October 2015

artus® VZV QS-RGQ Kit: Performance characteristics

artus VZV QS-RGQ Kit, Version 1 REF 4502363

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Analytical sensitivity – CSF

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

The analytical sensitivity in consideration of the purification of the *artus* VZV QS-RGQ Kit was determined using a dilution series of the VZV strain Ellen virus material from 1420 to nominal 4.5 VZV copies/ml spiked in clinical CSF specimens. These were subjected to DNA extraction using the QIAsymphony DSP Virus/Pathogen Mini Kit in combination with the Cellfree200_DSP protocol (extraction volume: 0.2 ml, elution volume: 60 µl). Each of the 8 dilutions was analyzed with the *artus* VZV QS-RGQ Kit on 3 different days in 3 runs with 11 replicates each. The results were determined by a probit analysis. A graphical illustration of the probit analysis is shown in Figure 1. The analytical detection limit in consideration of the purification of the *artus* VZV QS-RGQ Kit in combination with the Rotor-Gene Q is 80.67 copies/ml (p = 0.05). This means that there is a 95% probability that 80.67 copies/ml will be detected.

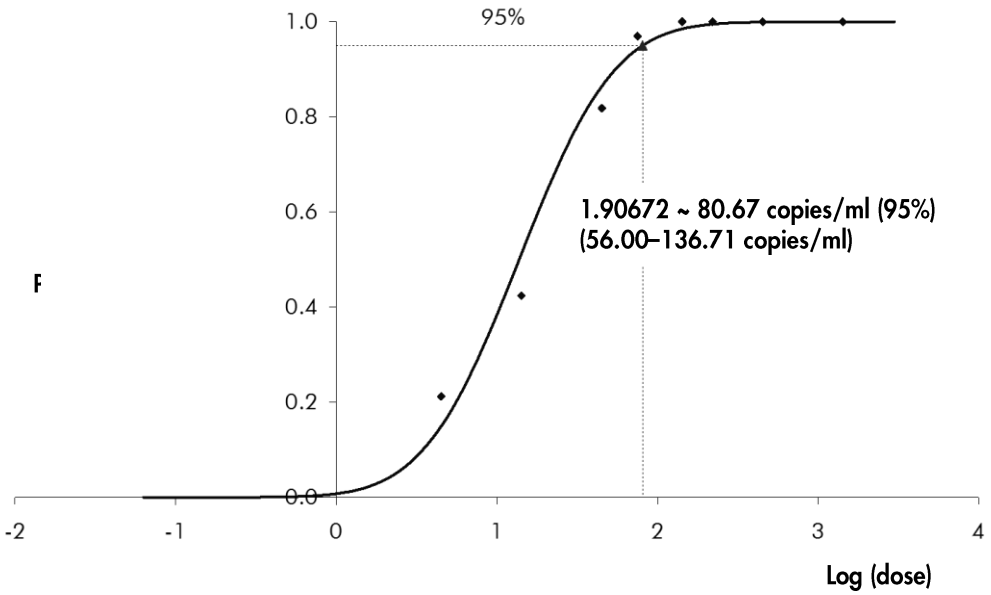

Figure 1. Probit analysis: CSF, VZV (Rotor-Gene Q). Analytical sensitivity in consideration of the purification (QIAsymphony DSP Virus/Pathogen Mini Kit) of the artus VZV QS-RGQ Kit on Rotor-Gene Q.

Specificity – CSF

The specificity of the *artus* VZV 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 30 different VZV negative CSF samples. These did not generate any signals with the VZV specific primers and probes, which are included in the VZV RG Master.

A potential cross-reactivity of the *artus* VZV QS-RGQ Kit was tested using the control group listed in Table 1. None of the tested pathogens has been reactive. No cross-reactivities appeared with mixed infections.

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

Control group	VZV (Cycling Green)	Internal control (Cycling Orange)
Human herpesvirus 1 (herpes simplex virus 1)	–	+
Human herpesvirus 2 (herpes simplex virus 2)	–	+
Human herpesvirus 4 (Epstein-Barr virus)	–	+
Human herpesvirus 5 (cytomegalovirus)	–	+
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	–	+
Enterovirus	–	+
Parvovirus B19	–	+
West Nile virus	–	+

Linear range – CSF

The linear range in consideration of the purification of the *artus* VZV QS-RGQ Kit was determined by analyzing a dilution series of ATCC® VR-1367 VZV strain Ellen standard material ranging from 1.00×10^8 copies/ml to 5.00×10^1 copies/ml. The purification was carried out in replicates (n = 4 for concentrations $\geq 1.00 \times 10^7$ copies/ml; n = 8 for concentrations $< 1.00 \times 10^7$ copies/ml) using the QIAsymphony DSP Virus/Pathogen Mini Kit in combination with the Cellfree200_DSP protocol (extraction volume: 0.2 ml, elution volume: 60 µl). Each of the samples was analyzed using the *artus* VZV QS-RGQ Kit. The linear range in consideration of the purification of the *artus* VZV QS-RGQ Kit has been determined to cover concentrations from 5.00×10^2 copies/ml to 1.00×10^8 copies/ml (Figure 2).

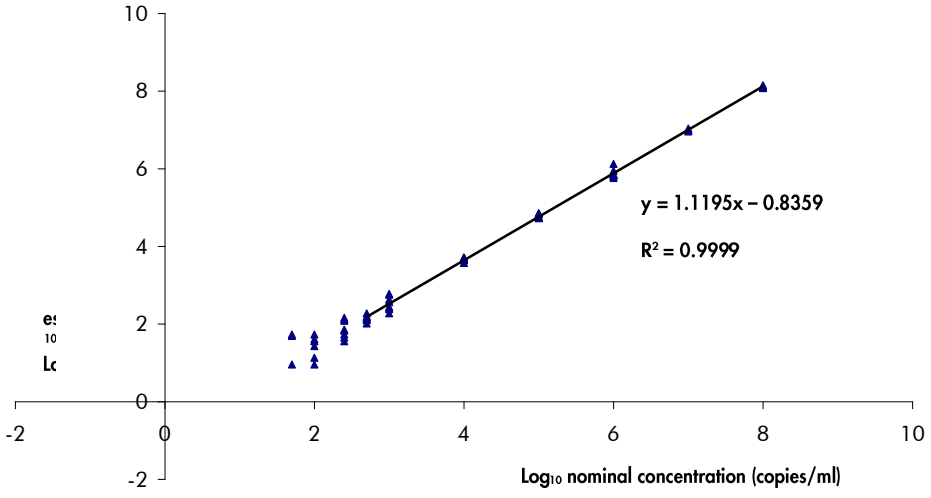

Figure 2. Linear range of the *artus* VZV QS-RGQ Kit (CSF). Calculation of the linear range. The straight line was determined by a linear regression of the $\log_{10}$ calculated concentrations with the $\log_{10}$ nominal concentrations. The equation of the regression line is included in the figure.

Robustness – CSF

The verification of the robustness allows the determination of the total failure rate of the *artus* VZV QS-RGQ Kit. To verify the robustness, 30 VZV negative samples of CSF were spiked with 300 copies/ml of VZV virus material (approximately threefold concentration of the analytical sensitivity limit). After extraction using the QIAsymphony DSP Virus/Pathogen Mini Kit in combination with the Cellfree200_DSP protocol (extraction volume: 0.2 ml, elution volume: 60 µl), these samples were analyzed with the *artus* VZV QS-RGQ Kit.

In addition, the robustness of the internal control was assessed by purification and analysis of the 30 spiked CSF samples. Inhibitions were not observed. Thus, the robustness of the *artus* VZV QS-RGQ Kit is ≥99%.

Interfering substances – CSF

Erythrocytes and genomic DNA are two endogenous substances that have the potential to interfere with the assays when present in CSF. To investigate their interfering potential, the effect of these substances on the performance of the assay was evaluated on CSF samples containing VZV at a concentration approximately 10-fold the limit of detection (LOD) value (1000 copies/ml). These tested substances demonstrated no interference with the *artus* VZV QS-RGQ Kit reagents (see Table 2).

Table 2. Interfering substances in CSF samples

VZV concentration (copies/ml)	Interfering substance		$C_{T(IC)}$			$C_{T(IC)\ IS} - C_{T(IC)\ Control}$
	Item	Concentration	Average C_T	SD	CV (%)	Absolute
1000	Erythrocytes	–	23.45	0.06	0.24	0.13
	gDNA	10,000	23.51	0.02	0.09	0.07
	gDNA	100,000	23.78	0.11	0.45	0.20
	Control	–	23.58	0.06	0.26	–

CV: coefficient of variation; IC: internal control; IS: interfering substance; SD: standard deviation.

Clinical evaluation – CSF

The clinical performance of the *artus* VZV QS-RGQ Kit was evaluated by testing a total of 163 contrived samples and analyzing the findings against the results from a comparable method at an external clinical laboratory. The results were analyzed in two parts: part one was a categorical agreement analysis of Positive Percent Agreement (PPA), Negative Percent Agreement (NPA), and Overall Percent Agreement (OPA); part two was an analysis of the results from a total of 75 CSF samples that fell within the common assay dynamic range using Deming and Passing-Bablok regression analyses, with the findings reported along with the corresponding intercept and slope (see Table 3 and Figure 3).

Table 3. Clinical performance study data for EDTA plasma samples

Measure of agreement	Frequencies	Percent agreement	Clopper-Pearson (exact) binomial lower two-sided 95% confidence limit	Clopper-Pearson (exact) binomial upper two-sided 95% confidence limit
Overall percent agreement	163/163	100.00	97.76	100.00
Positive percent agreement	100/100	100.00	96.38	100.00
Negative percent agreement	63/63	100.00	94.31	100.00

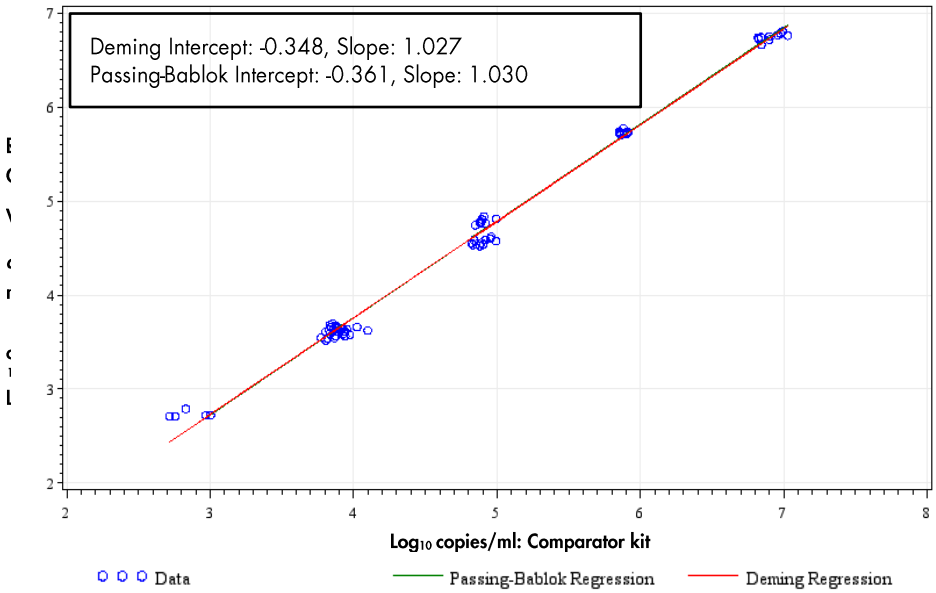

Figure 3. Regression plot with Passing-Bablok and Deming Lines for CSF samples. Samples that are between the lower limit of quantification and the upper limit of quantification for both kits were included in the analysis.

The Bland-Altman plot in Figure 4 shows that the approximate mean log difference observed between the kits is 0.22, and this difference is not influenced by the testing concentration.

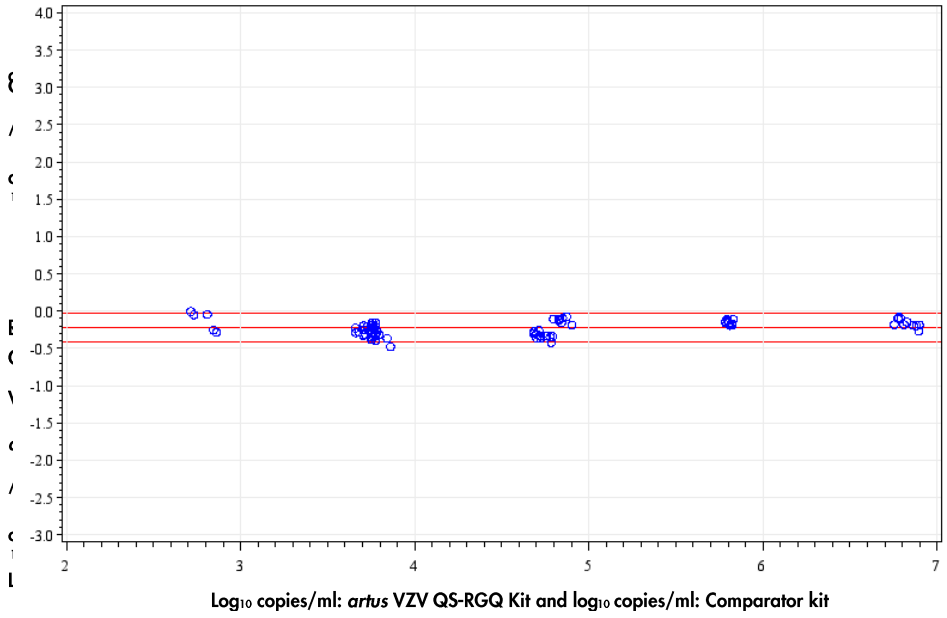

Figure 4. Bland-Altman plot (plasma). Horizontal reference lines are at –0.22, –0.42, and –0.03 and denote mean difference (log_{10} copies/ml: *artus* VZV QS-RGQ Kit – log_{10} copies/ml: comparator kit) and its corresponding 95% prediction interval. Samples that were between the lower limit of quantification and upper limit of quantification for both kits were included in the analysis.

Analytical sensitivity – plasma

For plasma, the analytical sensitivity in consideration of the purification of the *artus* VZV QS-RGQ Kit was determined using a dilution series of virus material spiked in human plasma from 100 to 0.316 copies/ml.

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 8 dilutions was analyzed with the *artus* VSV QS-RGQ Kit on 4 different days in 4 runs with 11 replicates each. The results were determined by a probit analysis.

A graphical illustration of the probit analysis is shown in Figure5. The analytical detection limit for VZV in consideration of the purification of the *artus* VZV QS-RGQ Kit in combination with the Rotor-Gene Q is 12.725 copies/ml (p = 0.05). This means that there is a 95% probability that 12.725 copies/ml will be detected.

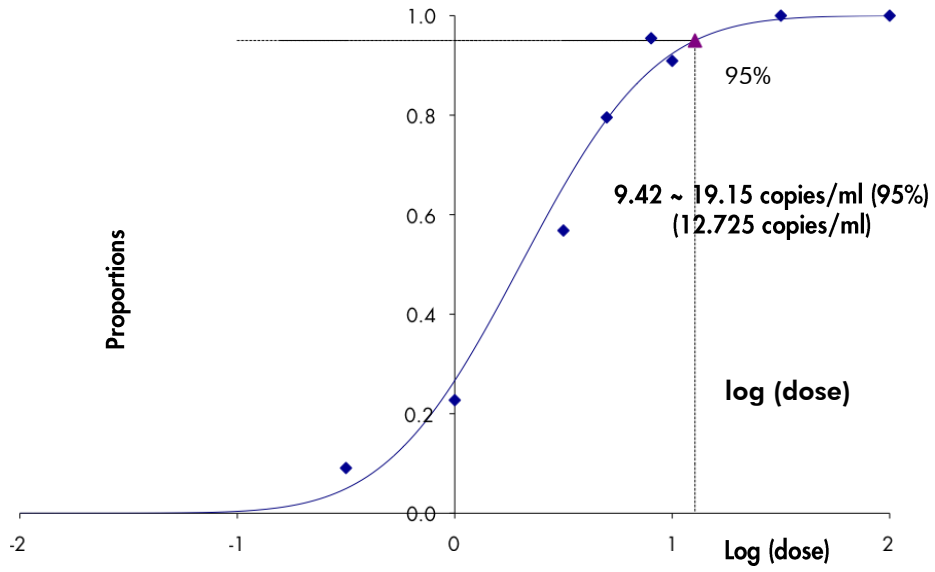

Figure 5. Probit analysis: plasma, VZV (Rotor-Gene Q). Analytical sensitivity in consideration of the purification (using the QIAsymphony DSP Virus/Pathogen Midi Kit) and the artus VZV QS-RGQ Kit on the Rotor-Gene Q.

Linear range – plasma

The linear range in consideration of the purification of the *artus* VZV QS-RGQ Kit was determined by analyzing a dilution series of virus material in plasma ranging from 6.92×10^6 copies/ml to 1.0×10^1 copies/ml. The purification was carried out in replicates (n = 4 for concentrations $\geq 1.00 \times 10^6$ copies/ml; n = 8 for concentrations $< 1.00 \times 10^6$ 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* VZV QS-RGQ Kit.

In plasma, the linear range in consideration of the purification of the *artus* VZV QS-RGQ Kit for VZV material has been determined to cover concentrations from 127 copies/ml to 6.92 x 10^6 copies/ml (Figure 6).

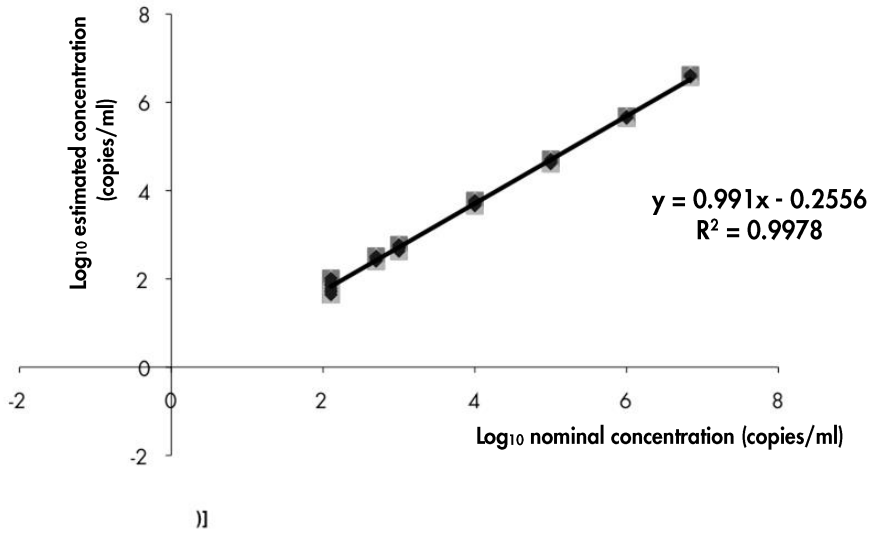

Figure 6. Linear range of the *artus* VZV QS-RGQ Kit (plasma). Calculation of the linear range. The straight line was determined by a linear regression of the log_{10} calculated concentrations with the log_{10} nominal concentrations. The equation of the regression line is included in the figure.

Robustness – plasma

The verification of the robustness in plasma allows the determination of the total failure rate of the *artus* VZV QS-RGQ Kit. To verify the robustness, 30 VZV negative samples of plasma were spiked with 38.175 copies/ml of VZV material (approximately threefold concentration of the analytical sensitivity limit). After extraction using the QIAsymphony DSP Virus/Pathogen Midi Kit in combination with the Cellfree1000_DSP protocol (extraction volume: 1 ml, elution volume: 60 µl), these samples were analyzed with the *artus* VZV QS-RGQ Kit. For robustness in VZV target testing, 100% (30/30) samples were detected positive for VZV.

In addition, the robustness of the internal control was assessed after purification and analysis of 116 spiked plasma samples. These samples were 100% negative for VZV, and 100% positive for internal control target. Inhibitions were not observed. Thus, the robustness of the *artus* VZV 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 plasma samples. Their effects were evaluated in plasma containing VZV at approximately 10-fold the LOD value (127.25 copies/ml). As a control, VZV 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_DSP protocol (extraction volume: 1 ml, elution volume: 60 µl). For samples containing elevated levels of endogenous inhibitors (bilirubin, hemoglobin, triglyceride, and albumin protein), no interference was observed for VZV detection.

Clinical evaluation – plasma

The clinical performance of the *artus* VZV QS-RGQ Kit was evaluated by testing a total of 161 contrived EDTA plasma samples and analyzing the findings against the results from a comparable method at an external site. The results were analyzed in two parts: part one was a categorical agreement analysis of Positive Percent Agreement (PPA), Negative Percent Agreement (NPA), and Overall Percent Agreement (OPA); part two was an analysis of the results from a total of 97 EDTA plasma samples that fell within the common assay dynamic range using Deming and Passing-Bablok regression analyses, with the findings reported along with the corresponding intercept and slope (see Table 4 and Figure 7).

Table 4. Clinical performance study data for EDTA plasma samples

Measure of agreement	Frequencies	Percent agreement	Clopper-Pearson (exact) binomial lower two-sided 95% confidence limit	Clopper-Pearson (exact) binomial upper two-sided 95% confidence limit
Overall percent agreement	161/161	100.00	97.73	100.00
Positive percent agreement	101/101	100.00	96.41	100.00
Negative percent agreement	60/60	100.00	94.04	100.00

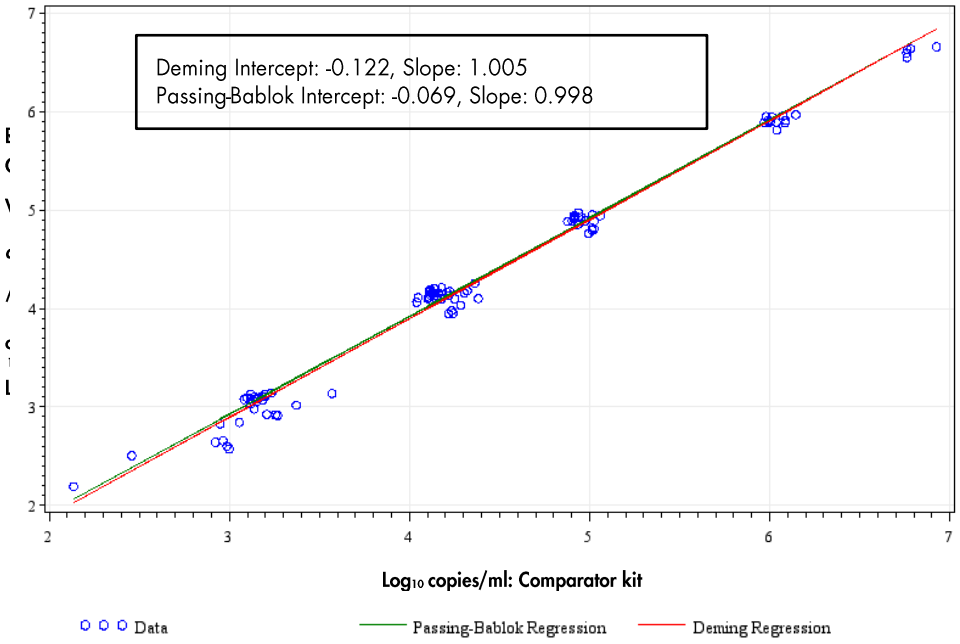

Figure 7. Regression plot with Passing-Bablok and Deming Lines for plasma samples. Samples that are between the lower limit of quantification and the upper limit of quantification for both kits were included in the analysis.

The Bland-Altman plot in Figure 8 shows that the approximate mean log difference observed between the kits is –0.10, and this difference is not influenced by the testing concentration.

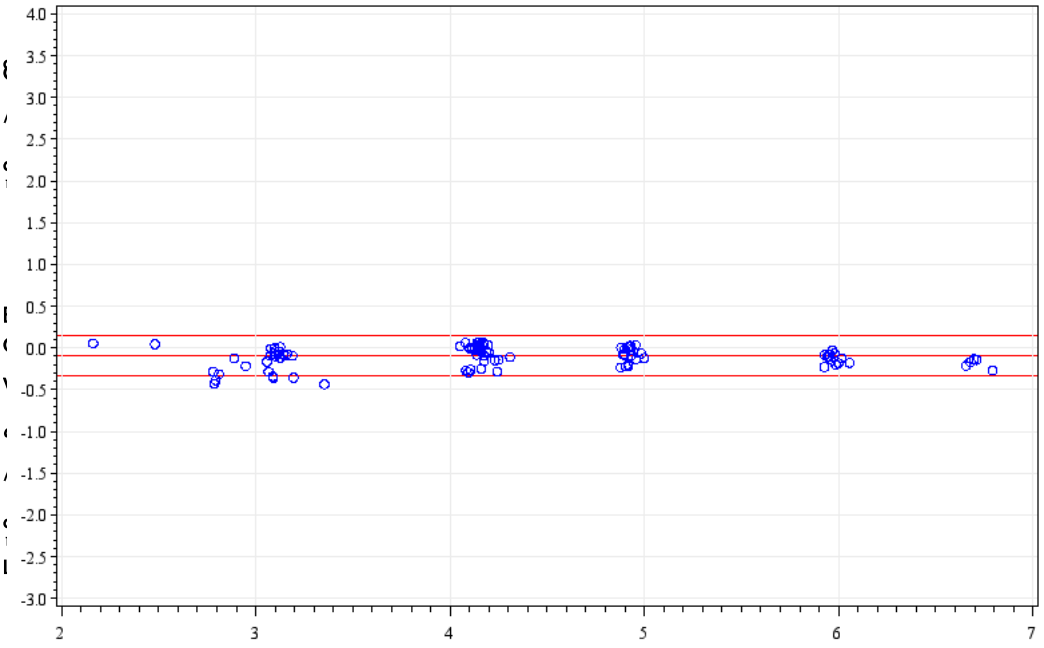

Figure 8. Bland-Altman plot (plasma). Horizontal reference lines are at –0.10, –0.34, and 0.14 and denote mean difference (log_{10} copies/ml: *artus* VZV QS-RGQ Kit – log_{10} copies/ml: Comparator kit) and its corresponding 95% prediction interval. Samples that were between the lower limit of quantification and upper limit of quantification for both kits were included in the analysis.

Precision

The precision data of the *artus* VZV 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* VZV 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). In addition, precision data for quantitative results in copies/µl were determined using the corresponding C_T values (Table 3). Based on these results, the overall statistical spread of any given sample with the mentioned concentration is 0.45% (C_T) or 8.32%

(concentration), and 2.81% (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 5. Precision data for VZV on basis of the C_T values

	Standard deviation	Variance	Coefficient of variation (%)
Intra-assay variability: VZV QS 4	0.08	0.01	0.26
Intra-assay variability: Internal control	0.04	0.002	0.17
Inter-assay variability: VZV QS 4	0.15	0.02	0.50
Inter-assay variability: Internal control	0.39	0.15	1.63
Inter-batch variability: VZV QS 4	0.10	0.01	0.34
Inter-batch variability: Internal control	0.66	0.43	2.65
Total variance: VZV QS 4	0.13	0.02	0.45
Total variance: Internal control	0.68	0.47	2.81

Table 6. Precision data for VZV on basis of the quantitative results (in copies/ml)

	Standard deviation	Variance	Coefficient of variation (%)
Intra-assay variability: VZV QS 4	0.50	0.25	5.46
Inter-assay variability: VZV QS 4	0.85	0.72	8.72
Inter-batch variability: VZV QS 4	0.75	0.56	7.67
Total variance: VZV QS 4	0.81	0.66	8.32

Reproducibility

Reproducibility data permit a regular performance assessment of the *artus* VZV 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.

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

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