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artus® HCV QS-RGQ Kit Handbook

72

Version 2

For use with QIAsymphony® SP/AS and Rotor-Gene® Q instruments

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Sample to Insight

QIAGEN

Contents

Intended Use

The *artus* HCV QS-RGQ assay is an in vitro nucleic acid amplification test based on reverse transcription-polymerase chain reaction (RT-PCR) technology for use with the QS-RGQ instruments for the quantitative detection of Hepatitis C virus (HCV) RNA (genotypes 1-6) in EDTA plasma from HCV-infected individuals.

The *artus* HCV QS-RGQ assay is intended to be used, in conjunction with clinical presentation and other laboratory markers, for disease prognosis, and as an aid in assessing viral response to antiviral treatment as measured by changes in HCV RNA levels in human EDTA plasma at baseline, during treatment and at end of treatment. The *artus* HCV QS-RGQ assay is not intended for screening blood, plasma or serum for HCV infection. The assay is not to be used as a diagnostic test to confirm the presence of HCV infection.

Summary and Explanation

The *artus* HCV QS-RGQ Kit constitutes a ready-to-use system for the detection of HCV RNA using PCR on Rotor-Gene Q instruments with sample preparation and assay setup using the QIAsymphony SP/AS instruments. The Hepatitis C Virus RG Master A and B contain reagents and enzymes for the specific amplification of a 69 basepair region of the HCV genome, and for the direct detection of the specific amplicon in fluorescence channel Cycling Green of the Rotor-Gene Q instrument.

In addition, the *artus* HCV QS-RGQ Kit contains a second heterologous amplification system to identify possible PCR inhibition. This is detected as an internal control (IC) in fluorescence channel Cycling Orange of the Rotor-Gene Q instrument. The detection limit of the analytical HCV PCR is not reduced. External positive controls

(Hepatitis C Virus RG QS 1–4) are supplied allowing the determination of the amount of viral RNA.

Pathogen information

Background

HCV is an RNA virus of the Flaviviridae family. Surrounded by an envelope structure and coding for only 10 mature proteins, HCV is responsible for severe pathologies ranging from liver inflammation (hepatitis) and cirrhosis to hepatocellular carcinoma (HCC), which is invariably fatal. There are over 200 million carriers of HCV worldwide, four million of whom are in Europe. Infection with HCV is one of the main causes of chronic liver disease worldwide with most individuals unaware of their infection. HCV is classified into six major genotypes (1–6) with genotype 1 (subtypes a and b) the most common subtype in North America and Western Europe (1). There is a nucleotide homology of only 55% to 70% between each genotype, and over 80 subtypes have been identified. The determination of genotype is recommended for proper clinical management and for predicting the likelihood of response to treatment (2).

Definition of clinical disease

Acute HCV infection remains for the vast majority of cases completely asymptomatic. The incubation period of HCV ranges between 6 to 10 weeks and the disease onset can have non-specific symptoms, including anorexia, vague abdominal discomfort, nausea and vomiting, fever and fatigue. In rarer cases, these initial symptoms may include icterus (jaundice). Only a small percentage (10–30%) of acutely infected individuals will clear the virus. In the majority of cases, HCV establishes lifelong infection and the patient become a chronic carrier.

Chronic HCV infection is defined as the continuation of disease without improvement for a period of more than 6 months and develops in around two thirds of infected individuals. In a further 10–20%, chronic HCV infection leads to cirrhosis and subsequently liver failure, with mortality rates of up to 25%. Only 1–5% of HCV carriers develop HCC and this tends to be rare in non-cirrhotic cases. Importantly, HCV infection can remain asymptomatic for up to 20 years before the development of serious complications.

Although the mechanisms behind disease progression are not entirely understood, several factors have been reported to influence the rate of HCV disease progression. These include age (increasing age associated with more rapid progression), gender (males have more rapid disease progression), alcohol consumption (associated with an increased rate of disease progression), and the presence of fat in liver cells. In addition, co-infection with hepatitis B virus (HBV) and human immunodeficiency virus-1 (HIV-1) has been well documented to markedly increase the disease progression rate (3).

Current therapeutic strategies

The goal of treatment is to eradicate HCV in chronically-infected individuals, leading to a Sustained Virological Response (SVR), which approximates to a cure. A SVR is defined as undetectable HCV RNA 12 weeks (SVR12) or 24 weeks (SVR24) after treatment completion as measured by a sensitive RNA assay (with an limit of detection [LOD] of ≤15 IU/ml). If this is achieved, HCV infection is cured in more than 99% of patients. SVR is generally associated with resolution of liver disease in patients without cirrhosis. Patients with cirrhosis remain at risk of life-threatening complications; however hepatic fibrosis may regress and the risk of complications such as hepatic failure and portal hypertension is reduced.

Until 2011, the combination of pegylated interferon alpha (PegIFN-a) and ribavirin for 24 or 48 weeks was the approved treatment for chronic HCV. With this regimen,

patients infected with HCV genotype 1 had SVR rates of approximately 40% in North America and 50% in Western Europe. Approximately 75% to 85% of people with genotype 2 or 3 had a SVR 6 months after finishing a course of treatment while for the other genotypes (4, 5 and 6) the proportion was between 50% and 75% (2).

In 2011, the protease inhibitors telaprevir (TEL) and boceprevir (BOC) were licensed for treatment in HCV genotype 1 infections. These were the first direct-acting antivirals (DAAs) active against HCV and targeted the HCV NS3-4A serine protease. Both TEL and BOC were administered in combination with PegIFN-a and ribavirin. Genotype 1 treatment-naïve patients treated with triple therapy regimens achieved higher SVR rates than PegIFN-a and ribavirin dual therapy alone (4).

Since then more efficacious pan-genotypic DAAs with fewer side effects have been licensed in the EU and the US (among other regions), for use as part of combination therapies for HCV infection. IFN-free combinations are now available for the first time, with ribavirin remaining for certain treatment combinations. The side effect profiles of BOC and TEL triple combination therapies and the costs per SVR means that they should ideally no longer be used in patients infected with HCV genotype 1 in high income countries. It should be noted that many middle-income countries have only recently received approval for the use of TEL and BOC, but these treatments are now being phased out in high-income countries in favor of second generation DAAs (2).

Materials Provided

Kit contents

artus HCV QS-RGQ Kit			(72)
Catalog number			4538366
Number of reactions			72
Blue	Hepatitis C Virus Master A	MASTER	3 x 820 µl
Violet	Hepatitis C Virus Master B	MASTER	3 x 200 µl
Red	Hepatitis C Virus RG QS 1 (10^4 IU/µl)		200 µl
Red	Hepatitis C Virus RG QS 2 (10^3 IU/µl)		200 µl
Red	Hepatitis C Virus RG QS 3 (10^2 IU/µl)		200 µl
Red	Hepatitis C Virus RG QS 4 (10^1 IU/µl)		200 µl
Green	Hepatitis C Virus RG Internal Control	IC	2 x 1000 µl
White	Water (PCR grade)		1900 µl
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QS: quantification standard.

The reagent volumes have been optimized for batches of 24 samples including the quantification standards (QS 1 to 4) and a no template control (NTC).

Fewer or a greater number of samples may be run, but there will be sub-optimal usage of the master mix due to the need to include a dead volume, which is required for the QIAsymphony SP/AS.

Materials Required but Not Provided

Prior to use, ensure that instruments have been checked and calibrated according to the manufacturer's recommendations. This kit requires the use of QIAsymphony SP/AS and Rotor-Gene Q MDx 5plex HRM instrument* with appropriate software (see below for details).

Sample preparation

- QIAsymphony DSP Virus/Pathogen Midi Kit (cat. no. 937055)

Adapters for 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)

Reagents and consumables for 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)

* If applicable, Rotor-Gene Q 5plex HRM instruments with a production date of January 2010 or later can be used as an alternative to Rotor-Gene Q MDx 5plex HRM instruments. The production date can be obtained from the serial number on the back of the instrument. The serial number is in the format 'mmyynnn' where 'mm' indicates the production month in digits, 'yy' indicates the last two digits of the production year, and 'nnn' indicates the unique instrument identifier.

- Microtubes 2.0 ml Type H or microtubes 2.0 ml Type I (Sarstedt®, cat. nos. 72.693 and 72.694, www.sarstedt.com) for use with samples and internal controls
- BD tubes 14 ml, 17 x 100 mm polystyrene round-bottom (Becton Dickinson, cat. no 352051) for internal control preparation

Adapters for QIAsymphony AS

- Reagent holder 1 QS (Cooling Adapter, Reagent Holder 1, Qsym, cat. no. 9018090)
- RG Strip Tubes 72 QS (Cooling Adapter, RG Strip Tubes 72, Qsym, cat. no. 9018092)

Reagents and consumables for QIAsymphony AS

- Strip Tubes and Caps, 0.1 ml (cat. no. 981103)
- Tubes, conical, 2 ml, Qsym AS (cat. no. 997102) or Microtubes 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)

Equipment

- Pipets (adjustable)* and sterile pipet tips with filters
- Vortex mixer*
- Benchtop centrifuge* with rotor for 2 ml reaction tubes, capable of centrifugation at 6800 x *g*
- Rotor-Gene Q MDx 5plex HRM*† (cat. no. 9002032) and Rotor-Gene Q software version 2.3, or higher
- QIAsymphony SP instrument (cat. no. 9001297)* and QIAsymphony AS instrument (cat. no. 9001301)* with QIAsymphony software version 4.0.3 or higher

External Full Process Controls

External full process controls (FPC) are not required to perform the *artus* HCV QS-RGQ assay; however, positive and negative controls should be routinely tested in each laboratory according to the guidelines or requirements of local, state and/or federal regulations or accrediting organizations.

A high positive full process control (H-FPC) and a low positive full process control (L-FPC) are intended to monitor the entire process. A negative full process control (N-FPC) detects reagent or environmental contamination by HCV.

It is recommended to test negative and positive process controls for HCV in each PCR run. The process controls should be treated as samples and subjected to the

* Ensure that instruments have been checked and calibrated according to the manufacturer's recommendations.

† If applicable, Rotor-Gene Q 5plex HRM instrument with a production date of January 2010 or later. The production date can be obtained from the serial number on the back of the instrument. The serial number is in the format 'mmyynnn' where 'mm' indicates the production month in digits, 'yy' indicates the last two digits of the production year, and 'nnn' indicates the unique instrument identifier.† International Air Transport Association. Dangerous Goods Regulations.

same RNA isolation procedure. Previously characterized samples may be used for this purpose.

Warnings and Precautions

For in vitro diagnostic use.

Read all instructions carefully before using the test.

For more information, please consult the appropriate safety data sheets (SDSs). These are available online in convenient and compact PDF format at **www.qiagen.com/safety** where you can find, view, and print the SDS for each QIAGEN kit and kit component.

For safety information for the purification kit used, see the relevant kit handbook. For safety information regarding instruments, see the relevant instrument user manual.

Warnings

- When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles.
- Use of this product is limited to personnel specially instructed and trained in the techniques of RT-PCR and in vitro diagnostic procedures.
- Specimens should always be treated as infectious and/or biohazardous in accordance with safe laboratory procedures.
- Wear protective disposable powder-free gloves, a laboratory coat and eye protection when handling specimens or kit components.
- It is recommended separated and segregated working areas are used for specimen preparation, reaction setup and amplification/detection activities following a 2-room concept that separates sample preparation

and assay setup from amplification. The workflow in the laboratory should proceed in a unidirectional manner. Always wear disposable gloves in each area, and change them before entering different areas.

- Dedicate supplies and equipment to the separate working areas and do not move them from one area to another.
- Avoid microbial and nuclease (DNase/RNase) contamination of the specimen and the components of the kit.
- Always use DNase/RNase-free disposable pipet tips with aerosol barriers.
- Store positive and/or potentially positive material separate from all other components of the kit.
- Do not open the reaction tubes post amplification to avoid contamination with amplicons.
- Do not mix components from kits with different lot numbers.
- Do not use components of the kit that have passed their expiration date.
- Discard sample and assay waste according to your local safety regulations.

General precautions

Always pay attention to the following:

- During manual steps, keep tubes closed when possible and avoid contamination.
- Thaw all components thoroughly at room temperature (15 to 25°C) before starting an assay.
- When thawed, mix the components by pipetting repeatedly up and down or by pulse vortexing and then centrifuge briefly.

  Note: Ensure that no foam or bubbles are present in the reagent tubes.

- Make sure that the required adapters are precooled to 2 to 8°C.
- Work quickly and keep PCR reagents on ice or in the cooling block before loading.
- Proceed continuously from one part of the workflow to the next. Do not exceed 30 minutes of transfer time between each module (QIAsymphony SP/AS to Rotor-Gene Q instrument).

Reagent Storage and Handling

The components of the *artus* HCV QS-RGQ Kit should be stored at –15 to –30°C. Master A and Master B can be reused, but should not exceed a maximum of two freeze thaw cycles. The tube volumes have been optimized for batches of 24 reactions.

The QS 1-4 and the IC have been verified to remain stable for up to six freeze/thaw cycles.

The reagents have been verified to be stable on-board the QIAsymphony SP/AS for the duration of the sample preparation when testing the maximum number of samples in one run (3-carrier run).

Procedure

Specimen collection

1. Blood should be withdrawn into standard specimen collection tubes containing EDTA.
2. The tube should be mixed by inverting 8 times without agitating the sample prior to centrifugation to separate the plasma.

Important: Heparinized human samples should not be used as heparin may be an interfering agent in this assay. This includes samples that have been collected in tubes containing heparin as well as samples from patients who are being treated with heparin.

Specimen transport and storage

Ship specimens within 24 hours of collection in a shatterproof transport container at a temperature of 2 to 8°C according to legal instructions for the transport of pathogen material.*

The stability of whole blood samples (before centrifugation) has been verified for the following storage conditions:

- Room temperature (15 to 25°C) for up to 24 hours

The stability of EDTA plasma samples (after centrifugation) has been verified for the following storage conditions (including the time needed for transport):

- Room temperature (15 to 25°C) for up to 24 hours
- 2 to 8°C for up to 3 days
- –15 to –30°C (or colder) for up to 6 weeks, including up to 3 freeze/thaw cycles

Specimen preparation

1. Place 1200 µl of EDTA plasma into a Sarstedt 2.0 ml Microtube Type H, without skirted base (cat. no. 72.693) or Sarstedt Microtube 2.0 ml Type I, with skirted base (cat. no. 72.694)
2. Load onto the QIAsymphony SP/AS taking care to avoid generating foam.

* International Air Transport Association. Dangerous Goods Regulations.

Detection of HCV-specific RNA

Table 1. General information about the *artus* HCV QS-RGQ Kit

Kit	*artus* HCV QS-RGQ Kit (cat. no. 4538366)
Sample material	EDTA plasma
Front-end purification	QIAsymphony DSP Virus/Pathogen Midi Kit (cat. no. 937055)
Sample volume (including excess volume)	1200 µl
Assay parameter set	170221_APS_HCV_v2_plasma1000_V2
Default assay control set	ACS_Cellfree1000_V7_DSP_artus_HCV_v2
Elution volume	90 µl
Internal Control (IC) volume per sample	9 µl
QIAsymphony software version	Version 4.0.3, or higher
Master mix volume	25 µl
Template volume	25 µl
Number of reactions	24–72* (including all controls to be loaded onto QIAsymphony SP and QIAsymphony AS; this corresponds to 19–67 clinical samples)
Runtime on QIAsymphony SP/AS	For 48 reactions: approximately 205 minutes
Runtime of Rotor-Gene Q instrument	Approximately 105 minutes

* 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 set-up and transfer to the Rotor-Gene Q instrument.

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 control (Hep. C Virus RG IC), supplied with the *artus* HCV QS-RGQ Kit, must be added to the carrier RNA (CARRIER)–Buffer AVE (AVE) mixture. The total volume of the internal control–carrier RNA (CARRIER)–Buffer AVE (AVE) mixture remains 120 µl per sample.

Table 2 gives the reaction mix for the internal control for the sample at a ratio of 0.1 µl per 1 µl elution volume. We recommend preparing fresh mixtures for each run just before use.

Table 2. Preparation of carrier RNA and internal control (Hep. C Virus RG Internal Control)

Component	Reactions	
	Volume (µl) for n≤13 in Sarstedt tubes*	Volume (µl) for n>13 in Corning® tubes†
Stock carrier RNA (CARRIER)	5	5
Internal Control (Hep. C Virus RG Internal Control)	9	9
Buffer AVE	106	106
Final volume per sample (excluding dead volume)	120	120
Total volume for n samples	(n × 120) + 360	(n × 120) + 600

* Microtubes 2.0 ml Type H and Microtubes 2.0 ml Type I (Sarstedt, cat. no. 72.693 and 72.694). 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). Optional if using more than 13 reactions, set up the internal control mixture in a larger tube and load in multiple in 2.0 ml microtubes. Make sure that for each tube the required excess volume of 3 additional reactions is added.

† If setting up more than 13 reactions, prepare the internal control mixture in a larger tube (14 ml, 17 x 100 mm polystyrene round-bottom, Corning, cat. no. 352051). An 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).

Getting started on the QIAsymphony SP/AS instruments

1. Close all drawers and the hoods.
2. Switch on the QIAsymphony SP/AS instruments, and wait until the 'Sample Preparation' screen appears and the initialization procedure has finished.
3. Log into the instrument (drawers will unlock).

Viral RNA purification

The *artus* HCV QS-RGQ Kit has been validated with a viral RNA purification step performed on the QIAsymphony SP using a QIAsymphony DSP Virus/Pathogen Kit. See the *QIAsymphony DSP Virus/Pathogen Handbook* for information on how to prepare the reagent cartridge for the sample purification step on the QIAsymphony SP.

Assay control sets and assay parameter sets

Assay control sets are the combination of a protocol plus additional parameters, such as internal control, for sample purification on the QIAsymphony SP. A default assay control set is preinstalled for each protocol.

Assay parameter sets are the combination of an assay definition with additional parameters defined, such as replicate count and number of assay standards, for assay setup on the QIAsymphony AS.

For integrated runs on the QIAsymphony SP/AS, the assay parameter set is directly linked to an upfront assay control set specifying the associated sample purification process.

Protocol: RNA isolation and assay setup on the QIAsymphony SP/AS

Important points before starting

- Ensure that you are familiar with operating the QIAsymphony SP/AS instruments. Refer to the user manuals supplied with your instrument and ensure the versions are as specified in the study protocol.
- Before using a reagent cartridge (RC) for the first time, check that Buffers QSL2 and QSB1 in the RC do not contain any precipitate. If necessary, remove the troughs containing Buffers QSL2 and QSB1 from the RC and incubate for 30 minutes at 37°C with occasional shaking to dissolve the precipitate. Make sure the troughs are replaced in the correct positions. If the RC is already pierced, make sure that the troughs are sealed with Reuse Seal Strips and incubate the complete RC for 30 minutes at 37°C with occasional shaking in a water bath.*
- Try to avoid vigorous shaking of the RC as otherwise foam may be generated that can lead to liquid-level detection problems.
- Work quickly and keep PCR reagents on ice or in the cooling block before loading.
- The reagent volumes are optimized for 3 x 24 reactions per kit. Fewer or more samples can be run, but sub-optimal usage of the available master mix volume will occur due to the calculated dead volume required for the QIAsymphony.
- Before each use, all reagents need to be thawed completely, mixed (by repeated up and down pipetting, inversion or by quick vortexing), and centrifuged for at least 3 seconds at 6800 x *g*. Avoid foaming of the reagents.

* Ensure that instruments have been checked, maintained, and calibrated regularly according to the manufacturer's instructions.

- Eluates from the sample preparation and all components of the *artus* HCV QS-RGQ Kit have been shown to be stable onboard the instrument for at least the normal time required for sample purification for 67 samples and assay setup of 72 reactions, including up to 30 minutes transfer time from the QIAsymphony SP/AS to the Rotor-Gene Q instrument.

Things to do before starting

- Prepare all required mixtures. If needed, prepare mixtures containing carrier RNA (CARRIER) and internal controls just before starting.
- Before starting the procedure, ensure that the magnetic particles are fully resuspended. Vortex the trough containing the magnetic particles vigorously for at least 3 minutes before first use.
- Before loading the RC, remove the cover from the trough containing the magnetic particles and open the enzyme tubes. Make sure that the enzyme rack has been equilibrated to room temperature (15 to 25°C).
- Make sure that the piercing lid (PL) is placed on the RC and the lid of the magnetic-particle trough has been removed or, if using a partially used RC, make sure the Reuse Seal Strips have been removed.
- If samples are bar coded, orient samples in the tube carrier so that the bar codes face the bar code reader within the 'Sample' drawer at the left side of the QIAsymphony SP.

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

Reagent cartridge (RC) position 1 and 2	**Load 1 reagent cartridge (RC) for up to 48 samples or 2 new reagent cartridges (RC) for up to 67 samples**
Tip rack holder position 1–4	Load sufficient racks of disposable filter-tips, 200 µl (see required plastic ware in the table overleaf)
Tip rack holder position 5–18	Load sufficient racks of disposable filter-tips, 1500 µl (see required plastic ware in the table overleaf)
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	**EDTA plasma**
Sample volume (including excess volume)	1200 µl
Sample tubes	Microtubes 2.0 ml Type H or Microtubes 2.0 ml Type I (Sarstedt, cat. no. 72.693 and 72.694)
Insert	Tube Insert 3B (cat. no. 9242083)

Required plastic ware for 1–3 sample batches

	One batch, 24 samples*	Two batches, 48 samples*	Three batches, 67 samples*
Disposable filter tips, 200 µl†‡	28	52	76
Disposable filter tips, 1500 µl†‡	113	206	309
Sample prep cartridges§	21	42	54
8-Rod Covers¶	3	6	9

* 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.

Procedure using the QIAsymphony SP/AS

Viral RNA purification on the QIAsymphony SP

1. Close all drawers and the hoods of the QIAsymphony SP/AS instrument.
2. Switch on the instrument, and wait until the 'Sample Preparation' screen appears and the initialization procedure has finished.

   The power switch is located at the bottom, left corner of the QIAsymphony SP.
3. Log in to the instrument.
4. Prepare the following drawers according to the section on "QIAsymphony SP setup" on page 24.
   - 'Waste' drawer, and when this has been prepared, perform an inventory scan.
   - 'Eluate' drawer, and when this has been prepared, perform an inventory scan.
   - 'Reagents and Consumables' drawer, and when this has been prepared, perform an inventory scan.

- ‘Sample’ drawer

5. Using the ‘Integrated run’ setup on the QIAsymphony touchscreen, enter the required information for each batch of samples to be processed. Select an Assay Parameter Set for the run, and assign it to the corresponding assay set up (AS) batch to the samples.
6. Information about the Assay Parameter Set and preselected elution volume is provided in Table 2.

   For more information about performing integrated runs using QIAsymphony SP, see the instrument user manual.
7. When setting up an integrated run, check for correct assignment of sample labware, sample type and volumes.

   Information about consumables and components that need to be loaded in each drawer is provided in the section above.
8. After information about all batches of the integrated run has been entered, click the ‘OK’ button to exit the ‘Integrated run’ setup. The status of all batches within the overview of the integrated run will change from ‘LOADED’ to ‘QUEUED’. As soon as one batch is queued, the ‘Run’ button appears, press the ‘Run’ button to start the procedure.

   All processing steps are fully automated.

QIAsymphony AS setup

Consumables

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

Consumables	Name on touchscreen	For use with adapter/reagent holder
Strip Tubes and Caps, 0.1 ml (250)	QIA#981103 Strip Tubes 0.1*	RG Strip Tubes 72 QS
Tubes, conical, 2 ml, Qsym AS (500)†‡	QIA#997102 T2.0 Screw Skirt*§	Reagent holder 1 QS
Tube, conical, 5 ml, Qsym AS (500)†‡	QIA#997104 T5.0 Screw Skirt*§	Reagent holder 1 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 barcode.
† 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 10, 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

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

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

Loading the QIAsymphony AS drawers for assay setup

1. After queuing an integrated run, open the QIAsymphony AS drawers. The required components to be loaded are shown on the touchscreen.
2. Always make sure to do the following before the integrated run.
   - Insert the tip chute
   - Discard the tip disposal bag
   - Install an empty tip disposal bag
3. Define and load assay rack(s). Assay rack(s), in precooled adapter(s), are loaded onto the 'Assay' slot(s). Information about the assay racks is provided in the previous section.
4. Check the temperature of the cooling positions.

   When the target cooling temperatures are reached, the small asterisk next to each slot will appear green.
5. Fill each reagent tube with the required volume of appropriate reagent according to the loading information given by the instrument software.

Note: Before each use, all reagents except Master Mix B need to be thawed completely, mixed (by repeated up and down pipetting, inversion or by quick vortexing), and centrifuged for at least 3 seconds at 6800 x *g*. Avoid bubbles or foaming as this could cause detection errors. Work quickly and keep PCR components on ice or in the cooling block before loading.

Note: Viscous reagents can be difficult to handle with manual pipets. Make sure to transfer the intended volume of the Master Mix in the tube.

6. It is recommended to scan in the assay kit information to allow for optimal reagent traceability. For this, follow these steps:
   - Press the 'Scan Kit Barcode' button on the touchscreen and press the light-blue kit bar code line.
   - Press the text field and, using the handheld bar code scanner, scan the kit bar code on the upper side of the *artus* HCV QS-RGQ Kit.
7. Load the reagent holder, and place the reagent tubes, without lids, into the appropriate positions of the precooled adapter for reagents.
8. Load disposable filter-tips into the 'Eluate and Reagents' and 'Assays' drawers, according to the required number of each tip type.
9. Close the 'Eluate and Reagents' and 'Assays' drawers.
10. Upon closing each drawer, press 'Scan' to start the inventory scan for each drawer.
    - The inventory scan checks the slots, adapters, filter-tips, and the tip chute, as well as the correct loading of specific reagent volumes. If required, correct any errors.
    - The assay setup will start automatically after the purification step on the QIAsymphony SP is completed and the eluate racks are transferred to the QIAsymphony AS.
11. After the run is finished, press 'Remove' in the assay setup 'Overview' screen. Open the 'Assays' drawer and unload the assay rack(s).

- Remove the residual *artus* HCV QS-RGQ Kit reagents from the QIAsymphony AS and dispose of as per local requirements.

12. Download the result and cycler files (optional).
13. Transfer the run cycler file to the Rotor-Gene Q instrument by using the QIAsymphony Management Console (QMC) or by downloading to a USB stick.
    - On the sample preparation user interface select the 'In-/Output Files' tab.
    - Insert USB stick and select 'Cycler files' and transfer.
    - The on screen prompt should confirm transfer, select OK and remove USB stick that now contains the downloaded files.
14. Proceed to "Protocol: RT-PCR on the Rotor-Gene Q instrument" on page 31.
15. Perform the regular maintenance of the QIAsymphony AS during the PCR run on the Rotor Gene Q instrument or later.
    - Since the workflow is an integrated operation, clean all instruments at the end of the completed workflow.
    - Follow the maintenance instructions in "*QIAsymphony SP/AS User Manual — General Description.*" Make sure maintenance is conducted regularly to minimize the risk of cross-contamination.

Protocol: RT-PCR on the Rotor-Gene Q instrument

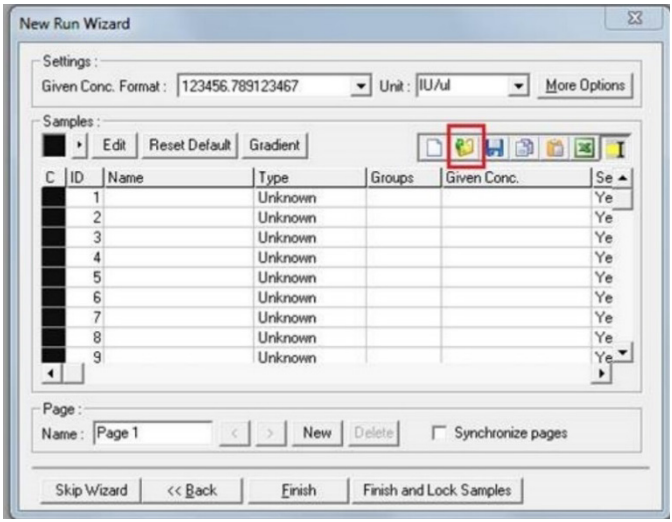
Important points before starting

- Take time to familiarize yourself with the Rotor-Gene Q instrument before starting the protocol. See the instrument user manual.
- The assay set up requires that all four quantitation standards as well as at least one negative control (PCR grade water) are included in each PCR run.

Procedure using the Rotor-Gene Q instrument

1. Select the 72-well rotor in the 'New Run Wizard' window.
2. Click on the 'Locking ring attached' check box in the setup page.
3. Click on the 'Next' button and confirm the run parameters.
4. Ensure the gain optimization is set on QS1
5. Enter the operator ID details and the reaction volume (50 µl)
6. Click on the 'Start' button to begin the PCR run.
7. Name the samples

   Note: It is recommended that the sample ID list is electronically transferred from the QIAsymphony SP/AS to the Rotor-Gene Q instrument to prevent data entry errors.
8. Transfer relevant cycler file to a local area on the computer
9. Select the 'Open file' icon (see screenshot overleaf) on the sample naming prompt, then locate and open the relevant cycler file.



10. Once samples are named, click 'Finish'.
11. Close the PCR tubes, and place the tubes in the 72-well rotor of the Rotor-Gene Q. Ensure the Rotor-Gene Q 4-strip tubes are transferred in the correct orientation such that the position of the cooling adapter and the rotor match.

    Note: Make sure the locking ring, which is an accessory of the Rotor-Gene Q instrument, is placed on top of the rotor to prevent accidental opening of the tubes during the run.
12. For the detection of HCV RNA, create a temperature profile as described in Table 3.
13. Ensure the gain optimization settings match the ones given in Table 4 and are applied to the tube position containing QS1 (this is the tube after the last test sample from the QIAsymphony SP).
14. Start the run.

Table 3. Run parameters for the Rotor-Gene Q instrument

Step	Temperature (°C)	Duration	Acquisition channels	Number of cycles
Reverse transcription (RT) step	50	10 min	N/A	1
Initial denaturation/ enzyme activation	95	2 min	N/A	1
Denaturing	95	10 sec	N/A	45
Annealing	55	20 sec	N/A	
Elongation	72	20 sec	Target: Green Internal control: Orange	

N/A: not applicable

Table 4. Gain optimization settings

Set temperature to:	**72°C**				
Perform optimization before first acquisition (ensure ticked)					
Auto-gain optimization channel settings					
Channel	**Tube position**	**Min. reading**	**Max. reading**	**Min. gain**	**Max. gain**
Green	QS1	5 FI	10 FI	–10	10
Orange	QS1	5 FI	10 FI	–10	10

Analysis Settings

This section describes the analysis settings in the Rotor-Gene Q software (2.3. or higher) after the run is finished. Using the same analysis settings ensures consistent assay performance and allows for comparison of results between different runs.

Table 5. Run analysis parameters for the *artus* HCV QS-RGQ Kit

Channel	Linear scale	Dynamic tube	Threshold	Ignore first	Slope correct	Outlier removal (reaction efficiency threshold)
Green (FAM)	Selected	Selected	0.02	10	off	N/A
Orange (Texas Red)	Selected	Selected	0.02	15	off	N/A

N/A: not applicable

Run and sample validity criteria

Result interpretation will be performed for all PCR runs using the Rotor-Gene Q software. The run and sample validity will be assessed as described in Table 6, Table 7, and Table 8 by examining the output from the Rotor-Gene Q instrument. Only valid sample results from valid runs must be used for the subsequent analysis.

Table 6. Run validity criteria

Control parameter	Green (FAM) channel criteria	Orange (Texas Red) channel criteria
No template control (NTC)	No amplification	C_T 26.30–33.60
QS1	Amplification	Amplification*
QS2	Amplification	Amplification
QS3	Amplification	Amplification
QS4	Amplification	Amplification

* In rare cases, a very high HCV viral load might cause the internal control (IC) to fail. If the IC of QS1 fails to amplify, but other validity criteria in the assay are met, the run is to be treated as valid.

Table 7. Run validity criteria for the standard curve

Control parameter	Criteria
R^2	≥0.990
Intercept ('B') C_T	30.75–34.43

The validity for the individual sample are shown in Table 8 and are applicable after the run has been determined as valid as per the criteria in Table 6 and Table 7.

Table 8. Sample validity criteria

Sample	Green (FAM) channel criteria	Orange (Texas Red) channel criteria
HCV not detected	No amplification	≥25.00 C_T*
HCV detected ≤2000 IU/ml	Amplification	≥25.00 C_T*
HCV detected >2000 IU/ml	Amplification	≥25.00 C_T†

* The delta between the internal control (IC) of the no template control (NTC) and the internal control (IC) of the sample must be <3.50 C_T ($\Delta C_T IC = C_T\ IC_{Sample} - C_T\ IC_{NTC}$).

† In rare cases, a very high HCV viral load might cause the IC to fail, but if the determined HCV concentration is within the linear range (≤1 x 10^8 IU/ml) for the assay, then sample is to be treated as valid.

Full process control results

External full process controls (FPC) are optional but recommended. The *artus* HCV QS-RGQ assay does not provide fixed rules for the analysis of the FPC as the FPCs are classified as samples and are to be supplied and included as per local, state and federal regulations.

If included, ensure:

- The high FPC (H-FPC) reports a HCV positive sample result within predefined specifications.
- The low FPC (L-FPC) reports a HCV positive sample result within predefined specifications.
- The negative FPC (N-FPC) reports a HCV negative sample result.

If results for the H-FPC, L-FPC or N-FPC fall outside the specifications predefined by the laboratory, follow established standard procedures for a root-cause analysis and the appropriate evaluation of the sample and run validity status.

Quantification

The quantification standards (Hep. C Virus RG QS 1–4) in the *artus* HCV QS-RGQ Kit are treated as previously purified samples and a sample volume of 25 µl is used. To generate a standard curve on Rotor-Gene Q Instruments, all four quantification 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 for more details).

Note: The quantification standards have been calibrated against the International Standard for HCV as determined by the World Health Organization (WHO). The values quoted are given in IU/µl, and the following equation must be used to convert the values obtained from the standard curve in IU/µl into IU/ml in order to report the HCV concentration in the sample.

$$\text{Result (IU/ml)} = \frac{\text{Result (IU/µl) x Initial elution volume (90 µl)}}{\text{Sample volume (1 ml)}}$$

Interpretation of Results

The *artus* HCV QS-RGQ Kit assay is intended to be used in conjunction with the patient's clinical presentation and determination of other laboratory markers. This kit can be used to ascertain disease prognosis and also as an aid in assessing viral response to antiviral treatment as measured by changes of HCV RNA levels in human EDTA plasma at baseline, during treatment and at the end of treatment.

Table 9. Interpretation of assay results with the *artus* HCV QS-RGQ Kit

Signal detected in the green channel	Signal detected in the orange channel	Quantitative result (IU/ml)	Interpretation
Yes	≥25.00*	<15	Valid result: HCV RNA detected, <15 IU/ml. Quantitation not possible since the quantitative result is below the linear range of the assay.
Yes	≥25.00*	≥15 and ≤2000	Valid result: HCV RNA detected at the calculated concentration. Quantitative result is within the linear range of the assay.
Yes	≥25.00†	>2000 and ≤1 x 10^8	Valid result: HCV RNA detected at the calculated concentration. Quantitative result is within the linear range of the assay.
Yes	Yes/No†	>1 x 10^8	Valid result: HCV RNA detected. Quantitation not possible since the quantitation is above the linear range of the assay.
No	≥25.00*	0	Valid result: No HCV RNA detected.
No	No	-	Invalid result: No results can be concluded.

* The delta between the internal control (IC) of the no template control (NTC) and the IC of the sample must be <3.50 C_t ($\Delta Ct_{IC} = C_t\ IC_{Sample} - C_t\ IC_{NTC}$).

† In rare cases, a very high HCV viral load might cause the IC to fail. If the determined HCV concentration is within the linear range of the assay, the sample is to be treated as valid.

Performance Characteristics

Limit of blank and specificity

The limit of blank (LOB) is defined as the highest measurement result that is likely to be observed for a blank sample. In the case of the *artus* HCV QS-RGQ Kit, an appropriate parameter to analyze for the LOB is the end-point fluorescence intensity in the Test Channel. The fluorescence levels of negative samples should remain below a given threshold value (e.g., 0.02) to generate the finding 'HCV RNA Not Detected'.

The performance of the test using negative samples determines the probability of potential false positive results.

A total of 120 HCV sero-negative EDTA plasma samples from individual donors were analyzed using the *artus* HCV QS-RGQ workflow. None of the 120 samples generated a C_t value before cycle 45 and all were determined as 'HCV RNA Not Detected'. The specificity of the *artus* HCV QS-RGQ Kit for HCV sero-negative samples was therefore 100% with a LOB at cycle 45 using a threshold set at 0.02.

Limit of detection (LOD)

The LOD for the *artus* HCV QS-RGQ Kit was determined using the 5th WHO International Standard for HCV (NIBSC code 14/150) and followed the Clinical and Laboratory Standards Institute (CLSI) Guideline EP17-A2 (5). The LOD was defined as the lowest amount of analyte in a sample that is detected with a 95% probability. The 5th WHO International Standard for HCV was used to prepare a set of six serial dilutions ranging from 69.5 IU/ml in EDTA plasma. The LOB was confirmed to be 0 IU/ml as determined by an analysis of HCV sero-negative samples.

A total of 102 replicates per concentration level (101 replicates each for 9 IU/ml and 15 IU/ml) were tested on seven QIAsymphony and seven Rotor-Gene Q instruments across three study days. All replicates of each dilution were tested in a single PCR run. The test was performed using three different lots of the *artus* HCV QS-RGQ Kit, with each lot used on the three different days, by three different operators.

A probit regression with SAS® Software was performed and the 95% LOD value was determined as well as the hit rates at 15 IU/ml. The results are shown in Table 10 and Table 11.

Table 10. Limit of detection estimate by probit analysis with two-sided 95% confidence limit

Limit of detection (LOD) estimate	Lower two-sided 95% confidence limit	Upper two-sided 95% confidence limit
10.66	8.90	14.21

Table 11. Hit rate summary with upper one-sided 95% confidence limit

Nominal IU/ml	Freq. hits/total no. reps	Hit rate (%)	Hit rate upper one-sided 95% conf. limit (%)	Mean calc. IU/ml	Mean calc. log_{10} IU/ml	SD calc. log_{10} IU/ml	Bias	FDD	TAE
5.40	84/102	82.35	88.27	7.87	0.90	0.243	0.16	4.86	0.65
9.00	91/101	90.10	94.53	12.30	1.09	0.312	0.14	7.64	0.76
15.00	99/101	98.02	99.65	19.31	1.29	0.295	0.11	6.85	0.70
25.00	102/102	100.00	100.00	36.67	1.56	0.191	0.17	3.48	0.55
41.70	102/102	100.00	100.00	56.55	1.75	0.187	0.13	3.39	0.51
69.50	102/102	100.00	100.00	103.64	2.02	0.178	0.17	3.18	0.53

Calc.: calculated; conf.: confidence; FFD: fold detectable difference; Freq.: frequency; no.: number; reps.: replicates; SD: standard deviation; TAE: total analytical error.

Limit of detection for hepatitis C virus genotypes 2 to 6

The verification strategy was based on the guidance provided in the CLSI guideline EP17-A2 (5). To verify the LOD and the lower limit of quantification (LLOQ) at 15 IU/ml, each HCV genotype from 2 to 6 was tested with 60 replicates at a concentration of 15 IU/ml. Clinical samples representing each genotype were diluted to give the intended concentration before being tested with the *artus* HCV QS-RGQ Kit. This testing was performed with three different lots of the *artus* HCV QS-RGQ Kit using three different QIAsymphony and Rotor-Gene Q instrument systems. The hit rates and the upper one-sided 95% confidence limit for HCV genotypes 2 to 6 at a nominal concentration of 15 IU/ml are shown in Table 12.

Table 12. Hit rate summary for hepatitis C virus genotypes 2 to 6 at 15 IU/ml, including the upper-one-sided 95% confidence limit.

HCV genotype	Nominal IU/ml	Frequency hits/ total no. reps	Proportion of hits (%)	Upper one-sided 95% confidence limit
2	15	58/60	96.67	99.40
3	15	60/60	100.00	100.00
4	15	58/60	96.67	99.40
5	15	55/59	93.22	97.65
6	15	56/58	96.55	99.38

HCV: hepatitis C virus.

Linear range and limit of quantitation

The linear range of the *artus* HCV QS-RGQ Kit was determined following recommendations of the CLSI Guideline EP06-A (6). This involved preparing 10 serial dilutions of incoated in vitro transcription (IVT) RNA constructs, which were representative for HCV genotypes 1 to 6. Each construct was serially diluted in

negative EDTA plasma to test the linear working range of the assay. Concentrations tested ranged from 15 IU/mL to 1 x 10^8 IU/ml. Samples were analyzed using the *artus* HCV QS-RGQ Kit and each dilution level was tested in six replicates. Figure 1 shows the graphical output and regression plot for HCV genotype 1 as an example as this is the most prevalent genotype in the European population.

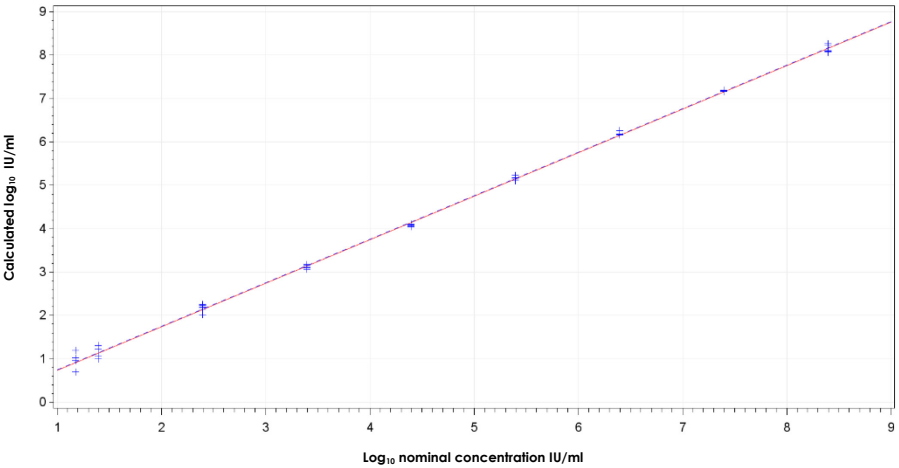


Figure 1. Calculated log_{10} IU/ml vs log_{10} IU/ml nominal concentration for HCV genotype 1. The red solid line represents the linear regression line and the blue dashed line represents the quadratic regression line.

The linear range of the *artus* HCV QS-RGQ V2 Kit was determined to cover concentrations from 15 IU/mL to 1 x 10^8 IU/ml HCV in EDTA plasma for genotypes 1-6. The LLOQ was defined as the lowest concentration within the linear range that has a total analytical error (TAE; 2 x standard deviation [SD] + [Bias]) of ≤1.0 log_{10} IU/ml. The data generated for the verification of the LOD in the assay was used to calculate the fold detectable difference [(FDD): 10^((Total SD)*sqrt(2)*2))] as well as

the TAE at 15 IU/ml. As shown in Table 13, HCV genotypes 1 to 6 demonstrated an TAE of ≤1.0 Log_{10} IU/ml at 15 IU/ml.

Table 13. Hit rate, calculated hepatitis C virus concentration (IU/ml), fold detectable difference (FDD) and total analytical error (TAE) at 15 IU/ml

HCV genotype	Nominal IU/ml	Freq. hits/total no. reps	Mean calc. IU/ml (Geometric mean)	Mean calc. log_{10} IU/ml	SD calc. log_{10} IU/ml	Bias	FDD	TAE
1 (WHO*)	15.00	99/101	19.31	1.29	0.295	0.11	6.85	0.70
2	15.00	58/60	21.00	1.32	0.258	0.15	5.37	0.66
3	15.00	60/60	10.77	1.03	0.403	-0.14	13.77	0.95
4	15.00	58/60	15.94	1.20	0.250	0.03	5.09	0.53
5	15.00	55/59	9.59	0.98	0.290	-0.19	6.61	0.77
6	15.00	56/58	17.10	1.23	0.273	0.06	5.94	0.60

* 5th World Health Organization (WHO) International Standard for HCV (NIBSC code 14/150).
FDD: fold detectable difference; Freq.: frequency; HCV: hepatitis C virus; SD: standard deviation; no.: number; reps.: replicates; TAE: total analytical error.

Precision, repeatability and lot-to-lot variability

The precision of the *artus* HCV QS-RGQ Kit was assessed by following the recommendations of the CLSI Guideline EP05-A3 (7). This involved testing a five-member panel, which included a negative sample, a sample with a concentration at 3 x LOD, a clinical sample diluted 1:100 in EDTA plasma, and two contrived samples within the linear range for the assay. The contrived samples contained an incoated IVT RNA construct representative of HCV genotype 3. All samples were in EDTA plasma. One integrated QS-RGQ run was performed by each operator over eight (non-)consecutive days with four replicates per panel member per run. This meant a total of 24 runs (8 days x 3 operators x 1 run per operator per day) were performed for this study generating 96 data points per test panel member across three different lots of the *artus* HCV QS-RGQ Kit. In addition, three different QS-RGQ

platforms were used for the testing, as were three different lots of DSP Virus/Pathogen Midi Kit, and three different operators performing the test.

The variance components from this study are shown in Table 14. The total SD was reported for Log_{10}(IU/ml) and this estimate represents within lab variability (i.e., intermediate precision). Table 14 demonstrates that the SD ranged from 0.131 at the highest concentration tested (5 x 10^6 IU/ml) to 0.222 at the lowest concentration tested (45 IU/ml).

Table 14. Log_{10} calculated IU/ml variance components standard deviation (SD) and log-normal percentage coefficient of variance (%CV)

Nominal conc. IU/ml	No. of observ -ations	Between run SD (%CV)	Between day SD (%CV)	Between operator SD (%CV)	Between kit lot SD (%CV)	Between extract- ion lot SD (%CV)	Within run SD (%CV)	Total SD (total %CV)
5 x 10^6	96	0.112 (26.30)	0.017 (3.82)	0.014 (3.34)	0.051 (11.86)	0.000 (0.00)	0.054 (12.38)	0.131 (30.96)
100	96	0.136 (32.04)	0.044 (10.21)	0.000 (0.00)	0.022 (5.05)	0.000 (0.00)	0.145 (34.22)	0.202 (49.14)
45	96	0.115 (26.86)	0.072 (16.60)	0.000 (0.00)	0.016 (3.68)	0.000 (0.00)	0.178 (42.86)	0.222 (54.62)
18.9 x 10^3 (clinical sample)	96	0.094 (21.97)	0.049 (11.24)	0.045 (10.46)	0.035 (7.96)	0.000 (0.00)	0.063 (14.69)	0.131 (30.74)

Conc.: concentration; CV: coefficient of variance; SD: standard deviation.

A model was fitted to the data with log_{10} IU/ml as the response variable and kit lot as a categorical fixed effect. The difference in mean log_{10} IU/ml between each pair of kit lots (i.e., three differences in total) were reported along with the corresponding standard error (SE) and 95% confidence interval (CI). The results are shown in Table 15.

Table 15. Difference in mean calculated log_{10} IU/ml between kit lots for each sample run

Nominal conc. IU/ml	Total no. observations	Diff. between kit lots	Diff. mean log_{10} IU/ml	Standard error (SE)	Lower 95% conf. limit	Upper 95% conf. limit	p-value
5 x 10^6	96	1-2	-0.046	0.030	-0.106	0.014	0.134
		1-3	-0.130	0.030	-0.190	-0.070	<0.001
		2-3	-0.085	0.030	-0.145	-0.025	0.006
100	96	1-2	-0.048	0.050	-0.146	0.050	0.336
		1-3	-0.117	0.050	-0.215	-0.018	0.021
		2-3	-0.069	0.050	-0.167	0.030	0.169
45	96	1-2	0.049	0.055	-0.060	0.159	0.371
		1-3	-0.058	0.055	-0.167	0.051	0.294
		2-3	-0.107	0.055	-0.217	0.002	0.054
18.9 x 10^3 (clinical sample)	96	1-2	-0.070	0.031	-0.132	-0.008	0.026
		1-3	-0.104	0.031	-0.166	-0.042	0.001
		2-3	-0.034	0.031	-0.096	0.028	0.278

Conc.: concentration; conf.: confidence; diff.: difference.

The maximum absolute difference between the different kit lots used was 0.130 in mean log_{10} IU/ml.

Reproducibility

The design of this study is based on CLSI guideline EP05-A3 (7). Precision is defined as "the closeness of agreement between measured values obtained by replicate measurements on the same or similar objects under specified conditions". Reproducibility, according to EP05-A3, is the multisite precision. Within this study, the laboratory conditions were varied by days, runs ('day' and 'run' are confounded) and the use of three different test sites (one internal and two external test locations).

At each external test site, one integrated *artus* HCV QS-RGQ Kit run was performed per day over a period of eight (non-)consecutive days with four replicates per sample per run. At each of the two external test sites, a single instrument was used for a total of 16 runs (8 days x 1 run per day x 2 test sites) in this study in addition to the internally generated data. The subset of the data generated for the precision and repeatability study (see page 43) where the kit lots match those in use in this study, accounted for the third test site in this reproducibility study.

Table 16. Summary statistics for calculated log_{10} IU/ml by nominal concentration of the sample for all three test sites

Nominal conc. IU/ml	Nominal log_{10} IU/ml	No. replicates	Mean	Median	Standard deviation (SD)	Minimum	Maximum
5×10^6	6.699	96	6.93	6.93	0.083	6.68	7.17
100	2.000	96	2.15	2.15	0.138	1.73	2.42
45	1.653	96	1.82	1.85	0.214	1.27	2.70
18.9×10^3 (clinical sample)	4.276	96	4.33	4.33	0.063	4.17	4.53

Conc.: concentration; No.: number.

As shown in Table 16, the maximum SD across all three test sites was 0.214 log_{10} IU/ml with the lowest concentration tested in this study, i.e., at 45 IU/ml (3 x LOD).

Cross-reactivity and mixed infections

This study was designed to test for any interference in HCV detection due to cross-reactivity with pathogens that are related or similar to HCV using the *artus* HCV QS-RGQ Kit. For samples positive for HCV, the absence of interference was defined as no significant difference in log_{10} IU/ml between the results obtained from the controls and the pathogen-spiked samples. If any significant difference was observed between samples, this was to be smaller than twice the intermediate precision of

the assay. In addition, samples that were negative for HCV were to test negative for HCV when tested in the presence of pathogens.

HCV positive samples were manufactured at a concentration of 45 IU/ml using incoated IVT material that was representative of HCV genotype 1a. A total of 34 different pathogens were spiked individually into the manufactured positive HCV samples as well as in samples negative for HCV. RNA was then extracted and tested in six replicates using the QIAsymphony SP/AS instrument and the Rotor-Gene Q 5Plex HRM instruments. Controls used for this study were pathogen-free HCV-negative plasma (control negative) and pathogen-free HCV-positive plasma at a concentration of 45 IU/mL (control HCV 45).

The pathogens were spiked into the samples to create a final concentration of 1 x 10^5 in their respective unit as stated on the certificate of analysis (e.g., IU, copies, particles, tissue culture infectious dose that will infect 50% [$TCID_{50}$], colony forming units [CFU], virus particles [VP]). Pathogens that were not sufficiently concentrated to create this final concentration in the sample were prepared at the highest concentration possible.

Table 17. Pathogens tested for cross-reactivity against control samples negative for hepatitis C virus and samples positive for hepatitis C virus at 45 IU/ml

Final test conc.	Species	HCV negative	HCV 45 IU/ml				
		Freq. negative calls/total negative samples	Diff. mean log_{10} IU/ml	Standard error (SE)	Lower 95% conf. limit	Upper 95% conf. limit	p-value
1 x 10^5 (NIU/ml)	Adenovirus type 5	6/6	0.251	0.182	-0.123	0.626	0.179
Neat (no stock conc. given)	BK human polyomavirus	6/6	0.022	0.182	-0.353	0.397	0.905
1 x 10^5 CFU/vial (/ml)	*Candida albicans*	6/6	0.148	0.182	-0.227	0.522	0.425
1 x 10^5 IFU/ml	*Chlamydia trachomatis*	6/6	0.348	0.182	-0.026	0.723	0.067
1 x 10^5 copies/ml	Cytomegalo virus	6/6	-0.079	0.161	-0.410	0.253	0.630
1 x 10^5 copies/ml	Dengue virus 1	6/6	-0.170	0.160	-0.499	0.159	0.297
1 x 10^5 copies/ml	Dengue virus 2	6/6	0.149	0.160	-0.180	0.478	0.361
1 x 10^5 copies/ml	Dengue virus 3	6/6	-0.044	0.160	-0.373	0.285	0.786
1 x 10^5 copies/ml	Dengue virus 4	6/6	0.126	0.160	-0.203	0.455	0.438
5 x 10^3 $TCID_{50}$/ml	Epstein-Barr virus	6/6	-0.209	0.161	-0.540	0.122	0.205

CFU: colony forming units; Conc.: concentration; conf.: confidence; diff.: difference; freq.: frequency; hepatitis C virus: HCV; NIU: normalized and adjusted standard (NAS) infectious units; PFU: plaque-forming units; $TCID_{50}$: tissue culture infectious dose that will infect 50%; vp: virus particle.

Cont

Final test conc.	Species	HCV negative	HCV 45 IU/ml				
		Freq. negative calls/total negative samples	Diff. mean log_{10} IU/ml	Standard error (SE)	Lower 95% conf. limit	Upper 95% conf. limit	p-value
1 x 10^5 $TCID_{50}$/ml	Hepatitis A virus	6/6	-0.275	0.161	-0.606	0.057	0.100
1 x 10^5 U/ml	Herpes simplex virus type 1	6/6	0.036	0.161	-0.295	0.367	0.823
1 x 10^5 $TCID_{50}$/ml	Herpes simplex virus type 2	6/6	0.332	0.146	0.027	0.637	0.034
5 x 10^3 $TCID_{50}$/ml	Human herpes virus type 8	6/6	0.265	0.146	-0.040	0.570	0.085
1 x 10^5 PFU/ml -	Influenza A	6/6	0.152	0.139	-0.136	0.440	0.286
1 x 10^5 CFU/vial (/ml)	*Mycobacterium gordonae*	6/6	-0.143	0.139	-0.431	0.145	0.315
1 x 10^4 CFU/vial (/ml)	*Mycobacterium smegmatis*	6/6	0.150	0.119	-0.095	0.395	0.220
1 x 10^4 CFU/vial (/ml)	*Neisseria gonorrhoeae*	6/6	-0.173	0.119	-0.418	0.072	0.158
1 x 10^5 CFU/vial (/ml)	*Propionibacterium acnes*	6/6	0.042	0.119	-0.203	0.287	0.728
1 x 10^5 CFU/vial (/ml)	*Staphylococcus aureus*	6/6	0.133	0.119	-0.112	0.378	0.275
1 x 10^5 CFU/vial (/ml)	*Staphylococcus epidermidis*	6/6	-0.156	0.186	-0.539	0.227	0.409

CFU: colony forming units; Conc.: concentration; conf.: confidence; diff.: difference; freq.: frequency; hepatitis C virus: HCV; NIU: normalized and adjusted standard (NAS) infectious units; PFU: plaque-forming units; $TCID_{50}$: tissue culture infectious dose that will infect 50%; vp: virus particle.

Cont

Final test conc.	Species	HCV negative	HCV 45 IU/ml				
		Freq. negative calls/total negative samples	Diff. in mean log_{10} IU/ml	Standard error (SE)	Lower 95% conf. limit	Upper 95% conf. limit	p-value
5 x 10^3 $TCID_{50}$/ml	Varicella-zoster virus (VZV)	6/6	-0.188	0.186	-0.571	0.195	0.321
1 x 10^5 IU/ml	Hepatitis B virus	6/6	-0.138	0.186	-0.521	0.245	0.464
1 x10^5 IU/ml	HIV-1 IIB	6/6	0.042	0.186	-0.341	0.424	0.825
1 x 10^5 U/ml	HIV-2 NIH-Z	6/6	0.097	0.158	-0.241	0.434	0.551
1 x 10^5 cells/ml	HPV16 CaSki	6/6	0.270	0.146	-0.035	0.575	0.080
1 x10^5 cells/ml	HPV18 HeLa	6/6	0.385	0.230	-0.093	0.864	0.109
1 x 10^5 copies/ml	Human Herpes Virus type 6A GS	6/6	-0.436	0.170	-0.787	-0.085	0.017
1 x 10^5 vp/ml	Human T-Cell Lymphotropic virus type 1	6/6	-0.154	0.170	-0.504	0.197	0.376
1 x 10^5 vp/ml	Human T-Cell Lymphotropic virus type 2	6/6	0.209	0.230	-0.269	0.688	0.373
1 x 10^5 U/ml	St. Louis Encephalitis Virus	6/6	0.148	0.164	-0.190	0.486	0.376
1 x 10^5 U/ml	West Nile Virus NY 2001-6263	6/6	-0.018	0.164	-0.356	0.320	0.913
1 x 10^5 U/ml	Yellow Fever Virus 17-D	6/6	0.208	0.230	-0.270	0.687	0.375
8.13 x 10^4 U/ml	Zika Virus MR 766	6/6	0.164	0.164	-0.174	0.501	0.328

CFU: colony forming units; Conc.: concentration; conf.: confidence; diff.: difference; freq.: frequency; hepatitis C virus: HCV; NIU: normalized and adjusted standard (NAS) infectious units; PFU: plaque-forming units; $TCID_{50}$: tissue culture infectious dose that will infect 50%; vp: virus particle.

As shown in Table 17, none of the pathogens tested demonstrated cross-reactivity with the *artus* HCV QS-RGQ Kit. This was defined as no significant difference in log_{10} IU/ml between the results obtained from the controls and the pathogen-spiked HCV 45 samples. In cases where significant differences were observed, they were smaller than 2 x total SD of the assay (<0.444 Log_{10} IU/ml, Table 17). In addition, 100% of the HCV-negative samples tested in the presence of pathogens generated negative results.

Interfering substances

Interference testing demonstrated the impact of potentially interfering substances that may be present in human EDTA plasma on the assay performance of the *artus* HCV QS-RGQ Kit. The CLSI guideline EP7-A2 (8) was used when designing this interference testing study. In this study, potentially interfering substances were drugs used for treatment of HCV infections (e.g., exogenous substances, Table 18 and Table 19) as well as blood components and hormones (e.g., endogenous substances, Table 20). Exogenous substances were spiked into the sample at three-times the maximum plasma level (C_{max}) for that drug. Endogenous substances were spiked at concentrations given in the CLSI guideline EP7-A2 (8). Substance interference was tested in human EDTA plasma negative for HCV and in a negative sample matrix spiked with HCV at 45 IU/mL (3 x LOD) using incoated IVT RNA representative of HCV genotype 1a.

Ten different exogenous substance pools were spiked into the two different experimental concentrations (HCV negative and HCV spiked at 45 IU/ml). The groupings of exogenous substances were based on the type of solvent used for resuspension (Table 18).

Table 18. Exogenous substances and their groupings generated for testing

Exogenous substance pool/solvent used for resuspension		Exogenous substances tested
DMSO	1	Boceprevir, efavirenz, emtricitabine, raltegravir, zidovudine
	2	Acyclovir, atazanavir, darunavir, fosamprenavir, indinavir
	3	Azithromycin, elbasvir, paritaprevir, saquinavir, tenofovir
	4	Clarithromycin, ganciclovir, lopinavir, telaprevir
Nuclease-free water	5	Abacavir, ciprofloxacin, enfurvitide, telbivudine, valganciclovir
	6	Adefovir, fluoxetine, interferon alpha 2a, interferon alpha 2b, stavudine
	7	Daclatasvir, didanosine, lamivudine, ribavirin, sofosbuvir
Ethanol	8	Entecavir, grazoprevir, ombitasvir, paroxetine, zalcitibine (DMSO)
	9	Amprenavir, nelfinavir, simeprevir, tipranavir
	10	Ledipasvir, ritonavir, sertraline, valacyclovir
DMSO	N/A	Nevirapine

DMSO: dimethyl sulfoxide; N/A: not applicable.

Table 19. Summary statistics for exogenous substances tested

Difference between control and interfering substance	Difference in mean calculated log_{10} IU/ml	Standard error (SE)	Lower 95% conf. limit	Upper 95% conf. limit	p-value
Group 1 - CONTROL	0.148	0.203	-0.272	0.567	0.474
Group 2 - CONTROL	0.286	0.213	-0.154	0.726	0.193
Group 3 - CONTROL	0.068	0.213	-0.372	0.509	0.751
Group 4 - CONTROL	0.302	0.203	-0.118	0.722	0.150
Group 5 - CONTROL	0.029	0.195	-0.375	0.432	0.884
Group 6 - CONTROL	0.250	0.195	-0.153	0.654	0.212
Group 7 - CONTROL	0.170	0.195	-0.234	0.573	0.393
Group 8 - CONTROL	0.307	0.204	-0.114	0.728	0.145
Group 9 - CONTROL	0.006	0.183	-0.380	0.391	0.976
Group 10 - CONTROL	0.174	0.192	-0.228	0.577	0.375
Nevirapine - CONTROL	0.014	0.183	-0.371	0.399	0.940

Conf.: confidence; SE: standard error.

As shown in Table 19, none of the exogenous substances tested in this study demonstrated a significant difference in log_{10} IU/ml when compared with the control samples (p-value >0.05). In addition, there was no amplification in samples negative for HCV when these negative samples were spiked with an exogenous substance or substance group (data not shown).

Table 20. Summary statistics for endogenous substances

Difference between control and interfering substance	Difference in mean calculated log_{10} IU/ml	Standard error (SE)	Lower 95% conf. limit	Upper 95% conf. limit	p-value
Triglycerides - CONTROL	0.373	0.125	0.115	0.631	0.006
Conjugated bilirubin - CONTROL	0.277	0.119	0.033	0.521	0.028
Hemoglobin - CONTROL	0.297	0.119	0.053	0.541	0.019
Unconjugated bilirubin - CONTROL	0.300	0.061	0.163	0.4370	<0.001
EDTA - CONTROL	0.005	0.144	-0.321	0.331	0.973
Globulin - CONTROL	0.256	0.058	0.124	0.387	0.002
hDNA - CONTROL	0.066	0.079	-0.112	0.244	0.425
hRNA - CONTROL	0.019	0.171	-0.368	0.405	0.915
Albumin - CONTROL	-0.080	0.162	-0.442	0.281	0.631

Table 20 shows that conjugated and unconjugated bilirubin, hemoglobin and globulin were statistically significantly different from the control samples (p=0.028, p<0.001 p=0.019 and p=0.002, respectively), but the difference in mean calculated log_{10} IU/ml was 0.277, 0.300, 0.297 and 0.256, respectively. This meant these substances passed the study acceptance criteria of <0.5 log_{10} IU/ml. In addition, there was no amplification in samples negative for HCV when these negative samples were spiked with the endogenous substances (data not shown).

Cross-contamination

The cross-contamination study was designed to test for cross-contamination between integrated QIAsymphony SP/AS runs using the *artus* HCV QS-RGQ workflow. Cross-contamination was defined as the amount of analyte carried over between

adjacent wells during automated runs. Instrument carryover, expressed as a percentage, was calculated as:

$$\left(\frac{\text{Number of negative samples where target is detected}}{\text{Total number of negative samples}}\right) \times 100$$

This study was performed using samples positive for HCV at clinically relevant concentrations (1 x 10^5, 1 x 10^6 and 1 x 10^7 IU/ml). In separate dilutions, an incoated IVT RNA representing HCV genotype 1a was diluted in EDTA plasma to provide the different concentrations. Each of these sample concentrations were tested with samples negative for HCV in an alternating order for five consecutive runs ('checkerboard runs'). For each concentration, a final (and sixth) run was performed to determine between-run contamination. The proportion of cross contamination (instrument carryover as defined above) was calculated and the result for each concentration is shown in Table 21 (below).

Table 21. Cross-contamination rate at clinically relevant concentrations

Sample concentration in checkerboard format	Frequency of cross-contamination	Proportion of cross-contamination (%)
1 x 10^7 IU/ml	4/170	2.35
1 x 10^6 IU/m1	3/170	1.76
1 x 10^5 IU/ml	0/170	0.00

Clinical Performance

The clinical performance of the *artus* HCV QS-RGQ Kit was evaluated during a comparison study at two clinical laboratories in the UK that tested 452 individual patient samples, which were either positive and negative for HCV. The samples were tested using the *artus* HCV QS-RGQ Kit in a routine clinical laboratory setting and the samples reflected the current HCV epidemiologic trends in European test population. Clinical samples of certain genotypes (4, 5 and 6) were obtained commercially in order to reach the full coverage of current HCV genotypes 1-6.

In this study, the patient samples were tested with the *artus* HCV QS-RGQ Kit and compared with either previously or in parallel generated results of a CE-marked comparator assay. A Deming and Passing-Bablok regression analysis was performed with the test results from the *artus* HCV QS-RGQ Kit on the y-axis and the test result of the comparator assay on the x-axis. The parameter estimates, along with their SEs and corresponding 95% CIs were reported. The regression analysis was performed including all samples between the LLOQ and upper limit of quantification (ULOQ) for both assays (n=165, Figure 2).

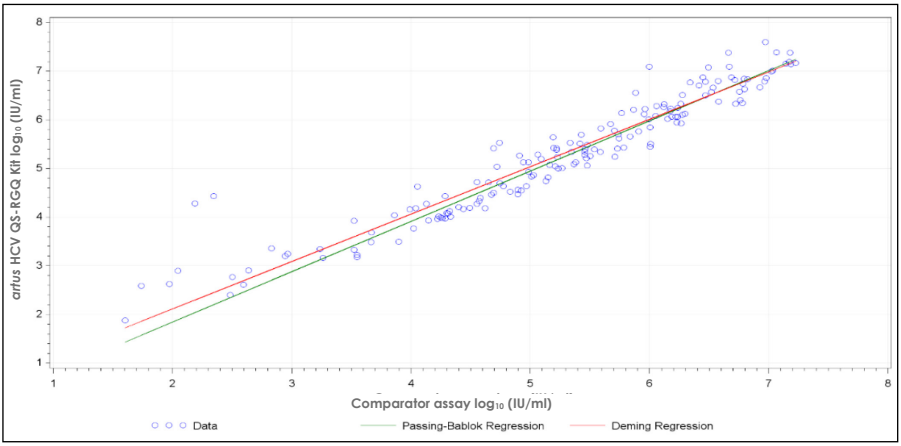


Figure 2: Regression Plot with Passing-Bablok and Deming lines (n=165).

Table 22. Regression analysis for the *artus* HCV QS-RGQ Kit and a comparator assay

Test	Response variable log_{10} (IU/ml)	Explan. variable log_{10} (IU/ml)	No. of obser-vations	Inter-cept	Inter-cept lower two-sided 95% conf. limit	Inter-cept upper two-sided 95% conf. limit	Slope	Slope lower two-sided 95% conf. limit	Slope upper two-sided 95% conf. limit
Deming	*artus* HCV QS-RGQ Kit	Compar-ator assay	165	0.164	-0.190	0.519	0.974	0.912	1.036
Passing-Bablok	*artus* HCV QS-RGQ Kit	Compar-ator assay	165	-0.222	-0.448	0.028	1.033	0.990	1.072

Conf.: confidence; Explan.: explanatory; No: number.

Table 22 shows that for both Deming and Passing-Bablok, the intercept is close to zero (0.164 and -0.222, respectively) and the slope is close to 1 (0.974 and 1.033, respectively). This demonstrates a close overall correlation between the *artus* HCV QS-RGQ Kit and the comparator assay.

Limitations

- Strict compliance with the user manual is required for optimal PCR results.
- Attention should be paid to expiration dates printed on the box and labels on all of the components. Do not use expired components.
- Fibrinous samples or samples that show other signs of clot accumulation may obstruct the pipet tips and result in false results due to insufficient volume transfer during the sample preparation process.
- Although rare, mutations within the highly conserved regions of the HCV genome covered by the kit's primers and/or probe may result in under-quantification of the viral load or failure to detect the presence of the HCV in affected samples.
- The product is intended to be used by professional users, such as technicians and physicians who are trained in in vitro diagnostic procedures.

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of the *artus* HCV QS-RGQ Kit is tested against predetermined specifications to ensure consistent product quality.

References

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3. European Association for Study of the Liver and Asociacion Latinoamericana para el Estudio del Higado (2015). EASL-ALEH Clinical Practice Guidelines: Non-invasive tests for evaluation of liver disease severity and prognosis. J. Hepatol., **63**, 237.
4. Harrington, P.R., Zeng, W., and Naeger, L.K. (2012) Clinical relevance of detectable but not quantifiable hepatitis C virus RNA during boceprevir or telaprevir treatment. Hepatology **55**, 1048.
5. Clinical and Laboratory Standards Institute (CLSI) Guideline EP17-A2, Vol. 32 No. 8, Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures, Approved Guideline – Second Edition 2012.
6. Clinical and Laboratory Standards Institute (CLSI) Guideline EP06-A, Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline 2003.
7. Clinical and Laboratory Standards Institute (CLSI) Guideline EP05-A3, Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline – Third Edition 2014.

8. Clinical and Laboratory Standards Institute (CLSI) Guideline EP7-A2, Vol. 25 No. 27, Interference Testing in Clinical Chemistry; Approved Edition – Second Edition 2005.

Symbols

The symbols in the following table are used in these instructions for use.

Symbol	Symbol definition
	Contains sufficient for 72 tests
	In vitro diagnostic
	CE mark
	Catalog number
	Lot number
	Material number
	Components

Symbol	Symbol definition
CONT	Contains
IC	Internal Control
GTIN	Global Trade Item Number
Rn	R is for the revision of the Instructions for Use (Handbook) and n is the revision number
	Temperature limitation
	Manufacturer
	Use by
	Consult instructions for use

Troubleshooting Guide

Refer to this section for error handling and troubleshooting any problems that may arise with the *artus* HCV QS-RGQ Kit. If the recommended steps do not resolve the problem, contact QIAGEN Technical Services for assistance, either via our Technical Support Center at **www.qiagen.com/support**, by calling 00800-22-44-6000, or by contacting one of the QIAGEN Technical Service Departments or your local distributors.

Possible problem or cause	Corrective action
General handling	
Error message displayed in the touchscreen	If an error message is displayed during a protocol run, refer to the user manuals supplied with your instruments.
Precipitate in reagent trough of opened cartridge of the QIAsymphony DSP Virus/Pathogen Kit	
a) Buffer evaporation	Excessive evaporation may lead to increased salt concentration or decreased alcohol concentrations in buffers. Discard reagent cartridge (RC). Make sure to seal buffer troughs of a partially used reagent cartridge (RC) with Reuse Seal Strips when not being used for purification.

Possible problem or cause	Corrective action
b) Storage of reagent cartridge (RC)	Storage of reagent cartridge (RC) under 15°C may lead to formation of precipitates. If necessary, remove the troughs containing Buffers QSL2 and QSB1 from the reagent cartridge (RC) and incubate in a water bath* at 37°C for 30 minutes with occasional shaking to dissolve precipitate. Make sure to replace the troughs in the correct positions. If the reagent cartridge (RC) is already pierced, make sure that the troughs are reclosed with Reuse Seal Strips and incubate the complete reagent cartridge (RC) in a water bath* at 37°C for 30 minutes with occasional shaking.
Low yield of nucleic acids	
a) Magnetic particles were not completely resuspended	Before starting the procedure, ensure that the magnetic particles are fully resuspended. Vortex for at least 3 minutes before use.
b) Frozen samples were not mixed properly after thawing	Thaw frozen samples with mild agitation to ensure thorough mixing
c) Carrier RNA (CARRIER) not added	Reconstitute carrier RNA (CARRIER) in Buffer AVE (AVE) and mix with appropriate volume of Buffer AVE (AVE) as described in the relevant section of this handbook. Repeat the purification procedure with new samples.
d) Degraded nucleic acids	Samples were stored incorrectly or subjected to too many freeze–thaw cycles. Repeat the purification procedure with new samples.

* Ensure that instruments have been checked, maintained, and calibrated regularly according to the manufacturer's instructions.

Possible problem or cause	Corrective action
e) Incomplete sample lysis	Before use, check that Buffer QSL2 and QSB1 do not contain precipitates. If necessary, remove the troughs containing Buffers QSL1 and QSB1 from the reagent cartridge (RC) and incubate for 30 minutes at 37°C with occasional shaking in a water bath* to dissolve precipitate. If the reagent cartridge (RC) is already pierced, make sure that the troughs are reclosed with Reuse Seal Strips, and incubate the complete reagent cartridge (RC) for 30 minutes at 37°C with occasional shaking in a water bath.*
f) Clogging of pipet tip due to insoluble material	Insoluble material was not removed from the sample prior to starting the QIAsymphony purification procedure. To remove insoluble material for viral applications, centrifuge the sample at 3000 x *g* for 1 minute, and transfer the supernatant to a new sample tube.

* Ensure that instruments have been checked, maintained, and calibrated regularly according to the manufacturer's instructions.

Possible problem or cause	Corrective action
QIAsymphony AS detects insufficient Master	
Not required volume of the Master transferred to tube	Combine the required volume of each reagent into one tube and place on to the QIAsymphony. Viscous reagents can be difficult to handle with manual pipets. Make sure to transfer the entire volume of the Master in the tube. For viscous reagents, we recommend aspirating an extra volume of 5% when using manual pipets (e.g., adjust the pipet to 840 µl for an 800 µl volume). Alternatively, after slowly dispensing the liquid and performing a blowout at the target tube's wall, remove the tip from the liquid, release the pipet plunger, and wait for an additional 10 seconds. Residual liquid will flow down the tip and can be blown out by pressing the pipet plunger a second time. The use of PCR grade filter-tips labeled as 'low retention' can improve the recovery of liquid.
No signal with positive controls (Hep. C Virus RG QS 1–4) in fluorescence channel Cycling Green	
a) The selected fluorescence channel for PCR data analysis does not comply with the protocol	For data analysis select the fluorescence channel Cycling Green for the analytical HCV PCR and the fluorescence channel Cycling Orange for the internal control PCR.
b) Incorrect programming of the temperature profile of the Rotor-Gene Q instrument	Compare the temperature profile with the protocol. See the relevant sections in this handbook regarding the Rotor-Gene Q cycling parameters (see Table 3 and the section on "Analysis Settings" on page 34)

Possible problem or cause	Corrective action
c) Incorrect configuration of the PCR	Make sure that assay setup was performed correctly and that the correct assay parameters were used (see Table 2). Repeat the PCR, if necessary
d) The storage conditions for one or more kit components did not comply with the instructions given in "Reagent Storage and Handling" on page **Error! Bookmark not defined.**)	Check the storage conditions and the expiration date (see the kit label) of the reagents and use a new kit, if necessary.
e) The *artus* HCV QS-RGQ Kit has expired	Check the storage conditions and the expiration date (see the kit label) of the reagents and use a new kit, if necessary.
Weak or no signal of the internal control of a negative or HCV low positive plasma sample subjected to purification using the QIAsymphony DSP Virus/Pathogen Kit in fluorescence channel Cycling Orange	
a) The PCR conditions do not comply with the protocol	Check the PCR conditions (see above) and repeat the PCR with corrected settings, if necessary.
b) The PCR was inhibited	Make sure that you use the validated isolation method (see "Protocol: RNA isolation and assay setup on the QIAsymphony SP/AS" on page 22) and closely follow the instructions.
c) RNA was lost during extraction	An absent signal of the internal control can indicate the loss of RNA during the extraction. Make sure that you use the validated isolation method (see "Protocol: RNA isolation and assay setup on the QIAsymphony SP/AS" on page 22) and closely follow the instructions. See also "Low yield of nucleic acids", above.

Possible problem or cause	Corrective action
d) The storage conditions for one or more kit components did not comply with the instructions given in "Reagent Storage and Handling" on page16)	Check the storage conditions (see the kit label) of the reagents and use a new kit, if necessary.
e) The *artus* HCV QS-RGQ Kit has expired	Check the storage conditions and the expiration date (see the kit label) of the reagents and use a new kit, if necessary.
Signals with the negative controls in fluorescence channel Cycling Green of the analytical PCR	
a) Contamination occurred during preparation of the PCR	Excessive evaporation may lead to increased salt concentration or decreased alcohol concentrations in buffers. Discard reagent cartridge (RC). Make sure to seal buffer troughs of a partially used reagent cartridge (RC) with Reuse Seal Strips when not being used for purification.
b) Contamination occurred during extraction	Repeat the extraction and PCR of the sample to be tested using new reagents. Make sure that work space and instruments are decontaminated at regular intervals.

Ordering Information

Product	Contents	Cat. no.
artus HCV QS-RGQ Kit	For 72 reactions: Master A and B, internal control, Hepatitis C Virus Quantification Standards 1–4, (Hepatitis C Virus RG QS 1–4), and PCR-grade water	4538366
Related Products		
QIAsymphony DSP Virus/Pathogen Midi Kit (96)	Includes 2 reagent cartridges and enzyme racks and accessories	937055
QIAsymphony SP	QIAsymphony sample prep module, 1-year warranty on parts and labor	9001297
QIAsymphony AS	QIAsymphony assay setup module, 1-year warranty on parts and labor	9001301
Rotor-Gene Q Software versions 2.3 or higher	Software for routine testing in combination with the Rotor-Gene Q and QIAsymphony RGQ instruments	9023241
Rotor-Gene Q MDx Cycler	Real-time PCR cycler and high resolution melt (HRM) analyzer with 5 channels (green, yellow, orange, red, crimson) plus HRM channel, laptop computer, software, accessories: includes 1-year warranty on parts and labor, installation and training not included	9002032

Trademarks: QIAGEN®, Sample to Insight®, QIAsymphony®, *artus*®, Rotor-Gene®, Rotor-Gene AssayManager® (QIAGEN Group); Corning® (Corning Inc.); Sarstedt® (Sarstedt AG and Co.); SAS® (SAS Institute Inc.).

Document Revision History	
R2 01/2019	Changes from the previous revision involved updates to the run validity criteria in Table 7 in the Assay Settings section as well as changes to clinical performance details, Figure 2 and Table 22 in the Performance Characteristics section.

Limited License Agreement for *artus* HCV QS-RGQ Kit

Use of this product signifies the agreement of any purchaser or user of the product to the following terms:

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