artus® HI Virus-1 QS-RGQ Kit

Performance Characteristics

artus HI Virus-1 QS-RGQ Kit, Version 1, REF 4513363, 4513366

CE 0197

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

Analytical sensitivity

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

The analytical sensitivity in consideration of the purification of the *artus* HI Virus 1 QS-RGQ Kit was determined using a dilution series of the 2nd WHO International Standard for HIV-1 RNA (NIBSC code 97/650) from 316 to nominal 5 IU/ml spiked in clinical plasma specimens. These were subjected to RNA 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 8 dilutions was analyzed with the *artus* HI Virus-1 QS-RGQ Kit on 4 different days in 5 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* HI Virus-1 QS-RGQ Kit in combination with the Rotor-Gene® Q is 76.4 IU/ml (p = 0.05). This means that there is a 95% probability that 76.4 IU/ml (corresponding to 34.4 copies/ml) will be detected.

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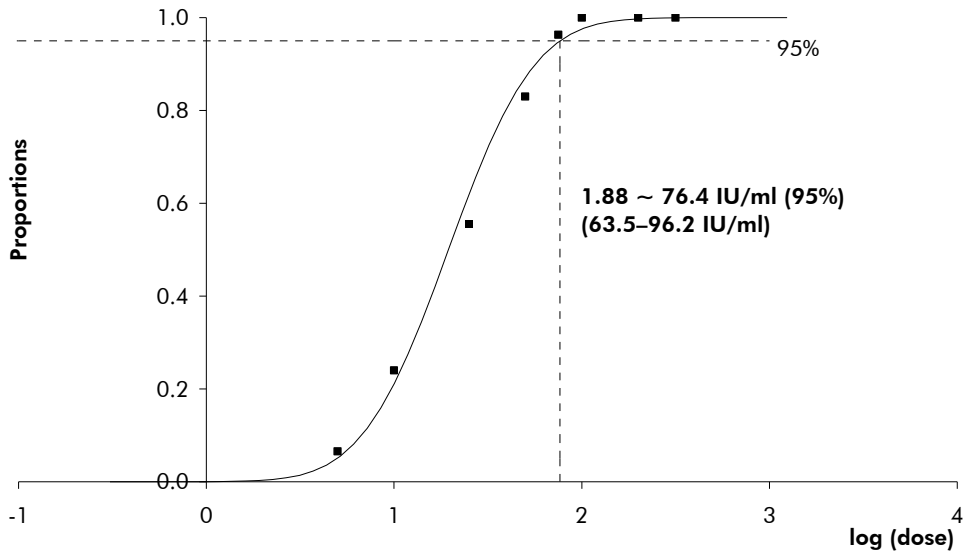

Figure 1. Probit analysis: HI Virus-1 (Rotor-Gene Q). Analytical sensitivity in consideration of the purification (QIAsymphony DSP Virus/Pathogen Kit) of the *artus* HI Virus-1 QS-RGQ Kit on the Rotor-Gene Q.

Specificity

The specificity of the *artus* HI Virus-1 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 an RT-PCR run on a Rotor-Gene instrument with the following genotypes (see Table 1).

Table 1. Testing of the specificity of relevant genotypes

Virus	Genotype	Source	HIV (Cycling Green)	Internal control (Cycling Orange)
HI virus-1	A	NIBSC*	+	+
HI virus-1	B	NIBSC	+	+
HI virus-1	C	NIBSC	+	+
HI virus-1	D	NIBSC	+	+
HI virus-1	E	NIBSC	+	+
HI virus-1	F	NIBSC	+	+
HI virus-1	G	NIBSC	+	+
HI virus-1	H	NIBSC	+	+

* National Institute for Biological Standards and Control, Hertfordshire.

For further specificity testing, HI virus-1 strains with known sequence differences in the pre-core region of the HI virus-1 genome (HI Virus-1 Pre-Core Mutant Panel, Teragenix, Florida, USA) were used. All 9 pre-core mutant strains of this panel could be detected using the *artus* HI Virus-1 QS-RGQ Kit.

Moreover, the specificity was validated with 100 different HIV negative plasma samples. These did not generate any signals with the HIV-1 specific primers and probes, which are included in the HI Virus-1 RG Masters.

A potential cross reactivity of the *artus* HI Virus-1 QS-RGQ Kit was tested using the control group listed in Table 2 (page 4). 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	HIV (Cycling Green)	Internal control (Cycling Orange)
Hepatitis A virus	–	+
Hepatitis B virus	–	+
Hepatitis C virus	–	+
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 type 1 and type 2	–	+
Enterovirus	–	+
Parvovirus B19	–	+
Yellow fever	–	+
Aspergillus flavus	–	+
Aspergillus fumigatus	–	+
Candida albicans	–	+
Chlamydia trachomatis	–	+
Cryptosporidium parvum	–	+
Filobasidiella neoformans	–	+
Mycoplasma pneumoniae	–	+
Pneumocystis cariniie	–	+
Staphylococcus sp.	–	+
Streptococcus agalactiae	–	+
Staphylococcus aureus	–	+
Streptococcus pyogenes	–	+

Linear range

The linear range in consideration of the purification of the *artus* HI Virus-1 QS RGQ Kit was determined by analyzing a dilution series of Acrometrix® HIV standard material ranging from 1.00 x 10^8 IU/ml to 2.50 x 10^1 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* HI Virus-1 QS-RGQ Kit. The linear range in consideration of the purification of the *artus* HI Virus-1 QS-RGQ Kit has been determined to cover concentrations from 1.00 x 10^2 IU/ml to 1.00 x 10^8 IU/ml (corresponding to 4.5 x 10^1 to 4.5 x 10^7 copies/ml).

Precision

The precision data of the *artus* HI Virus-1 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* HI Virus-1 QS-RGQ Kit (without consideration of the purification) were collected using the quantitation standard of the lowest concentration (QS4; 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). Based on these results, the overall statistical spread of any given sample with the mentioned concentration is 1.66% (C_T), and 2.15% (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 3. Precision data on basis of C_T values

	C_T value	Standard deviation	Coefficient of variation (%)
Intra-assay variability: HI Virus-1 RG QS 4	35.62	0.45	1.26
Intra-assay variability: Internal control	31.24	0.18	0.58
Inter-assay variability: HI Virus-1 RG QS 4	35.75	0.56	1.55
Inter-assay variability: Internal control	31.65	0.36	1.13
Inter-batch variability: HI Virus-1 RG QS 4	35.40	0.61	1.73
Inter-batch variability: Internal control	31.20	0.55	1.76
Total variance: HI Virus-1 RG QS 4	35.58	0.59	1.66
Total variance: Internal control	31.40	0.67	2.15

Precision data in consideration of the purification of the *artus* HI Virus-1 QS-RGQ Kit was collected using Acrometrix HIV 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* HI Virus-1 QS-RGQ Kit. Based on these results, the overall statistical spread of any given sample with the mentioned concentration is 1.45% (C_T) or 31.34% (concentration), and 1.47% (C_T) for the detection of the internal control (Tables 4 and 5). These values are based on the totality of all single values of the determined variabilities in consideration of the purification.

Table 4. Precision data (total variance) on basis of the C_T values

	Standard deviation	Variance	Coefficient of variation (%)
Acrometrix HIV standard (1.00×10^3 IU/ml)	0.48	0.24	1.45
Internal control (HIV, 1.00×10^3 IU/ml)	0.51	0.26	1.47

Table 5. Precision data (total variance) on basis of the quantitative results (in IU/ml)

	Mean	Standard deviation	Coefficient of variation (%)
Acrometrix HIV standard (1.00×10^3 IU/ml)	1.54×10^3	4.84×10^2	31.34

Robustness

The verification of the robustness allows the determination of the total failure rate of the *artus* HI Virus-1 QS-RGQ Kit. To verify the robustness, 100 HIV negative samples of plasma were spiked with 230 IU/ml of HIV (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* HI Virus-1 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* HI Virus-1 QS-RGQ Kit is ≥99%.

Reproducibility

Reproducibility data permit a regular performance assessment of the *artus* HI Virus-1 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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