artus® HBV QS-RGQ Kit

Performance Characteristics

artus HBV QS-RGQ Kit, Version 1, REF 4506363, 4506366

CE 0197

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Analytical sensitivity — plasma

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

The analytical sensitivity in consideration of the purification of the *artus* HBV QS-RGQ Kit was determined using a dilution series of the 2nd WHO International Standard for Hepatitis B Virus DNA Nucleic Acid Amplification Techniques (NIBSC code 97/750) from 316 to nominal 0.316 HBV IU/ml spiked in clinical plasma specimens. These were subjected to DNA extraction using the QIAsymphony DSP Virus/Pathogen Kit in combination with the Cellfree1000 protocol (extraction volume: 1 ml, elution volume: 60 µl). Each of the 9 dilutions was analyzed with the *artus* HBV 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 analytical detection limit in consideration of the purification of the *artus* HBV QS-RGQ Kit in combination with the Rotor-Gene Q is 10.22 IU/ml (p = 0.05). This means that there is a 95% probability that 10.22 IU/ml will be detected.

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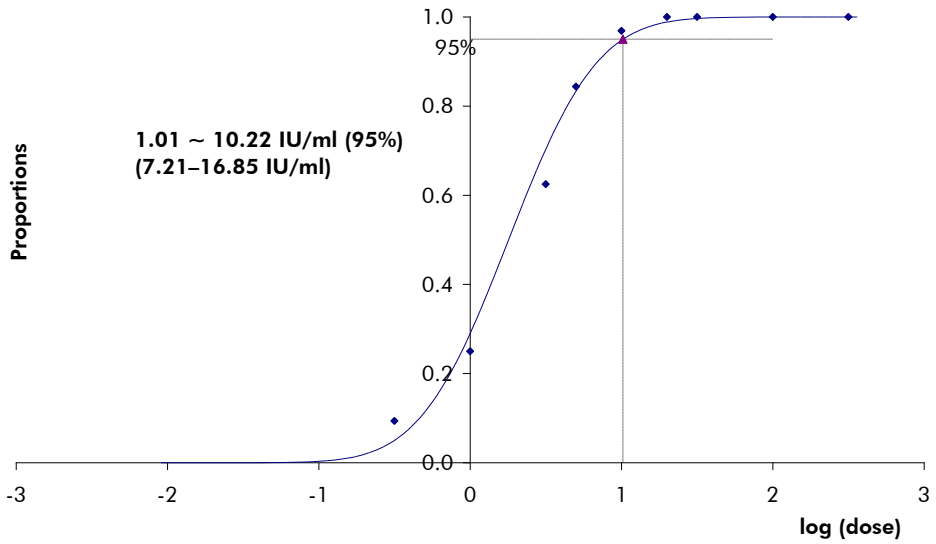

Figure 1. Probit analysis: plasma, HBV (Rotor-Gene Q). Analytical sensitivity in consideration of the purification (plasma, using the QIAsymphony DSP Virus/Pathogen Midi Kit) of the *artus* HBV QS-RGQ Kit on the Rotor-Gene Q.

Specificity — plasma

The specificity of the *artus* HBV 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 a PCR run on Rotor-Gene instruments with the following genotypes (see Table 1).

Table 1. Testing of the specificity of relevant strains

Virus	Genotype	Source	BK Virus (Cycling Green)	Internal control (Cycling Yellow)
HBV	A (USA)	Teragenix*	+	+
HBV	B (Indonesia)	Teragenix	+	+
HBV	C (indonesia)	Teragenix	+	+
HBV	C (Venezuela)	Teragenix	+	+
HBV	D (USA)	Teragenix	+	+
HBV	E (Cote D'Ivoire	Teragenix	+	+
HBV	F (Venezuela)	Teragenix	+	+
HBV	G (USA)	Teragenix	+	+
HBV	H (Nicaragua)	Teragenix	+	+

* Teragenix Corporation, Florida, USA.

For further specificity testing, HBV strains with known sequence differences in the pre-core region of the HBV genome (HBV Pre-Core Mutant Panel, Teragenix, Florida, USA) were used. All 9 pre-core mutant strains of this panel could be detected using the *artus* HBV QS-RGQ Kit.

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

A potential cross-reactivity of the *artus* HBV 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 mixed infections.

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

Control group	HBV (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 5 (cytomegalovirus)	–	+
Human herpesvirus 6	–	+
Human immunodeficiency virus 1	–	+
Hepatitis A virus	–	+
Hepatitis C virus	–	+
Parvovirus B19	–	+
Yellow fever virus	–	+
Human T cell leukemia virus type 1 and type 2	–	+
Coxsackie virus B3	–	+
Dengue virus 1–4	–	+
Escherichia coli	–	+

Linear range

The linear range in consideration of the purification of the *artus* HBV QS-RGQ Kit was determined by analyzing a dilution series of Acrometrix® HBV standard material ranging from 2.00 x 10^7 IU/ml to 3.16 x 10^0 IU/ml. The purification was carried out in replicates (n = 4 for concentrations ≥1.00 x 10^7 IU/ml; n = 8 for concentrations <1.00 x 10^7 IU/ml) using the QIAsymphony DSP Virus/Pathogen Kit in combination with the Cellfree1000 protocol (extraction volume: 1 ml, elution volume: 60 µl). Each of the samples was analyzed using the *artus* HBV QS-RGQ Kit. The linear range in consideration of the purification of the *artus* HBV QS-RGQ Kit has been determined to cover concentrations from 3.16 x 10^1 IU/ml to 2.00 x 10^7 IU/ml (Figure 2).

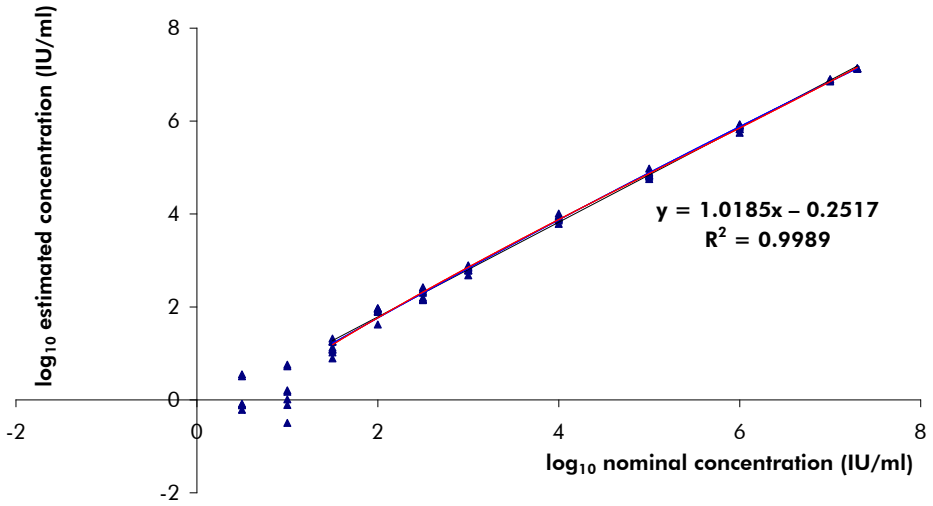

Figure 2. Linear range of the *artus* HBV QS-RGQ Kit. Calculation of the linear range. The straight line was determined by a linear regression of the $\log_{10}$ calculated concentrations with the $\log_{10}$ nominal concentrations. The equation of the regression line is included in the figure.

Precision

The precision data of the *artus* HBV 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* HBV QS-RGQ Kit (without consideration of the purification) were collected using the quantitation standard of the lowest concentration (QS 5; 10 IU/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 3).

Table 3. Precision data on basis of the C_T values

	Standard deviation	Variance	Coefficient of variation (%)
Intra-assay variability: HBV RG/TM QS 5	0.09	0.01	0.32
Intra-assay variability: Internal control	0.10	0.01	1.06
Inter-assay variability: HBV RG/TM QS 5	0.14	0.02	0.49
Inter-assay variability: Internal control	0.29	0.08	1.00
Inter-batch variability: HBV RG/TM QS 5	0.38	0.15	1.39
Inter-batch variability: Internal control	0.62	0.39	2.23
Total variance: HBV RG/TM QS 5	0.36	0.13	1.29
Total variance: Internal control	0.52	0.27	1.87

In addition, precision data for quantitative results in IU/µl were determined using the corresponding C_T values (Table 4). Based on these results, the overall statistical spread of any given sample with the mentioned concentration is 1.29% (C_T) or 8.99% (concentration), and 1.87% (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 4. Precision data on basis of the quantitative results (in IU/µl)

	Standard deviation	Variance	Coefficient of variation (%)
Intra-assay variability: HBV RG/TM QS 5	0.93	0.87	9.28
Inter-assay variability: HBV RG/TM QS 5	0.79	0.63	7.92
Inter-batch variability: HBV RG/TM QS 5	1.03	1.05	10.21
Total variance: HBV RG/TM QS 5	0.90	0.81	8.99

Precision — plasma

Precision data in consideration of the purification of the *artus* HBV QS-RGQ Kit was collected using Acrometrix HBV standard material with a concentration of 1.00 x 10^3 IU/ml spiked in clinical plasma specimens. Testing was performed using the QIAsymphony DSP Virus/Pathogen Kit in combination with the Cellfree1000 protocol (extraction volume: 1 ml, elution volume: 60 µl). Testing was performed on 36 replicates using a matrix of various batches of the QIAsymphony DSP Virus/Pathogen Kit and the *artus* HBV QS-RGQ Kit. Based on these results, the overall statistical spread of any given sample with the mentioned concentration is 1.22% (C_T) or 20.56% (concentration), and 1.29% (C_T) for the detection of the internal control (Tables 5 and 6). These values are based on the totality of all single values of the determined variabilities in consideration of the purification.

Table 5. Precision data (total variance) on basis of the C_T values

	Standard deviation	Variance	Coefficient of variation (%)
Acrometrix HBV standard (1.00 x 10^3 IU/ml)	0.37	0.13	1.22
Internal control (HBV, 1.00 x 10^3 IU/ml)	0.37	0.14	1.29

Table 6. Precision data (total variance) on basis of the quantitative results (in IU/ml)

	Mean	Standard deviation	Coefficient of variation (%)
Acrometrix HBV standard (1.00×10^3 IU/ml)	1.12×10^3	2.29×10^2	20.56

Robustness

The verification of the robustness allows the determination of the total failure rate of the *artus* HBV QS-RGQ Kit. To verify the robustness, 100 HBV negative samples of plasma were spiked with 30 IU/ml of HBV (approximately threefold concentration of the analytical sensitivity limit). After extraction using the QIAsymphony DSP Virus/Pathogen Kit in combination with the Cellfree1000_DSP protocol (extraction volume: 1 ml, elution volume: 60 µl), these samples were analyzed with the *artus* HBV 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* HBV QS-RGQ Kit is ≥99%.

Reproducibility

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

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.

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