QIAsymphony® RGQ Application Sheet

QIAsymphony RGQ application *artus*® HCV QS-RGQ Kit (sample type: plasma)

IVD CE 0197

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General information

Kit	*artus* HCV QS-RGQ Kit, Version 1, REF 4518363, 4518366
Validated sample material	Human EDTA plasma
Front-end purification	QIAsymphony DSP Virus/Pathogen Midi Kit (cat. no. 937055)
Sample volume (including excess volume)	1200 µl
Assay Parameter Set	artus_HCV_plasma1000_V4
Default Assay Control Set	Cellfree1000_V6_DSP_artus_HCV
Elution volume	60 µl
Required software version	Version 4.0 or higher
Master mix volume	30 µl
Template volume	20 µl
Number of reactions	6–24 or 6–72*
Runtime on AS module	For 6 reactions: approximately 9 minutes For 72 reactions: approximately 35 minutes

* When running multiple assay runs, ensure that the limit of 72 reactions and 1 assay rack adapter is not exceeded. Avoid extended incubation time (>30 minutes) between completion of the assay run and transfer to the Rotor-Gene® Q.

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Sample & Assay Technologies

Materials required but not provided

Purification kit	■	QIAsymphony DSP Virus/Pathogen Midi Kit (cat. no. 937055)
Adapters for the QIAsymphony SP	■	Elution Microtube Rack QS (Cooling Adapter, EMT, v2, Qsym, cat. no. 9020730)
	■	Tube Insert 3B (Insert, 2.0ml v2, samplecarr. (24), Qsym, cat. no. 9242083)
Consumables for the QIAsymphony SP	■	Sample Prep Cartridges, 8-well (cat. no. 997002)
	■	8-Rod Covers (cat. no. 997004)
	■	Filter-Tips, 1500 µl (cat. no. 997024)
	■	Filter-Tips, 200 µl (cat. no. 990332)
	■	Elution Microtubes CL (cat. no. 19588)
	■	Tip disposal bags (cat. no. 9013395)
	■	Micro tubes 2.0 ml Type H or Micro tubes 2.0 ml Type I (Sarstedt, cat. nos. 72.693 and 72.694, www.sarstedt.com) for use with samples and internal controls
Adapters and reagent holders for the QIAsymphony AS	■	Reagent holder 1 QS (Cooling Adapter, Reagent Holder 1, Qsym, cat. no. 9018090)
	■	Reagent holder 2 QS (Cooling Adapter, Reagent Holder 2, Qsym, cat. no. 9018089)
	■	RG Strip Tubes 72 QS (Cooling Adapter, RG Strip Tubes 72, Qsym, cat. no. 9018092)
Consumables for the QIAsymphony AS	■	Strip Tubes and Caps, 0.1 ml (cat. no. 981103)
	■	Tubes, conical, 2 ml, Qsym AS (cat. no. 997102)* or Micro tubes 2.0 ml Type I (Sarstedt, cat. no. 72.694.005)
	■	Tube, conical, 5 ml, Qsym AS (cat. no. 997104)* or Tubes with flat base from PP (Sarstedt, cat. no. 60.558.001)
	■	Reagent Bottles, 30 ml, Qsym AS (cat. no. 997108)
	■	Elution Microtubes CL (cat. no. 19588)
	■	Filter-Tips, 1500 µl (cat. no. 997024)
	■	Filter-Tips, 200 µl (cat. no. 990332)
	■	Filter-Tips, 50 µl (cat. no. 997120)
	■	Tip disposal bags (cat. no. 9013395)

* Please inquire for availability.

Specimen handling and storage

Sample collection	Blood sample 5–10 ml EDTA blood 8x overhead mix — no agitation! Heparinized human samples must not be used
Sample storage	Separation: 20 minutes centrifugation, 800–1600 x *g* within 24 hours post-collection Transfer the isolated plasma into a sterile polypropylene tube Virus encapsulated RNA stable at:* 4°C days –20°C weeks –70°C months
Sample transport	Shatterproof transport Shipment within 24 hours Mail shipment according to legal instructions for the transport of pathogen material† Blood samples should be shipped cool (2 to 8°C)
Interfering substances	Heparin (≥10 IU/ml) affects the PCR. Samples collected in tubes containing heparin as an anticoagulant or samples from heparinized patients must not be used. Elevated levels of albumin (≤6 g/dl), bilirubin (≤30 mg/dl), lipids (≤1 g/dl triglyceride), and hemolytic samples (≤2 g/dl hemoglobin) do not influence the system.

* Arbeitskreis Blut, V17 (09.1997), Bundesgesundheitsblatt 11/1997, p. 452–456.

† International Air Transport Association (IATA). Dangerous Goods Regulations.

Procedure

Preparation of carrier RNA and addition of the internal control to the samples

Using the QIAsymphony DSP Virus/Pathogen Midi Kit in combination with the *artus* HCV QS-RGQ Kit requires introduction of the internal control (Hep. C Virus RG IC) into the purification procedure to monitor the efficiency of sample preparation and downstream assay.

Internal controls must be added with carrier RNA (CARRIER)–Buffer AVE (AVE) mixture, and the total volume of the internal control–carrier RNA (CARRIER)–Buffer AVE (AVE) mixture remains 120 µl.

The table represents the addition of internal control to the isolation at a ratio of 0.1 µl per 1 µl elution volume. We recommend preparing fresh mixtures for each run just before use.

Component	Volume (µl) (Sarstedt® tubes)*	Volume (µl) (BD™ tubes)†
Stock carrier RNA (CARRIER)	5	5
Internal control‡	9	9
Buffer AVE	106	106
Final volume per sample (excluding dead volume)	**120**	**120**
Total volume for n samples	**(n x 120) + 360§**	**(n x 120) + 600¶**

* Micro tubes 2.0 ml Type H and Micro tubes 2.0 ml Type I, Sarstedt cat. nos. 72.693 and 72.694.

† Tubes 14 ml, 17 x 100 mm polystyrene round-bottom (Becton Dickinson, cat. no. 352051).

‡ The calculation of the amount of internal control is based on the initial elution volumes (90 µl). Additional void volume depends on the type of sample tube used.

§ Internal control mixture corresponding to 3 additional samples (i.e., 360 µl) is required. Do not fill more than 1.92 ml total volume (corresponding to a maximum of 13 samples. These volumes are specific for Micro tubes 2.0 ml Type H and Micro tubes 2.0 ml Type I, Sarstedt cat. nos. 72.693 and 72.694).

¶ Internal control mixture corresponding to 5 additional samples (i.e., 600 µl) is required. Do not fill more than 13.92 ml total volume (corresponding to a maximum of 111 samples. These volumes are specific for Tubes 14 ml, 17 x 100 mm polystyrene round-bottom, Becton Dickinson, cat. no. 352051).

QIAsymphony SP setup

"Waste" drawer

Unit box holder 1–4	Empty unit boxes
Waste bag holder	Waste bag
Liquid waste bottle holder	Empty and install liquid waste bottle

"Eluate" drawer

Elution rack	Use slot 1, cooling position
Elution volume*	Preselected elution volume: 60 µl Initial elution volume: 90 µl

* The elution volume is preselected for the protocol. This is the minimum accessible volume of eluate in the final elution tube. The initial volume of elution solution is required to ensure that the actual volume of eluate is the same as the preselected volume.

"Reagents and Consumables" drawer

RC Position 1 and 2	Load 1 reagent cartridge (RC) for up to 48 samples or 2 new reagent cartridges (RC) for up to 96 samples
Tip rack holder position 1–4	Load sufficient racks of disposable filter-tips, 200 µl (see "Required plasticware for 1–4 sample batches", page 6)
Tip rack holder position 5–18	Load sufficient racks of disposable filter-tips, 1500 µl (see "Required plasticware for 1–4 sample batches", page 6)
Unit box holder position 1–3	Load 3 unit boxes containing sample prep cartridges
Unit box holder position 4	Load 1 unit box containing 8-Rod Covers

"Sample" drawer

Sample type	Plasma
Sample volume (including excess volume)	1200 µl
Sample tubes	Micro tubes 2.0 ml Type H or Micro tubes 2.0 ml Type I (Sarstedt, cat. nos. 72.693 and 72.694)
Insert	Tube Insert 3B (cat. no. 9242083)

Required plasticware for 1–4 sample batches

	One batch, 24 samples*	Two batches, 48 samples*	Three batches, 72 samples*	Four batches, 96 samples*
Disposable filter-tips, 200 µl†‡	28	52	76	100
Disposable filter-tips, 1500 µl†‡	113	206	309	402
Sample prep cartridges§	21	42	54	72
8-Rod Covers¶	3	6	9	12

* Use of more than one internal control tube per batch and performing more than one inventory scan requires additional disposable filter tips.

† There are 32 filter-tips/tip rack.

‡ Number of required filter-tips includes filter-tips for 1 inventory scan per reagent cartridge.

§ There are 28 sample prep cartridges/unit box.

¶ There are twelve 8-Rod Covers/unit box.

QIAsymphony AS setup

Consumables

During the setup, the appropriate positions for each consumable on the QIAsymphony AS module are indicated on the touchscreen of the instrument.

Consumables	Name on touchscreen	For use with adapter/reagent holder
Strip Tubes and Caps, 0.1 ml (250)	QIA#981103 *StripTubes 0.1	RG Strip Tubes 72 QS
Tubes, conical, 2 ml, Qsym AS (500)†‡	QIA#997102 *T2.0 ScrewSkirt§	Reagent holder 1 QS Reagent holder 2 QS
Tube, conical, 5 ml, Qsym AS (500)†‡	QIA#997104 *T5.0 ScrewSkirt§	Reagent holder 1 QS Reagent holder 2 QS
Reagent Bottles, 30ml, Qsym AS (50)†	QIA#997108 *Bottle 30ml§	Reagent holder 2 QS
Elution Microtubes CL (24 x 96)	QIA#19588 * EMTR	Elution Microtube Rack QS

* Indicates labware that can be cooled using a cooling adapter with bar code.

† For master mix components, system-prepared master mix, assay standards, and assay controls.

‡ Alternatively, the Sarstedt tubes described in "Materials required but not provided", page 2, can be used.

§ The suffix "(m)" in the touchscreen indicates that liquid level calculations for the respective tube have been optimized for reagents forming a concave meniscus.

Adapters and reagent holders

Rack/reagent holder	Name	Number required¶
Sample rack	Elution Microtube Rack QS	1
Reagent holders	Reagent holder 1 QS	1
Assay racks	RG Strip Tubes 72 QS	1

¶ Calculated for an assay run with 72 reactions.

Filter-tips

Load tip racks starting with tip slots 1, 2, and 3 in the "Eluate and Reagents" drawer, and then load tip racks into tip slots 7, 8, and 9 in the "Assays" drawer.

Consumable	Name on touchscreen	Minimum number for 24 reactions	Minimum number for 72 reactions
Filter-Tips, 1500 µl (1024)	1500 µl	5	6
Filter-Tips, 200 µl (1024)	200 µl	10	10
Filter-Tips, 50 µl (1024)	50 µl	25	73
Tip Disposal Bags	–	1	1

RT-PCR on the Rotor-Gene Q

The *artus* HCV QS-RGQ Kit can be run on the Rotor-Gene Q using manual analysis with Rotor-Gene Q software 2.1 or higher or using automatic analysis with Rotor-Gene AssayManager®. The following sections describe the settings and setup using the 2 different softwares.

RT-PCR using Rotor-Gene Q software 2.1 or higher

Set the following parameters for the run.

Reaction Volume (µL)	50
Hold	Hold Temperature: 50 deg Hold Time: 30 mins
Hold 2	Hold Temperature: 95 deg Hold Time: 15 mins
Cycling	50 time(s) 95 deg for 30 secs 50 deg for 60 secs 72 deg for 30 secs
Auto-Gain Optimisation Setup	50 degrees (Samples: Green; IC: Orange)

For more detailed instructions, refer to the protocol sheet "Settings to run *artus* QS-RGQ Kits" at www.qiagen.com/products/artushcvrgpcrkitce.aspx.

RT-PCR using Rotor-Gene AssayManager

For automatic analysis using the *artus* HCV QS-RGQ Kit with Rotor-Gene AssayManager, the following files must be installed in your Rotor-Gene AssayManager database.

- *artus* basic plug-in (available for download from www.qiagen.com/Products/Rotor-GeneAssayManager.aspx)
- *artus* HCV QS-RGQ AssayProfile for plasma samples (AP_artus_HCV_plasma1000_QS_V1.iap) (available for download from www.qiagen.com/products/artushcvrgpcrkitce.aspx)

For a description of how to install these files, refer to the *Rotor-Gene AssayManager Core Application User Manual*.

After installing these files, Rotor-Gene AssayManager can use the information given in the QIAsymphony AS result file to set up a run for real-time PCR amplification and subsequent automatic analysis. For a description of how to import QIAsymphony AS result files into Rotor-Gene AssayManager, refer to the *Rotor-Gene AssayManager Core Application User Manual*. Please note that the export of cycler files is not needed with the Rotor-Gene AssayManager.

Interpretation of results

This section describes interpretation of results on the Rotor-Gene Q. Review also the sample status information from the QIAsymphony SP/AS result files for analysis of the complete sample-to-result workflow. Only samples with a valid status should be used.

The *artus* HCV QS-RGQ Kit can be run on the Rotor-Gene Q using manual analysis with Rotor-Gene Q software 2.1 or higher or using automatic analysis with Rotor-Gene AssayManager. The following sections describe interpretation of results using the 2 different softwares.

Interpretation of results using Rotor-Gene Q software 2.1 or higher

Signal detection and conclusions

Signal in channel Cycling Green	Signal in channel Cycling Orange	Quantitative result (IU/ml)	Interpretation
Yes	Yes	<21	Valid result: HCV RNA detected, <35 IU/ml Quantitation not possible since the quantitative result is below limit of detection. Reproducibility of the positive result is not assured.
Yes	Yes	≥21 and <35	Valid result: HCV RNA detected, <35 IU/ml Quantitation not possible since the quantitative result is below the linear range of the assay.
Yes	Yes/No*	≥35 and ≤1.77 x 10^7	Valid result: HCV RNA detected at the calculated concentration Quantitative result is within the linear range of the assay.
Yes	Yes/No*	>1.77 x 10^7	Valid result: HCV RNA detected, >1.77 x 10^7 IU/ml Quantitation not possible since the quantitative result is above the linear range of the assay.†
No	Yes	–	Valid result: No HCV RNA is detectable.‡
No	No	–	Invalid result: No result can be concluded.§

* In this case, the detection of a signal in the Cycling Orange channel is dispensable, since high initial concentrations of HCV RNA (positive signal in the Cycling Green channel) can lead to a reduced or absent fluorescence signal of the internal control in the Cycling Orange channel (competition).

† If quantitation is desired, dilute the sample with HCV-free plasma and reprocess. Multiply the quantitative result from the reprocessed sample by the dilution factor.

‡ If the C_T value for the internal control of a negative sample is more than 3 cycles higher than the C_T value for the internal control of the no template control in the run ($C_{T\ IC\ Sample} - C_{T\ IC\ NTC} > 3$), then the sample should be treated as invalid. No result can be concluded.

§ Information regarding error sources and their solution can be found in "Troubleshooting guide" of the artus *HCV QS-RGQ Kit Handbook*.

Threshold setup for the PCR analysis

The optimal threshold settings for a given combination of Rotor-Gene Q instrument and *artus* QS-RGQ Kit should be set empirically by testing each individual combination since it is a relative value depending on the overall diagnostic workflow. The threshold can be set at a preliminary value of 0.04 for the analysis of the first PCR run, but this value should be fine-tuned in a comparative analysis of the next runs of the workflow. The threshold should be set manually just above the background signal of the negative controls and negative samples. The mean threshold value calculated from these experiments will most likely work for the majority of future runs, but the user should nevertheless review the generated threshold value at regular intervals. The threshold value will usually be in the range of 0.03–0.05 and should be rounded to no more than three decimal places.

Quantitation

The quantitation standards (Hep. C Virus RG QS 1–4) in the *artus* HCV QS-RGQ Kit are treated as previously purified samples and the same volume is used (20 µl). To generate a standard curve on Rotor-Gene Q Instruments, all 4 quantitation standards should be used and defined in the "Edit Samples" dialog box on the Rotor-Gene Q instrument as standards with the specified concentrations (see the instrument user manual).

Note: The quantitation standards are defined as IU/µl.* The following equation has to be applied to convert the values determined using the standard curve into IU/ml of sample material

$$\text{Result (IU/ml)} = \frac{\text{Result (IU/µl) x Initial Elution Volume (90 µl)}^{\dagger}}{\text{Sample Volume (ml)}}$$

As a matter of principle the initial sample volume should be entered in the equation above. This has to be considered when the sample volume has been changed prior to the nucleic acid extraction (e.g., reducing the volume by centrifugation or increasing the volume by adding to the volume required for the isolation).

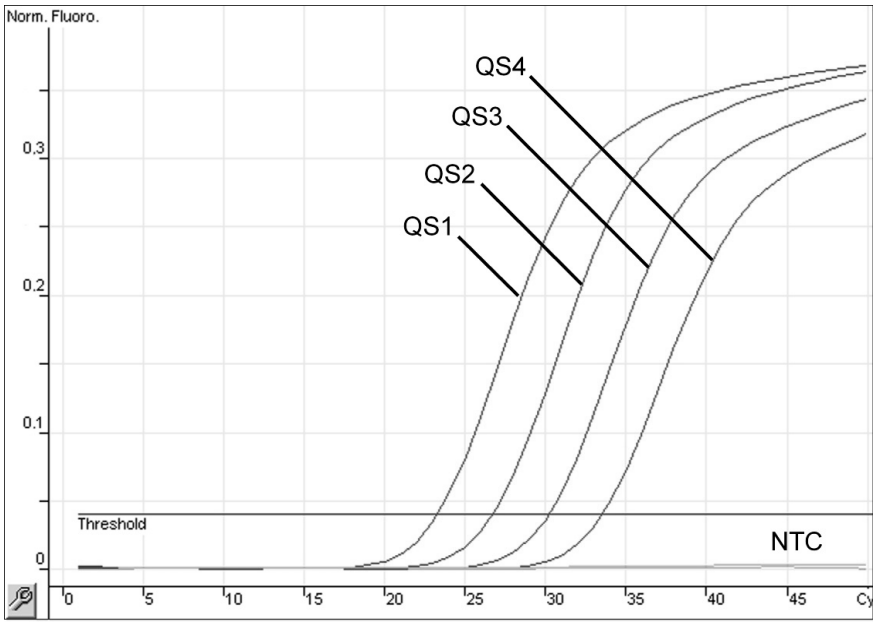
Conversion factor

1 IU/ml corresponds to 1.21 copies/ml for detection of HCV RNA on the Rotor-Gene Q. The conversion factor is an approximation based on an average factor across the assay's dynamic range.

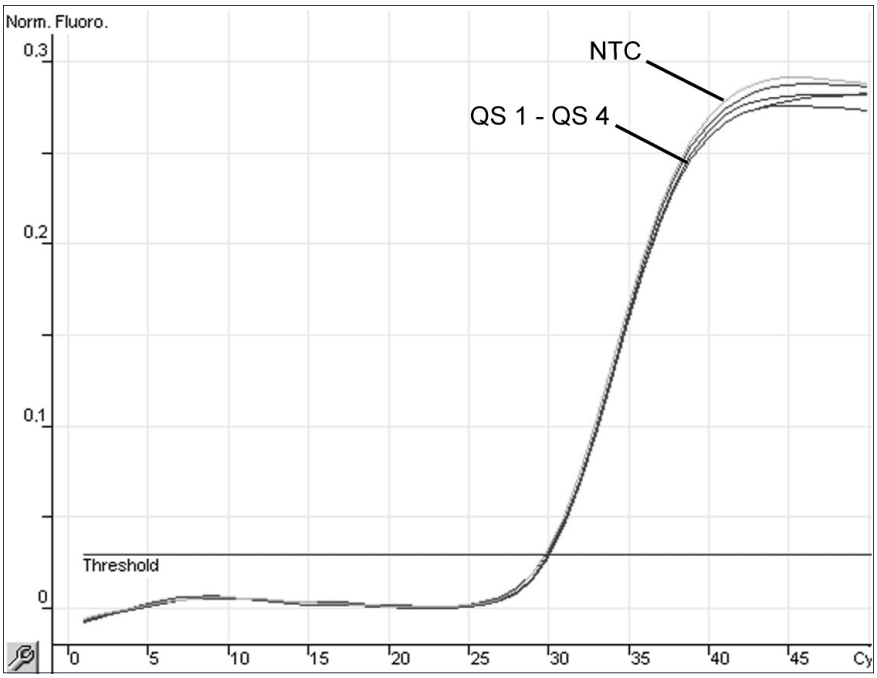
* The standard has been calibrated using the International HCV standard (WHO).

† The calculation is based on the initial elution volumes (90 µl).

Examples of positive and negative PCR reactions



Detection of the quantitation standards (Hep. C Virus QS 1–4) in fluorescence channel Cycling Green. NTC: No template control (negative control).



Detection of the internal control (IC) in fluorescence channel Cycling Orange with simultaneous amplification of the quantitation standards (Hep. C Virus QS 1–4). NTC: No template control (negative control).

Interpretation of results using Rotor-Gene AssayManager

The *artus* HCV QS-RGQ AssayProfile for plasma samples contains all rules for interpreting the assay results automatically. Based on these, the software will assess the validity or invalidity of samples and controls. This automatic analysis may provide the following corresponding flags.

Flag	Behavior	Description
ASSAY_INVALID	Invalid	Assay is set to invalid because at least one external control is invalid.
CORRESPONDING_ CONTROL_INVALID	Invalid	Target is set to invalid because at least one corresponding external control is invalid.
CORRESPONDING_ POSITIVE_CONTROL_ TARGET_INVALID	Invalid	The target result is set to invalid because the corresponding positive control is invalid.
CT_ABOVE_ ACCEPTED_RANGE	Invalid	The detected C_T value is higher than the defined cut-off C_T.
CT_BELOW_ ACCEPTED_RANGE	Invalid	The detected C_T value is lower than the defined cut-off C_T.
CURVE_SHAPE_ ANOMALY	Invalid	The raw data amplification curve shows a shape that is deviating from the established behavior for this assay. There is a high likelihood for wrong results or result misinterpretation.
FLAT_BUMP	Invalid	The amplification curve shows a shape like a flat bump, deviating from the established behavior for this assay. There is a high likelihood for wrong results or result misinterpretation (wrong C_T value determination).
FLUORESCENCE_ TOO_LOW	Invalid	The fluorescence signal is lower than the defined fluorescence cut-off.
IC_INVALID	Invalid	An internal control in the same tube is invalid.
IC_NO_SIGNAL	Invalid	No signal is detected for an internal control in the same tube.
INHIBITION_BY_CT	Warning	The defined maximum C_T range between the C_T for the internal control of that sample and the C_T for the internal control of the NTC is exceeded.

Flag	Behavior	Description
INHIBITION_BY_ FLUORESCENCE	Warning	The defined maximum fluorescence difference between the internal control fluorescence of the NTC and the internal control fluorescence of that sample for the last cycle is exceeded.
MULTI_THRESHOLD_ CROSSING	Invalid	The amplification curve crosses the threshold more than once. An unambiguous C_T cannot be determined. This flag corresponds to the "NEG (Multi Ct)" flag of the Rotor-Gene Software. For more details refer to the *Rotor-Gene Q User Manual*.
NO_CT_DETECTED	Invalid	No C_T is detected for this target.
NORM_FACTOR_ ALTERATION	Warning	Normalization failed. The amplification curve is displayed without normalization. Results should be manually checked for correctness.
OUT_OF_ COMPUTATION_ RANGE	Invalid	The calculation of the concentration for this sample exceeds the technical limit.
SATURATION	Invalid	The raw data fluorescence is saturating strongly before the inflection point of the amplification curve.
SATURATION_ IN_PLATEAU	Warning	The raw data fluorescence is saturating in the plateau phase of the amplification curve.
SPIKE	Warning	A spike in the raw data fluorescence is detected in the amplification curve but outside the region where the C_T is determined.
SPIKE_CLOSE_TO_CT	Invalid	A spike is detected in the amplification curve close to the C_T.
STEEP_BASELINE	Invalid	A steeply rising baseline for the raw data fluorescence is detected in the amplification curve.
STRONG_BASELINE_ DIP	Invalid	A strong drop in the baseline for raw data fluorescence is detected in the amplification curve.
STRONG_NOISE	Invalid	Strong noise is detected outside the growth (exponential) phase of the amplification curve.
STRONG_NOISE_ IN_GROWTH_PHASE	Invalid	Strong noise is detected in the growth (exponential) phase of the amplification curve.

Flag	Behavior	Description
TOO_LESS_ CORRELATION_IN_ STANDARD_CURVE	Invalid	Either a lower limit for the R^2 value or a lower limit for the R value is not reached.
UNCERTAIN	Warning	Results from the automatic data scan (AUDAS) are conflicting with results from the core analysis. An unambiguous automatic assessment of data validity is not possible.
UPSTREAM	Variable	Sample status was set to invalid or unclear by an upstream process (e.g., QIAsymphony Assay Setup). **Note**: For "unclear" flags from upstream processes, the behavior of Rotor-Gene AssayManager is defined in the "Configuration" environment. For "invalid" flags from upstream processes Rotor-Gene AssayManager always invalidates such samples.
WAVY_BASE_ FLUORESCENCE	Invalid	A wavy baseline for the raw data fluorescence is detected in the amplification curve.

The results of Rotor-Gene AssayManager need approval/rejection by a user with the user role "Approver". For more information on the approval process, refer to the Rotor-Gene AssayManager artus *Basic Plug-in User Manual*.

Threshold setup for PCR analysis

The *artus* HCV QS-RGQ AssayProfile for plasma samples automatically sets the threshold.

Quantitation

The *artus* HCV QS-RGQ AssayProfile for plasma samples contains all information on the quantitation standards needed for calculating the concentration of the target in the sample or the eluate. Rotor-Gene AssayManager also allows direct conversion into other concentration units. Refer to the Rotor-Gene AssayManager artus *Basic Plug-in User Manual* for more information.

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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