September 2015

artus® HSV-1/2 QS-RGQ Kit: Performance Characteristics

artus HSV-1/2 QS-RGQ Kit, Version 1 REF 4500363

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Sample to Insight

QIAGEN

Analytical sensitivity – CSF

The analytical detection limit in consideration of the purification (sensitivity limit) was assessed for the *artus* HSV-1/2 QS-RGQ Kit using HSV-positive clinical specimens in combination with the extraction on the QIAsymphony® SP.

The analytical sensitivity in consideration of the purification of the *artus* HSV-1/2 QS-RGQ Kit was determined using a dilution series of ATCC® virus material for herpes simplex virus 1 and 2 (ATCC code VR-260™/VR-734™) from 450 to nominal 1.42 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* HSV-1/2 QS-RGQ Kit on 3 different days in 3 runs with 11 replicates each. The results were determined by a probit analysis.

The analytical detection limit of the *artus* HSV-1/2 QS-RGQ Kit in combination with the Rotor-Gene Q is 57.27 copies/ml (p = 0.05) for HSV-1. This means that there is a 95% probability that 57.27 copies/ml of HSV 1 DNA will be detected. A graphical illustration of the probit analysis for HSV-1 is shown in Figure 1.

The analytical detection limit of the *artus* HSV-1/2 QS-RGQ Kit in combination with the Rotor-Gene Q is 65.74 copies/ml (p = 0.05) for HSV 2. This means that there is a 95% probability that 65.74 copies/ml of HSV-2 DNA will be detected. A graphical illustration of the probit analysis for HSV-2 is shown in Figure 2.

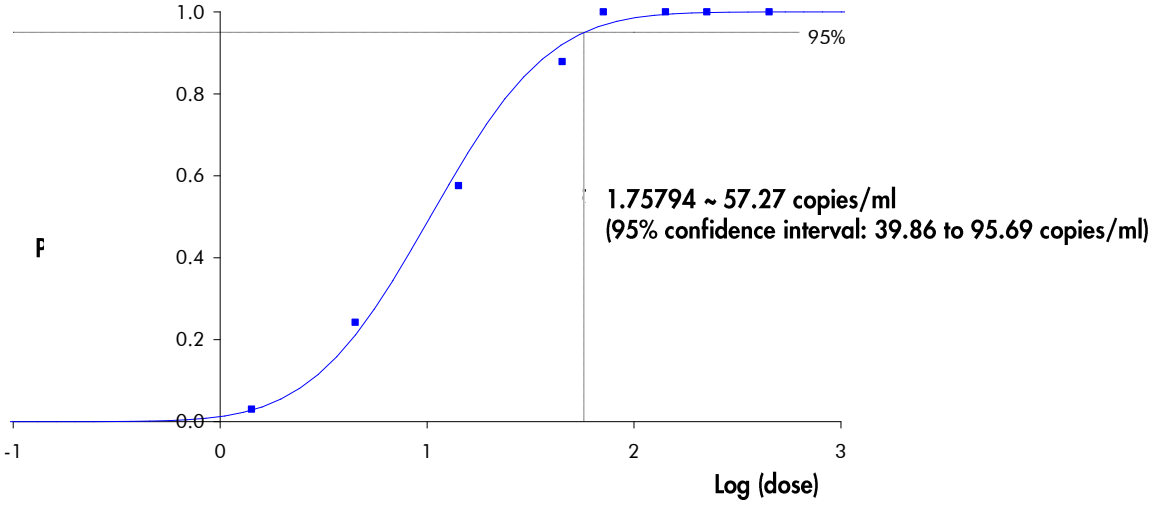

Figure 1. Probit analysis: CSF, HSV-1 (Rotor-Gene Q). Analytical sensitivity in consideration of the purification (QIAsymphony DSP Virus/Pathogen Mini Kit) of the *artus* HSV-1/2 QS-RGQ Kit on the Rotor-Gene Q.

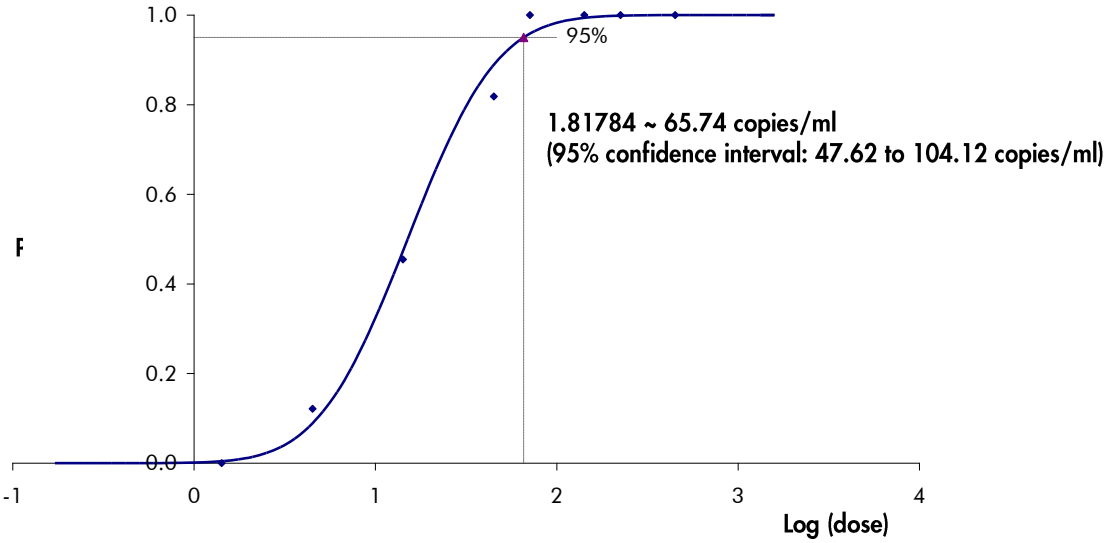

Figure 2. Probit analysis: CSF, HSV-2 (Rotor-Gene Q). Analytical sensitivity in consideration of the purification (QIAsymphony DSP Virus/Pathogen Mini Kit) of the *artus* HSV-1/2 QS-RGQ Kit on the Rotor-Gene Q.

Specificity – CSF

The specificity of the *artus* HSV-1/2 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 by a database alignment and by PCR runs on Rotor-Gene instruments with the genotypes as shown in Table 1.

Moreover, the specificity was validated with 30 different HSV-1 and HSV-2 negative CSF samples. These did not generate any signals with the HSV-1 and HSV-2 specific primers and probes, which are included in the HSV-1/2 RG Master.

A potential cross-reactivity of the *artus* HSV-1/2 QS-RGQ Kit was tested using the control group listed in Table 2. None of the tested pathogens has been reactive. No cross-reactivities appeared with the mixed infections tested.

Table 1. Testing of the specificity of relevant genotypes (CSF)

Virus	Strain	Source	HSV-1 (Cycling Green)	HSV-2 (Cycling Orange)	Internal control (Cycling Yellow)
HSV-1	HF	ATCC*	+	–	+
HSV-1	KOS	INSTAND†	+	–	+
HSV-1	MacIntyre	QCMD‡	+	–	+
HSV-2	HG-52	NCPV§	–	+	+
HSV-2	G	ATCC*	–	+	+
HSV-2	MS	QCMD‡	–	+	+

* American Type Culture Collection.
† Society for Promotion of Quality Assurance in Medical Laboratories.
‡ Quality Control for Molecular Diagnostics.
§ National Collection of Pathogenic Viruses.

Table 2. Testing the specificity of the kit with potentially cross-reactive pathogens (CSF)

Control group	HSV-1 (Cycling Green)	HSV-2 (Cycling Orange)	Internal control (Cycling Yellow)
Human herpesvirus 3 (varicella-zoster virus)	–	–	+
Human herpesvirus 4 (Epstein-Barr virus)	–	–	+
Human herpesvirus 5 (cytomegalovirus)	–	–	+
Human herpesvirus 2 (herpes simplex virus 2)	–	–	+
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 (HIV)	–	–	+
Human T cell leukemia virus 1	–	–	+
Human T cell leukemia virus 2	–	–	+
Enterovirus	–	–	+
Parvovirus B19	–	–	+
West Nile virus	–	–	+

Robustness – CSF

The verification of the robustness allows the determination of the total failure rate of the *artus* HSV-1/2 QS-RGQ Kit. To verify the robustness, 32 HSV negative CSF samples were spiked with 172 copies/ml of HSV-1 and 30 HSV negative CSF samples were spiked with 200 copies/ml HSV-2 (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* HSV-1/2 QS-RGQ Kit. In addition, the robustness of the internal control was assessed by purification and analysis of the all spiked CSF samples in this study. Inhibitions were not observed. Thus, the robustness of the *artus* HSV-1/2 QS-RGQ Kit was ≥99%.

Interfering substances – CSF

The tested potential interfering substances show no interference with the *artus* HSV-1/2 RG PCR Kit, see Tables 3 and 4.

Table 3. Results for interfering substances study for HSV-1

HSV-1 concentration (copies/ml)	Interfering substance		$C_{T\ (HSV-1)}$			$C_{T\ (HSV-1)\ IS} - C_{T\ (HSV-1)\ control}$
	Item	Concentration (copies/ml)	Average C_T	SD	CV (%)	Absolute
572.7	Erythrocytes	–	31.68	0.37	1.17	0.01
	gDNA	10,000	31.60	0.26	0.82	0.06
	gDNA	100,000	31.95	0.29	0.90	0.29
	Control	*572.7*	31.67	0.23	0.72	–

CV: coefficient of variation; IS: interfering substance; SD: standard deviation

Table 4. Results for interfering substances study for HSV-2

HSV-2 concentration (copies/ml)	Interfering substance		$C_{T\ (HSV-2)}$			$C_{T\ (HSV-2)\ IS} - C_{T\ (HSV-2)\ control}$
	Item	Concentration (copies/ml)	Average C_T	SD	CV (%)	Absolute
657.4	Erythrocytes	–	31.59	0.22	0.69	0.13
	gDNA	10,000	31.34	0.39	1.25	0.38
	gDNA	100,000	31.48	0.37	1.17	0.24
	Control	572.7	31.72	0.37	1.16	–

CV: coefficient of variation; IS: interfering substance; SD: standard deviation

Clinical evaluation – CSF

The clinical performance of the *artus* HSV-1/2 QS-RGQ assay was also evaluated by testing contrived CSF specimens and analyzing the findings against the results of a comparative CE-IVD method. A total of 524 specimens of human CSF were prepared (HSV-1/2 positive: n = 404; HSV-1/2 negative: n = 120) and tested with the *artus* HSV-1/2 QS-RGQ Kit and with a comparator method at an external clinical diagnostic laboratory. The results were analyzed for the analytical sensitivity and analytical specificity, and the results reported for both assays to demonstrate equivalent performance.

Table 5. Asymptotic two-sided, 95% confidence interval: Newcombe score method for negative samples (CSF)

Type of assay by call Frequencies	Negative	Positive	Total
artus HSV-1/2 QS-RGQ Kit	60	0	60
Comparator Kkit	59	1	60
Total	119	1	120

Table 6. Asymptotic two-sided, 95% confidence interval: Newcombe score method for positive samples (CSF)

Type of assay by call Frequencies	Negative	Positive	Total
artus HSV-1/2 QS-RGQ Kit	14	188	202
Comparator kit	29	173	202
Total	43	361	404

Table 7. Proportion of correct calls for each assay (CSF)

Sample set	Sample type	*artus* HSV-1/2 QS-RGQ Kit: proportion of correct calls	Comparator kit: proportion of correct calls	Difference in proportions (*artus* HSV-1/2 QS-RGQ Kit – comparator kit)	Lower 95% confidence interval	Upper 95% confidence interval
All	Negative	1.000	0.983	0.017	–0.045	0.089
All	Positive	0.931	0.856	0.074	0.014	0.136

The analytical sensitivity and analytical specificity values for the *artus* HSV-1/2 QS-RGQ Kit were 93.1% and 100% respectively. The sensitivity and analytical specificity values for the comparator kit were 85.6% and 98.3% respectively. The difference in analytical specificity between the *artus* HSV-1/2 QS-RGQ Kit and the comparator kit was 1.7% (95% confidence interval: –4.5 to 8.9%). The difference in analytical sensitivity between the *artus* HSV-1/2 QS-RGQ Kit and the comparator kit was 7.4% (95% confidence interval: 1.4 to 13.6%). Overall, data indicates there is a less than 2% difference in the analytical specificity between the *artus* HSV-1/2 QS-RGQ Kit and the comparator kit. There is a difference of almost 8% in the analytical sensitivity between the kits, with the *artus* HSV-1/2 QS-RGQ Kit demonstrating improved sensitivity compared with the comparator kit.

Analytical sensitivity – plasma

For plasma, the analytical sensitivity in consideration of the purification of the *artus* HSV-1/2 QS-RGQ Kit was determined using a dilution series of ATCC virus material spiked in human plasma for HSV-1 from 1000 to 3.16 copies/ml, and HSV-2 from 316 to 1.00 copies/ml.

These samples 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 dilutions (9 for HSV-1; 8 for HSV-2) was analyzed with the *artus* HSV-1/2 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 for HSV-1 is shown in Figure 3. The analytical detection limit for HSV-1 in consideration of the purification of the *artus* HSV-1/2 QS-RGQ Kit in combination with the Rotor-Gene Q is 37.16 copies/ml ($p = 0.05$). This means that there is a 95% probability that 37.16 copies/ml will be detected.

A graphical illustration of the probit analysis for HSV-2 is shown in Figure 4. The analytical detection limit for HSV-2 in consideration of the purification of the *artus* HSV-1/2 QS-RGQ Kit in combination with the Rotor-Gene Q is 43.24 copies/ml (p = 0.05). This means that there is a 95% probability that 43.24 copies/ml will be detected.

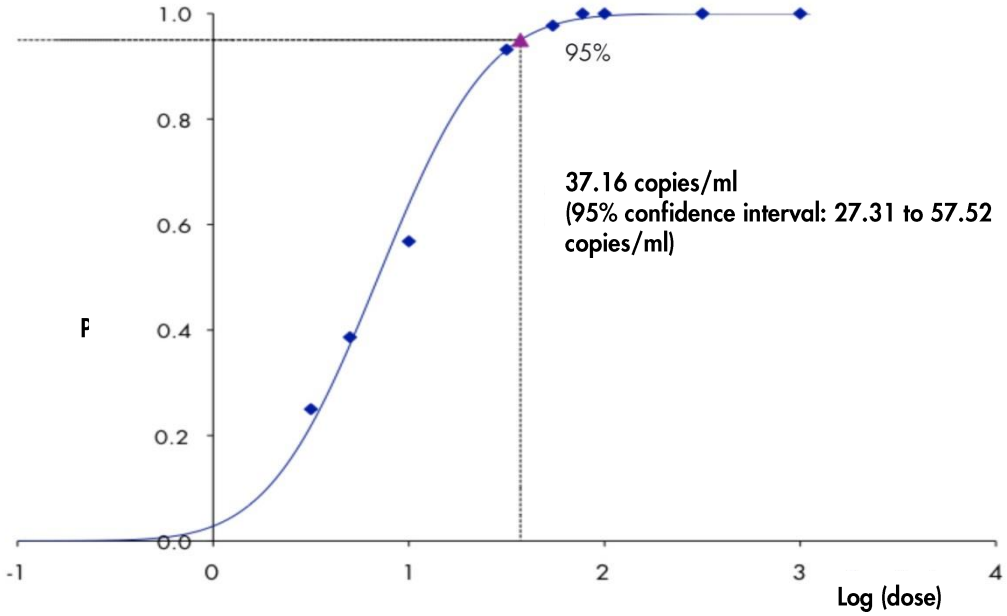

Figure 3. Probit analysis: plasma, HSV-1 (Rotor-Gene Q). Analytical sensitivity in consideration of the purification (urine, using the QIAsymphony DSP Virus/Pathogen Midi Kit) of the *artus* HSV-1/2 QS-RGQ Kit on Rotor-Gene Q.

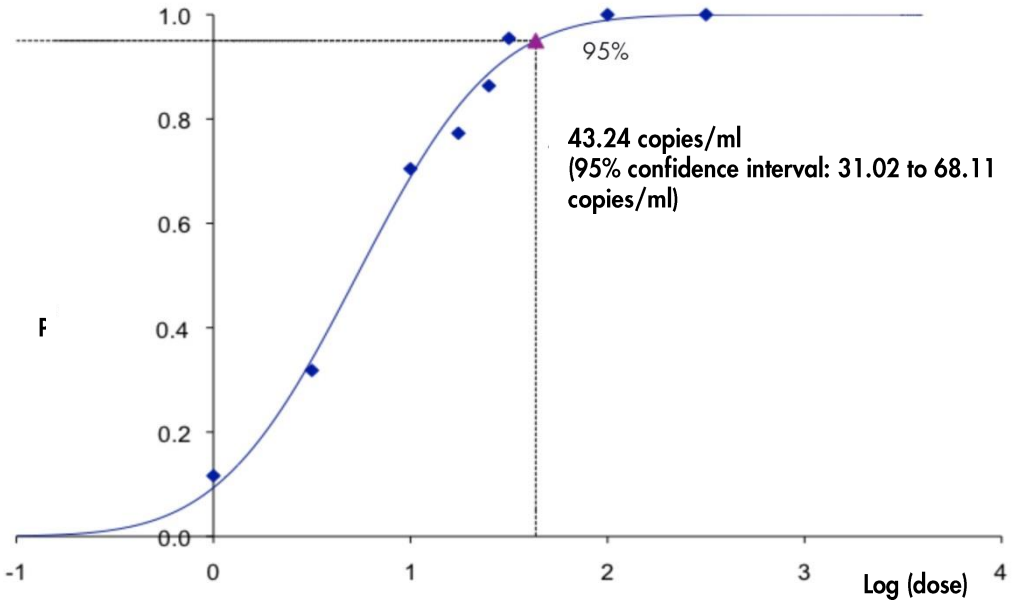

Figure 4. Probit analysis: plasma, HSV-2 (Rotor-Gene Q). Analytical sensitivity in consideration of the purification (urine, using the QIAsymphony DSP Virus/Pathogen Midi Kit) of the *artus* HSV-1/2 QS-RGQ Kit on Rotor-Gene Q.

Linear range – plasma

The linear range in consideration of the purification of the *artus* HSV-1/2 QS-RGQ Kit was determined by analyzing a dilution series of virus material in plasma ranging from 2.89×10^7 copies/ml to 2.97×10^1 copies/ml for HSV-1, and 1.51×10^7 copies/ml to 3.45×10^1 copies/ml for HSV-2 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* HSV-1/2 QS-RGQ Kit.

In plasma, the linear range in consideration of the purification of the *artus* HSV-1/2 QS-RGQ Kit for HSV-1 has been determined to cover concentrations from 37.3 copies/ml to 2.89×10^7 copies/ml (Figure 5).

In plasma, the linear range in consideration of the purification of the *artus* HSV-1/2 QS-RGQ Kit for HSV-2 has been determined to cover concentrations from 43.2 copies/ml to 1.51×10^7 copies/ml (Figure 6).

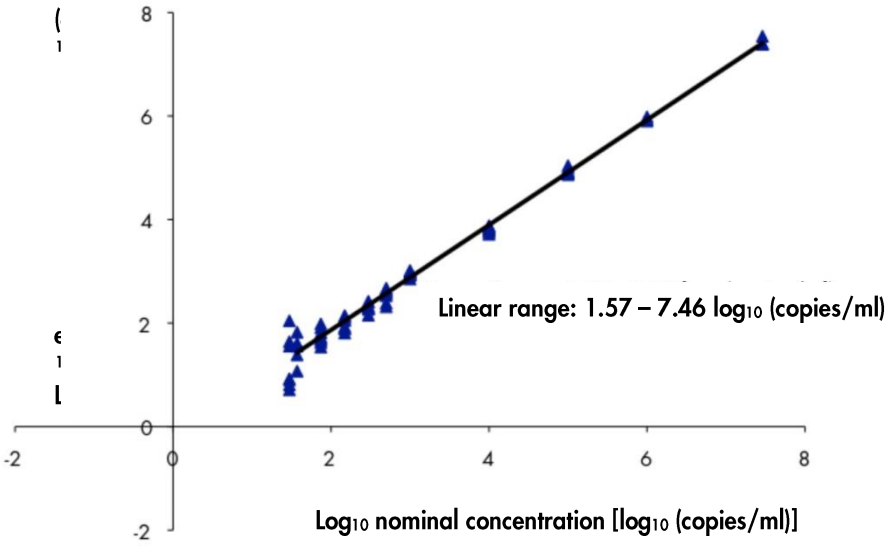

Figure 5. Linear range of the *artus* HSV-1/2 QS-RGQ Kit (HSV-1, plasma). Calculation of the linear range. The straight line was determined by a linear regression of the log10 calculated concentrations with the log10 nominal concentrations.

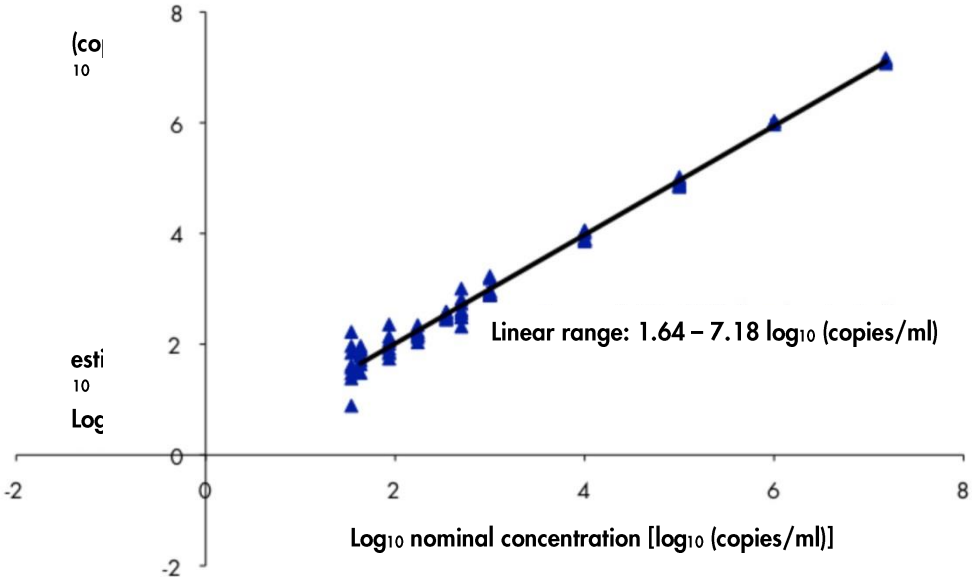

Figure 6. Linear range of the *artus* HSV-1/2 QS-RGQ Kit (HSV-2, plasma). Calculation of the linear range. The straight line was determined by a linear regression of the log10 calculated concentrations with the log10 nominal concentrations.

Robustness – plasma

The verification of the robustness in plasma allows the determination of the total failure rate of the *artus* HSV-1/2 QS-RGQ Kit. To verify the robustness for HSV-1, 30 HSV-1 negative samples of plasma were spiked with 111.5 copies/ml of HSV-1 material (approximately threefold concentration of the analytical sensitivity limit). To verify the robustness for HSV-2, 30 HSV-2 negative samples of plasma were spiked with 129.7 copies/ml of HSV-2 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* HSV-1/2 QS-RGQ Kit. For robustness in HSV-1 and HSV-2 target testing, 100% (30/30) samples were detected positive for HSV-1 and HSV-2 in each respective study. In addition, the robustness of the internal control was assessed after purification and analysis of 48 spiked plasma samples. These samples were 100% negative for HSV-1 and HSV-2 targets, and 100% positive for internal control target. Inhibitions were not observed. Thus, the robustness of the *artus* HSV-1/2 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 either HSV-1 or HSV-2 at approximately 10-fold the limit of detection (LOD) value (371.65 copies/ml and 432.39 copies/ml respectively). As a control, HSV-1 and HSV-2 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 HSV-1 and HSV-2 detection.

Clinical evaluation – plasma

The clinical performance of the *artus* HSV-1/-2 QS-RGQ Kit was evaluated by testing contrived specimens and analyzing the findings against the results with a comparative CE-IVD method. A total of 464 specimens of human EDTA plasma were prepared (HSV-1/-2 positive: n = 386; HSV-1/-2 negative: n = 78) and tested with the *artus* HSV-1/-2 QS-RGQ Kit and with a comparator kit at an external clinical diagnostic laboratory. The results were analyzed for the analytical sensitivity and analytical specificity, with the results reported for both assays to demonstrate equivalent performance.

Table 8. Asymptotic two-sided, 95% confidence interval: Newcombe score method for negative samples (plasma)

Type of assay by call frequencies	Negative	Positive	Total
artus HSV-1/2 QS-RGQ Kit	39	0	39
Comparator assay	39	0	39
Total	78	0	78

Table 9. Asymptotic two-sided, 95% confidence interval: Newcombe score method for positive samples (plasma)

Type of assay by call frequencies	Negative	Positive	Total
artus HSV-1/2 QS-RGQ Kit	0	193	193
Comparator assay	0	193	193
Total	0	386	386

Table 10. Proportion of correct calls for each assay (plasma)

Sample set	Sample type	*artus* HSV-1/2 QS-RGQ Kit: proportion of correct calls	Comparator kit: proportion of correct calls	Difference in proportions (*artus* HSV-1/2 QS-RGQ Kit – comparator)	Lower 95% confidence interval	Upper 95% confidence interval
All	Negative	1.000	1.000	0.000	–0.090	0.090
All	Positive	1.000	1.000	0.000	–0.020	0.020

The analytical sensitivity and analytical specificity values for the *artus* HSV-1/2 QS-RGQ Kit were both found to be 100%. The sensitivity and specificity values for the comparator kit were both also 100%. The difference in analytical specificity between the *artus* HSV-1/2 QS-RGQ Kit and comparator kit was 0% (95% confidence interval: –9 to 9%). The difference in analytical sensitivity between the *artus* HSV-1/2 QS-RGQ Kit and comparator kit was 0% (95% confidence interval: –2 to 2%). Overall, the data show the estimated difference in the analytical specificity and sensitivity between the *artus* HSV-1/2 QS-RGQ Kit and the comparator kit was zero.

Precision

The precision data of the *artus* HSV-1/2 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* HSV-1/2 QS-RGQ Kit (without consideration of the purification) were collected using HSV-1 and HSV-2 DNA with the concentration of 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 Tables 11 and12). Based on these results, the overall statistical spread of any given sample with the mentioned concentration is 1.82% (C_T) for HSV-1, 0.67% (C_T) for HSV-2, and 1.24% (C_T) and 1.58% (C_T) respectively for the detection of the internal control. These values are based on the totality of all single values of the determined variability.

Table 11. Precision data for HSV-1 on basis of the C_T values

	C_T value	Standard deviation	Coefficient of variation (%)
Intra-assay variability: HSV-1 10 copies/µl	30.46	0.25	0.81
Intra-assay variability: Internal control	25.29	0.08	0.30
Inter-assay variability: HSV-1 10 copies/µl	29.69	0.69	2.05
Inter-assay variability: Internal control	24.97	0.31	1.25
Inter-batch variability: HSV-1 10 copies/µl	29.95	0.40	1.35
Inter-batch variability: Internal control	24.90	0.30	1.20
Total variance: HSV-1 10 copies/µl	29.91	0.55	1.82
Total variance: Internal control	24.99	0.31	1.24

Table 12. Precision data for HSV-2 on basis of the C_T values

	C_T value	Standard deviation	Coefficient of variation (%)
Intra-assay variability: HSV-2 10 copies/µl	29.85	0.15	0.50
Intra-assay variability: Internal control	25.17	0.39	1.55
Inter-assay variability: HSV-2 10 copies/µl	29.92	0.15	0.49
Inter-assay variability: Internal control	25.11	0.41	1.63
Inter-batch variability: HSV-2 10 copies/µl	29.80	0.23	0.79
Inter-batch variability: Internal control	24.89	0.33	1.32
Total variance: HSV-2 10 copies/µl	29.88	0.20	0.67
Total variance: Internal control	25.07	0.40	1.58

Reproducibility

Reproducibility data permit a regular performance assessment of the *artus* HSV-1/2 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* HSV-1/2 QS-RGQ Kit

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.