September 2017

artus® EBV QS-RGQ Kit: Performance characteristics

IVD

CE

REF 4501363 *artus* EBV QS-RGQ Kit, Version 1.

Check availability of new electronic labeling revisions at **www.qiagen.com/products/artusebvpcrkitce.aspx** before test execution. The current revision status is indicated by the issue date (format: month/year).

Limit of Detection – plasma

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

For plasma, the limit of detection in consideration of the purification of the *artus* EBV QS-RGQ Kit was determined using a dilution series of EBV material from 3160 to nominal 1 EBV copy/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* EBV 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 limit of detection in consideration of the purification of the *artus* EBV QS-RGQ Kit in combination with the Rotor-Gene® Q is 157 copies/ml (p = 0.05). This means that there is a 95% probability that 157 copies/ml (corresponding to 22.29 IU/ml) will be detected.

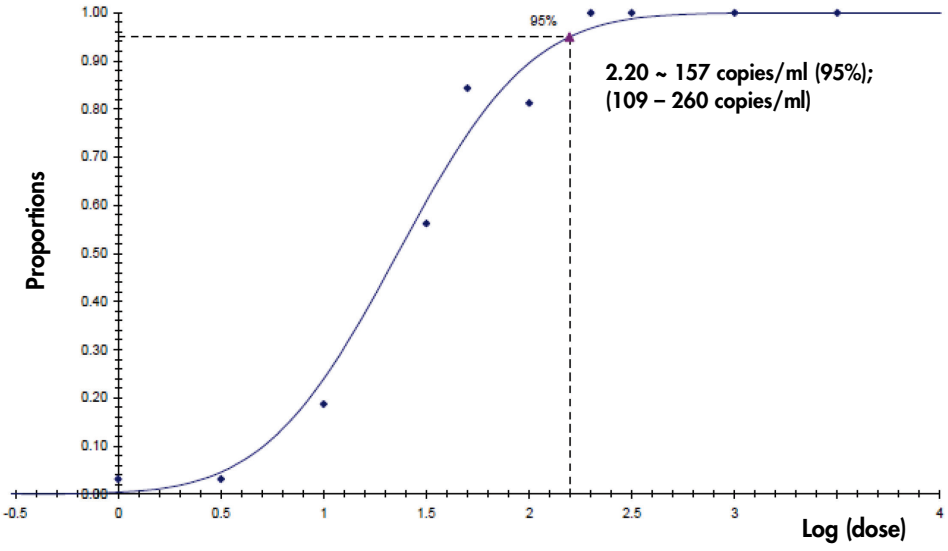

Figure 1. Probit analysis: plasma, EBV (Rotor-Gene Q). Limit of detection in consideration of the purification (plasma, using the QIAsymphony DSP Virus/Pathogen Midi Kit) and the *artus* EBV QS-RGQ Kit on Rotor-Gene Q

Specificity – plasma

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

A potential cross-reactivity of the *artus* EBV QS-RGQ Kit was tested using the control group listed in Table 1 below. 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

Control group	EBV (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 5 (Cytomegalovirus)	–	+
Human T cell leukemia virus 1	–	+
Human T cell leukemia virus 2	–	+

Linear range – plasma

The linear range in consideration of the purification of the *artus* EBV QS-RGQ Kit was determined by analyzing a dilution series of EBV material ranging from 1.00 x 10^7 copies/ml to 6.31 x 10^2 copies/ml in plasma. The purification was carried out in replicates (n = 4 for concentrations ≥1.00 x 10^6 copies/ml; n = 8 for concentrations <1.00 x 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* EBV QS-RGQ Kit.

The linear range in consideration of the purification of the *artus* EBV QS-RGQ Kit has been determined to cover concentrations from 6.31 x 10^2 copies/ml to 1.00 x 10^7 copies/ml (corresponding to 8.96 x 10^1 to 1.42 x 10^6 IU/ml) for plasma (Figure 2).

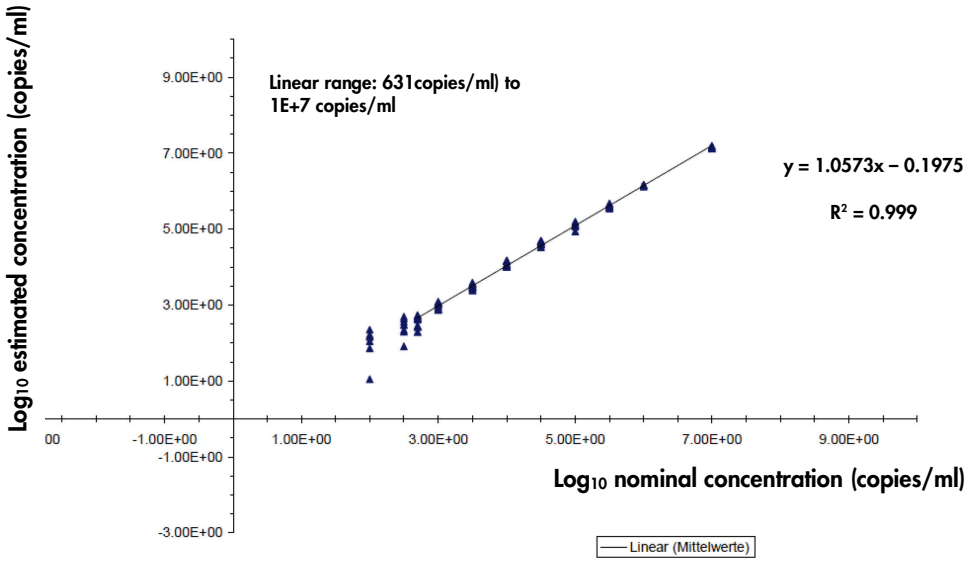

Figure 2. Linear range of the *artus* EBV QS-RGQ Kit (plasma). Calculation of the linear range. The straight line was determined by a linear regression of the $\log_{10}$ calculated concentrations with the $\log_{10}$ nominal concentrations. The equation of the regression line is included in the figure.

Robustness – plasma

The verification of the robustness allows the determination of the total failure rate of the *artus* EBV QS-RGQ Kit. To verify the robustness, 30 EBV negative samples of plasma were spiked with 500 copies/ml of EBV (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* EBV QS-RGQ Kit. In addition, the robustness of the internal control was assessed by purification and analysis of the 30 spiked plasma samples. Inhibitions were not observed. Thus, the robustness of the *artus* EBV QS-RGQ Kit is ≥99%.

Interfering substances – plasma

Bilirubin, hemoglobin and triglycerides showed no interference with the *artus* EBV QS-RGQ Kit at concentrations as shown in Table 2.

Table 2. Interfering substances in EDTA plasma samples

EBV concentration (copies/ml)	Interfering substance		$C_{T(EBV)}$			$C_{T(EBV)\ IS} - C_{T(EBV)\ Control}$
	Item	Concentration	Average C_T	SD	CV (%)	Absolute
1600	Bilirubin	30 mg/dl	32.30	0.37	1.14	0.58
	Hemoglobin	2 g/dl	32.82	0.20	0.60	0.06
	Triglyceride	1 g/dl	32.42	0.28	0.87	0.46
	Albumin	4 g/dl	31.71	0.54	1.69	1.15
	Control	-	32.88	0.33	0.99	-

CV: coefficient of variation; EBV: Epstein-Barr virus; IS: interfering substance; SD: standard deviation

Clinical evaluation – plasma

The clinical performance of the *artus* EBV QS-RGQ Kit was evaluated by testing clinical specimens and analyzing the findings against the results from a comparable method. A total of 166 specimens of EDTA plasma collected from EBV infected patients as well as from negative controls were tested with the *artus* EBV QS-RGQ Kit and the 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 83 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 correlation coefficient (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	154/166	92.77	87.71	96.21
Positive percent agreement	100/102	98.04	93.10	99.76
Negative percent agreement	54/64	84.38	73.14	92.24

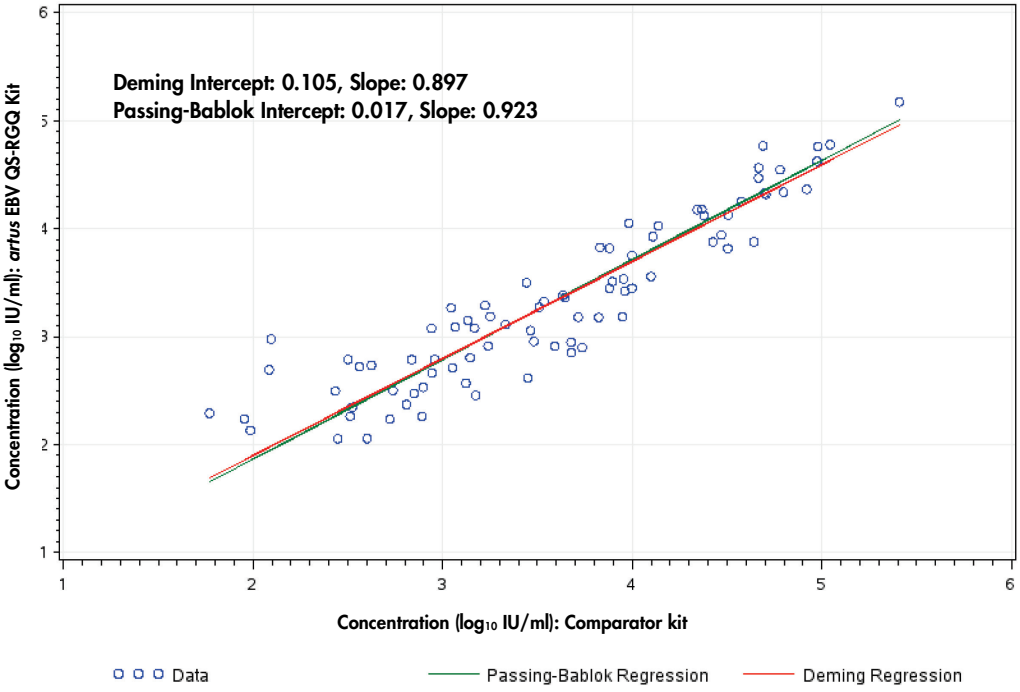

Figure 3. Regression plot with Passing-Bablok and Deming Lines. Samples that are between the lower limit of quantification and the upper limit of quantification for both kits are included in the analysis.

Linear regression analysis between the two assays resulted in a Pearson Correlation Coefficient of 0.922 and Spearman Correlation Coefficient of 0.928.

Limit of Detection – whole blood

For whole blood, the limit of detection in consideration of the purification of the *artus* EBV QS-RGQ Kit was determined using a dilution series of EBV material from 3160 to nominal 3.16 EBV 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 10 dilutions was analyzed with the *artus* EBV 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 4.

The limit of detection in consideration of the purification of the *artus* EBV QS-RGQ Kit in combination with the Rotor-Gene Q is 288.29 copies/ml (p = 0.05). This means that there is a 95% probability that 288.29 copies/ml (corresponding to 40.36 IU/ml) will be detected.

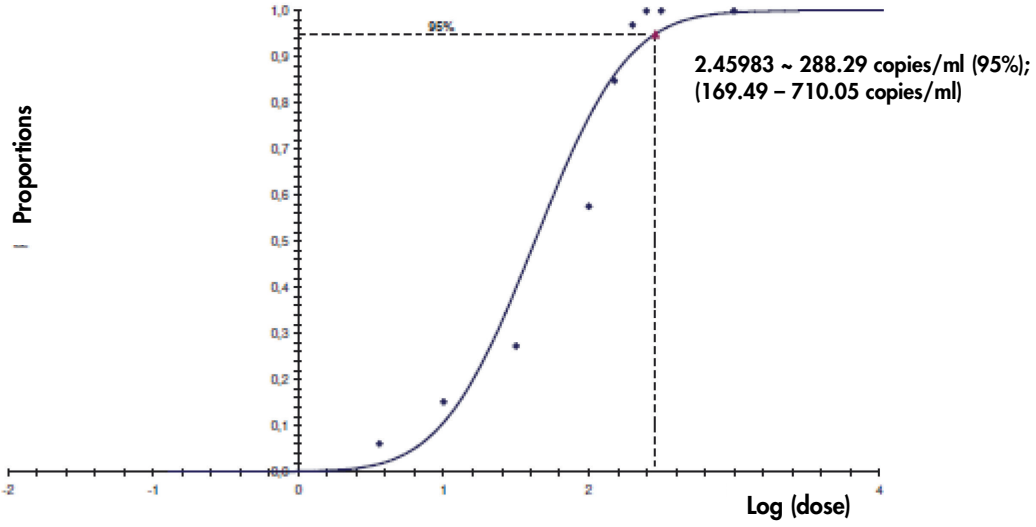

Figure 4. Probit analysis: whole blood, EBV (Rotor-Gene Q). Limit of detection in consideration of the purification (whole blood, using the QIAsymphony DNA Mini Kit) of the *artus* EBV QS-RGQ Kit on the Rotor-Gene Q.

Specificity – whole blood

The specificity of the *artus* EBV 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 EBV negative whole blood samples. These did not generate any signals with the EBV specific primers and probes, which are included in the EBV RG Master.

A potential cross-reactivity of the *artus* EBV 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 cross-reactivities appeared with mixed infections.

Linear range – whole blood

The linear range in consideration of the purification of the *artus* EBV QS-RGQ Kit was determined by analyzing a dilution series of EBV material ranging from 5.00 x 10^7 copies/ml to 1.00 x 10^3 copies/ml in whole blood. The purification was carried out in replicates (n = 4 for concentrations ≥1.00 x 10^7 copies/ml; n = 8 for concentrations <1.00 x 10^7 copies/ml) using 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* EBV QS-RGQ Kit. The linear range in consideration of the purification of the *artus* EBV QS-RGQ Kit has been determined to cover concentrations from 1.00 x 10^3 copies/ml to 5.00 x 10^7 copies/ml (corresponding to 1.4 x 10^2 to 7.0 x 10^6 IU/ml) for whole blood (Figure 5).

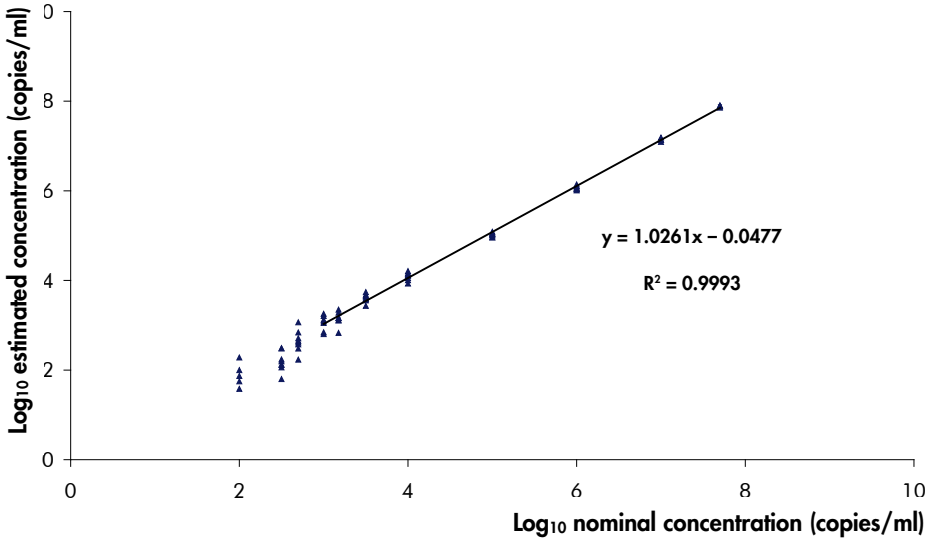

Figure 5. Linear range of the *artus* EBV QS-RGQ Kit (whole blood). Calculation of the linear range. The straight line was determined by a linear regression of the $\log_{10}$ calculated concentrations with the $\log_{10}$ nominal concentrations. The equation of the regression line is included in the figure.

Robustness – whole blood

The verification of the robustness allows the determination of the total failure rate of the *artus* EBV QS-RGQ Kit. To verify the robustness, 51 EBV negative whole blood samples were spiked with 750 copies/ml of EBV (approximately threefold concentration of the analytical sensitivity limit). After extraction using the QIAsymphony DNA Mini Kit in combination with the VirusBlood200_DSP protocol (extraction volume: 200 µl, elution volume: 60 µl), these samples were analyzed with the *artus* EBV QS-RGQ Kit. In addition, the robustness of the internal control was assessed by purification and analysis of the 51 spiked whole blood samples. Inhibitions were not observed. Thus, the robustness of the *artus* EBV QS-RGQ Kit is ≥99%.

Interfering substances – whole blood

Substances that could potentially interfere with the results of the *artus* EBV QS-RGQ Kit were tested and the concentrations of these substances that did not interfere with the kit are shown in Table 4.

Table 4. Interfering substances in whole blood samples

EBV concentration (copies/ml)	Interfering substance		$C_{T(EBV)}$			$C_{T(EBV)\ IS} - C_{T(EBV)\ Control}$
	Item	Concentration	Average C_T	SD	CV (%)	Absolute
2500	Bilirubin	30 mg/dl	34.44	0.27	0.78	0.73
	Triglyceride	1 g/dl	34.58	0.32	0.91	0.59
	gDNA	3 µg/sample	34.79	0.18	0.52	0.38
	gDNA	2.5 µg/sample	34.57	0.39	1.13	0.60
	gDNA	2 µg/sample	34.73	0.49	1.41	0.44
	gDNA	1 µg/sample	34.86	0.22	0.62	0.31
	Control	–	35.17	0.40	1.13	–

CV: coefficient of variation; EBV: Epstein-Barr virus; gDNA: genomic DNA; IS: interfering substance; SD: standard deviation

Clinical evaluation – whole blood

The clinical performance of the *artus* EBV QS-RGQ Kit was evaluated by testing clinical specimens and analyzing against a comparable method. A total of 178 specimens of whole blood collected from EBV infected patients as well as from negative controls, were tested with the *artus* EBV QS-RGQ Kit and with a comparable method at an external site. The results were analyzed in two parts: part one was a categorical agreement analysis of PPA, NPA, and OPA; part two was an analysis of the results from a total of 98 whole blood samples that fell within the common assay dynamic range using Deming and Passing-Bablok regression analyses, with the findings were reported along with the corresponding correlation coefficient (see Table 5 and Figure 6).

Table 5. Clinical performance study data for whole blood 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	169/178	94.94	90.62	97.66
Positive percent agreement	115/119	96.64	91.62	99.08
Negative percent agreement	54/59	91.53	81.32	97.19

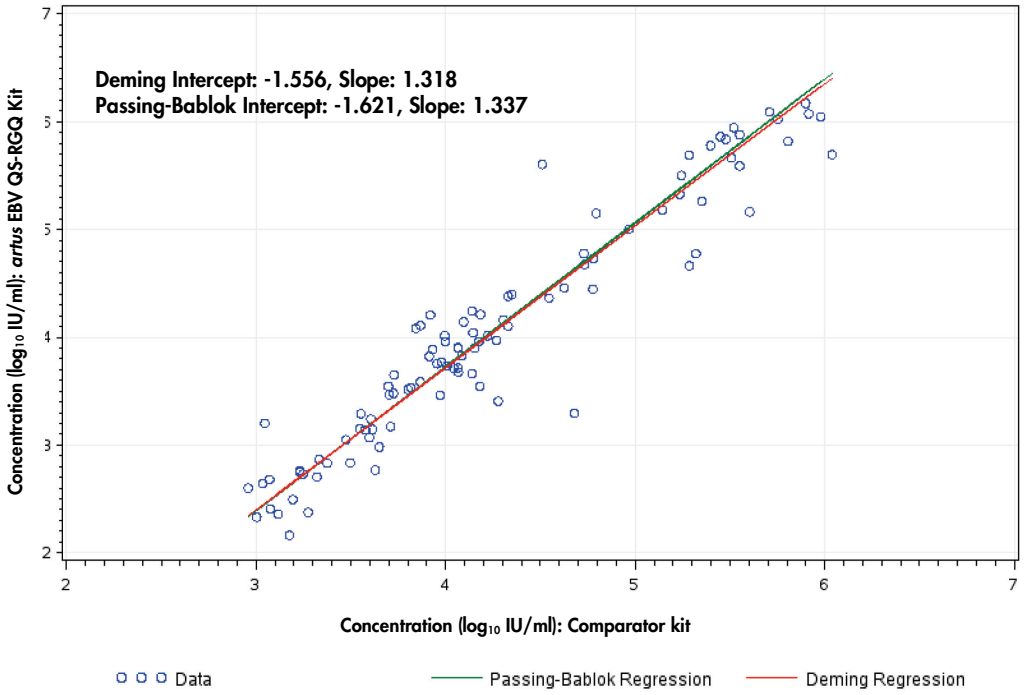

Figure 6. Regression plot with Passing-Bablok and Deming Lines. Samples that are between the lower limit of quantification and the upper limit of quantification for both kits are included in the analysis.

Linear regression analysis between the two assays resulted in a Pearson Correlation Coefficient of 0.956 and Spearman Correlation Coefficient of 0.945.

Reproducibility

Reproducibility data permit a regular performance assessment of the *artus* EBV 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* EBV QS-RGQ Kit.

Document revision history	
September 2017	Updated Table 5 Clinical performance study data for whole blood samples. Added IU/ml as well as copies/ml concentration units throughout.

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