclusion criteria:

- Subjects must have had a kidney transplant and present to a hospital, clinic or physician's office for post-transplantation care.
- Subjects must be equal or greater than 18 years of age.
- Subjects providing informed consent.
- Subjects must have a CMV DNAemia as demonstrated by a positive result by the site's CMV-PCR-standard of care test.
- Subjects must be candidates for, and will be treated with, ganciclovir and/or valganciclovir antiviral therapy.

Subjects were not permitted to enroll in the study, and specimens were not used in the study, if they met any of the following exclusion criteria:

- Subjects wherein the HIV status is positive.
- Specimens with less than 1.0 mL EDTA plasma for *artus* CMV RGQ MDx Kit testing.
- Subjects, from whom samples were collected, handled and/or stored inappropriately and/or determined to be unsatisfactory for processing/testing with the *artus* CMV RGQ MDx Kit (for which an explanation is provided in the case of subject exclusion).
  - EDTA plasma specimens that have been stored inappropriately which include the following storage conditions: whole blood that has been frozen; whole blood processed for plasma more than 24 hours after collection; plasma stored at room temperature for more than 24 hours or 4°C for more than 5 days or -20°C for more than 6 months; frozen plasma with more than two freeze/thaw cycles;
  - Extracted nucleic acid that has been stored inappropriately which include the following storage conditions: extracted DNA stored for more than 5 days at 4°C, or longer than six months at -20°C; frozen nucleic acid with more than two freeze/thaw cycles.
- Specimens that had been stored inappropriately for testing with that test used by the site to demonstrate a CMV DNAemia. (A site specific memo was provided to QIAGEN on appropriate specimen storage conditions.)
- Subjects currently on antiviral therapy, or who have been treated for CMV infection with ganciclovir and/or valganciclovir within 30 days prior to enrollment.

2. Follow-up Schedule/Study Visits

Each subject enrolled in the study was required to come for follow-up visits after initial enrollment. The total number of visits depended on the medical practice at each site; however the clinical protocol defined a minimal number of visits and time points. Table 27 below shows a summary of the required visits for the study protocol.

Table 27: Summary of Study Visits

Visit Type	Description	Collection Schedule
Enrollment Visit/Baseline	Therapy starts	±1 day from initiation of therapy
Initial Follow-up Testing	Follow-up Testing Period #1 (FTP#1)	• Either 7± 2 days, or every 3 ± 1 days after the initiation of antiviral therapy. • Collections during FTP#1 will continue for 5 weeks after the initiation of therapy, or until the termination of antiviral therapy, whichever comes first.

Visit Type	Description	Collection Schedule
Long-Term Follow-up Testing	Follow-up Testing Period #2 (FTP#2)	• Every 7 ± 3 days after the beginning of week 5 of antiviral therapy. • Collections during FTP#2 will continue for the next 7 weeks, or until the termination of antiviral therapy, whichever comes first.
Study Exit for treatment responders	Study Exit	Termination of antiviral therapy, or 84 ± 7 days after the initiation of therapy
Follow-up testing and Study Exit for treatment non-responders/treatment failures	Collection when medically indicated as per standard medical practice at each site	
	Study Exit	84 ± 7 days after the Enrollment Visit
Early Study Exit	Therapy change unrelated to clinical assessments	anytime

3. Clinical Endpoints

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 comparator test results.

4. Statistical Methods and Data Analysis

The statistical analysis for the clinical studies included analysis of the data from the prospective clinical study to show agreement between the *artus* CMV RGQ MDx Kit and the FDA-approved comparator test with regards to when to initiate antiviral therapy and when to stop antiviral treatment or resolution of CMV DNAemia. In addition, a method comparison and a negative (IgG Negative) cohort analysis was performed.

4.1. Prospective Clinical Study Statistical Methods

For the prospective clinical study, the *artus* CMV RGQ MDx Kit was compared to the FDA-approved comparator test and data were presented in 2x2 matrices. The data was presented in 2x2 tabular format to compare agreement at the different baseline thresholds, and to compare agreement at resolution of CMV episode by Day 14, 21, 28, 35 and where available >35 days. Resolution of CMV

DNAemia episode was defined by two consecutive CMV viral load measurements below the LOQ, on different days. In addition to the 2x2 tables, data were presented in 6x6 matrices for all evaluable specimens in the study for Day 14, 21, 28, 35 and where available >35 days, and combined.

4.2. Method Comparison Statistical Methods

In the method comparison analysis, the data from the clinical study were used. In addition to the data from the clinical study, data from a panel of 88 samples, made up of cultured CMV diluted in human plasma and tested at 3 of the clinical testing sites, were used for the method comparison analysis. The method comparison analysis included the following:

- Bias scatter plots based on the *artus* CMV RGQ MDx Kit results (Y axis) and FDA-approved test results (X axis) for each of the 3 testing sites and all sites combined. Regression analyses were performed, and the slope and intercept with 95% Confidence Intervals (CIs) were calculated. The regression analysis included both Deming Regression and Passing-Bablok Regression.

  To assess potential correlations due to data obtained from the same subject (even if at different time points) the 95% confidence intervals for the regression analyses were re-estimated using bootstrap sampling at the subject level.

- 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 were generated, 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.

4.3. Negative (CMV IgG Neg) Arm Statistical Methods

For the negative arm of the study, the *artus* CMV RGQ MDx Kit was compared to the FDA-approved comparator test and data were presented in 3x3 tables.

B. Accountability of PMA Cohort

1. Number of Subjects Enrolled/Eligible

A total of 53 subjects from five clinical sites with a diverse geographical distribution throughout the US was enrolled in the prospective clinically significant arm of this evaluation and a total of 58 subjects was enrolled in the negative CMV arm.

In the clinically significant arm, 44 subjects were eligible and 9 were excluded. Of these 9 subjects excluded, seven were found to not meet the inclusion criteria, one had the specimens not collected according to study instructions, and for one the initiation of antiviral start and stop could not be verified.

For the CMV negative arm, out of the 58 subjects enrolled, there were 16 subjects that were excluded due to not meeting the enrollment criterion of being CMV IgG negative. Therefore, there were 42 eligible subjects in the negative CMV arm.

There were challenges encountered during the enrollment process throughout the clinical evaluation: in 2009 during the site selection process, approximately 60 sites were contacted and, of these sites, eight collection sites showed interest and agreed to participate. Of the eight collection sites, three dropped out in the first year with no patients enrolled, one of them being a large kidney transplant center in the United States. The prophylactic treatment of subjects was the primary reason for not meeting enrollment criteria. Table 28 below shows a summary of the number of subjects enrolled in the clinical study.

Table 28: Summary of Subjects Enrolled/Eligible

Site	Subjects Enrolled	Subjects Excluded	Subjects Eligible
Clinically Significant Arm			
Site 1	36	5	31
Site 2	5	0	5
Site 3	4	1	3
Site 4	3	0	3
Site 5	5	3	2
Total	53	9	44
CMV Negative Arm			
Site 1	6	0	6
Site 2	37	7	30
Site 3	0	0	0
Site 4	15	9	6
Site 5	0	0	0
Total	58	16	42

2. Number of Specimens Collected/Evaluable

A total of 424 specimens were collected in the clinical significant arm and a total of 58 specimens were collected in the CMV negative arm.

Of the 424 specimens in the clinically significant arm, 398 were from eligible subjects. Specimens from the eligible subjects were considered themselves eligible if:

- the plasma volume was sufficient for analysis as specified in the clinical protocol, and
- the specimen was collected, stored and analyzed properly as specified in the clinical protocol.

Based on these aforementioned criteria, of the 398 specimens, 18 were not eligible for the following reasons: 9 did not have the plasma collected within 24 hours of blood collection; for one the collection time was unknown; two had less than 1 mL of blood collected; four were outside the window of the baseline visit and two specimens were either not collected or misplaced. This left a total of 380 eligible specimens.

Specimens were considered evaluable if they met the eligibility criteria and there was a complete data set for both, the *artus* CMV RGQ MDx Kit and the FDA-approved testing.

Of the 380 eligible specimens, there were 12 specimens that were not evaluable. For six (6) of the 12 specimens there was insufficient volume to perform the FDA-approved comparator test. For one there was insufficient volume to repeat the *artus* CMV RGQ MDx Kit test due to an invalid result. For two there was insufficient volume and both the *artus* CMV RGQ MDx Kit and the FDA-approved comparator test were not performed. Three specimens did not have a valid FDA-approved test result.

Consequently, there was a total of 368 specimens that were considered eligible and evaluable.

For the CMV negative arm, of the 58 specimens collected (one per subject), 16 were excluded due to a positive serological CMV status of the subject. Therefore, there were a total of 42 evaluable specimens.

Table 29 below presents a summary of the specimens collected in the clinical study.

Table 29: Summary of Specimens Collected/Evaluable

Site	Specimens Collected	Specimens Excluded	Specimens Evaluable
Clinically Significant Arm			
Site 1	281	15	258
Site 2	41	0	37
Site 3	32	0	32
Site 4	33	3	30
Site 5	11	0	11
Total	398	18	368

CMV Negative Arm			
Site 1	6	0	6
Site 2	37	7	30
Site 3	0	0	0
Site 4	15	9	6
Site 5	0	0	0
Total	58	16	42

C. Study Population Demographics and Baseline Parameters

The demographics of the study population are typical for this type of study performed in the US.

1. Age Distribution

The following tables present the distribution of age at time of transplant and at baseline visit, respectively, among the 44 evaluable subjects at the 5 collection sites participating in this clinical study. For one subject of the 44 evaluable subjects, the exact date of transplant was unknown and therefore was not included in the age at transplant analysis.

Table 30: Distribution of Subject Age at Time of Transplant Stratified by Collection Site among Evaluable Subjects

Site	N	Mean	Med	Min	Max
Site 1	30	49	47	22	71
Site 2	5	34	35	19	47
Site 3	3	49	43	42	62
Site 4	3	51	48	36	67
Site 5	2	45	45	26	64
Overall	43	47	46	19	71

Table 31: Distribution of Subject Age at Baseline Visit Stratified by Collection Site among Evaluable Subjects

Site	N	Mean	Med	Min	Max
Site 1	31	49	49	22	72
Site 2	5	46	46	44	47
Site 3	3	57	58	51	63
Site 4	3	52	49	39	68
Site 5	2	46	46	27	65
Overall	44	49	48	22	72

2. Race Distribution

Table 32 shows the distribution of race among the 44 evaluable subjects enrolled at the 5 collection sites participating in this clinical evaluation. The race data reflects the expected demographic patterns for the locations where the specimens were collected.

Table 32: Distribution of Subject Race

Race	**N**	**%**
Asian	6	13.6%
Black/African American	14	31.8%
Hispanic or Latino	13	29.5%
Native Hawaiian/Other Pacific Islander	1	2.3%
White/Caucasian/Not Hispanic or Latino	9	20.5
Other	1	2.3%

3. Gender Distribution

Table 33 shows the distribution of gender among the 44 evaluable subjects (25 males, 19 females) enrolled at the 5 collection sites participating in this clinical evaluation.

Table 33: Distribution of Subject Gender

Gender	**Male**		**Female**	
Site	**N**	**%**	**N**	**%**
Site 1	18	58.1%	13	41.9%
Site 2	2	40.0%	3	60.0%
Site 3	1	33.3%	2	66.7%
Site 4	2	66.7%	1	33.3%
Site 5	2	100.0%	0	0.0%
Overall	25	56.8%	19	43.2%

D. Safety and Effectiveness Results

1. Safety Results

The *artus* CMV RGQ MDx Kit is an *in-vitro* diagnostic test that requires specimens derived from a subject's blood sample; therefore it may involve removal of blood from an individual for testing purposes. The test presents safety hazards similar to those for any other test where blood is drawn.

Risks of false positive and false negative results are discussed in Section VIII. Briefly, a false negative result may lead to inappropriate patient management decisions, a premature discontinuation of antiviral therapy, or may instill a false sense of security in a patient or clinician. Theoretically, a less sensitive assay

could lead to earlier discontinuation of treatment relative to an alternative assay, with perhaps greater risk of relapse. A false positive result may contribute to a change in therapy, unnecessary treatment, prolonged duration of therapy, or create anxiety in the patient.

There were no significant adverse effects of the device related to these risks reported while the study was conducted.

2. Effectiveness Results

Prospective Clinical Study Results

Agreement at Baseline Threshold Values

Since no definitive baseline threshold value of CMV for initiation of anti-viral therapy is available, the applicant selected arbitrary values based on the fact that most of the collected data were in the lower range. The selected values analyzed were "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. Although these lower values might indicate differences between the two tests which would be insignificant when using a higher threshold value, due to clinical practice with prophylactic treatment and surveillance of CMV DNAemia, subjects with a higher CMV threshold value seemed to be low in numbers. Tables 34 and 35 below show the agreement data at baseline threshold values for the clinical study.

Table 34: Summary of *artus* CMV RGQ MDx Kit vs. FDA-approved Test by Threshold – Clinical Study

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 35: Statistical Summary of *artus* CMV RGQ MDx Kit vs. FDA-approved Test by Viremia Threshold – Clinical Study

Clinical Study Threshold	≤ Threshold Agreement (%) (n/N)	> Threshold Agreement (%) (n/N)	Overall Agreement (%) (n/N)
Not Detected	100 (1/1)	100 (43/43)	100 (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 (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%. The 86.36% is at the LOQ threshold where some differences are expected given the two different LOQs 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

The tables below show the data for the agreement analysis for resolution of CMV episode as defined by two consecutive CMV viral load measurements below the LOQ, on different days. Resolution date is then taken as the date of the first of these measurements.

Tables 36 and 37 below show the agreement data for resolution of CMV episode in the clinical study. There was 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 36: 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

			FDA-approved Test		
	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 37: 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% across all days. For the no resolution of CMV episode, the agreement ranges from 50.00% to 100.00%. There were 29.1% (7 out of 24) subjects that were discordant.

Overall Agreement at Different Viral Load Levels and Time Windows

The overall agreement clinical study included the 44 evaluable subjects with the 368 evaluable specimens. 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 and arbitrarily determined viral load values of <LOQ; 500; 1,000; and 10,000 IU/mL, and across different time windows. The different time windows were: at baseline, between day 1 and day 14, between day 15 and day 28, between day 29 and day 42, between day 43 and day 56 and between day 57 and day 70. The number of specimens falling into the respective categories is presented in Table 38. Tables 39 through 45 present the comparison of the *artus* CMV RGQ MDx Kit to the FDA-approved test assessing the CMV viral load across different time points.

There were 13 specimens that fell within a time window that had very low numbers. The overall analysis included the 13 specimens (Table 39) but the time window these specimens fell in was not analyzed separately.

Table 38: 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 39: *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

	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**
≥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 LOQ: 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 40: *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 41: *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 42: *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 43: *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

	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**
≥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 44: *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 45: *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 39 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.

In conclusion, the positive percent agreement (PPA) and negative percent agreement (NPA) for the different CMV thresholds were as follows:
For threshold LOQ: 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)

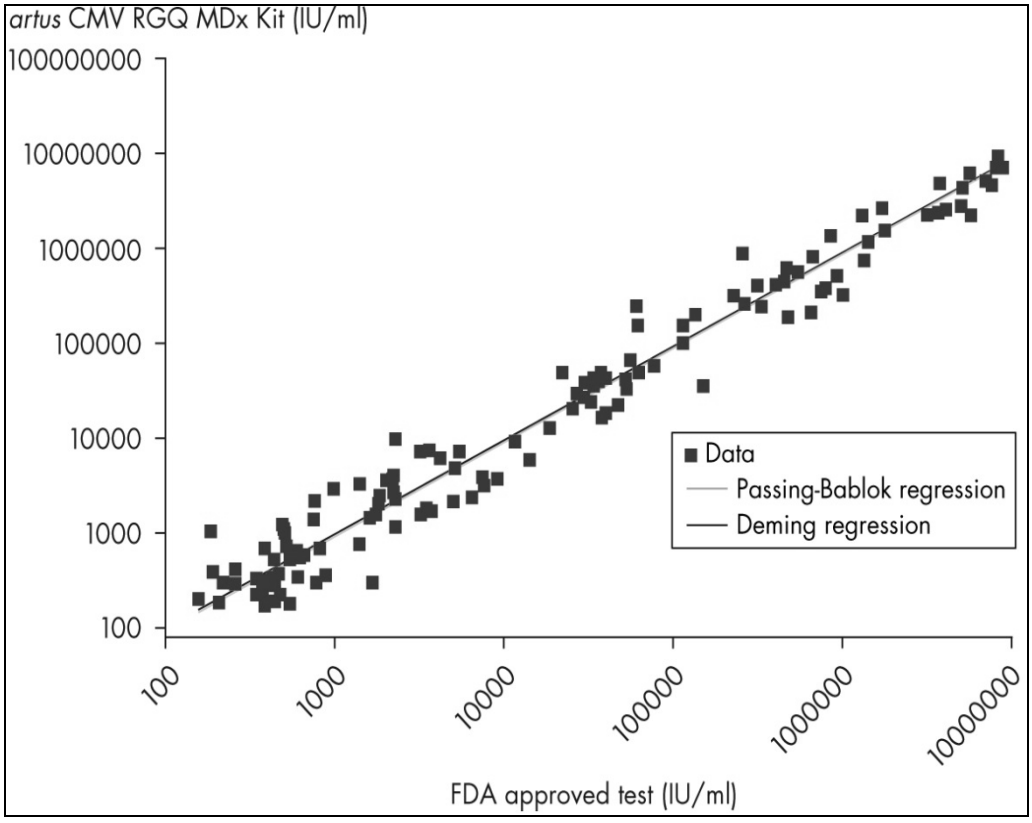
Overall, the *artus* CMV RGQ MDx Kit results and the FDA-approved test results were comparable as far as clinical relevance of the result. In the clinical study, out of the 368 specimens, 343 (93.2%) specimens had results that were comparable to the FDA-approved test with regards to clinical relevance 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 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

For the method comparison study, a total of 73 specimens corresponding to 25 subjects out of the prospective clinical study that had a result above the limit of quantitation (LOQ) of both, the *artus* CMV RGQ MDx Kit and the FDA-approved test were used in the analysis. In order to supplement the clinical specimens with samples across the linear range, 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 test were equally distributed for testing by the *artus* test at three of the five testing sites and by the FDA-approved test at one site.

Figure 5 shows a scatter plot with the results from testing this panel and the positive specimens from the clinical prospective study for all sites combined.

Figure 5: *artus* CMV RGQ MDx Kit vs. FDA approved test scatter plot for $\log_{10}$ IU/mL, all sites



Deming and Passing-Bablok regression analyses were performed. Tables 46 and 47 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 46: Deming Regression Estimates for the *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 47: 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.

Tables 48 and 49 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 48: Passing-Bablok Regression Estimates for the *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 49: 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/mL0	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 multiple time points from the same subject.

As described in the Statistical Methods section above, in addition to the bias scatter plots, the method comparison was analyzed assessing the Allowable Total Difference (ATD) zone 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 $\log_{10}$ 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 50 and 51 show the analyses at the different subintervals.
Figure 6: Allowable Total Difference (ATD) Plot, All Sites Combined

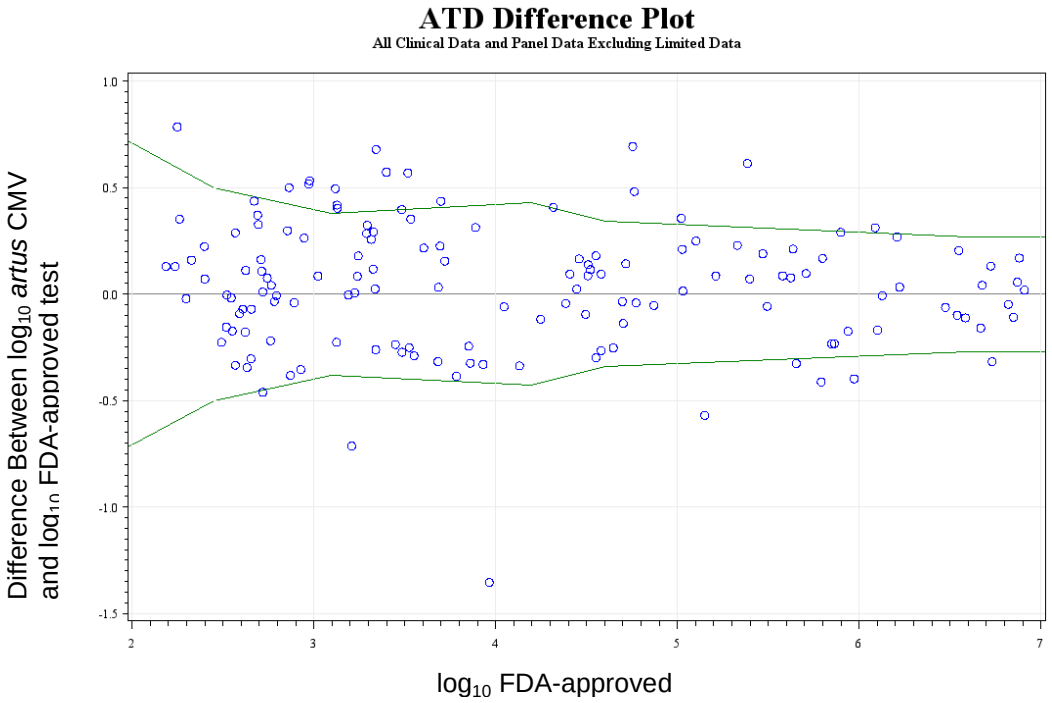


Table 50 below presents the total difference at 2.5th, 5.0th, 95.0th and 97.5th percentiles for the following three measuring subintervals for the FDA-approved test: less than 10,000 IU/mL (4.00 on the $\log_{10}$ scale); between 10,000 and 1,000,000 IU/mL (4.00 and 6.00 on the $\log_{10}$ scale respectively) and greater than 1,000,000 IU/mL (6.00 on the $\log_{10}$ scale).

Table 50: 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 51 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 51: 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 allowable total difference (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 the two 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).

The magnitude of the fold differences for the percentiles illustrate that for those samples lying outside of the ATD Zone there is still reasonable agreement between the *artus* CMV and FDA-approved test and that these differences are not clinically relevant.

Negative (CMV IgG Neg) Arm Results

For the negative arm of the study, a total of 42 evaluable subjects out of the 58 enrolled was analyzed. The *artus* CMV RGQ MDx Kit was compared to the FDA-approved test and data was presented in 3x3 matrices (Table 52).

Table 52: 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.

3. Subgroup Analyses

Apart from the parameters described above, there were no additional characteristics evaluated for potential association with outcomes.

Conclusion

The clinical usefulness study and the statistical analysis of clinical data in this application has shown that serial CMV DNA levels measured with the *artus* CMV RGQ MDx Kit are informative for assessing the CMV virological response to treatment in solid organ transplant patients who are undergoing anti-CMV drug therapy. The clinical studies presented also support the reasonable assurance of safety and effectiveness of this device when used in accordance with the indications for use, the instructions for use as stated in the labeling, the warnings and precautions, and the limitations sections of the labeling.

The data demonstrate that the *artus* CMV RGQ MDx Kit tracks well to the FDA-approved test to assess CMV viral load response to antiviral drug therapy. The overall agreement between the *artus* CMV RGQ MDx Kit and the FDA-approved test is 92.0% averaged over days 7 through 42 in the clinical study for CMV episode resolution status.

The method comparison regression analysis estimates (Deming and Passing-Bablok) show that there is high concordance between the *artus* CMV RGQ MDx Kit and FDA-approved test, with systematic difference less than 0.04 $\log_{10}$ IU/mL.

For the ATD analysis the magnitude of the fold differences for the percentiles illustrate that for those samples lying outside of the ATD Zone (85.2%) there is still reasonable agreement between the *artus* CMV RGQ MDx Kit and FDA-approved test and that these differences are not clinically relevant.

E. Financial Disclosure

The Financial Disclosure by Clinical Investigators regulation (21 CFR 54) requires applicants who submit a marketing application to include certain information concerning the compensation to, and financial interests and arrangement of, any clinical investigator conducting clinical studies covered by the regulation. The pivotal clinical study included six investigators. None of the clinical investigators had disclosable financial interests/arrangements as defined in sections 54.2(a), (b), (c), and (f). The information provided does not raise any questions about the reliability of the data.

XI. SUMMARY OF SUPPLEMENTAL CLINICAL INFORMATION

Guidelines for CMV DNA Testing in Clinical Practice

Published guidelines and the medical literature support the importance of measuring CMV levels prior to treatment and at intervals during treatment [5][6]. The International Consensus Guidelines on the Management of Cytomegalovirus in Solid Organ Transplantation [7] state that laboratory monitoring of CMV should be applied weekly during the treatment phase with a quantitative nucleic acid test (QNAT) or antigenemia-based assay to monitor response and the possible development of resistance. Trends of serial monitoring are easier to interpret than an individual test result. Two consecutive negative samples (preferably sampled one week apart) have been recommended as a virological endpoint for treatment of acute CMV episodes. Periodic viral load monitoring should also be performed during secondary prophylaxis. This document highlights the importance of serial testing of CMV DNA levels, and describes the issues associated with using a "universal" cutoff value for initiating therapy.

XII. PANEL MEETING RECOMMENDATION AND FDA'S POST-PANEL ACTION

In accordance with the provisions of section 515(c)(2) of the act as amended by the Safe Medical Devices Act of 1990, this PMA was not referred to the Microbiology Advisory Panel, an FDA advisory committee, for review and recommendation because the information in the PMA substantially duplicates information previously reviewed by this panel.

XIII. CONCLUSIONS DRAWN FROM PRECLINICAL AND CLINICAL STUDIES

A. Effectiveness Conclusions

The effectiveness of the *artus* CMV RGQ MDx Kit has been demonstrated when used for the quantitation of cytomegalovirus (CMV) DNA in human EDTA plasma. A reasonable determination of effectiveness of the *artus* CMV RGQ MDx Kit for aiding in the management of solid-organ transplant patients who are undergoing anti-CMV therapy, by serially measuring CMV DNA levels at baseline and during treatment to assess virological response to treatment, in conjunction with other laboratory results and clinical information, has been demonstrated.

B. Safety Conclusions

Based on the results of the analytical and clinical laboratory studies, the *artus* CMV RGQ MDx Kit, when used according to the provided directions and in conjunction with other laboratory results and clinical information, should be safe and pose minimal risk to the patient due to false test results.

C. Benefit-Risk Conclusions

The probable benefits of the device are based on data collected in a clinical study and in the analytical studies conducted to support PMA approval as described above. When used for the intended use, benefits to the clinicians and patients include: 1) an

assessment of both the likely time to symptom resolution and the likely time to a decrease in CMV viral load below the lower limit of quantitation in patients post-transplantation being treated for symptomatic CMV infection, and 2) confirmation that CMV viral load is responding to treatment as anticipated.

The risks for the intended use are relatively low. When the assay is used for predicting rapidity of response, the assay would not be used to alter treatment and would therefore essentially pose no risk. The risk is greater when assessing response, i.e., an inaccurate result could lead to either initiating an alternative treatment (i.e., falsely assuming the patient is not responding), or prematurely stopping treatment (i.e., falsely assuming that the patient is below the test lower limit of quantitation), or inadequately prolonging the treatment (e.g., if the device is so sensitive that the event of two consecutive CMV viral load measurements below detection takes longer to occur, or occurrence of false positive result). These risks, however, are substantially mitigated by recommendations for serial measurement of CMV viral load, or by the labelling requirement for sites adopting this test to compare assay results with previously used CMV assays to understand the relationship between the two assays for managing patients accordingly.

In conclusion, given the available information presented above, the data support that, for the *artus* CMV RGQ MDx Kit, the probable benefits of approving the test strongly outweigh the probable risks from the use of the test.

D. Overall Conclusions

The data in this application support the reasonable assurance of safety and effectiveness of this device when used in accordance with the indications for use. The data from the preclinical studies demonstrated acceptable analytical sensitivity, traceability, linearity, precision, and analytical specificity of the *artus* CMV RGQ MDx Kit when used according to the instructions for use as stated in the labeling, the warnings and precautions, and limitations sections of the labeling. The clinical usefulness study and the statistical analysis of clinical data in this application has shown that serial CMV DNA levels measured with the *artus* CMV RGQ MDx Kit are informative for assessing the virological response to treatment in solid organ transplant patients who are undergoing anti-CMV drug therapy, and that the test is safe and effective when used according to the directions for use in the labeling.

XIV. CDRH DECISION

CDRH issued an approval order on June 2, 2014. The final conditions of approval can be found in the approval order.

The applicant's manufacturing facilities have been inspected and found to be in compliance with the device Quality System (QS) regulation (21 CFR 820).

XV. APPROVAL SPECIFICATIONS

Directions for use: See device labeling.

Hazards to Health from Use of the Device: See Indications, Contraindications, Warnings, Precautions, and Adverse Events in the device labeling.

Post-approval Requirements and Restrictions: See approval order.

XVI. REFERENCES

[1] Fryer JF, Heath AB, Anderson R, Minor PD and The Collaborative Study Group. 2010. Collaborative Study to Evaluate the Proposed 1st WHO International Standard for Human Cytomegalovirus (HCMV) for Nucleic Acid Amplification (NAT)-Based Assays. WHO ECBS Report WHO/BS/10.2138

[2] Clinical and Laboratory Standards Institute (CLSI) (2004) Protocols for Determination of Limits of Detection and Limits of Quantitation. CLSI document EP17-A2. CLSI. 940 West Valley Road; Suite 1400, Wayne; Pennsylvania 19087-1898 USA.

[3] Clinical and Laboratory Standards Institute (CLSI) (2004) Interference Testing in Chemistry; Approved Guideline. CLSI document EP07-A2. CLSI. 940 West Valley Road; Suite 1400, Wayne; Pennsylvania 19087-1898 USA.

[4] Clinical and Laboratory Standards Institute (CLSI) (2004) Evaluation of Precision Performance of Quantitative Measurement Methods. Approved Guideline-Second Edition. CLSI document EP05-A2. CLSI. 940 West Valley Road; Suite 1400, Wayne; Pennsylvania 19087-1898 USA.

[5] Mandell L. et al. Principles and Practice of Infectious Diseases. Churchill Livingstone; 7th edition, 2009

[6] Razonable RR, Emery VC. International Herpes Management Forum (IHMF) Management Recommendations: Management of CMV Infection and Disease in Transplant Patients. HERPES 2004; 11 (3): 77-86

[7] Kotton CN, Kumar D, Caliendo AM, et al, Transplantation Society International CMV Consensus Group. International consensus guidelines on the management of cytomegalovirus in solid organ transplantation. *Transplantation*. 2010;89(7):779-795.