

artus[®] CT/NG QS-RGQ Kit Handbook



Version 1

IVD

Qualitative in vitro diagnostics

For use with QIASymphony[®] SP/AS and Rotor-Gene[®] Q instruments

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Intended Use

The *artus* CT/NG QS-RGQ Kit is an in vitro real-time polymerase chain reaction (PCR) assay for the direct qualitative detection of *Chlamydia trachomatis* plasmid and genomic DNA and *Neisseria gonorrhoeae* genomic DNA to aid in the diagnosis of chlamydial and/or gonococcal urogenital disease. This diagnostic test is configured for use with the QIASymphony SP/AS and Rotor-Gene Q instruments for target amplification and detection.

The *artus* CT/NG QS-RGQ Kit is intended for use in conjunction with clinical presentation and other laboratory markers for disease prognosis.

QIAGEN continues to develop and validate further applications for *artus* QS-RGQ Kits, such as use with additional sample types. The most current version of this handbook and associated Application Sheets are available online at www.qiagen.com/products/artusctngqsrqgqkitce.



For more information about specific human biological samples with which the kit has been validated, see the Application Sheets, available online at www.qiagen.com/products/artusctngqsrqgqkitce.

As QIAGEN continuously monitors the assay's performance and validates new claims, the users are required to ensure that they work with the latest revision of the instruction for use.



Check availability of new electronic labeling revisions at www.qiagen.com/products/artusctngqsrqgqkitce before test execution.

All kits can be used with the respective instruction elements as long as the version number of the handbook and other labeling information matches with the kit version number. The version number is visible on each kit box label. QIAGEN ensures compatibility between all test kit lots under the same version number.

Summary and Explanation

The *artus* CT/NG QS-RGQ Kit constitutes a ready to use system for the detection of *C. trachomatis* (CT) and/or *N. gonorrhoeae* (NG) DNA using polymerase chain reaction (PCR) on Rotor-Gene Q instruments with sample preparation and assay setup using the QIASymphony SP/AS instruments. The CT/NG RG Master contains reagents and enzymes for the specific amplification of a 86 bp region of the *C. trachomatis* cryptic plasmid, a 66 bp region of the *C. trachomatis* genome, and a 74 bp target of the *N. gonorrhoeae* genome for the direct detection of the specific amplicon in fluorescence channel Cycling Green and Cycling Orange of the Rotor-Gene Q.

In addition, the *artus* CT/NG QS-RGQ Kit contains a fourth heterologous amplification system to identify possible PCR inhibition. This is detected as an

internal control (IC) in fluorescence channel Cycling Yellow of the Rotor-Gene Q. The detection limit of the analytical CT/NG PCR is not reduced by this control. External positive/negative controls (Control CT+/NG– and Control NG+/CT–) and a no template control (NTC) are supplied.

For further information, see the relevant Application Sheet at www.qiagen.com/products/artusctngqsrqgkitce.

Pathogen information

***Chlamydia trachomatis* (CT)**

Bacteria of the genus *Chlamydia* (C.) are of great epidemiological importance, and the 16 *C. trachomatis* serovars cause different diseases. *Chlamydia trachomatis* (serovars D–L) is one of the most frequent causes of sexually transmitted diseases (STD) worldwide. Serovars A–C are responsible for trachoma, a chronic, recurring disease of the conjunctiva and cornea found in the tropics. Serovars D–K are responsible for sexually transmitted urogenital and eye infections as well as newborn infant infections following perinatal transmission. Serovars LGV I–III are responsible for lymphogranuloma venereum, a sexually transmitted disease predominantly found in the tropics (1).

Trachoma occurs almost exclusively in tropical countries with insufficient hygienic conditions. Worldwide, it is the most frequent eye disease and, after cataracts, the second most frequent cause of blindness. It is estimated that approximately 150 million people are infected, and, of these, approximately 6 million have gone blind (1).

In the industrialized nations, chlamydia are the most frequent bacterial cause of urogenital infections. In Germany, the number of new genital infections is estimated to be 300,000 per year. The incidence of lymphogranuloma venereum (lymphogranuloma inguinale, Durand-Nicolas-Favre disease) is decreasing worldwide. However, this sexually transmitted disease is still endemic in Asia, Africa, South America, and parts of the Caribbean (1).

***Neisseria gonorrhoeae* (NG)**

Neisseria gonorrhoeae is a human pathogen that is transmitted via sexual intercourse only: the organism does not survive outside the human body since it is sensitive to drying. The major source of infection is asymptotically infected women. Symptoms develop within 2–7 days after infection and become noticeable by vaginal discharge. Nevertheless about 50% of infected women have mild symptoms or are asymptomatic. In male patients an infection with *N. gonorrhoeae* causes urethral discharge and pain while passing urine (1).

In the United States, gonorrhea is the second most commonly reported sexually transmitted disease. The rate of infection in 2010 was 100.8 per 100,000 population with 309,341 cases reported in the United States (2).

Materials Provided

Kit contents

artus CT/NG QS-RGQ Kit			(2 x 48)
Catalog no.			4569365
Number of reactions			96
Blue	CT/NG RG Master	RG MASTER	2 x 660 μ l
Yellow	CT/NG RG Mg-Sol*	RG MG-SOL	4 x 200 μ l
Red	CT/NG Control CT+/NG-	CONTROL CT+NG-	4 x 500 μ l
Brown	CT/NG Control NG+/CT-	CONTROL NG+CT-	4 x 500 μ l
Green	CT/NG RG IC [†]	RG IC	2 x 1000 μ l
Black	CT/NG RG NTC [‡]	RG NTC	4 x 100 μ l
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* Magnesium solution.

[†] Internal control.

[‡] No template control.

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.

- 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

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

For specimen handling and storage

- Urine specimen collection cup
- Swabs for collection of vaginal or cervical samples (Copan, cat. no. 502CS01, www.copaninnovation.com)
- Swabs for collection of urethral samples (Copan, cat. no. 525CS01)
- Transport Tube (Copan, cat. no. 606C 2ml)

For sample preparation

- QIASymphony SP instrument (cat. no. 9001297), *software version 4.0.1 or higher
- QIASymphony AS instrument (cat. no. 9001301), *software version 4.0.1 or higher

For PCR

- Rotor-Gene Q MDx 5plex HRM instrument*†
- Rotor-Gene Q software version 2.1 or higher

Note: Additional information about materials required for specific applications is contained in the relevant Application Sheet at www.qiagen.com/products/artusctngqsrqgqkitce.

Warnings and Precautions

For in vitro diagnostic use.

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 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.

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

*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 "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.

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.
- 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.
- Proceed continuously from one part of the workflow to the next. Do not exceed 30 minutes of transfer time between each module (QIAasymphony SP to QIAasymphony AS to Rotor-Gene Q).

Reagent Storage and Handling

The components of the *artus* CT/NG QS-RGQ Kit should be stored at –15°C to –30°C and are stable until the expiration date stated on the label. Repeated thawing and freezing (>2 x) should be avoided, as this may reduce assay performance. If the reagents are to be used only intermittently, they should be frozen in aliquots. Storage at 2–8°C should not exceed a period of 5 hours. All reagents that are loaded on the assay setup module 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 specific applications is contained in the relevant Application Sheet at www.qiagen.com/products/artusctngqsrgqkitce.

Urine collection

The patient should collect the first 20 ml of voided urine into a collection cup. From this urine, transfer 4 ml into a sterile eNAT™ collection tube using a sterile, disposable transfer pipet. Invert the tube to ensure uniform mixing. Do not vortex or shake vigorously, so as to avoid extensive foaming.

Swab collection

The *artus* CT/NG QS-RGQ Kit was validated with vaginal, cervical, and male urethral swabs collected by a clinician using the swabs described in “Materials Required but Not Provided”, page 8.

After collection, place the swabs in a 2 ml eNAT tube and break the shaft at the breaking point. Close the tube and ship it according to the sample transport instructions (see the relevant Application Sheet at www.qiagen.com/products/artusctngqsrqgkitce).

Specimen handling

The *artus* CT/NG QS-RGQ Kit was designed to be used with the QIASymphony RGQ system for automated sample preparation and assay setup. Samples can be processed either in eNAT primary tubes or secondary tubes. Swabs should be removed from the eNAT tubes or the samples have to be transferred to secondary tubes (Micro tubes 2.0 ml Type I, with skirted base (Sarstedt, cat. no. 72.694, www.sarstedt.com) before loading onto the QIASymphony SP module.

Before transferring the sample into the secondary tube, make sure to prepare urine samples by carefully vortexing urine samples and thoroughly vortexing swab samples for approximately 15 seconds.

Procedure

Getting started on the QIASymphony SP/AS instruments

Close all drawers and the hoods.

Switch on the QIASymphony SP/AS instruments, and wait until the “Sample Preparation” screen appears and the initialization procedure has finished.

Log into the instrument (drawers will unlock).

Bacterial DNA purification

The *artus* CT/NG QS-RGQ Kit has been validated with a bacterial DNA purification step performed on the QIASymphony SP using the QIASymphony DSP Virus/Pathogen Midi Kit. See the *QIASymphony DSP Virus/Pathogen Handbook* for all the information on how to prepare the reagent cartridge for the sample purification step on the QIASymphony SP.

Using an internal control and carrier RNA (CARRIER)

Using QIASymphony DSP Virus/Pathogen Kits in combination with the *artus* CT/NG QS-RGQ Kit requires introduction of the internal control (CT/NG RG IC) into the purification procedure to monitor the efficiency of sample preparation and downstream assay. In addition, QIASymphony DSP Virus/Pathogen Kits may require the preparation of carrier RNA (CARRIER). For specific information regarding the internal control and the use of carrier RNA (CARRIER), see the relevant Application Sheet at www.qiagen.com/products/artusctngqsrqgkitce.

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: DNA isolation and assay setup on the QIASymphony SP/AS

The following description is a general protocol for using QIASymphony DSP Virus/Pathogen Kits. Detailed information for a specific application, including volumes and tubes, is provided in the relevant Application Sheet at www.qiagen.com/products/artusctngqsrqgkitce.

Important points before starting

- Ensure that you are familiar with operating the QIASymphony 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 for operating instructions.
- Before using a reagent cartridge (RC) from the QIASymphony DSP Virus/Pathogen Midi Kit for the first time, check that Buffers QSL2 and QSB1 in the 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.*
- 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 CT/NG PCR reagent volumes are optimized for 2 x 48 reactions per kit per run.
- For use on the QIASymphony AS module, the CT/NG RG Master and CT/NG RG Mg-Sol must be provided in QIAGEN 2 ml (cat. no. 997102) or 5 ml (cat. no. 997104) tubes, depending on the number of reactions.
- The *artus* CT/NG QS-RGQ Kit contains 4 controls each. This is sufficient for 4 separate PCR runs.

*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* CT/NG QS-RGQ Kit have been shown to be stable onboard the instrument for at least the normal time required for sample purification for 96 samples and assay setup of 72 assays, including the transfer time from the QIA Symphony SP to the QIA Symphony AS and up to 30 minutes transfer time from the QIA Symphony AS to the Rotor-Gene Q.

Things to do before starting

- Before each use, all reagents need to be thawed completely, mixed (by repeated up and down pipetting or by quick vortexing), and centrifuged for at least 3 seconds at 6800 x g. Avoid foaming of the reagents.
- Prepare all required mixtures. If needed, prepare mixtures containing carrier RNA (CARRIER) and internal controls just before starting. For more information, see the relevant Application Sheet at www.qiagen.com/products/artusctngqsrqgkitce.
- 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 *QIA Symphony SP/AS User Manual — General Description*, *QIA Symphony SP/AS User Manual — Operating the QIA Symphony SP*, *QIA Symphony SP/AS User Manual — Operating the QIA Symphony AS*, and *QIA Symphony Management Console User Manual* supplied. Make sure to carry out maintenance regularly to minimize the risk of cross-contamination.
- 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 reagent cartridge (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 (15–25°C).
- Make sure that the piercing lid (PL) is placed on the reagent cartridge (RC) and the lid of the magnetic-particle trough has been removed or, if using a partially used reagent cartridge (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 QIA Symphony SP.

Procedure

Bacterial DNA purification on the QIASymphony SP

- 1. Close all drawers and the hoods of the QIASymphony SP/AS instruments.**
- 2. Switch on the instruments, 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 instruments.**
- 4. Prepare the following drawers according to the relevant Application Sheet at www.qiagen.com/products/artusctngqsrqgkitce.**
 - “Waste” drawer; when prepared, perform an inventory scan.
 - “Eluate” drawer; when prepared, perform an inventory scan.
 - “Reagents and Consumables” drawer; when 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 the *artus* CT/NG Assay Parameter Set for the run, and assign it and the corresponding AS batch to the samples.**

Information about the Assay Parameter Set and preselected elution volume is provided on the relevant Application Sheet.

For more information about integrated runs on the QIASymphony SP/AS, see the instrument user manuals.

Note: The QIASymphony SP/AS allows the user to specify the number of controls and samples (i.e., replicates) in the “Specifications” menu. For the CT/NG protocol, the maximum allowed value for replicates is 2.
- 6. When setting up an integrated run, check for correct assignment of sample labware and sample type (sample, EC+ for CT/NG Control CT+/NG–, and EC+ for CT/NG Control NG+/CT–).**

Information about consumables and components to load in each drawer is provided on the relevant Application Sheet.
- 7. Make sure that the internal control (CT/NG RG IC) was set up and loaded onto the system as described in the relevant purification kit handbook.**

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 changes from “LOADED” to “QUEUED”. As soon as one batch is queued the “Run” button appears. Press the “Run” button to start the procedure.**

Note: Do not forget to assign an AS Batch to the respective SP batch(es).

All processing steps are fully automated.

Loading the QIASymphony AS drawers for assay setup

9. **After queuing an integrated run, open the QIASymphony AS drawers. The required components to be loaded are shown on the touchscreen.**
10. **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
11. **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 on the relevant Application Sheet at www.qiagen.com/products/artusctngqsrqgkitce.**
12. **Check the temperature of the cooling positions.**

When the target cooling temperatures are reached, the small asterisk next to each slot will appear green.
13. **Provide the CT/NG RG Master volume needed by the QIASymphony AS module in one tube before use. One tube is sufficient for 48 reactions.**

Note: Viscous reagents can be difficult to handle with manual pipets. Make sure to transfer the required volume of the Master into the respective tube.
14. **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 need to be thawed completely, mixed (by repeated up and down pipetting or by quick vortexing), and centrifuged for at least 3 seconds at 6800 x g. Avoid bubbles or foaming, which could cause detection errors. Work quickly and keep PCR components on ice or in the cooling block before loading.
15. **Load the reagent rack, and place the reagent tubes, without lids, into the appropriate positions of precooled adapters for reagents according to the relevant Application Sheet.**

- 16. Scan the kit bar code on the upper side of the *artus* CT/NG QS-RGQ Kit by pressing the “Scan Kit Barcode” button within the “Loading Reagents” screen.**
- 17. Load disposable filter-tips into the “Eluate and Reagents” and “Assays” drawers, according to the required number of each tip type indicated on the relevant Application Sheet.**
Note: It is recommended to load more than the requested amount of each tip size.
- 18. Close the “Eluate and Reagents” and “Assays” drawers.**

19. 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.

20.  After the run is finished, open the “Assays” drawer and unload the assay rack(s). Afterwards press “Scan” to confirm that the assay rack was unloaded. In the assay setup “Overview” screen, press “Remove” to finally remove the assay setup run. Download the result and cyclor files using the QIASymphony Management Console or a USB flash drive.

21. If multiple batches on the QIASymphony AS are configured in an integrated run, reload the QIASymphony AS drawers, starting at step 9.

22. Proceed to “Protocol: PCR on the Rotor-Gene Q”, page 18.

23. If all integrated runs are finished, remove them by pressing the “Integrated batch” button in the “Integrated Overview” screen.

24. Perform the regular maintenance of the QIASymphony SP and AS during the PCR run on the Rotor-Gene Q or later.

Since the workflow is an integrated operation, clean all instruments at the end of the completed workflow.

Follow the maintenance instructions in the *QIASymphony SP/AS User Manual — General Description*. Make sure to carry out maintenance regularly to minimize the risk of cross-contamination.

Protocol: PCR on the Rotor-Gene Q

Important points before starting

- Take time to familiarize yourself with the Rotor-Gene Q before starting the protocol. See the instrument user manual.
- For automatic interpretation of the PCR results, Rotor-Gene AssayManager® may be used instead of Rotor-Gene Q software.

Procedure

1. Close the PCR tubes, and place them in the 72-Well Rotor of the Rotor-Gene Q. Make sure to transfer the Rotor-Gene Q 4-strip tubes in the correct orientation, so that the position indices of the cooling adapter and the rotor match. Make sure that the locking ring (accessory of the Rotor-Gene Instrument) is placed on top of the rotor to prevent accidental opening of the tubes during the run.
2. Download the cycler file from the QIASymphony AS and transfer it to the Rotor-Gene Q computer.
3. For the detection of CT/NG DNA, create a temperature profile and start the run according to the relevant Application Sheet at www.qiagen.com/products/artusctngqsrqgkitce. Software-specific information about programming the Rotor-Gene Q is provided in the relevant Protocol Sheet “Settings to run artus QS-RGQ Kits” at www.qiagen.com/products/artusctngqsrqgkitce.

Interpretation of Results

See the relevant Application Sheet at www.qiagen.com/products/artusctngqsrgqkitce for detailed information about interpretation of results.

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. 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).

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.

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.

Comments and suggestions

- b) Storage of reagent cartridge (RC) Storage of reagent cartridge (RC) 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 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 Application Sheet at www.qiagen.com/products/artusctngqsrqgqkitce. 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.

Comments and suggestions

- 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 QIASymphony SP 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.

QIASymphony AS detects insufficient Master

Insufficient Master transferred to tube

Make sure that the required volume of CT/NG RG Master is available. If necessary combine the contents of both CT/NG RG Master tubes from the kit (i.e., each sufficient for 45 samples and 3 controls). Viscous reagents can be difficult to handle with manual pipets. Make sure to transfer the required 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 μ l for an 800 μ l 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.

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

Comments and suggestions

No signal with positive controls (CT/NG) in fluorescence channel Cycling Green and/or Cycling Orange

- | | |
|---|--|
| 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 CT and Cycling Orange for analytical NG. |
| b) Incorrect programming of the temperature profile of the Rotor-Gene instrument | Compare the temperature profile with the protocol. See the relevant Application Sheet and Protocol Sheet at www.qiagen.com/products/artusctngqsrgqkitce . |
| c) 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 the relevant Application Sheet at www.qiagen.com/products/artusctngqsrgqkitce . |
| d) The storage conditions for one or more kit components did not comply with the instructions given in "Reagent Storage and Handling" (page 10) | 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> CT/NG 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 QIASymphony DSP Virus/Pathogen Kit in fluorescence channel Cycling Orange, Cycling Green, and simultaneous absence of a signal in channel Cycling Yellow

- | | |
|---|--|
| 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 QIASymphony SP/AS", page 13) and closely follow the instructions. |

Comments and suggestions

- 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 QIASymphony SP/AS”, page 13) and closely follow the instructions.
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 10)
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* CT/NG 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
Repeat the PCR with new reagents in replicates.
If possible, close the PCR tubes directly after addition of the sample to be tested.
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.
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* CT/NG QS-RGQ Kit is tested against predetermined specifications to ensure consistent product quality.

Limitations

All reagents may exclusively be used in in vitro diagnostics.

The product is to be used by personnel specially instructed and trained in the in vitro diagnostics procedures only.  It is important that the operator read the instructions for use thoroughly before using the system. The *artus* CT/NG QS-RGQ Kit is to be used by laboratory professionals trained in the use of the QIAGEN QIASymphony RGQ system, Rotor-Gene AssayManager, and the *artus* CT/NG system.

Strict compliance with the instructions for use is required for optimal PCR results. Attention should be paid to expiration dates printed on the box and labels of all components. Do not use expired components.

Although rare, mutations within the highly conserved regions of the bacterial genome covered by the kit's primers and/or probe may result in failure to detect the presence of the bacteria in these cases. Validity and performance of the assay design are revised at regular intervals.

Any diagnostic results that are generated must be interpreted in conjunction with other clinical or laboratory findings.

For information about further limitations, see the specific Application Sheets available online at www.qiagen.com/products/artusctngqsrqgqkitce.

Note about specific risk

In case of an infection with *Chlamydia trachomatis* not only the tested persons themselves could be harmed by false negative results but also the unborn fetus or neonate if the tested woman is gravid.

Performance Characteristics

See www.qiagen.com/products/artusctngqsrqgqkitce for performance characteristics of the *artus* CT/NG QS-RGQ Kit.

References

1. Mims, C.A., Playfair, J.H.L., Roitt, I., Wakelin, D., and Williams, R. (1998) *Medical Microbiology*, 2nd ed. London: Mosby.
2. CDC, 2010 Sexually Transmitted Diseases Surveillance www.cdc.gov/std/stats10/gonorrhea.htm (accessed April 15, 2013)

Symbols

The following symbols may appear on the packaging and labeling:

	Contains reagents sufficient for <N> reactions
	Use by
	In vitro diagnostic medical device
	Catalog number
	Lot number
	Material number
	Components
	Contains
	Number
	Global Trade Item Number
	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, 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).

Ordering Information

Product	Contents	Cat. no.
<i>artus</i> CT/NG QS-RGQ Kit (2 x 48)	For 96 reactions: Master, Magnesium Solution, Positive/Negative Controls, Internal Control, NTC	4569365
QIASymphony RGQ system		
QIASymphony RGQ, System	QIASymphony SP, QIASymphony AS, Rotor-Gene Q 5plex HRM, required accessories and consumables, installation and training	9001850

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at www.qiagen.com or can be requested from QIAGEN Technical Services or your local distributor.

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