

# **artus<sup>®</sup> VanR QS-RGQ Kit Handbook**



For Research Use Only. Not for use in diagnostic procedures.

For use with QIA Symphony<sup>®</sup> SP/AS and Rotor-Gene<sup>®</sup> Q instruments

**REF** 4574306



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## Intended Use

The *artus* VanR QS-RGQ Kit is intended for research use only. Not for use in diagnostic procedures. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of a disease.

All due care and attention should be exercised in the handling of the products. We recommend all users of QIAGEN products to adhere to the NIH guidelines that have been developed for recombinant DNA experiments, or to other applicable guidelines.

## Summary and Explanation

The *artus* VanR QS-RGQ Kit constitutes a ready-to-use system for the detection of *vanA* and *vanB* DNA using polymerase chain reaction (PCR) on Rotor-Gene Q instruments with sample preparation and assay setup using the QIAasymphony SP and AS instruments.

## Principle of the Procedure

The VanR Master A and VanR Master B contain reagents and enzymes for the specific amplification of target regions within the *vanA*, and *vanB* genes associated with vancomycin-resistant enterococci, and for the direct detection of the specific amplicon in fluorescence channels Cycling Green and Cycling Orange of Rotor-Gene Q instruments.

In addition, the *artus* VanR QS-RGQ Kit contains a second heterologous control system to identify potential failures during the entire assay process. This is detected as an internal control (IC) in fluorescence channel Cycling Crimson of Rotor-Gene Q instruments.

## Materials Provided

The contents of the *artus* VanR QS-RGQ Kit are sufficient for 72 tests in one to three batches of 24 reactions on the QIAsymphony RGQ. The Rotor-Gene Q instrument rotor holds up to 72 reaction tubes.

### Kit contents

<b><i>artus</i> VanR QS-RGQ Kit</b>			<b>(72)</b>
<b>Catalog no.</b>			<b>4574306</b>
<b>Number of reactions</b>			<b>72</b>
Blue	VanR Master A	<b>MASTER A</b>	3 x 330 µl
Violet	VanR Master B	<b>MASTER B</b>	3 x 600 µl
Green	VanR Internal Control	<b>IC</b>	3 x 540 µl
Red	VanR Positive Control	<b>CONTROL +</b>	3 x 330 µl
White	VanR Negative Control	<b>CONTROL -</b>	3 x 330 µl
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## Materials Required but Not Provided

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate safety data sheets (SDSs), available from the product supplier.

### Consumables and reagents for sample collection

- BD™ ESwab Collection Kit (Becton Dickinson, cat. no. 220245)

### Adapters for the QIAsymphony SP

- Elution Microtube Rack QS (Cooling Adapter, EMT, v2, Qsym, cat. no. 9020730) in combination with the QIAsymphony SP/AS Transfer Frame
- Tube Insert 3B (Insert, 2.0 ml v2, samplecarr. (24), Qsym, cat. no. 9242083)

## **Consumables and reagents for the QIA Symphony SP**

- QIA Symphony DSP Virus/Pathogen Mini Kit (cat. no. 937036)
- Buffer ATL (4 x 50 ml) (cat. no. 939016)
- 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 (EMTR) (cat. no. 19588)
- Tip disposal bags (cat. no. 9013395)
- Micro tubes 2.0 ml Type H, without skirted base (cat. nos. 72.693) or Micro tubes 2.0 ml Type I, with skirted base (Sarstedt®, cat. nos. 72.694, [www.sarstedt.com](http://www.sarstedt.com)) for use with samples and internal controls
- Tubes 14 ml, 17 x 100 mm polystyrene round-bottom (Becton Dickinson, cat. no. 352051) for use with internal controls

## **Adapters and reagent holders for the QIA Symphony 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)

## **Consumables for the QIA Symphony AS**

- Strip Tubes and Caps, 0.1 ml (cat. no. 981103)
- Tubes, conical, 2 ml, Qsym AS (cat. no. 997102)
- Tubes, conical, 5 ml, Qsym AS (cat. no. 997104)
- 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)

## General laboratory equipment

- Pipets (adjustable)\* and sterile pipet tips with filters
- Vortex mixer\*
- Benchtop centrifuge\* with rotor for 2 ml reaction tubes

## Equipment for sample preparation and assay setup

- QIAasymphony SP (cat. no. 9001297), \* software version 4.0 or higher
- QIAasymphony AS (cat. no. 9001301), \* software version 4.0 or higher

## Equipment for PCR

- Rotor-Gene Q 5plex HRM instrument\*†
- Rotor-Gene AssayManager® version 1.0 or higher

# Warnings and Precautions

## Safety information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. 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](http://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 QIAasymphony DSP Virus/Pathogen Mini Kit, see the *QIAasymphony DSP Virus/Pathogen Kit Instructions for Use (Handbook)* supplied with this kit. For safety information regarding the instruments, see the *QIAasymphony SP/AS User Manual – General Description*, *QIAasymphony SP/AS User Manual – Operating the QIAasymphony SP*, *QIAasymphony SP/AS User Manual – Operating the QIAasymphony AS*, *QIAasymphony Management Console User Manual*, *Rotor-Gene AssayManager Core Application User*

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

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† 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 "mmyyynn" where "mm" indicates the production month in digits, "yy" indicates the last two digits of the production year, and "ynn" indicates the unique instrument identifier.

*Manual, artus Basic Plug-in User Manual, and the user manual supplied with the Rotor-Gene Q instrument.*

Discard sample and assay waste according to your local safety regulations.

The following risk and safety phrases apply to components of the *artus* VanR QS-RGQ Kit:

### VanR Positive Control:



Xi

Contains ProClin®: Irritant. Risk and safety phrases: \* R43, S24-36/37/39-46.

### 24-hour emergency information

Chemical emergency or accident assistance is available 24 hours a day from: CHEMTREC

**Outside USA & Canada** ■ Tel: +1-703-527-3887 (collect calls accepted)

## General precautions

Always pay attention to the following:

- Use sterile pipet tips with filters.
- During manual steps, keep tubes closed when possible and avoid contamination.
- Thaw all components thoroughly at room temperature (15–25°C) before starting an assay.
- When thawed, mix the components (by pipetting repeatedly up and down or by pulse vortexing) and centrifuge briefly. Ensure that no foam or bubbles are present in the reagent tubes.
- Do not mix components from kits with different lot numbers.
- Follow universal precautions. All specimens should be considered potentially infectious and handled accordingly.
- Make sure that the required adapters are precooled to 2–8°C.
- Work quickly and keep PCR reagents on ice or in the cooling block before loading.

\* R43: May cause sensitization by skin contact; S24: Avoid contact with the skin; S36/37/39: Wear suitable protective clothing, gloves, and eye/face protection; S46: If swallowed, seek medical advice immediately and show container or label.



- Proceed continuously from one part of the workflow to the next. Do not exceed 30 minutes of transfer time between QIAasymphony AS and the Rotor-Gene Q instrument.
- Check that maintenance has been performed and replaceable parts (e.g., tip guards) have been reinstalled.

## Reagent Storage and Handling

The components of the *artus* VanR QS-RGQ Kit should be stored at –10 to –30°C and are stable until the expiration date stated on the label. Repeated thawing and freezing (>3 x) should be avoided, as this may reduce assay performance. All reagents that are loaded on QIAasymphony AS are for use in that run only. Do not remove the residual components to use them for a second PCR.

## Specimen Handling and Storage

Information about specimen handling and storage for rectal or perianal swabs is given in Table 1.



All specimens must be treated as potentially infectious material.

**Table 1. Specimen handling, storage, and preparation for rectal or perianal swabs**

Specimen collection	Rectal or perianal swabs collected in Liquid Amies transport medium using a BD ESwab Collection Kit (Becton Dickinson, cat. no. 220245)
Specimen transport	Shatterproof transport Shipment within 24 hours of collection Mail shipment according to legal instructions for the transport of pathogen material* Samples should be shipped cool (2 to 8°C)
Specimen storage (including time needed for transport)	2–8°C for up to 7 days –10 to –30°C for up to 30 days
Sample preparation	Place 300 µl of liquid from swab collected in Liquid Amies transport medium using a BD ESwab Collection Kit (Becton Dickinson, cat. no. 220245) into a Sarstedt 2.0 ml Micro tube Type H, without skirted base (cat. no. 72.693) or Sarstedt Micro tube 2.0 ml Type I, with skirted base (cat. no. 72.694) and load onto the QIAasymphony SP.

\*International Air Transport Association (IATA). Dangerous Goods Regulations.

# Procedure

**Table 2. General information**

Kit	<i>artus</i> VanR QS-RGQ Kit, <b>REF</b> 4574306
Sample material	Rectal or perianal swabs
Front-end purification	QIAasympyony DSP Virus/Pathogen Mini Kit (cat. no. 937036)
Sample volume (including excess volume)	300 µl
Assay Parameter Set	<i>artus_VanR_rec-swab200_V1</i>
Default Assay Control Set	<i>Complex200_V6_DSP_artus_VanR</i>
Elution volume	110 µl
Required QIAasympyony software version	Version 4.0 or higher
Required QIAasympyony SP/AS configuration profile	Default profile 1
Master mix volume	25 µl
Template volume	15 µl
Number of reactions	24–72* (including all controls to be loaded onto QIAasympyony SP and QIAasympyony AS)
Runtime on QIAasympyony SP/AS	For 24 reactions: approximately 90 minutes For 72 reactions: approximately 280 to 290 minutes
Runtime on Rotor-Gene Q instrument	Approximately 120 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 run and transfer to the Rotor-Gene Q instrument.

## Controls

### Positive control

The VanR Positive Control (supplied with the *artus* VanR QS-RGQ Kit) monitors the efficiency of sample preparation and the downstream assay. This positive control is loaded onto QIAasymphony SP before DNA purification (see page 20 for further details on loading the positive control).

### Negative control

The VanR Negative Control (supplied with the *artus* VanR QS-RGQ Kit) is loaded onto the QIAasymphony SP before DNA purification in place of a swab sample and assists in identifying contamination during sample preparation and/or the downstream assay (see page 20 for further details on loading the negative control).

## Preparation of carrier RNA (CARRIER) and internal control (VanR Internal Control)

Using QIAasymphony DSP Virus/Pathogen Kits in combination with the *artus* VanR QS-RGQ Kit requires introduction of the internal control (VanR Internal Control), consisting of inactivated, intact *Geobacillus stearothermophilus*, into the purification procedure to monitor the efficiency of sample preparation and downstream assay.

The internal control (VanR Internal Control), supplied with the *artus* VanR QS-RGQ Kit, must be added with carrier RNA (CARRIER)–Buffer AVE (AVE) mixture. The total volume of the internal control–carrier RNA (CARRIER)–Buffer AVE (AVE) mixture is 120 µl per sample.

To prepare the carrier RNA (CARRIER)–Buffer AVE (AVE) mixture, add 1350 µl Buffer AVE (AVE), supplied with the QIAasymphony DSP Virus/Pathogen Mini Kit, to resuspend the lyophilized carrier RNA (CARRIER). Invert tube to mix.

For internal control (IC) calculation, the “IC Calculator” within the QIAasymphony Management Console (QMC) should be used.

Table 3 represents the addition of internal control to 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 3. Preparation of carrier RNA (CARRIER) and internal control (VanR Internal Control)**

<b>Component</b>	<b>n = number of samples and controls</b>	
	<b>n ≤ 13 Volume (µl) (Sarstedt tubes)*</b>	<b>n ≥ 13 Volume (µl) (BD tubes)†</b>
Stock carrier RNA (CARRIER)	$(n + 3) \times 3$	$(n + 5) \times 3$
Internal control (VanR Internal Control)	$(n + 3) \times 14$	$(n + 5) \times 14$
Buffer AVE (AVE)	$(n + 3) \times 103$	$(n + 5) \times 103$
<b>Final volume per sample (excluding dead volume)</b>	<b>120</b>	<b>120</b>
<b>Total volume for n samples</b>	<b><math>(n + 3) \times 120</math></b>	<b><math>(n + 5) \times 120</math></b>

\* Micro tubes 2.0 ml Type H and Micro tubes 2.0 ml Type I (Sarstedt, cat. nos. 72.693 and 72.694). If preparing the internal control as a stock solution in a larger tube, multiply the total volume of each component by the number of internal control tubes 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).

If using more than 13 reactions in Micro tubes 2.0 ml, set up the reactions in a larger tube and load in multiple tubes. Make sure that for each tube the required excess volume of 3 additional reactions is added.

† Tubes 14 ml, 17 x 100 mm polystyrene round-bottom (Becton Dickinson, cat. no. 352051). Internal control mixture corresponding to 5 additional samples (i.e., 600 µl) is required.

### Calculation of mixture by “IC Calculator”

1. Open the QMC.
2. Select the IC Calculator icon.
3. Select “Complex200\_V6\_DSP\_artus\_VanR” from the ACS drop-down list.
4. Enter the required number of samples.
5. Select the labware used for the internal control.
6. Select an elution volume of 110 µl.
7. Select “Internal Control/Eluate” and “0.1 µl” for internal control mode.
8. Press “Calculate” to start calculation of internal control mixture.

The IC calculator displays the different volumes of reagents to be mixed for the internal control mixture and the tube type to be used on the right side of the screen.

## **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 QIAasymphony 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 QIAasymphony AS.

For the integrated run on the QIAasymphony SP/AS, the Assay Parameter Set, *artus\_VanR\_rec-swab200\_V1*, is directly linked to the upfront Assay Control Set, *Complex200\_V6\_DSP\_artus\_VanR*, specifying the associated sample purification process.

# Protocol: DNA isolation and assay setup on the QIAasympphony SP/AS

## Important points before starting

- Ensure that you are familiar with operating the QIAasympphony SP/AS instruments. Refer to the user manuals supplied with your instruments and the most current versions available online at [www.qiagen.com/products/qiasymphonyrgq.aspx](http://www.qiagen.com/products/qiasymphonyrgq.aspx) for operating instructions.
- Download the Application Package from “Protocol Files” on the “Resources” tab of the *artus* VanR QS-RGQ Kit web catalog page ([www.qiagen.com/p/artus-VanR-QS-RGQ-Kit-RUO](http://www.qiagen.com/p/artus-VanR-QS-RGQ-Kit-RUO)).
- Before using a reagent cartridge (RC) for the first time, check that Buffers QSL2 and QSB1 in the reagent cartridge (RC) do not contain a precipitate. If necessary, remove the troughs containing Buffers QSL2 and QSB1 from the reagent cartridge (RC) and incubate for 30 minutes at 37°C 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 sealed with Reuse Seal Strips and incubate the complete reagent cartridge (RC) for 30 minutes at 37°C with occasional shaking in a water bath.\* Allow the reagents to cool down to room temperature (15–25°C).
- Check that Buffer ATL (ATL) does not contain a precipitate. If a precipitate has formed, dissolve by heating the buffer at 70°C with gentle agitation in a water bath.\* Aspirate bubbles from the surface, and let the buffer cool to room temperature (15–25°C).
- Try to avoid vigorous shaking of the reagent cartridge (RC). Otherwise foam may be generated, which 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 batches of 24 reactions per kit per run.
- Make sure that eluates from the sample preparation and all components of the *artus* VanR QS-RGQ Kit remain on the instrument for no more than the normal time required for sample purification and assay setup of 72 assay

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

reactions, including up to 30 minutes transfer time from the QIAsymphony AS to the Rotor-Gene Q instrument.

### Things to do before starting

- Before each use, all assay reagents in the *artus* VanR QS-RGQ Kit need to be thawed completely, mixed (by repeated up and down pipetting or by quick vortexing), and centrifuged for at least 3 seconds. Avoid bubbling or foaming of the reagents.
- Prepare all required mixtures. If needed, prepare mixtures containing RNA (CARRIER) and internal controls just before starting. For more information, see “Preparation of carrier RNA (CARRIER) and internal control (VanR Internal Control)”, page 12.
- Before starting an integrated run, make sure that all instruments are clean and that the replaceable parts have been loaded (e.g., tip guards) as described in the maintenance instructions in the *QIAsymphony SP/AS User Manual – General Description*, *QIAsymphony SP/AS User Manual – Operating the QIAsymphony SP*, *QIAsymphony SP/AS User Manual – Operating the QIAsymphony AS*, and *QIAsymphony Management Console User Manual* supplied. Make sure to carry out maintenance regularly to minimize the risk of cross-contamination.
- Ensure that QIAsymphony process profile “Default Profile 1” is active. The selected profile is shown at the bottom, right corner of the touchscreen. The profile may be changed in the “Configuration” menu of the “Tools” tab by a user logged in as “Supervisor”.

### Procedure

- 1. Close all drawers and the hoods of the QIAsymphony SP/AS.**
- 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 “Waste” drawer of the QIAsymphony SP.**
  - Open the “Waste” drawer.
  - Empty and install liquid waste bottle. Make sure to remove the lid before placing the liquid waste bottle into the drawer.
  - Insert tip chute.



Note: Different tip chutes must be used for benchtop and QIAasympphony Cabinet SP/AS operation.

- Insert tip park station.
- Insert empty unit boxes (see Table 4 and Figure 1). Make sure that there is at least one empty unit box in slot 4 (closest to you).
- Install empty tip disposal bag (below waste drawer for benchtop operation or in the waste bin for QIAasympphony Cabinet SP/AS operation).
- Close the “Waste” drawer and perform an inventory scan.

**Table 4. Required plasticware for 1–3 sample batches**

	One batch, 24 samples	Two batches, 48 samples	Three batches, 72 samples
<b>Empty unit boxes</b>	2	3	4



**Figure 1. Position of unit boxes.**

## **5. Load the “Eluate” drawer.**

- Place the adapter (Elution Microtubes Rack QS) onto the transfer frame.
- Open the “Eluate” drawer.
- Place the assembly of adapter and transfer frame onto slot 1 of the “Eluate” drawer.
- Select “Elution Slot 1” on the touchscreen.
- Remove the bottom from the Elution Microtubes CL by twisting the rack until the bottom comes out.

- Scan the bar code on the Elution Microtubes CL rack using the handheld bar code scanner.
- Insert the rack in the adapter on "Elution Slot 1".
- Remove the lid of the Elution Microtubes CL rack.
- Close the "Eluate" drawer.
- Press "OK".
- Wait until the scan has finished.

## 6. Load the "Reagents and Consumables" drawer (Figure 2).

- Open the "Reagents and Consumables" drawer.
- Take the reagent cartridge (RC) and before using for the first time, check that Buffers QSL2 and QSB1 in the cartridge do not contain a precipitate. If Buffers QSL2 and QSB1 contain a precipitate, follow the instructions on page 15.

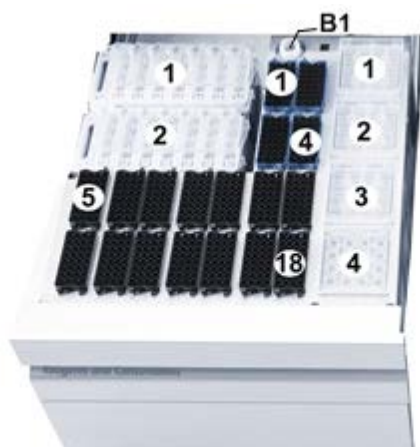
**Note:** Try to avoid vigorous shaking of the reagent cartridge (RC) otherwise foam may be generated, which can lead to liquid-level detection problems.

- Place the reagent cartridge (RC) in the gray reagent cartridge holder.
- Ensure that the magnetic particles are fully resuspended. Vortex the trough containing the magnetic particles vigorously for at least 3 minutes before use. Place the trough containing the magnetic particles back in to the reagent cartridge (RC).
- Before loading the reagent cartridge (RC), remove the cover from the trough containing the magnetic particles.
- Open the enzyme tubes. Place the lids of the enzyme tubes onto the cap holders on the gray reagent cartridge holder.

**Note:** If enzyme tubes contain air bubbles, aspirate bubbles from the surface.

- Mount the enzyme rack (ER) on the reagent cartridge (RC).
- Mount the piercing lid (PL) onto the reagent cartridge (RC) and gently click into place.
- Place prepared reagent cartridge(s) (RC) onto position RC 1 and/or RC 2. One new reagent cartridge (RC) is sufficient for up to 96 samples.
- Press the "R+C" button on the touchscreen.
- Press the "Bottle ID" button.

- Press the text field and scan the bar code of the Buffer ATL (ATL) bottle using the handheld bar code scanner.



**Figure 2. Position of the reagents and consumables on the QIAasymphony SP.**

- Open the bottle of Buffer ATL (ATL) and check that it does not contain a precipitate. If Buffer ATL (ATL) contains a precipitate, follow the instructions on page 15.
- Place the bottle of Buffer ATL (ATL) into position B1, which is next to the reagent cartridge slot 1 (RC 1) (position 1 in the photo above).
- Load sufficient racks of disposable 200  $\mu$ l filter-tips in tip rack holder positions 1–4 (See Table 5, page 20).
- Load sufficient racks of 1500  $\mu$ l disposable filter-tips in tip rack holder positions 5–18 (See Table 5, page 20).
- Make sure to click all racks into place.

**Note:** We recommend loading more than the required number of filter-tips of each size so that sufficient filter-tips are available for automated error handling.

- Remove lid of unit boxes for sample prep cartridges and load sufficient sample prep cartridges in unit box holder positions 1–3 (See Table 5, page 20).
- Remove lid of unit box for 8-Rod Covers and load unit box with sufficient 8-Rod Covers in unit box holder position 4 (See Table 5, page 20).

**Note:** Plastic consumables may shift during transit or storage. Check that all plastics are aligned properly inside the unit box before loading on the QIAasymphony SP.

- Press "OK" in the consumables screen.

- Close the “Reagents and Consumables” drawer and perform an inventory scan.

**Table 5. Required plasticware for 1–3 sample batches**

	<b>One batch, 24 samples*</b>	<b>Two batches, 48 samples*</b>	<b>Three batches, 72 samples*</b>
<b>Disposable filter-tips, 200 µl<sup>†‡</sup></b>	34 (1 rack)	60 (2 racks)	86 (3 racks)
<b>Disposable filter-tips, 1500 µl<sup>†‡</sup></b>	123 (4 racks)	205 (7 racks)	295 (10 racks)
<b>Sample prep cartridges</b>	18	36	54
<b>8-Rod Covers</b>	3	6	9

\* Performing more than one inventory scan requires additional disposable filter-tips.

† There are 32 filter-tips/tip rack, 28 sample prep cartridges/unit box, and twelve 8-Rod Covers/unit box.

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

## **7. Load the “Sample” drawer (tube carrier) with the positive and negative controls.**

- Place the tube with the VanR Positive Control (supplied with the *artus* VanR QS-RGQ Kit) in position 1 of the first sample carrier (use Tube Insert 3B for 2 ml Micro tubes).
- Place the tube with the VanR Negative Control (supplied with the *artus* VanR QS-RGQ Kit) in position 2 of the first sample carrier (use Tube Insert 3B for 2 ml Micro tubes).

Note: Make sure to load the positive and negative controls in the correct position. Rotor-Gene AssayManager will not import the result file if the positive and negative controls are placed in any other position. Do not load controls into additional carriers for the same AS batch.

## **8. Load the “Sample” drawer (tube carrier) with the samples.**

- Load prepared samples (see page 10) in 2 ml micro tubes in the sample tube carrier already containing the controls (use Tube Insert 3B for 2 ml micro tubes).

- If required, prepare further sample tube carriers in the same way, but without controls.

Note: If samples contain bar codes, orient samples in the tube carrier so that the bar codes are completely visible.

- Check that sample and control tubes are correctly loaded and clicked into place.
- Insert all sample carriers in "Sample" drawer slots 1–4. The LED light turns orange if loaded correctly.

**9. Using the "Integrated run" setup on the QIAasympy touchscreen, enter the required information for each batch of samples to be processed.**

- Press the "Integrated Run" tab on the touchscreen.
- Press "Define run".
- Select "SP Batch 1" (or appropriate batch number of sample carrier with "Full Process Controls", if performing continuous loading).
- Press "Edit samples".
- Make sure that the correct labware is assigned to the samples. If necessary, correct the labware assignment.
- Press "ID/Type".
- Select the first position and press "Sample ID".
- Press the text field and enter VanR Positive Control, then press "OK".
- Select the first position and press "EC+".
- Select the second position and press "Sample ID".
- Press the text field and enter VanR Negative Control, then press "OK".
- Select the second position and press "EC-".
- If necessary, resolve any bar code errors for sample and insert IDs.
- Press "OK".

Note: Do not assign the Sample Type "EC+" or "EC-" to tubes other than the positive and negative control supplied with the *artus* VanR QS-RGQ Kit. Rotor-Gene AssayManager will reject runs with incorrect control patterns. If you are additionally processing previously characterized samples along with the test samples, make sure to assign the "sample type" "sample" to these samples.

**10. Define the assay(s) to run.**

- Press the corresponding "SP Batch" button.

- Press "Define assays".
- Select the samples to be processed with the assay.
- Select the assay "artus\_VanR rec-swab200\_V1" under the category "artus QS-RGQ".
- Press "OK".
- Repeat step 10 for all batches and samples to be processed.

#### **11. Define the QIAasymphony AS batch.**

- Select all batches that should be processed in one integrated QIAasymphony RGQ run.
- Press "Create AS batch".

Note: All QIAasymphony SP batches assigned to the same QIAasymphony AS batch (integrated QIAasymphony RGQ run) will be processed in the same assay setup procedure.

- Press "OK" to queue the run.

#### **12. Load the "Sample" drawer with the internal control mixture.**

- Place the previously prepared tube(s) of internal control mixture (see page 12) into the sample carrier (use Tube Insert 3B for 2 ml micro tubes).
- Insert sample carrier in slot A of the "Sample" drawer.

#### **13. Define the internal control positions.**

- Press the "Define ICs" button.
- Select the positions of the internal control mixture.
- Select the corresponding internal control "Complex200\_V6\_DSP\_artus\_VanR" from the folder "Required".
- Make sure that the correct labware is assigned. If not, correct labware assignment by pressing "IC Tubes".
- Press "OK".

#### **14. Start the run.**

- To start the run press the "Run" button.
- Read and confirm the message that appears.
- We recommend waiting by the instrument until it has performed liquid level detection of the internal control tubes (QIAasymphony SP carrier status changes to "running").

**Note:** Do not pause or stop the run during processing (unless an emergency occurs), as this will lead to the respective samples and assay reactions being flagged as “unclear”. Rotor-Gene AssayManager will invalidate “unclear” assay reactions.

**Note:** It is possible to continuously load samples and add them to this run (until reagents are loaded) or to a new QIASymphony RGQ run.

## **Loading the QIASymphony AS drawers for assay setup**

### **15. Install an empty tip disposal bag and tip chutes.**

- Install an empty tip disposal bag below the “Waste” drawer for benchtop operation or in the waste bin for QIASymphony SP/AS operation.
- Open the “Eluate and Reagents” drawer and the “Assays” drawer of QIASymphony AS.
- Open the hood and insert the tip chute inside the instrument.

**Note:** Different tip chutes must be used for benchtop and QIASymphony Cabinet SP/AS operation.

- Close hood, and read and confirm message.

### **16. Load “Assays” drawer with assay rack.**

- Press slot 5 “Assay” (yellow).
- Fill the required number of strip tubes (4 tubes = 1 segment) in a precooled Rotor-Gene Strip Tubes 72 QS cooling adapter as indicated on the touchscreen.

**Note:** Load complete strip tubes. Do not break strip tubes.

- Load adapter with strip tubes on slot 5 of the “Assays” drawer.
- Press “Rack ID” on the touchscreen, enter a user-defined rack ID, and press “OK”.

**Note:** It is also possible to use the automatic ID function.

- Press “Load”.

### **17. Load “Assays” drawer with filter-tips.**

- Load at least the number of filter-tips provided in the “Assay Setup | Loading Information” screen.

**Note:** Start loading tip racks from the positions at the back (near the cooling adapters). In rare cases, the pipetting head may not be able to reach some positions toward the hood and this may cause the instrument to

automatically pause. We recommend loading more than the required number of filter-tips of each size so that sufficient filter-tips are available for automated error handling.

## **18. Load “Eluate and Reagents” drawer with reagents.**

- Before each use, all assay reagents need to be thawed completely, mixed, and centrifuged for at least 3 seconds. Avoid bubbling or foaming of the reagents (see procedure described in “Important points before starting”, page 15).
- Press slot 3 “Reagent” (yellow) on the touchscreen.
- Prepare a precooled reagent holder as requested on the touchscreen.
- Select the tube positions on the touchscreen, load an empty tube for the master mix, and fill at least the required volume of the correct reagents in the required tubes in the corresponding positions as indicated on the touchscreen.

Note: It may be necessary to combine the same reagent types (VanR Master A or VanR Master B) into one tube if required volume exceeds filling volume of the corresponding reagents. One tube each of VanR Master A and VanR Master B is sufficient for 24 QIAasympphony SP eluates (including controls).

Note: Viscous reagents can be difficult to handle with manual pipets. Make sure to transfer the entire volume of the VanR Master A and VanR Master B into the respective tubes.

Note: Alternatively, select “List View” on the touchscreen and prepare the reagent adapter accordingly. A “Loading Information File” can also be downloaded via the QMC or USB port (and printed) after the QIAasympphony AS batch is defined and queued.

- Press the “Scan Kit Barcode” button on the touchscreen and press the light-blue kit bar code line.
- Press the text field and scan the kit bar code on the upper side of the *artus* VanR QS-RGQ Kit using the handheld bar code scanner.
- Load the prepared reagent adapter onto slot 3 of the “Eluate and Reagents” drawer.
- Press the “Load” button.
- Close both drawers.
- Press “Scan” to enter the scan dialog.
- Press “Scan” to perform an inventory scan of all QIAasympphony AS components.



Note: We recommend waiting beside the instrument until the scan is completed.

- Assay setup will start automatically when sample preparation on the QIAasympphony SP has finished.

#### **19. Check the time for the end of the QIAasympphony AS batch to remove assay rack.**

- After the QIAasympphony AS scan has finished, the calculated integrated run time is shown on the "Integrated Run Overview" screen. The maximum time permitted from the end of the QIAasympphony AS run until the start of the Rotor-Gene Q instrument run is 30 minutes. Make sure to transfer the assay rack to the Rotor-Gene Q instrument within 30 minutes of the assay run finishing.

### **Removal of assay rack and transfer of result file**

#### **20. Remove the QIAasympphony AS batch and the assay rack.**

- Open the "Assays" and the "Eluate and Reagents" drawers.
- Remove the adapter with the strip tubes and close the tubes with the appropriate caps.
- Press slot 5 "Assay".
- Press the "Remove" button.
- Remove the reagent adapter and discard the reagents according to your local safety regulations.
- Press slot 3 "Reagent".
- Press the "Remove" button.
- Close the "Assays" and the "Eluate and Reagents" drawers.
- Press "Scan" to enter the scan dialog.
- Press "Scan" to perform an inventory scan for adapters on the left and right (typically preselected).
- Press the "Integrated Batch" button (green) to remove the integrated run.
- Read and confirm the message.
- The final QIAasympphony AS result file is created and can be transferred to either a USB stick or to a defined folder (\log\Results\AS) via the QMC.

**21. Transfer the result file to a defined folder. To transfer the result file using the USB stick, follow step 21a. To transfer the result file using the QMC, follow step 21b.**

**21a. Transfer result file using the USB stick.**

- Insert the USB stick.
- Select "Tools".
- Select "File Transfer".
- Select "Result Files" in the "Save to USB Stick" column.
- Press the "Transfer" button.
- Read and confirm the message.
- After successful transfer, press "OK" and remove the USB stick.
- Proceed to "Protocol: PCR on the Rotor-Gene Q instrument", page 27.

**21b. Transfer result file using the QMC.**

- Log in to the correct QIAasympyony SP/AS.
- Select the transfer file icon.
- Choose file format "Result File AS".
- Select result file with the correct time stamp and batch ID from the list of "Remote Site" files (right column).
- Transfer result file to the "Local Site" (the file is saved under the path defined in "Tools", "Options", "File Transfer", under \log\Results\AS).

Note: If multiple batches on the QIAasympyony AS are configured in an integrated run, reload the QIAasympyony AS drawers, starting at step 15.

- Proceed to "Protocol: PCR on the Rotor-Gene Q instrument", page 27.

Note: We recommend marking the strip tube caps to ensure correct positioning and to use a cooled transport frame to avoid contamination.

## Protocol: 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.
- Rotor-Gene AssayManager enables automated interpretation of the PCR results.
- The *artus* VanR QS-RGQ Kit must be run on the Rotor-Gene Q instrument using automated interpretation of results with Rotor-Gene AssayManager. The cycling parameters are locked for the run.
- Download the Application Package from “Protocol Files” on the “Resources” tab of the *artus* VanR QS-RGQ Kit web catalog page ([www.qiagen.com/p/artus-VanR-QS-RGQ-Kit-RUO](http://www.qiagen.com/p/artus-VanR-QS-RGQ-Kit-RUO)).
- After installing the plug-in and importing the assay profile (see “Things to do before starting”, below), 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 automated interpretation of results.
- For system-wide process safety it is necessary to activate the following settings for the closed mode: “Material number required”, “Valid expiry date required”, and “Lot number required”.

### Things to do before starting

- For automated interpretation of results using the *artus* VanR QS-RGQ Kit with Rotor-Gene AssayManager, the latest *artus* Basic plug-in must be installed to your Rotor-Gene AssayManager.  
Start the installation process by double-clicking the **ArtusBasic.Installation.msi**, and follow the installation instructions. For a detailed description refer to “Installing Plug-ins” (see the *Rotor-Gene AssayManager Core Application User Manual* supplied).
- To use the *artus* VanR QS-RGQ Kit for rectal or perianal samples, the file **AP\_artus\_VanR\_rec-swab200\_QS\_V1\_0\_X.iap** must be imported to Rotor-Gene AssayManager.  
To import the assay profile into Rotor-Gene AssayManager, navigate to the “Configuration Environment” and change to the “Assay Profile” tab. Click “Import” and select the **AP\_artus\_VanR\_rec-swab200\_QS\_V1\_0\_X.iap** file in the open file dialog. Click “Open”, and the assay profile is loaded and added to the list of available assay profiles.  
**Note:** The same version of an assay profile cannot be imported twice.

## Procedure

### 1. Prepare the rotor and start the run on the Rotor-Gene Q instrument.

- Place a 72-Well Rotor on the Rotor Holder.
- Fill the rotor with strip tubes. Make sure to start at position 1 and to fill the strip tubes in the correct orientation.
- Use empty capped strip tubes to fill all unused positions.
- Attach the locking ring.
- Load the Rotor-Gene Q instrument with the rotor and locking ring.
- If using a USB stick for data transfer direct from the QIAasympphony SP/AS, unzip the result file from the QIAasympphony AS. The result files are stored under \log\Results\AS.

Note: On most computers, files can be unzipped by right-clicking the file and then clicking "Extract" in the menu that opens.

- Start Rotor-Gene AssayManager.
- Log in to the closed mode.
- Select the "Setup" environment, if not already preselected.
- Import the QIAasympphony AS result file at the bottom of the screen. Select the source "QIAasympphony" as "Import type".
- In the "Select file" dialog, open the corresponding QIAasympphony AS result file and click "Open".
- Read and confirm the message.
- After successful import, select the corresponding work list from the work list manager list and click the "Apply" button.
- Enter an experiment name.
- Select the cyclor to be used in the "Cyclor selection" dialog.
- Check correct attachment of locking ring and confirm on the screen that the locking ring is attached.
- Close the Rotor-Gene Q instrument lid.
- Click the "Start run" button.

Note: If using multiple cyclor runs, change to the corresponding cyclor environment to see the progress of the run.

- When the run is finished, click "Finish run...".
- For users logged in with the Operator role: Click "Release".

- For users logged in with the Approver role: Click “Release and go to approval”.

## 2. Release and report results.

- If you have used “Release” before, select the “Approval” environment.
- Press “Apply filter” (or choose own filter options beforehand).
- Select experiment.
- Click “Start approval”.
- Approve the results of each test sample: Use the “Accepted” button for test samples whose results analyzed by Rotor-Gene AssayManager you agree with. Use the “Rejected” button if the test sample result evaluated by Rotor-Gene AssayManager is not acceptable for any reason.

Note: A result automatically set to “Invalid” by Rotor-Gene AssayManager cannot be converted to a valid result anymore, even if the result is rejected.

- Optional: Enter comments.
- Click “Release /report data...”.
- Choose a report profile and click “OK”. The report will be generated and stored automatically.

Note: The user needs approval rights to release an assay.

- Unload the Rotor-Gene Q instrument and discard the strip tubes according to your local safety regulations.

## 3. Perform maintenance.

- When all QIAasympphony AS batches of the integrated QIAasympphony SP/AS run have finished, perform the regular maintenance as described in the *QIAasympphony SP/AS User Manual – General Description*.

Note: This can be performed at any time before the start of the next integrated run, according to local regulations or priorities.

- Perform daily, weekly, and annual preventive maintenance as described in the *QIAasympphony SP/AS User Manual – General Description*.

## Interpretation of Results

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

The *artus* VanR QS-RGQ Kit Assay Profile for rectal or perianal swabs contains rules for interpreting the assay results automatically.

Every sample and control displays an independent result for each target: *vanA*, *vanB*, and internal control. Each result is reported as "Signal detected", "No signal", or "INVALID".

Positive/negative control results:

- All targets for the positive control ("Positive Control") and negative control ("Negative Control") must be valid in order to confirm that the assay status is successful and the test results may be reported. If any target of the positive or negative control is invalid, results for every sample in the run will display "INVALID". The entire assay run must be retested.
- The positive control must report a "Signal detected" result for *vanA*, *vanB*, and the internal control.
- The negative control must report a "Signal detected" result for the internal control and "No signal" for the specific targets.

Sample results:

- See Table 6 (page 31) for a summary of results interpretation.
- Since results are reported independently for *vanA* and *vanB* targets, a sample is considered positive for vancomycin resistance if either target is detected.
- The internal control signal must be detected in samples where no *vanA* and/or *vanB* signal is detected. If the internal control signal is not detected or is "INVALID", all targets for the sample will be displayed as "INVALID". The sample must be retested.
- The internal control target may be reported as "No signal" or "INVALID" in samples where *vanA* and/or *vanB* signal is detected. In these cases, *vanA* and *vanB* will be reported. No retesting is necessary.
- Note: It is expected that in some positive vancomycin-resistant samples the internal control PCR may be inhibited, which will cause a "No signal" or "INVALID" result for the internal control.

- In some samples, a result for *vanA* or *vanB* may be reported as "INVALID". In these cases, we recommend retesting the sample.
- If *vanA* or *vanB* is reported as "INVALID" and the flag says CT\_ABOVE\_ACCEPTED\_RANGE, this sample does not need to be retested and is considered negative, if the internal control is valid.

**Table 6. Summary of results interpretation**

Target result			Vancomycin resistance detected in sample
<i>vanA</i>	<i>vanB</i>	Internal control	
Signal detected	Signal detected	Signal detected/ No signal/ INVALID	Yes
Signal detected	No signal	Signal detected/ No signal/ INVALID	Yes
No signal	Signal detected	Signal detected/ No signal/ INVALID	Yes
No signal	No signal	Signal detected	No

Table continued on next page

**Table 6. Continued**

Target result			Vancomycin resistance detected in sample
<i>vanA</i>	<i>vanB</i>	Internal control	
No signal	No signal	No signal/ INVALID	Error, retest sample
Signal detected/ No signal	INVALID*	Signal detected/ No signal/ INVALID	Error, retest sample
INVALID*	Signal detected/ No signal	Signal detected/ No signal/ INVALID	Error, retest sample

\* If *VanA* or *VanB* is reported as invalid with the flag "CT\_ABOVE\_ACCEPTED\_RANGE", the target is considered valid, but negative.

This automated analysis may provide the following corresponding flags.

Flag	Behavior	Description
ASSAY_INVALID	Invalid	Assay is invalid because at least one external control is invalid.
CT_ABOVE_ACCEPTED_RANGE	Invalid	The detected C <sub>T</sub> value is higher than the defined cutoff C <sub>T</sub> .
CT_BELOW_ACCEPTED_RANGE	Invalid	The detected C <sub>T</sub> value is lower than the defined cutoff C <sub>T</sub> .
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.



Flag	Behavior	Description
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).
IC_INVALID	Invalid	The internal control is invalid. Target and internal control share the same tube.
IC_NO_SIGNAL	Invalid	No internal control signal detected. Target and internal control share the same tube.
MULTI_THRESHOLD_CROSSING	Invalid	The amplification curve crosses the threshold more than once. An unambiguous $C_T$ cannot be determined.
NO_CT_DETECTED	Invalid	No $C_T$ is detected for this target.
NORM_FACTOR_ALTERATION	Warning	<p>Curve not normalized properly due to low signal.</p> <p><b>Note:</b> If a valid sample is tagged with this flag, the approver is asked to pay special attention to the information provided by this flag before deciding to accept or reject the result.</p>
OTHER_TARGET_INVALID	Invalid	Another target for the same sample is invalid.
SATURATION	Invalid	The raw data fluorescence is saturating strongly before the inflection point of the amplification curve.

Flag	Behavior	Description
SATURATION_ IN_PLATEAU	Warning	<p>The raw data fluorescence is saturating in the plateau phase of the amplification curve.</p> <p>Note: If a valid sample is tagged with this flag, the approver is asked to pay special attention to the information provided by this flag before deciding to accept or reject the result.</p>
SPIKE	Variable	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.
UNCERTAIN	Invalid	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.

Flag	Behavior	Description
UPSTREAM	Invalid	<p>Sample status was set to invalid or unclear by an upstream process (e.g., QIA Symphony Assay Setup).</p> <p><b>Note:</b> For “unclear” flags from upstream processes, the behavior of Rotor-Gene AssayManager is defined in the “Configuration” environment.</p> <p>For “invalid” flags from upstream processes Rotor-Gene AssayManager always invalidates such samples.</p>
WAVY_BASE_FLUORESCENCE	Invalid	A wavy baseline for the raw data fluorescence is detected in the amplification curve.

## Troubleshooting guide

This troubleshooting guide may be helpful in solving any problems that may arise. For more information, see also the Frequently Asked Questions page at our Technical Support Center: [www.qiagen.com/FAQ/FAQList.aspx](http://www.qiagen.com/FAQ/FAQList.aspx). The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information and protocols in this handbook or sample and assay technologies (for contact information, see back cover or visit [www.qiagen.com](http://www.qiagen.com)).

### Comments and suggestions

#### General handling

Error message displayed in the touchscreen	If an error message is displayed during an integrated run, refer to the user manuals supplied with your instruments.
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## Comments and suggestions

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### **Precipitate in reagent trough of opened cartridge of the QIA Symphony 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.  |
| b) Storage of reagent cartridge (RC) | Storage of reagent cartridge (RC) at less than 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 vigorously 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.  |

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

### Comments and suggestions

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- |  |   |
|--|---|
| 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 "Preparation of carrier RNA (CARRIER) and internal control (VanR Internal Control)", page 12. 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.  |
| e) Incomplete sample lysis                         | Before use, check that Buffers QSL2 and QSB1 do not contain precipitates. If necessary, remove the troughs containing Buffers QSL2 and QSB1 from the reagent cartridge (RC) and incubate for 30 minutes at 37°C with occasional shaking 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 QIAasympy purification procedure. To remove insoluble material for bacterial 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.

## Comments and suggestions

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### **QIAsymphony AS detects insufficient Master**

Insufficient Master transferred to tube

Combine the contents of an appropriate number of VanR Master A tubes into one tube before use. Combine the contents of an appropriate number of VanR Master B tubes into one tube before use. 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 control (VanR Positive Control) in fluorescence channel Cycling Orange and Cycling Green**

a) Incorrect configuration of the PCR

Make sure that assay setup was performed correctly and that the correct Assay Parameter Set was used. Repeat the PCR, if necessary. See "Assay Control Sets and Assay Parameter Sets", page 14.

b) The storage conditions for one or more kit components did not comply with the instructions given in "Reagent Storage and Handling" (page 9)

Check the storage conditions and the expiration date (see the kit label) of the reagents and use a new kit, if necessary.

## Comments and suggestions

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- |   |   |
|---|---|
| c) The <i>artus</i> VanR 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 sample subjected to purification using the QIAAsymphony DSP Virus/Pathogen Kit in fluorescence channel Cycling Crimson and simultaneous absence of a signal in channel Cycling Orange or channel Cycling Green**

- |  |   |
|--|---|
| a) The PCR conditions do not comply with the protocol  | Check the PCR conditions (see above) and repeats the PCR with corrected settings, if necessary.   |
| b) The PCR was inhibited   | Make sure that you use the validated isolation method (see "Protocol: DNA isolation and assay setup on the QIAAsymphony SP/AS", page 15) and closely follow the instructions.   |
| c) DNA was lost during extraction  | An absent signal of the internal control can indicate the loss of DNA during the extraction. Make sure that you use the validated isolation method (see "Protocol: DNA isolation and assay setup on the QIAAsymphony SP/AS", page 15) and closely follow the instructions.<br><br>See also "Low yield of nucleic acids", above. |
| d) The storage conditions for one or more kit components did not comply with the instructions given in "Reagent Storage and Handling" (page 9) | Check the storage conditions and the expiration date (see the kit label) of the reagents and use a new kit, if necessary.   |
| e) The <i>artus</i> VanR 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.   |

## Comments and suggestions

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### Signals with the negative controls in fluorescence channel Cycling Green of the analytical PCR

- |   |   |
|---|---|
| a) Contamination occurred during preparation of the PCR | Repeat the PCR with new reagents in replicates.<br>If possible, close the PCR tubes directly after addition of the sample to be tested.<br>Make sure that work space and instruments are decontaminated at regular intervals. |
| b) Contamination occurred during extraction             | Repeat the extraction and PCR of the sample to be tested using new reagents.<br>Make sure that work space and instruments are decontaminated at regular intervals.  |

## Quality Control

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

## References













QIAGEN maintains a large, up-to-date online database of scientific publications utilizing QIAGEN products. Comprehensive search options allow you to find the articles you need, either by a simple keyword search or by specifying the application, research area, title, etc.

For a list of references, visit the QIAGEN Reference Database online at [www.qiagen.com/RefDB/search.asp](http://www.qiagen.com/RefDB/search.asp) or contact QIAGEN Technical Services or your local distributor.



## Symbols

The following symbols may appear on the packaging and labeling:

 $\Sigma$	$\langle N \rangle$	Contains reagents sufficient for $\langle N \rangle$ reactions
		Use by
		Catalog number
		Lot number
		Material number (i.e., component labeling)
		Components (i.e., a list of what is included)
		Contains (contents)
		Number (i.e., vials, bottles)
		Temperature limitation
		Manufacturer
		Consult instructions for use
		Caution

## Contact Information

For technical assistance and more information, please see our Technical Support Center at [www.qiagen.com/Support](http://www.qiagen.com/Support), call 00800-22-44-6000, or contact one of the QIAGEN Technical Service Departments or local distributors (see back cover or visit [www.qiagen.com](http://www.qiagen.com)).

## Ordering Information

Product	Contents	Cat. no.
<i>artus</i> VanR QS-RGQ Kit (72)	For 72 reactions: 2 Masters, Positive Control, Internal Control, Negative Control	4574306
<b>QIAasymphony DSP Virus/Pathogen Kit</b>		
QIAasymphony DSP Virus/Pathogen Mini Kit	For 192 preps (200 µl each): includes 2 reagent cartridges and enzyme racks and accessories	937036
<b>QIAasymphony SP/AS Instruments</b>		
QIAasymphony SP	QIAasymphony sample prep module: includes 1-year warranty on parts and labor	9001297
QIAasymphony SP System	QIAasymphony sample prep module: includes installation and training, 1-year warranty on parts and labor	9001751
QIAasymphony AS	QIAasymphony assay setup module: includes 1-year warranty on parts and labor	9001301
<b>Rotor-Gene Q</b>		
Rotor-Gene Q 5plex HRM Platform	Real-time PCR cyclers and High Resolution Melt 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	9001580
Rotor-Gene Q 5plex HRM System	Real-time PCR cyclers and High Resolution Melt 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	9001650

Product	Contents	Cat. no.
<b>Rotor-Gene AssayManager — for routine testing with Rotor-Gene Q and QIAsymphony RGQ instruments</b>		
Rotor-Gene AssayManager	Software for routine testing in combination with the Rotor-Gene Q and QIAsymphony RGQ instruments; single license software for installation on one computer	9022737
Rotor-Gene AssayManager (10)	Software for routine testing in combination with the Rotor-Gene Q and QIAsymphony RGQ instruments; multi-license software for installation on up to 10 computers	9022739

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## Notes

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