

QIA Symphony RGQ Application Sheet

artus[®] CT/NG QS-RGQ Kit

(sample type: swabs in eNaT[™], 400 µl)

July 2017

Version management

This document is the artus CT/NG QS-RGQ Kit Application Sheet for swabs, Version 1, R3.



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

General information

Kit	artus CT/NG QS-RGQ Kit, Version 1, REF 4569365
Validated sample material	Vaginal, cervical, and male urethral swabs in eNaT
Front-end purification	QIA Symphony DSP Virus/Pathogen Midi Kit (cat. no. 937055)
Sample volume (including excess volume)	500 µl
Assay Parameter Set	artus_CT_NG 400_V1
Default Assay Control Set	Complex400_V4_DSP artus CT_NG
Internal control name on SP module	Complex400_V4_DSP artus CT_NG
Elution volume	60 µl
Required software version	Version 4.0 or higher
Master mix volume	10 µl
Template volume	15 µl
Number of reactions	6–96
Runtime on AS module	For 6 reactions: approximately 8 minutes For 72 reactions: approximately 35 minutes

Materials required but not provided

Sample collection	■	2 ml eNaT tubes (Copan, cat. no. 606C, www.copaninnovation.com)
Purification kit	■	QIASymphony DSP Virus/Pathogen Midi Kit (cat. no. 937055)
Adapters for the QIASymphony SP	■	Elution Microtube Rack QS (Cooling Adapter, EMT, v2, Qsym, cat. no. 9020730)
	■	Tubes, conical, 2 ml, Qsym AS (cat. no. 997102) for use with samples and internal controls
Consumables for the QIASymphony SP	■	Sample Prep Cartridges, 8-well (cat. no. 997002)
	■	8-Rod Covers (cat. no. 997004)
	■	Filter-Tips, 1500 µl (cat. no. 997024)
	■	Filter-Tips, 200 µl (cat. no. 990332)
	■	Elution Microtubes CL (cat. no. 19588)
	■	Tip disposal bags (cat. no. 9013395)
	■	Micro tubes 2.0 ml Type I, with skirted base (Sarstedt, cat. no. 72.694, 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 internal controls
Adapters and reagent holders for the QIASymphony AS	■	Reagent holder 1 QS (Cooling Adapter, Reagent Holder 1, Qsym, cat. no. 9018090)
	■	Reagent holder 2 QS (Cooling Adapter, Reagent Holder 2, Qsym, cat. no. 9018089)
	■	RG Strip Tubes 72 QS (Cooling Adapter, RG Strip Tubes 72, Qsym, cat. no. 9018092)
Consumables for the QIASymphony AS	■	Strip Tubes and Caps, 0.1 ml (cat. no. 981103)
	■	Tubes, conical, 2 ml, Qsym AS (cat. no. 997102)
	■	Tube, conical, 5 ml, Qsym AS (cat. no. 997104)
	■	Elution Microtubes CL (cat. no. 19588)
	■	Filter-Tips, 1500 µl (cat. no. 997024)
	■	Filter-Tips, 200 µl (cat. no. 990332)
	■	Filter-Tips, 50 µl (cat. no. 997120)
	■	Tip disposal bags (cat. no. 9013395)
For sample preparation (eNaT)	■	Buffer ATL, GPR (cat. no. 939016)

Specimen handling and storage

Sample collection	2 ml eNaT tubes (Copan, cat. no. 606C, www.copaninnovation.com)
Sample transport	Shatterproof transport Shipment at 20°C within 6 hours of sample collection Mail shipment according to legal instructions for the transport of pathogen material*
Sample preparation	Prevent formation of foam in or on the samples. Samples should be equilibrated to room temperature (15–25°C) before starting the run.
Sample storage	Short term (up to 7 days from arrival at testing site): 20°C or 4°C depending on local conditions Long term (up to 2 weeks): 4°C Longer storage: –20°C

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

Procedure

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

Using the QIASymphony DSP Virus/Pathogen Midi Kit in combination with the *artus* 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.

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

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

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

Component	Volume (µl) (SAR tubes)*	Volume (µl) (BD™ tubes)†
Stock carrier RNA (CARRIER)	3	3
Internal control‡	9	9
Buffer AVE	108	108
Final volume per sample (excluding dead volume)	120	120
Total volume for n samples	$(n \times 120) + 360^{\S}$	$(n \times 120) + 600^{\parallel}$

* Micro tubes 2.0 ml Type I, with skirted base (Sarstedt, cat. no. 72.694, www.sarstedt.com).

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

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

§ Internal control mixture corresponding to 3 additional samples (i.e., 360 µl) is required. Do not fill more than 1.92 ml total volume (corresponding to a maximum of 13 samples). These volumes are specific for Micro tubes 2.0 ml Type I, with skirted base (Sarstedt, cat. no. 72.694, www.sarstedt.com).

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

QIA Symphony SP setup

“Waste” drawer

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

“Eluate” drawer

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

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

“Reagents and Consumables” drawer

Position A1 and/or A2	Load 1 reagent cartridge (RC) for up to 72 samples or 2 new reagent cartridges (RC) for up to 144 samples
Position B1	Buffer ATL (ATL), Scan the bar code of the bottle by press the “Bottle ID” button within the “Reagent and Consumable” drawer
Tip rack holder positions 1–17	Load sufficient racks of disposable filter-tips, 200 µl and 1500 µl (see page 6)
Unit box holder position 1–4	Load unit boxes containing sample prep cartridges and 8-Rod Covers (see page 6)

“Sample” drawer

Sample type	eNaT transport medium
Sample volume (including excess volume)	500 µl
Sample tubes (primary)	2 ml eNaT tubes (Copan, cat. no. 606C, www.copaninnovation.com)*
Sample tubes (secondary)	Micro tubes 2.0 ml Type I, with skirted base (Sarstedt, cat. no. 72.694, www.sarstedt.com)
Insert	Tube Insert 3B (cat. no. 9242083)

* Make sure to remove swabs from primary tubes before loading onto QIASymphony SP.

Required plasticware for 1–4 sample batches

	One batch, 24 samples*	Two batches, 48 samples*	Three batches, 72 samples*	Four batches, 96 samples*
Disposable filter-tips, 200 µl†‡	28	52	74	100
Disposable filter-tips, 1500 µl†‡	93	178	263	348
Sample prep cartridges§	18	36	54	72
8-Rod Covers¶	3	6	9	12

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

† There are 32 filter-tips/tip rack.

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

§ There are 28 sample prep cartridges/unit box.

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

Loading of samples and controls

Make sure that the 2 controls (CT/NG Control CT+/NG– and CT/NG Control NG+/CT–) are located at the beginning of your samples in the QIASymphony sample input. When preparing more than 69 samples, 2 additional controls must be available (see table below for an example). This is important because one PCR run contains 72 reactions (69 samples + 2 controls in the sample prep modules and 1 NTC in the assay setup module). When testing more than 69 samples, a second PCR run will be pipetted by the AS module automatically. To ensure that this run is valid, 2 controls must be in PCR positions 1 and 2. Therefore make sure that the 2 controls for the sample prep are always at the beginning of the Rotor-Gene Q run. When testing more than 45 samples, we recommend to split the samples into 2 batches on the AS module and, correspondingly, into 2 separate runs on the Rotor-Gene Q MDx 5plex HRM instrument. For more information see the 2 tables below. Please keep in mind that the NTC is processed by the AS module but not by the SP module.

Note: We do not recommend changing the number of NTC replicates manually. Rotor-Gene AssayManager will reject the run if the number of NTC replicates is changed.

Distribution of samples and controls (example for 96 reactions)

	SP batch 1 Positions	SP batch 2 Positions	SP batch 3 Positions	SP batch 4 Positions
CT/NG Controls	1: CT+/NG– 2: NG+/CT–	–	49: CT+/NG– 50: NG+/CT–	–
Samples	3–24	25–48	51–72	73–96

After each set of samples (1–71 and 72–96) the AS module will add a NTC sample (No Template Control).

The recommended workflow for 96 samples (including controls) is shown in the table below. In this example 2 x 46 samples (+ 2 controls) will be processed in 2 AS batches and 2 PCR runs. The first PCR run, with 46 samples, 2 controls, and 1 NTC, is finished while SP batches 3 and 4 are being processed.

Recommended workflow for 96 samples using the integrated run

	AS batch 1		AS batch 2	
	SP batch 1 Positions	SP batch 2 Positions	SP batch 3 Positions	SP batch 4 Positions
CT/NG Controls	1: CT+/NG– 2: NG+/CT–	–	49: CT+/NG– 50: NG+/CT–	–
Samples	3–24	25–48	51–72	73–96

QIAasymphony AS setup

Consumables

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

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

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

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

[‡] Alternatively, Tubes, conical, 2 ml, Qsym AS (cat. no. 997102) can be used.

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

Adapters and reagent holders

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

* Calculated for an assay run with 72 reactions.

Filter-tips

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

Consumable	Name on touchscreen	Minimum number for 24 reactions	Minimum number for 72 reactions
Filter-Tips, 1500 µl (1024)	1500 µl	2	2
Filter-Tips, 200 µl (1024)	200 µl	6	6
Filter-Tips, 50 µl (1024)	50 µl	24	72
Tip Disposal Bags	–	1	1

Master mix splitting

Even though the kit is optimized for 2 x 48 reactions, different combinations are possible. Because automated pipetting systems always have a certain amount of dead volume, a split 48 reaction tube does not contain 2 x 24 reactions. See the table below for an overview of possible combinations.

Component(s)	Master mix tubes	PCR runs	Reactions per PCR run*	Patient samples	Controls†
2 x 48 reaction tube	2	2	49	2 x 46	2 x 3
1 x 48 reaction tube	1	1	49	1 x 46	1 x 3
1 x 48 reaction tube	1	2	17	2 x 14	2 x 3

* Calculated as n patient samples + 2 CT/NG Controls (CT+/NG– and NG+/CT–) + 1 NTC per PCR run.

† CT/NG Control CT+/NG–, CT/NG Control NG+/CT–, and NTC (added by the assay setup module).

Real-time PCR on the Rotor-Gene Q MDx 5plex HRM*

The *artus* CT/NG QS-RGQ Kit can be run on the Rotor-Gene Q MDx 5plex HRM using manual analysis with Rotor-Gene Q software 2.1 or higher or using automatic analysis with Rotor-Gene AssayManager®. The following sections describe the settings and setup using the 2 different software packages.

Prepare the rotor for the run on the Rotor-Gene Q MDx 5plex HRM 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 MDx 5plex HRM instrument with the rotor and locking ring.

Real-time PCR using Rotor-Gene AssayManager

For automatic analysis using the *artus* CT/NG QS-RGQ Kit with Rotor-Gene AssayManager, the *artus* Basic plug-in V1.0.3 (available for download from www.qiagen.com/shop/automated-solutions/accessories/rotor-gene-assaymanager) must be installed to your Rotor-Gene AssayManager.

Start the installation process by double-clicking the ArtusBasic.Installation.msi file, and follow the installation instructions. For a detailed description, refer to “Installing Plug-ins” in the *Rotor-Gene AssayManager Core Application User Manual*.

To use the assay profile *artus_CTNG_sample400_QS* (short name: *CTNG_a*) with the *artus* CT/NG QS-RGQ Kit, the file *AP_artus_CTNG_sample400_QS_V2_0_0.iap* (available for download from www.qiagen.com/products/artusctngqsrgqkitce) must be imported to Rotor-Gene AssayManager.

To import the assay profile into Rotor-Gene AssayManager:

1. Navigate to the “Configuration Environment” and change to the “Assay Profile” tab.
2. Click “Import” and select the *AP_artus_CTNG_sample400_QS_V2_0_0.iap* file in the open file dialog.
3. Click “Open”, and the profile is loaded and added to the list of available assay profiles.

Note: The same version of an assay profile cannot be imported twice.

Starting a run using Rotor-Gene AssayManager

After installing the plug-in and importing the assay profile, Rotor-Gene AssayManager can use the information given in the QIASymphony AS result file to set up a run for real-time PCR amplification and subsequent automatic interpretation

* 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.

of results.

QIASymphony AS result files can either be downloaded using a USB stick or using the QIASymphony Management Console. If the QIASymphony AS result file is downloaded using a USB stick, it is stored in .zip format in the folder x:\Log\results\AS.

Note: Before importing the QIASymphony AS result file the .zip file needs to be extracted. If the QIASymphony AS result file is transferred using the QIASymphony Management Console (QMC), this step is not required.

To perform a PCR run:

1. Start Rotor-Gene AssayManager.
2. Change to the "Setup" environment and select the source "QIASymphony" as "Import type". In the "Select file" dialog, open the corresponding QIASymphony AS result file and click "Open". The work list is then added to the list of available work lists.
3. The run can be started from the "Available work lists" table by clicking "Apply" in the button bar in the appropriate work list entry (Insert naming of imported QS worklists).
4. Enter an experiment name.
5. Select a cycler and confirm that the locking ring is attached.
6. Click the green "Start run" button.

Finishing and releasing a run

To see the progress of the run, change to the corresponding cycler screen. When the run is finished, click "Finish run" to release the cycler and to approve the sample in the "Approval" environment.

1. Select the "Approval" environment.
2. Click "Apply filter" (or choose own filter options beforehand).
3. Select experiment.
4. Click "Start approval".
5. 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.

6. Click "Release /report data..."
7. Choose a report profile and click "OK". The report will be generated and stored automatically.

Note: The user needs approval rights to approve a run.

8. Unload the Rotor-Gene Q MDx 5plex HRM instrument and discard the strip tubes according to your local safety regulations.

Interpretation of results using Rotor-Gene AssayManager

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

IMPORTANT: A cut-off of 40 C_T is applied in the NG channel, which will produce an “INVALID” result with a flag of “CT_ABOVE_ACCEPTED_RANGE”. The following instruction should be followed carefully.

- If NG is reported as invalid with the flag “CT_ABOVE_ACCEPTED_RANGE”, and the IC is detected and valid, the sample can be treated as a **valid NG-negative sample**. No retest is required.
- If NG is reported as invalid with any other flag, the sample should be retested.
- If CT is reported as invalid with any flag, the sample should be retested.

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_T value is higher than the defined cut-off C_T . IMPORTANT: if NG is reported as invalid with this flag, the sample can be treated as a valid NG-negative sample provided the IC is valid.
CT_BELOW_ACCEPTED_RANGE	Invalid	The detected C_T value is lower than the defined cut-off C_T .
CURVE_SHAPE_ANOMALY	Invalid	The raw data amplification curve shows a shape that is deviating from the established behavior for this assay. There is a high likelihood for wrong results or result misinterpretation.

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).
FLUORESCENCE_TOO_LOW	Invalid	The fluorescence signal is lower than the defined fluorescence cut-off.
IC_INVALID	Invalid	The internal control is invalid. Target and internal control share the same tube.
IC_NO_SIGNAL	Invalid	No internal control signal is detected. Target and internal control share the same tube.
INHIBITION_BY_CT	Invalid	The defined maximum C_T range between the C_T for the internal control of that sample and the C_T for the internal control of the NTC is exceeded.
INHIBITION_BY_FLUORESCENCE	Invalid	The defined maximum fluorescence difference between the internal control fluorescence of the NTC and the internal control fluorescence of that sample for the last cycle is exceeded.

Flag	Behavior	Description
LOW_FLUORESCENCE_CHANGE	Warning	<p>The percentage fluorescence change for this sample relative to the sample tube with the largest fluorescence change is lower than a defined limit.</p> <p>Note: If a valid sample is tagged with this flag, the approver is asked to pay special attention to the fact described by this flag before they decide to accept or reject the result.</p>
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	Deviation during the normalization procedure. The amplification curve is displayed with a default normalization; results should be manually checked for correctness.
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 fact described by this flag before they decide to accept or reject the result.</p>
SPIKE	Warning	<p>A spike in the raw data fluorescence is detected in the amplification curve but outside the region where the CT is determined.</p> <p>Note: If a valid sample is tagged with this flag, the approver is asked to pay special attention to the fact described by this flag before they decide to accept or reject the result.</p>
SPIKE_CLOSE_TO_CT	Invalid	<p>A spike is detected in the amplification curve close to the C_T.</p>
STEEP_BASELINE	Invalid	<p>A steeply rising baseline for the raw data fluorescence is detected in the amplification curve.</p>

Flag	Behavior	Description
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 phase of the amplification curve.
STRONG_NOISE_IN_GROWTH_PHASE	Invalid	Strong noise is detected in the growth (exponential) phase of the amplification curve.
UPSTREAM	Variable	<p>Sample status was set to invalid or unclear by an upstream process (e.g., QIASymphony Assay Setup).</p> <p>Note: 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.

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

Real-time PCR using Rotor-Gene Q software 2.1 or higher

Specific settings for the *artus* CT/NG QS-RGQ Kit

With Rotor-Gene software 2.1, the specific settings are shown below.

Reaction Volume (µL)	25
Hold	Hold Temperature: 95 deg
	Hold Time: 15 mins
Cycling	45 times
	95 deg for 11 secs
	60 deg for 20 secs
	72 deg for 20 secs
Auto-gain optimization setup	60 degrees
	(Samples: CT: Green, NG: Orange; IC: Yellow)

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

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

The *artus* CT/NG QS-RGQ Kit can be run on the Rotor-Gene Q MDx 5plex HRM using manual analysis with Rotor-Gene Q software 2.1 or higher. This section describes interpretation of results on the Rotor-Gene Q MDx 5plex HRM. Review also the sample status information from the QIASymphony SP/AS result files for analysis of the complete sample-to-result workflow. Only samples with a valid status should be used.

Signal detection and conclusions

Signal in channel Cycling Green	Signal in channel Cycling Orange ≤40 Cts	Signal in channel Cycling Orange >40 Cts	Signal in channel Cycling Yellow	Interpretation
Yes	No	No	Yes/No*	Valid result: CT DNA detected, NG DNA not detected
Yes	No	Yes	Yes/No*	Valid result: CT DNA detected, NG DNA not detected
No	Yes	No	Yes/No*	Valid result: CT DNA not detected, NG DNA detected
Yes	Yes	No	Yes/No*	Valid result: CT and NG DNA detected
No	No	Yes	Yes	Valid result: no CT or NG DNA detected [†]
No	No	No	Yes	Valid result: no CT or NG DNA detected [†]
No	No	Yes	No	Invalid result: No result can be concluded [‡]
No	No	No	No	Invalid result: No result can be concluded [‡]

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

† If the C_T value for the internal control of a negative sample is more than 5 cycles higher than the C_T value for the internal control of the no template control in the run ($C_{T\text{ IC Sample}} - C_{T\text{ IC NTC}} > 5$), then the sample should be treated as inhibited. No result can be concluded.

‡ Information regarding error sources and their solution can be found in "Troubleshooting guide" of the artus CT/NG QS-RGQ Kit Handbook.

Threshold setup for the PCR analysis

The recommended threshold settings for the *artus* CT/NG assay are given in the table below.

Recommended threshold settings

Fluorescence channel	Threshold setting
Cycling Green	0.07
Cycling Orange	0.10
Cycling Yellow	0.03

Examples of positive and negative PCR reactions

The *artus* CT/NG QS-RGQ Kit contains 2 controls to monitor the extraction procedure and the PCR: the CT/NG Control CT+/NG- and the CT/NG Control NG+/CT-. These controls are loaded on the QIASymphony SP/AS and treated like the other samples. The internal control (CT/NG RG IC) is added to the sample during the DNA extraction process and is present in all samples and the NTC.

The controls are used in the PCR setup process and should produce specific results in the PCR similar to the results shown in the figures below.

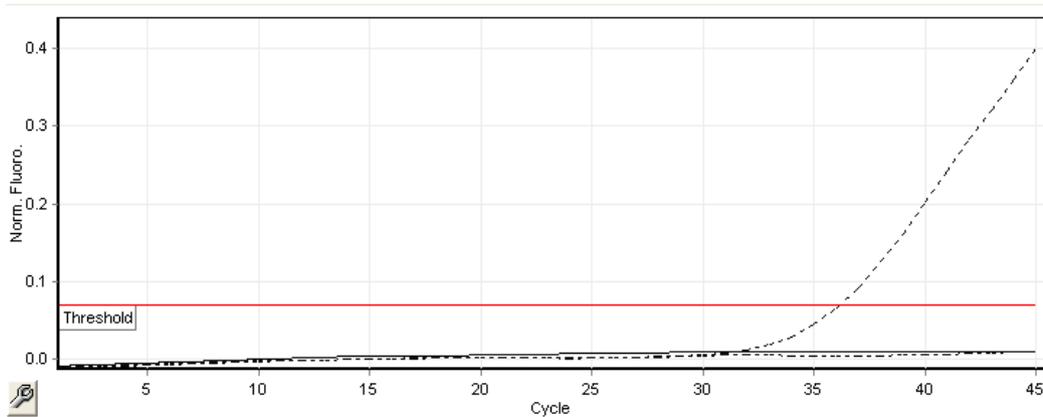


Figure 1. Cycling Green: CT positive control. Results of a run with the CT/NG Control CT+/NG-.

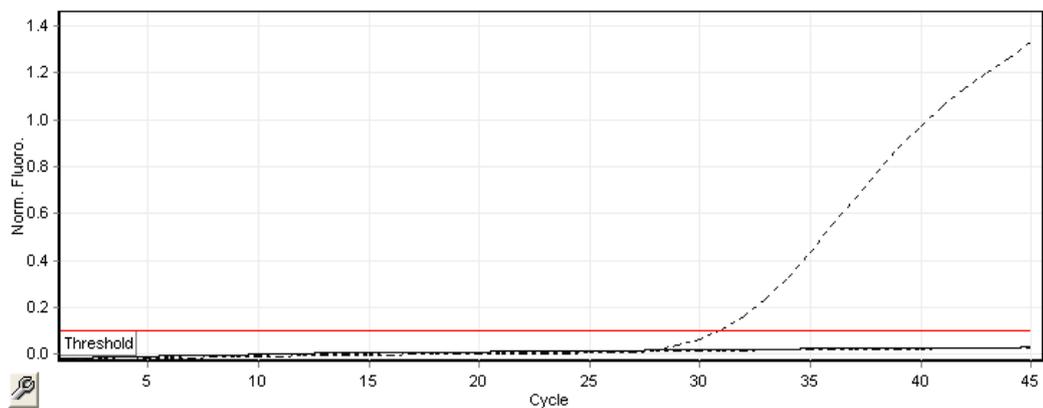


Figure 2. Cycling Orange: NG positive control. Results of a run with the CT/NG Control NG+/CT-.

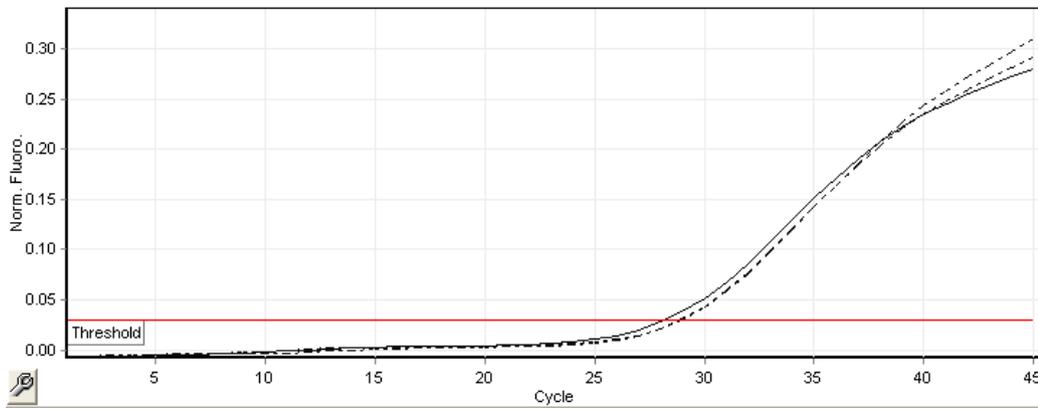


Figure 3. Cycling Yellow: internal control. Results of a run with the CT/NG RG IC.

Expected C_T values for the controls for a successful and valid PCR experiment are shown in the following table.

Expected C_T values

Control/sample	C_T range (minimum – maximum)		
	Cycling Green	Cycling Yellow	Cycling Orange
Control CT+/NG–	28.99–37.94	≤ 33.44	–
Control NG+/CT–	–	≤ 33.44	27.22–35.08
NTC	–	≤ 33.44	–
Patient sample	Any	$\leq C_T$ value from NTC in the current run + 5 C_T	Any

If one of the controls or the corresponding IC signal fails, the run must be rated as invalid.

Limitations

A study was conducted to assess the performance of the *artus* CT/NG QS-RGQ Kit with samples containing high concentrations of CT or NG in the presence of the other pathogen in low copy numbers. The results are shown in the table below.

Performance of the *artus* CT/NG QS-RGQ Kit with differing concentrations of target DNA

Pathogen A	Pathogen B	Hit rate pathogen B (%)
1.00×10^6 cfu/ml <i>N. gonorrhoeae</i>	14.6 EB/ml <i>C. trachomatis</i>	100
1.00×10^5 cp/ml <i>C. trachomatis</i>	2.2 cfu/ml <i>N. gonorrhoeae</i>	100

Note: Lower concentrations of “Pathogen B” can result in lower hit rates.

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