artus® HCV QS-RGQ Kit

Performance Characteristics

artus HCV QS-RGQ Kit, Version 1, REF 4518363, 4518366

CE 0197

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Analytical sensitivity

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

The analytical sensitivity in consideration of the purification of the *artus* HCV QS-RGQ Kit was determined using a dilution series of HCV standard from Acrometrix® (the standard was calibrated to 2nd WHO International standard) from 150 to nominal 0.316 HCV 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* HCV QS-RGQ Kit on 4 different days in 4 runs with up to 15 replicates each. The limit of detection (LOD) value was determined by a probit analysis and verified using additional lots of the QIAsymphony DSP Virus/Pathogen Kit and *artus* HCV QS-RGQ Kit at 20 IU/ml (analyzed on 4 different days in 4 runs with 15 replicates per run). The hit rates of the probit experiment and verification experiment are shown in Table 1. The analytical detection limit in consideration of the purification of the *artus* HCV QS-RGQ Kit in combination with the Rotor-Gene Q using probit analysis is 21 IU/ml (p = 0.05; 95% confidence interval of 16–33 IU/ml). This means that there is a 95% probability that 21 IU/ml will be detected.

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Table 1. Hit rate analysis for HCV LOD study (data was used for probit analysis and verification study)

HCV titer (IU/ml)	Total replicate number	Total number positive	Percentage of positives
Probit analysis			
150	12	12	100
100	12	12	100
50	12	12	100
30	32	32	100
20	60	59	98
15	60	51	85
5	60	40	67
0.316	57	3	5
Verification			
20	60	57	95.00

Specificity

The specificity of the *artus* HCV 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 2).

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

A potential cross-reactivity of the *artus* HCV QS-RGQ Kit was tested using the control group listed in Table 3 (page 4). None of the tested pathogens has been reactive. No cross-reactivities appeared with mixed infections.

Table 2. Testing of the specificity of relevant genotypes

Virus	Genotype	Source	HCV (Cycling Green)	Internal control (Cycling Orange)
Hepatitis C virus	1	NIBSC, HemaCare, University of Essen	+	+
Hepatitis C virus	2	NIBSC, HemaCare, University of Essen	+	+
Hepatitis C virus	3	NIBSC, HemaCare, University of Essen	+	+
Hepatitis C virus	4	NIBSC, HemaCare, University of Essen	+	+
Hepatitis C virus	5	NIBSC, HemaCare, University of Essen	+	+
Hepatitis C virus	6	NIBSC, HemaCare, University of Essen	+	+

* National Institute for Biological Standards and Control, Hertfordshire.

Table 3. Testing the specificity of the kit with potentially cross-reactive pathogens

Control group	HCV (Cycling Green)	Internal control (Cycling Orange)
Human immunodeficiency virus 1	–	+
Hepatitis A virus	–	+
Hepatitis B 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	–	+
Human herpesvirus 6A	–	+
Human herpesvirus 6B	–	+
Human herpesvirus 8 (Kaposi's sarcoma herpesvirus)	–	+
Enterovirus	–	+
Parvovirus B19	–	+
Dengue fever	–	+
Yellow fever	–	+
Aspergillus flavus	–	+
Aspergillus fumigatus	–	+
Candida albicans	–	+
Chlamydia trachomatis	–	+
Cryptosporidium parvum	–	+
Filobasidiella neoformans	–	+

Table continues on next page

Table 3. Continued

Control group	HCV (Cycling Green)	Internal control (Cycling Orange)
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* HCV QS RGQ Kit was determined by analyzing dilution series of Acrometrix HCV standard material ranging from 1.77 x 10^7 IU/ml to 2.50 x 10^1 IU/ml. The purification was carried out in replicates (n = 4 for concentrations ≥1.00 x 10^5 IU/ml; n = 8 for concentrations <1.00 x 10^5 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* HCV QS-RGQ Kit. The linear range in consideration of the purification of the *artus* HCV QS-RGQ Kit has been determined to cover concentrations from 3.50 x 10^1 IU/ml to 1.77 x 10^7 IU/ml.

Precision

The precision data of the *artus* HCV 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* HCV QS-RGQ Kit (without consideration of the purification) were collected using the quantitation standard of the lowest concentration (QS 4; 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 4). In addition, precision data for quantitative results in IU/µl were determined using the corresponding C_T values (Table 5, page 7). Based on

these results, the overall statistical spread of any given sample with the mentioned concentration is 1.52% (C_T) or 25.71% (concentration), and 0.75% (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 C_T values

	C_T value	Standard deviation	Coefficient of variation (%)
Intra-assay variability: Hep. C Virus RG QS 4	32.81	0.09	0.28
Intra-assay variability: Hep. C Virus RG IC	30.04	0.08	0.27
Inter-assay variability: Hep. C Virus RG QS 4	32.14	0.5	1.57
Inter-assay variability: Hep. C Virus RG IC	30.23	0.22	0.71
Inter-batch variability: Hep. C Virus RG QS 4	32.56	0.48	1.46
Inter-batch variability: Hep. C Virus RG IC	30.28	0.24	0.78
Total variance: Hep. C Virus RG QS 4	32.41	0.49	1.52
Total variance: Hep. C Virus RG IC	30.29	0.29	0.75

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

	Standard deviation	Variance	Coefficient of variation (%)
Intra-assay variability: Hep. C Virus RG QS 4	0.64	0.41	6.34
Inter-assay variability: Hep. C Virus RG QS 4	1.00	1.00	9.93
Inter-batch variability: Hep. C Virus RG QS 4	3.92	15.34	37.35
Total variance: Hep. C Virus RG QS 4	2.63	6.93	25.71

Precision data in consideration of the purification of the *artus* HCV QS-RGQ Kit was collected using Acrometrix HCV 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 Midi 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 Midi Kit and the *artus* HCV QS-RGQ Kit. Based on these results, the overall statistical spread of any given sample with the mentioned concentration is 0.95% (C_T) or 20.07% (concentration), and 1.26% (C_T) for the detection of the internal control (Tables 6 and 7). These values are based on the totality of all single values of the determined variabilities in consideration of the purification.

Table 6. Precision data (total variance) on basis of the C_T values

	Standard deviation	Variance	Coefficient of variation (%)
Acrometrix HCV standard (1.00 x 10^3 IU/ml)	0.30	0.09	0.95
Internal control (HCV, 1.00 x 10^3 IU/ml)	0.43	0.18	1.26

Table 7. Precision data (total variance) on basis of the quantitative results (in IU/ml)

	Mean	Standard deviation	Coefficient of variation (%)
Acrometrix HCV standard (1.00 x 10^3 IU/ml)	2.37 x 10^3	4.76 x 10^2	20.07

Robustness

The verification of the robustness allows the determination of the total failure rate of the *artus* HCV QS-RGQ Kit. To verify the robustness, 100 HCV negative samples of plasma were spiked with 110 IU/ml of HCV (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* HCV 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* HCV QS-RGQ Kit is ≥99%.

Reproducibility

Reproducibility data permit a regular performance assessment of the *artus* HCV 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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