February 2018

artus® CMV QS-RGQ Kit: Performance characteristics

R4

IVD

CE
0197

REF

4503363, *artus* CMV QS-RGQ Kit, Version 1.

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

Limit of detection – plasma

The limit of detection (LOD) in consideration of the purification (sensitivity limit) was assessed for the *artus* CMV QS-RGQ Kit using CMV-positive clinical specimens in combination with the extraction on the QIAsymphony® SP.

For plasma, the LOD in consideration of the purification of the *artus* CMV QS-RGQ Kit was determined using a dilution series of CMV virus material from 1000 to nominal 0.316 CMV copies/ml spiked in clinical plasma specimens. These were subjected to DNA extraction using the QIAsymphony DSP Virus/Pathogen Midi Kit in combination with the Cellfree1000_DSP protocol (extraction volume: 1 ml, elution volume: 60 µl). Each of the 10 dilutions was analyzed with the *artus* CMV QS-RGQ Kit on 4 different days in 4 runs with 8 replicates each. The results were determined by a probit analysis. A graphical illustration of the probit analysis is shown in Figure 1. The LOD in consideration of the purification of the *artus* CMV QS-RGQ Kit in combination with the Rotor-Gene Q is 42.5 copies/ml (p = 0.05). This means that there is a 95% probability that 42.5 copies/ml (corresponding to 69.7 IU/ml) will be detected.

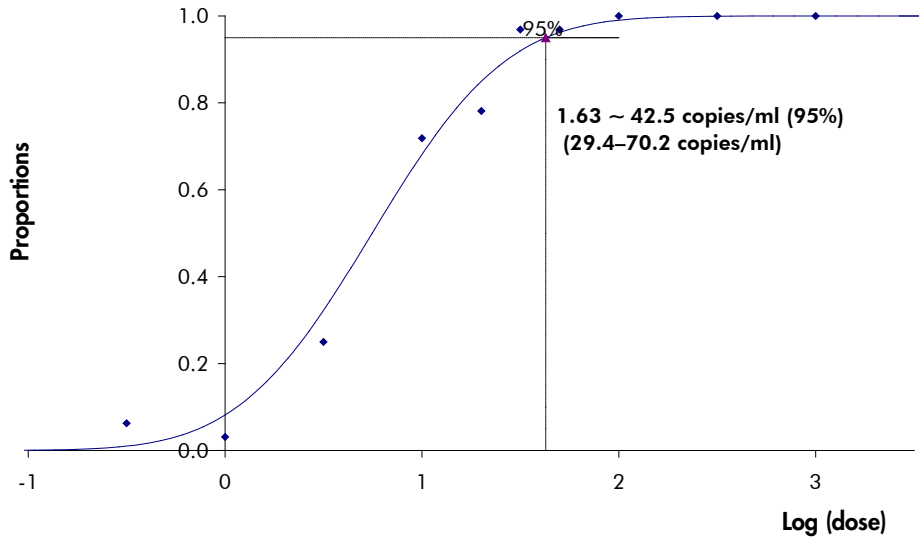

Figure 1. Probit analysis: plasma, CMV (Rotor-Gene Q). Limit of detection in consideration of the purification (plasma, using the QIAsymphony DSP Virus/Pathogen Midi Kit) of the *artus* CMV QS-RGQ Kit on the Rotor-Gene Q.

Specificity – plasma

The specificity of the *artus* CMV QS-RGQ Kit is first and foremost ensured by the selection of the primers and probes, as well as the selection of stringent reaction conditions. The primers and probes were checked for possible homologies to all sequences published in gene banks by sequence comparison analysis. The detectability of all relevant genotypes has thus been ensured.

Moreover, the specificity was validated with 100 different CMV negative plasma samples. These did not generate any signals with the CMV specific primers and probes, which are included in the CMV RG Master.

A potential cross-reactivity of the *artus* CMV QS-RGQ Kit was tested using the control group listed in Table 1 (below). None of the tested pathogens has been reactive. No incidences of cross-reactivities were observed with mixed infections.

Table 1. Testing the specificity of the kit with potentially cross-reactive pathogens

Control group	CMV (Cycling Green)	Internal control (Cycling Yellow)
Human herpesvirus 1 (Herpes simplex virus 1)	–	+
Human herpesvirus 2 (Herpes simplex virus 2)	–	+
Human herpesvirus 3 (Varicella-zoster virus)	–	+
Human herpesvirus 4 (Epstein-Barr virus)	–	+
Human herpesvirus 6A	–	+
Human herpesvirus 6B	–	+
Human herpesvirus 7	–	+
Human herpesvirus 8 (Kaposi's sarcoma-associated herpesvirus)	–	+
Hepatitis A virus	–	+
Hepatitis B virus	–	+
Hepatitis C virus	–	+
Human immunodeficiency virus 1	–	+
Human T cell leukemia virus 1	–	+
Human T cell leukemia virus 2	–	+
West Nile virus	–	+
Enterovirus	–	+
Parvovirus B19	–	+

Linear range — plasma

The linear range in consideration of the purification of the *artus* CMV QS-RGQ Kit was determined by analyzing a dilution series of CMV virus material ranging from 1.00 x 10^8 copies/ml to 3.16 x 10^1 copies/ml in plasma. The purification was carried out in replicates (n = 4 each for concentrations ≥1.00 x 10^7 copies/ml; n = 8 each for concentrations <1.00 x 10^7 copies/ml) using the QIAsymphony DSP Virus/Pathogen Midi Kit in combination with the Cellfree1000_DSP protocol (extraction volume: 1 ml, elution volume: 60 µl). Each of the samples was analyzed using the *artus* CMV QS-RGQ Kit. The linear range in consideration of the purification of the *artus* CMV QS-RGQ Kit has been determined to cover concentrations from 7.94 x 10^1 copies/ml to 1.00 x 10^8 copies/ml (corresponding to 1.30 x 10^2 to 1.64 x 10^8 IU/ml) for plasma (Figure 2).

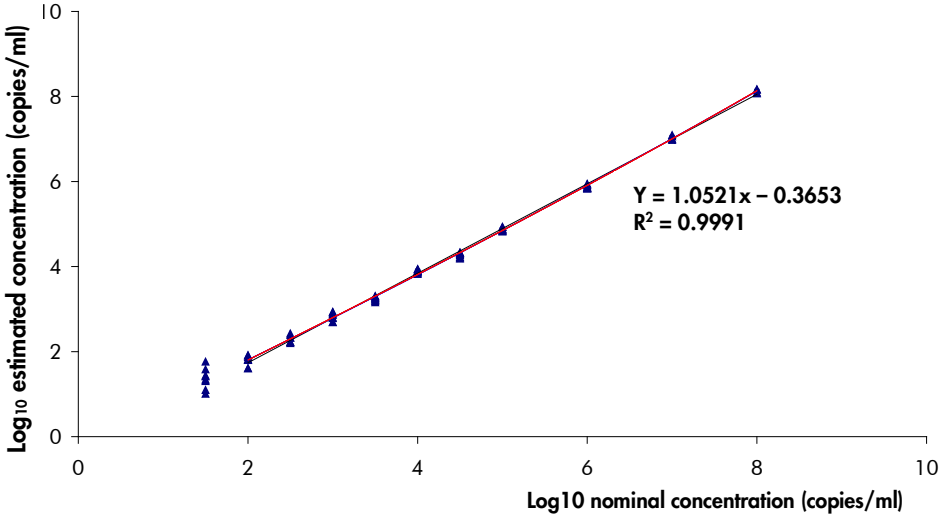

Figure 2. Linear range of the *artus* CMV QS-RGQ Kit (plasma). Calculation of the linear range. The straight line was determined by a linear regression of the $\log_{10}$ calculated concentrations with the $\log_{10}$ nominal concentrations. The equation of the regression line is included in the figure.

Robustness — plasma

The verification of the robustness allows the determination of the total failure rate of the *artus* CMV QS-RGQ Kit. To verify the robustness, 100 CMV negative samples of plasma were spiked with 130 copies/ml of CMV (approximately 3-fold concentration of the LOD). After extraction using the QIAsymphony DSP Virus/Pathogen Midi Kit in combination with the Cellfree1000_DSP protocol for plasma (extraction volume: 1 ml, elution volume: 60 µl), these samples were analyzed with the *artus* CMV QS-RGQ Kit. In addition, the robustness of the internal

control was assessed by purification and analysis of the 100 spiked plasma samples. Inhibitions were not observed. Thus, the robustness of the *artus* CMV QS-RGQ Kit is ≥99%.

Interfering substances – plasma

Four endogenous substances (bilirubin, hemoglobin, triglyceride and albumin protein) at an elevated concentration have been identified as potential interfering substances present in EDTA-plasma samples. Their effects were evaluated in plasma containing CMV at approximately 10-fold the LOD value (425 copies/ml). As a control, CMV spiked plasma samples without addition of any interfering substance were included. All samples, with or without addition of interfering substances, were analyzed in 4 replicates using the QIAsymphony DSP Virus/Pathogen Midi Kit in combination with the Cellfree1000 protocol (extraction volume: 1 ml, elution volume: 60 µl). For samples containing elevated levels of endogenous inhibitors (bilirubin 30mg/dl, hemoglobin 2g/dl, triglyceride 1g/dl, and albumin protein 6g/dl), no interference was observed for CMV detection.

Clinical evaluation – plasma

The clinical performance of the *artus* CMV QS-RGQ Kit was evaluated by testing clinical specimens and analyzing the findings against the results from a comparable method. A total of 174 plasma specimens collected in EDTA tubes from CMV infected patients or prepared artificially using the first WHO standard for CMV as well as from negative controls were tested with the *artus* CMV QS-RGQ Kit and the comparable method at an external site. The qualitative agreement of both kits was 100%. Deming and Passing-Bablok regression analysis was performed with the QIAGEN kit test result on the Y-axis and the comparator test result on the X-axis (see Figure 3). The estimated difference in $\log_{10}$ (IU/mL) at the medical decision point (1000 IU/mL) between the QIAGEN kit and comparator kit was 0.074 $\log_{10}$ IU/ml, as calculated from the Deming regression.

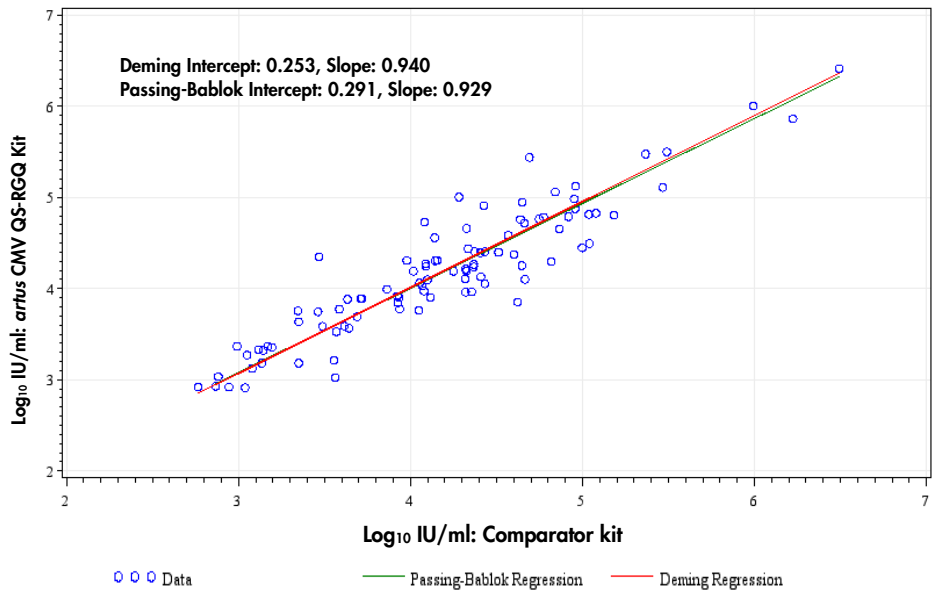

Figure 3. Regression plot with Passing-Bablok and Deming lines (plasma). Samples that were between the lower limit of quantification (LLOQ) and the upper limit of quantification (ULOQ) for both kits were included in the analysis.

A Bland-Altman plot was produced to look at the difference in calculated $\log_{10}$ (IU/ml). Furthermore, the mean $\log_{10}$ (IU/ml) difference and its corresponding 95% range was calculated, and overlaid on the plot (see Figure 4).

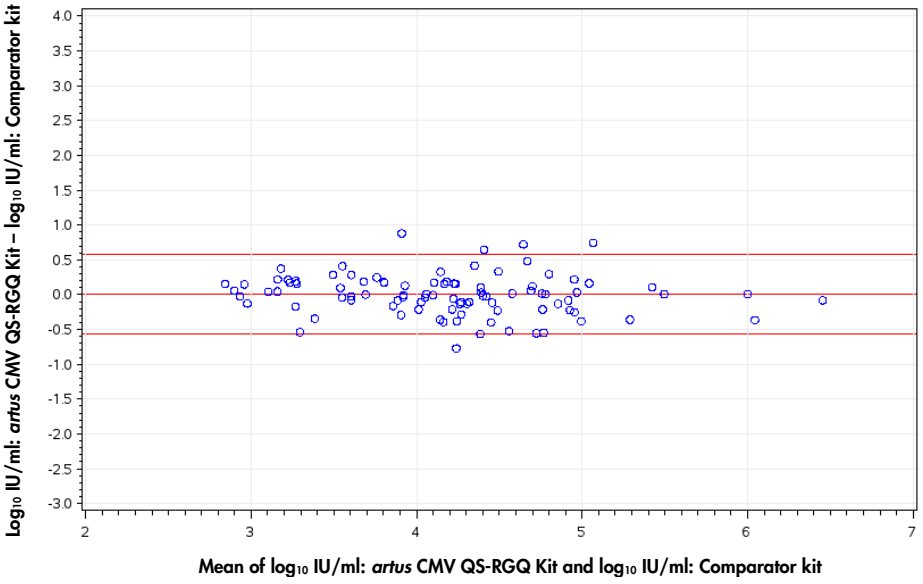

Figure 4. Bland-Altman plot (plasma). Horizontal reference lines are at 0.00, –0.57, and 0.58 and denote mean difference ($\log_{10}$ IU/ml: *artus* CMV QS-RGQ Kit – $\log_{10}$ IU/ml: Comparator kit) and its corresponding 95% prediction interval. Samples that were between the lower limit of quantification and upper limit of quantification for both kits were included in the analysis.

Limit of detection – whole blood

The LOD in consideration of the purification (sensitivity limit) was assessed for the *artus* CMV QS-RGQ Kit using CMV-positive clinical specimens in combination with the extraction on the QIAsymphony SP.

For whole blood, the LOD in consideration of the purification of the *artus* CMV QS-RGQ Kit was determined using a dilution series of CMV virus material from 1000 to nominal 3.16 CMV copies/ml spiked in human whole blood specimens.

These were subjected to DNA extraction using the QIAsymphony DNA Mini Kit in combination with the VirusBlood200_DSP protocol (extraction volume: 200 µl, elution volume: 60 µl). Each of the 8 dilutions was analyzed with the *artus* CMV QS-RGQ Kit on 3 different days in 6 runs with 11 replicates each. The results were determined by a probit analysis.

A graphical illustration of the probit analysis is shown in Figure 5. The LOD in consideration of the purification of the *artus* CMV QS-RGQ Kit in combination with the Rotor-Gene Q is 164.55 copies/ml (p = 0.05). This means that there is a 95% probability that 164.55 copies/ml (corresponding to 122.59 IU/ml) will be detected.

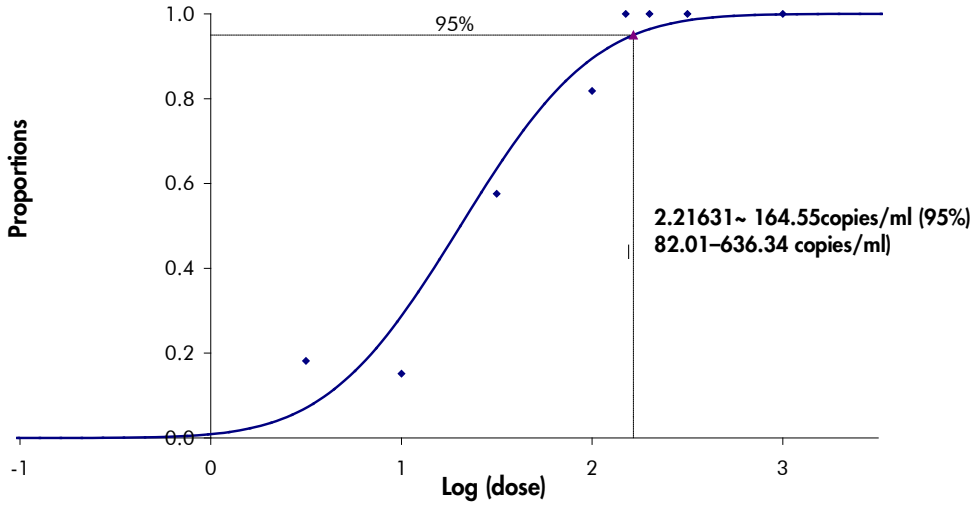

Figure 5. Probit analysis: whole blood, CMV (Rotor-Gene Q). Limit of detection in consideration of the purification (whole blood, using the QIAsymphony DNA Mini Kit) of the *artus* CMV QS-RGQ Kit on Rotor-Gene Q.

Specificity – whole blood

The specificity of the *artus* CMV QS-RGQ Kit is first and foremost ensured by the selection of the primers and probes, as well as the selection of stringent reaction conditions. The primers and probes were checked for possible homologies to all sequences published in gene banks by sequence comparison analysis. The detectability of all relevant genotypes has thus been ensured.

Moreover, the specificity was validated with 100 different CMV negative whole blood samples. These did not generate any signals with the CMV specific primers and probes, which are included in the CMV RG Master.

A potential cross-reactivity of the *artus* CMV QS-RGQ Kit was tested using the control group listed in Table 1 (see page 3). None of the tested pathogens has been reactive. No incidences of cross-reactivity were observed with mixed infections.

Linear range – whole blood

The linear range in consideration of the purification of the *artus* CMV QS-RGQ Kit was determined by analyzing a dilution series of CMV virus material ranging from 5.00×10^7 to 1.00×10^2 in whole blood. The purification was carried out in replicates (n = 4 each for concentrations $\geq 1.00 \times 10^7$ copies/ml; n = 8 each for concentrations $< 1.00 \times 10^7$ copies/ml) using the QIAsymphony DNA Mini Kit in combination with the VirusBlood200_DSP protocol (extraction volume: 200 µl, elution volume: 60 µl). Each of the samples was analyzed using the *artus* CMV QS-RGQ Kit. The linear range in consideration of the purification of the *artus* CMV QS-RGQ Kit has been determined to cover concentrations from 1.00×10^3 copies/ml to 5.00×10^7 copies/ml (corresponding to 7.45×10^2 to 3.73×10^7 IU/ml) for whole blood (Figure 6).

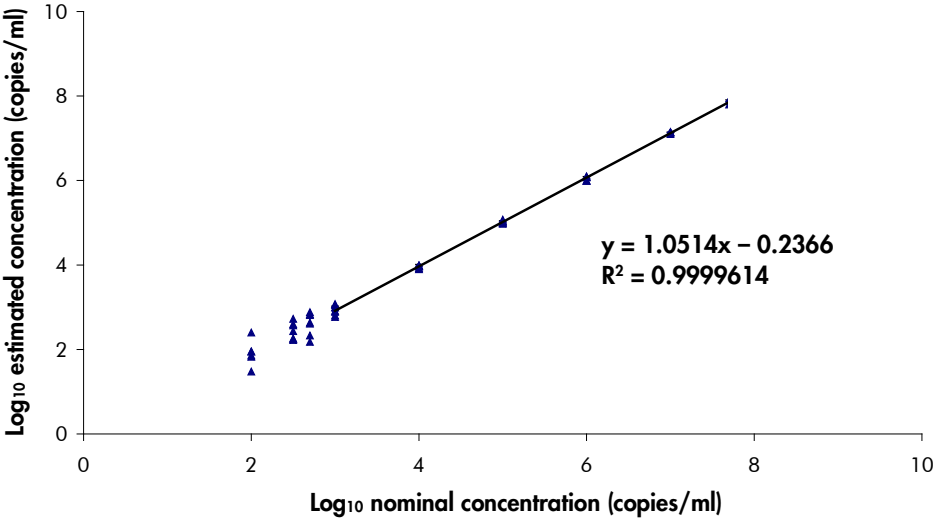

Figure 6. Linear range of the *artus* CMV QS-RGQ Kit (whole blood). Calculation of the linear range. The straight line was determined by a linear regression of the $\log_{10}$ calculated concentrations with the $\log_{10}$ nominal concentrations. The equation of the regression line is included in the figure.

Robustness – whole blood

The verification of the robustness allows the determination of the total failure rate of the *artus* CMV QS-RGQ Kit. To verify the robustness, 100 CMV negative whole blood samples were spiked with 500 copies/ml of CMV (approximately 3-fold concentration of the LOD). After extraction using the QIAsymphony DNA Mini Kit in combination with the VirusBlood200_DSP protocol for whole blood, these samples were analyzed with the *artus* CMV QS-RGQ Kit. In addition, the robustness of the internal control was assessed by purification and analysis of the 100 spiked whole blood samples. Inhibitions were not observed. Thus, the robustness of the *artus* CMV QS-RGQ Kit is ≥99%.

Interfering substances – whole blood

Three endogenous substances (bilirubin, triglyceride and gDNA) at an elevated concentration have been identified as potential interfering substances present in EDTA-whole blood samples. Their effects were evaluated in whole blood containing CMV at approximately 10-fold the LOD value (1650 copies/ml). As a control, CMV spiked whole blood samples without addition of any interfering substance were included. All samples, with or without addition of interfering substances, were analyzed in 4 replicates using the QIAsymphony DNA Mini Kit in combination with the VirusBlood200_DSP protocol (extraction volume: 0.2 ml, elution volume: 60 µl). For samples containing elevated levels of endogenous inhibitors (bilirubin 30mg/dl, triglyceride 1g/dl and gDNA up to 3µg/sample), no interference was observed for CMV detection.

Clinical evaluation – whole blood

The clinical performance of the *artus* CMV QS-RGQ Kit was evaluated by testing clinical specimens and analyzing the findings against the results from a comparable method. A total of 115 clinical specimens of whole blood collected from CMV infected patients as well as from negative controls were tested with the *artus* CMV QS-RGQ Kit and the comparable method at an external site. Deming and Passing-Bablok regression analysis was performed with QIAGEN kit test result on the Y-axis and the comparator test result on the X-axis (see Figure 7).

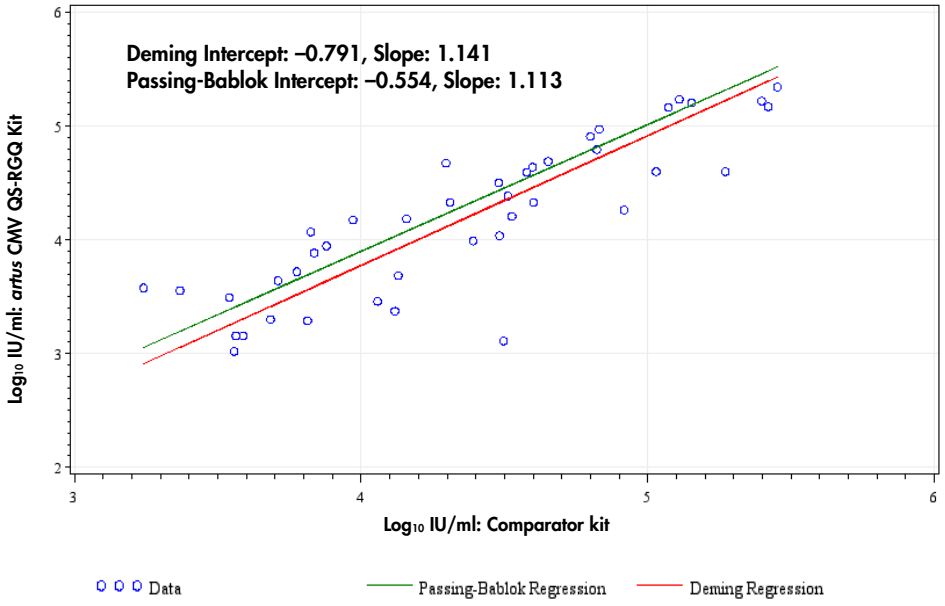

Figure 7. Regression plot with Passing-Bablok and Deming lines (whole blood). Only clinical samples included in the analysis. Samples that were between the lower limit of quantification (LLOQ) and the upper limit of quantification (ULOQ) for both kits were included in the analysis.

A Bland-Altman plot was produced to look at the difference in calculated log_{10}(IU/ml). Furthermore, the mean log_{10}(IU/ml) difference and its corresponding 95% range was calculated, and overlaid on the plot (see Figure 8).

The mean difference in log_{10} (IU/mL) between the QIAGEN kit and comparator kit was 0.18 log_{10} IU/ml. The qualitative agreement of both kits was 100%.

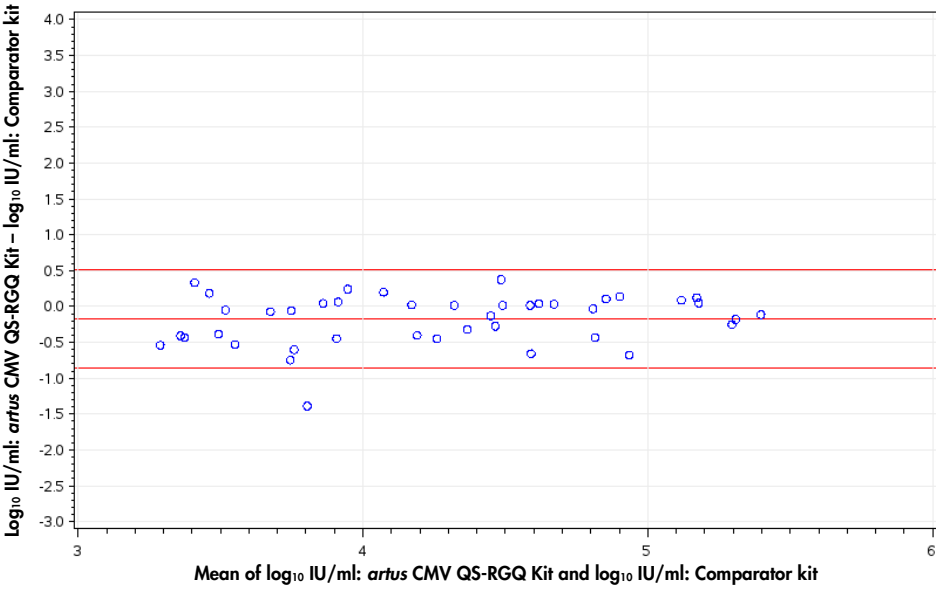

Figure 8. Bland-Altman plot (whole blood). Horizontal reference lines are at –0.18, –0.86 and 0.51 and denote mean difference (log10 IU/ml: *artus* CMV QS-RGQ Kit – log10 IU/ml: comparator kit) and its corresponding 95% prediction interval. Only clinical samples were included in the analysis. Samples that were between the lower limit of quantification and upper limit of quantification for both kits were included in the analysis.

Precision

The precision data of the *artus* CMV QS-RGQ Kit allow determination of the total variance of the assay. The total variance consists of the intra-assay variability (variability of multiple results of samples of the same concentration within one experiment), the inter-assay variability (variability of multiple results of the assay generated on different instruments of the same type by different operators within one laboratory) and the inter-batch variability (variability of multiple results of the assay using various batches). The data obtained were used to determine the standard deviation, the variance and the coefficient of variation for the pathogen specific and the internal control PCR.

Analytical precision data of the *artus* CMV QS-RGQ Kit (without consideration of the purification) were collected using the quantitation standard of the lowest concentration (QS 4; 10 copies/µl). Testing was performed with 8 replicates. The precision data were calculated on basis of the C_T values of the amplification curves (C_T: threshold cycle, see Table 2, page 12). In addition, precision data for quantitative results in copies/µl were determined using the corresponding C_T values (Table 3, page 12). Based on these results, the overall statistical spread of any given sample with the mentioned concentration is 1.21% (C_T) or 14.38% (concentration), and 1.93% (C_T) for the detection of the internal control. These values are based on the totality of all single values of the determined variabilities.

Table 2. Precision data on basis of the C_T values

	Standard deviation	Variance	Coefficient of variation (%)
Intra-assay variability: CMV QS 4	0.17	0.03	0.57
Intra-assay variability: Internal control	0.31	0.10	1.16
Inter-assay variability: CMV QS 4	0.38	0.14	1.27
Inter-assay variability: Internal control	0.47	0.22	1.77
Inter-batch variability: CMV QS 4	0.33	0.11	1.10
Inter-batch variability: Internal control	0.53	0.28	2.02
Total variance: CMV QS 4	0.36	0.13	1.21
Total variance: Internal control	0.51	0.26	1.93

Table 3. Precision data on basis of the quantitative results (in copies/µl)

	Standard deviation	Variance	Coefficient of variation (%)
Intra-assay variability: CMV QS 4	1.34	1.80	13.30
Inter-assay variability: CMV QS 4	1.54	2.38	15.25
Inter-batch variability: CMV QS 4	1.46	2.12	14.41
Total variance: CMV QS 4	1.45	2.11	14.38

Reproducibility

Reproducibility data permit a regular performance assessment of the *artus* CMV QS-RGQ Kit as well as an efficiency comparison with other products. These data are obtained by the participation in established proficiency programs.

Cross-contamination

Absence of cross-contamination between samples for the entire workflow was proven by the correct detection of all known positive and negative samples in alternating positions (checkerboard pattern) for a representative *artus* QS-RGQ system.

Related products and ordering information are listed in the handbook for the *artus* CMV QS-RGQ Kit.

Document revision history	
R4, February 2018	"Analytical sensitivity" changed to "limit of detection" or "LOD"; Added "Interfering substances" information; added values in IU/ml (in addition to existing data in copies/ml) based on the conversion factor information in the respective Application Sheets.

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