December 2019

# *artus*® Infl. A/B/H1 QS-RGQ Kit
# Instructions for use (Handbook)

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72

Version 1
For in vitro diagnostic use

For use with QIAsymphony® SP/AS, Rotor-Gene® Q MDx 5plex HRM instrument and Rotor-Gene AssayManager®

IVD

CE

REF    4523366

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Sample to Insight

QIAGEN

# Contents

# Intended Use

The *artus* Infl. A/B/H1 QS-RGQ Kit is a multiplex reverse transcription–real time polymerase chain reaction (RT-qPCR) in vitro diagnostic test for the separate qualitative detection and identification of influenza A and/or influenza B virus RNA and influenza A – H1 Pandemic 2009 strain (H1pdm2009) RNA. Samples to be tested should be human nasal swab specimens analyzed using the QIAsymphony Sample Preparation module and the QIAsymphony Assay Setup module, Rotor-Gene Q MDx 5plex HRM instruments and Rotor-Gene AssayManager software.

The *artus* Infl. A/B/H1 QS-RGQ Kit is intended for use as an aid in the differential diagnosis of viral upper respiratory tract infection in symptomatic patients when used in conjunction with other clinical and laboratory findings.

Negative results do not preclude influenza virus infection and should not be used as the sole basis for treatment or other management decisions. The *artus* Infl. A/B/H1 QS-RGQ Kit is not intended for the detection of influenza C virus RNA. Positive results do not exclude co-infection with other pathogens. The pathogen(s) detected may not be the definite cause of disease. Other laboratory testing and assessment of clinical presentation must be included in the final diagnosis.

The *artus* Infl. A/B/H1 QS-RGQ Kit is intended for in vitro diagnostic use by laboratory professionals only.

# Summary and Explanation

The *artus* Infl. A/B/H1 QS-RGQ Kit constitutes a ready-to-use system for the detection of influenza A and B viral RNA and influenza A – H1 Pandemic 2009 strain (H1pdm2009) in nasal swab specimens. This uses reverse transcription–real time polymerase chain reaction (RT-qPCR) on Rotor-Gene Q MDx 5plex HRM instruments. The *artus* Infl. A/B/H1 QS-RGQ system uses the QIAsymphony Sample Preparation (SP) module and the QIAsymphony Assay Setup (AS) module automated sample preparation and Rotor-Gene Q MDx 5plex HRM instrument for target amplification and detection (see Figure 1).
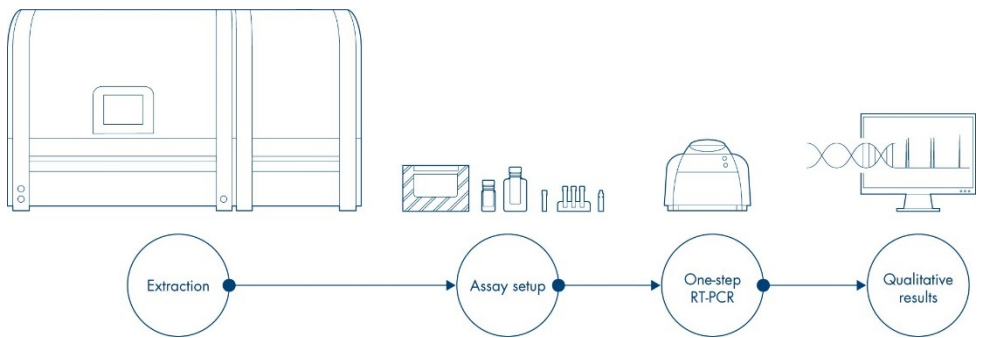


**Figure 1: Schematic description of artus Infl. A/B/H1 QS-RGQ Kit workflow.**

The *artus* Infl. A/B/H1 QS-RGQ Kit contains two influenza master mixes for detection of (1) influenza A and B viruses and (2) influenza A – H1 Pandemic 2009 H1N1 strain. To validate and control the full automated workflow, i.e. RNA extraction, plate setup and RT-qPCR reaction, an internal control sequence is automatically added into each sample at the beginning of the RNA extraction. Each influenza master mix includes primers and probe to detect this internal control sequence.

# Pathogen Information

Influenza viruses (A, B and C) have a worldwide distribution. Influenza viruses are enveloped single-stranded RNA viruses that belong to the *Orthomyxoviridae* family. The major surface antigens are hemagglutinin (H) and neuraminidase (N). The surface antigens change continuously (antigenic drift). The influenza A viruses are further divided into subtypes determined by two glycoproteins: hemagglutinin (H1–H18) and neuraminidase (N1–N11). Type B does not have subtypes, but two antigenically distinct lineages: Victoria and Yamagata. Type C viruses cause some human disease, but only comparatively few outbreaks.

The incubation time for seasonal influenza is on average 2 days, and ranges from 1 to 4 days. In most cases, virus can be found in nose and throat swabs 24 hours before the onset of symptoms to 4 to 5 days after.

Seasonal influenza is a vaccine preventable infectious disease caused by the influenza virus. Manifesting with mostly respiratory symptoms, it is easily transmitted via infectious droplets and by indirect spread from respiratory secretions. In the northern hemisphere, seasonal influenza generally occurs in epidemics between November and April each year, and in the southern hemisphere between June and October. At irregular intervals, there are influenza pandemics, the most recent one caused by influenza A/H1N1pdm09 in 2009.

The clinical diagnosis of influenza remains a challenge, especially in the emergency department (ED) where short, episodic visits, leave emergency clinicians to make diagnostic and treatment decisions with limited, insufficient information.

# Principles of the Procedure

The *artus* Infl. A/B/H1 QS-RGQ Kit is an RT-qPCR assay for detection and identification of influenza A, B, and A – H1 Pandemic 2009 strain (H1pdm2009). The *artus* Infl. A/B/H1 QS-RGQ Kit contains reagents and enzymes to perform three separate RT-qPCR runs for the detection and identification of influenza A, B, and A/H1pdm2009 virus RNA.

The RT-qPCR template is RNA extracted from human nasal swab specimens using the QIAsymphony Sample Preparation module. After RNA extraction, RT-qPCR plates are automatically prepared by the QIAsymphony Assay Setup module. The RT-qPCR runs are then performed in Rotor-Gene Q MDx 5plex HRM instruments. Samples are tested with AB Master mix and H1 Master mix in a single integrated run (combined workflow) (see Figure 2).

**QIAsymphony Sample Purification**

**Sample collection:**
Human nasal swab

**RNA extraction:**
- QIAsymphony DSP Virus/Pathogen Midi Kit (cat. No. 937055)
- Buffer ATL (cat. No. 939016)
- QIAsymphony Software version 4.0 (or higher)

**QIAsymphony Assay Setup**

**RT-qPCR Automated reaction setup with AB and H1 Master:**
- *artus* Infl.A/B/H1 QS-RGQ Kit (cat. no. 4523366)
- QIAsymphony Software version 4.0 (or higher)

**RotorGene Q MDX 5plex HRM**

**RT-qPCR Run with AB and H1 Master:**
- RGAM Software version 1.0.4
- (or higher)
- Epsilon Plug-in version 1.0.1
- (or higher)

**Automated data analysis:**
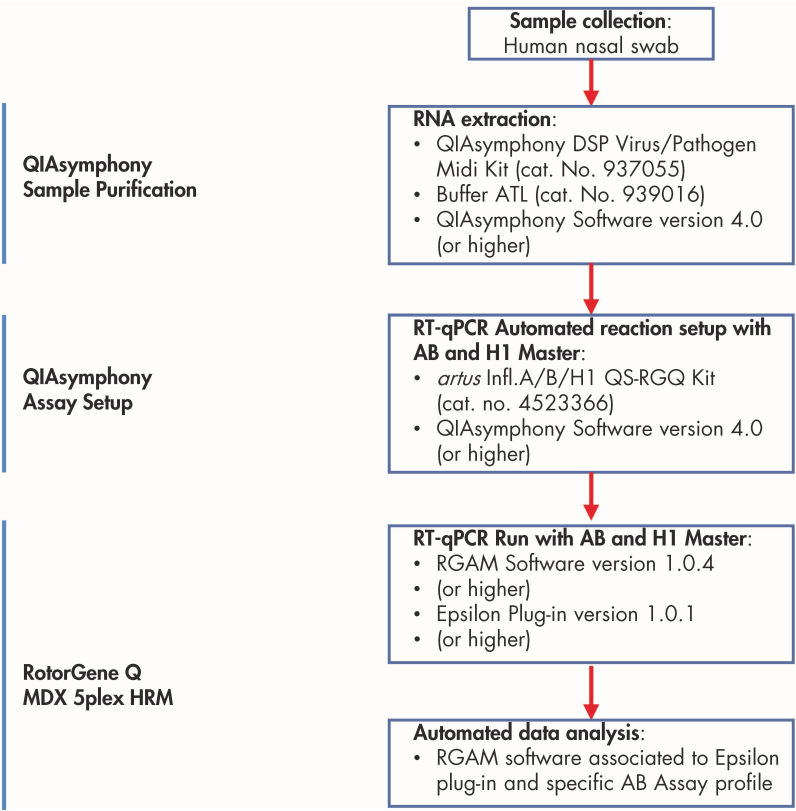- RGAM software associated to Epsilon plug-in and specific AB Assay profile

**Figure 2: artus Infl. A/B/H1 QS-RGQ Kit workflow.**

The *artus* Infl. A/B/H1 QS-RGQ Kit contains two master mixes. The Influenza A/B Master contains reagents and enzymes for the specific amplification of two targets: 1) 143 bp region of the influenza virus A genome in Green channel and 2) 94 bp region of the influenza virus B genome in Yellow channel. The Influenza H1 Master contains reagents and enzymes for the specific amplification of an 80 bp region of influenza virus A – H1 Pandemic 2009 strain (H1pdm2009) genome in Green channel. Both master mixes contain primer and probes to detect the internal control. Indeed, to validate and control the full automated workflow, i.e. RNA extraction, plate setup and RT-qPCR reaction, an internal control sequence is automatically added into each sample at the beginning of the RNA extraction.

The *artus* Infl. A/B/H1 QS-RGQ Kit also contains three external positive controls (Influenza Control A, Influenza Control B, and Influenza H1 Control). Influenza Control A, Influenza Control B, and Influenza H1 Control are tested within the same RT-qPCR run. These controls verify that each qPCR assay was performed correctly.

The validity of each control (internal and external controls) is automatically assessed by the specific Assay Profile.

# Materials Provided

The contents of the *artus* Infl. A/B/H1 QS-RGQ Kit is sufficient for 72 reactions. The reagent volumes have been optimized for batches of 24 reactions including controls. Fewer or more samples may be run, but sub-optimal usage of reagents will occur due to the calculated dead volumes required for the QIAsymphony. The Rotor-Gene Q MDx 5plex HRM rotor holds up to 72 reaction tubes (controls included).

## Kit contents

| | *artus* Infl. A/B/H1 QS-RGQ Kit | | |
|---|---|---|---|
| **Catalog number** | | | **4523366** |
| **Number of reactions** | | | **(72)** |
| **Color** | **Identity** | **Symbol** | **Volume** |
| Blue | Influenza AB Master | MASTER | 3 x 600 µl (3 x 24 reactions) |
| Violet | Influenza H1 Master | MASTER | 3 x 600 µl (3 x 24 reactions) |
| Yellow | Influenza Mg-Sol* | Mg-Sol | 3 x 800 µl |
| Red | Influenza A Control† | CONTROL + | 3 x 200 µl |
| Orange | Influenza B Control | CONTROL + | 3 x 200 µl |
| Brown | Influenza H1 Control | CONTROL + | 3 x 200 µl |
| Green | Influenza IC‡ | IC | 3 x 1000 µl |
| Clear | Water for no template control (NTC), PCR grade | CONTROL – | 3 x 1900 µl |

\* Magnesium solution.

† Note that the Influenza Control is the Influenza A Control.

‡ Internal 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.

**Important**: Ensure that instruments have been checked and calibrated according to the manufacturer's recommendations. Ensure that all kits reagents have not expired and have been transported and stored under the correct conditions

## Adapters for 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 QIAsymphony SP

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

- Microtubes 2.0 ml Type I, with skirted base (Sarstedt®, cat. no. 72.694) for use with samples and internal control*
- Tubes, 14 ml, 17 x 100 mm polystyrene round-bottom (Corning®, cat. no. 352051, for use with internal control*

## Adapters and reagent holders for QIAsymphony AS

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

## Consumables for QIAsymphony 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)

* For internal control, both tube sizes (2mL or 14 ml) can be used.

## General laboratory equipment

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

## Equipment and software for sample preparation and assay setup

- QIAsymphony SP (cat. no. 9001297, module of the QIAsymphony RGQ system, cat. no. 9001850)‡
- QIAsymphony AS (cat. no. 9001301, module of the QIAsymphony RGQ system, cat. no. 9001850)‡
- QIAsymphony software version 4.0 or higher
- The required scripts and files for the use of the QIAsymphony software are available online at **www.qiagen.com** and listed below
  - Bioscript: Complex_Influenza_V1.xml
  - Assay control set (ACS): ACS_Complex_Influenza_V1.xml
  - Assay parameters set (APS) from file
    - APS_artus InflAB_400_V2.xml
    - APS_artus InflH1_400_V2.xml
    - APS_artus_InflAB_400_Indep_V2.xml
    - APS_artus_InflH1_400_Indep_V2.xml
  - Assay definition (AD)
    - AD_artus_InflAB_400_V2
    - AD_artus_InflH1_400_V2

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

## Equipment and software for PCR

- Rotor-Gene Q MDx 5plex HRM System (cat. no. 9002033, module of the QIAsymphony RGQ system, cat. no. 9001850)*

- 72-well rotor (cat. no. 9018903)

- Locking ring 72 well rotor (cat. no. 9018904)

- Rotor holder (cat. no. 9018908)

- Rotor-Gene AssayManager version 1.0 (cat. no. 9022737 and 9022739, module of the QIAsymphony Rotor-Gene Q System, cat. no. 9001850)

- Rotor-Gene AssayManager Epsilon CE Plug-in installed, version 1.0.x (where x is ≥1)

- Influenza AB Assay Profile (version 1.0.x where x is ≥3) from file "AP_artus_Infl.AB_CE_V1_0_x.iap" for integrated runs and "AP_artus_Infl.AS_AB_CE_V1_0_x.iap" for independent runs

- Influenza H1 Assay Profile (version 1.0.x where x is ≥3) from file "AP_artus_Infl.H1_CE_V1_0_x.iap" for integrated runs and "AP_artus_Infl.AS_H1_CE_V1_0_x.iap" for independent runs

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

# 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 QIAsymphony DSP Virus/Pathogen Midi Kit, see the QIAsymphony DSP Virus/Pathogen Midi Kit Instructions for Use (Handbook) supplied. For safety information regarding instrument modules, see QIAsymphony RGQ System User Manual and UDT (CE) Plug-in User Manual.

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

## General precautions

Always pay attention to the following:

- Use of this product is limited to personnel specially instructed and trained in the techniques of real-time PCR.
- Strict compliance with the user manual is required for optimal RT-qPCR results.

    **Important**: Follow universal safety precaution. Specimens should always be considered as infectious and/or biohazardous and handled in accordance with safe laboratory procedures.

**Note**: 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.

All kits can be used with the respective instruction elements if 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.

- Attention should be paid to expiration dates printed on the box and labels of all components. Do not use expired components.
- Use sterile pipette tips with filters.
- Store positive and/or potentially positive material separate from all other components of the kit.
- Thaw all components thoroughly at room temperature (15–25°C)* before starting an assay. Check regularly whether the material has thawed, especially the Masters as they contain dNTPs that are sensitive to temperature.

  **Note**: Masters should be protected from light as they contain dye nucleotides.
- When thawed, mix the components by pulse vortexing and centrifuge briefly. Make sure that no foam or bubbles are present in the reagent tubes.
- Work quickly and keep the PCR reagents on ice or in the cooling block before loading.
- Discard sample and assay waste according to your local safety regulations.
- Wear protective disposable powder-free gloves, a laboratory coat, and eye protection when handling specimens.
- Avoid microbial and nuclease (DNase/RNase) contamination of the specimen and the components of the kit.
- Always use DNase/RNase-free disposable pipette tips with aerosol barriers.

* Note that the operating conditions of the QIAsymphony RGQ system are from 18–26°C.

- Always wear protective disposable powder-free gloves when handling kit components.

- Use separated and segregated working areas for specimen preparation, reaction setup, and amplification/detection activities. The workflow in the laboratory should proceed in a unidirectional manner. Always wear disposable gloves in each area and change them before entering different areas.

- Dedicate supplies and equipment to the separate working areas and do not move them from one area to another.

- Repeated thawing and freezing of the eluates should be avoided, as this may reduce assay performance.

- Do not open the reaction tubes post amplification to avoid contamination with amplicons.

- Additional controls may be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations.

- Check that maintenance has been performed and replaceable parts (e.g., tip guards) have been reinstalled.

- Make sure that the required adapters are precooled to 2–8°C.

- Keep tubes closed until loading on QIAsymphony SP to avoid contamination.

- Proceed continuously from one part of the workflow to the next. Do not exceed 30 minutes of transfer time between the QIAsymphony AS and the Rotor-Gene Q MDx 5plex HRM instrument. Reaction mixes may be altered if exposed to light.

- Do not mix components from kits with different lot numbers.

- Make sure that the Application Process files and required Rotor-Gene AssayManager plug-ins are installed. If not installed, refer to the applicable user manual or contact QIAGEN customer support or technical services for advice.

# Reagent Storage and Handling

The components of the *artus* Infl. A/B/H1 QS-RGQ Kit should be shipped on dry ice and stored immediately upon receipt at –15°C to –30°C in a constant temperature freezer and protected from light. They are stable until the expiration date stated on the label. For these components, a maximum of 2 thaw-freeze cycles are allowed. If the reagents are thawed and re-frozen more often than this, assay sensitivity may be reduced. Storage at 2 to 8°C should not exceed a period of 5 hours.

# Specimen Collection and Preparation

Information about specimen handling and storage for human nasal swabs is given in Table 1.

⚠️ **All specimens must be treated as potentially infectious material.**

**Table 1. Specimen handling, storage, and preparation for human nasal swabs**

| | |
|---|---|
| Specimen collection | Human nasal swab specimens can be collected following the user institution's standard procedures and placed into Σ virocult® transport medium (MW951S, MW950S2) |
| Specimen transport | Samples should be shipped following the user institution's standard procedures* |
| Specimen storage (including time needed for transport) | Room temperature or +2/+8°C for 3 to 72 hours (-80°C if longer) |
| Specimen preparation | Place a minimum of 500 µl of nasal swabs into a Sarstedt tube (cat.no. 72.694). |

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

**Important Note**: Repeated thawing and freezing of the specimens should be avoided, as this may reduce the viral load in the test sample.

**Note**: Do not store samples adjacent to the reagents to avoid cross-contamination of samples or the kit.

**Table 2. Kit requirements**

| Kit | *artus* Infl. A/B/H1 QS-RGQ Kit (cat. no. 4523366) |
|---|---|
| Sample material | Human nasal swab |
| Front-end purification | QIAsymphony DSP Virus/Pathogen Midi Kit (cat. no. 937055) |
| Sample volume (including excess volume) | Minimum 500 µl |
| Assay parameter set (APS)* | APS_artus InflAB_400_V2.xml<br>APS_artus InflH1_400_V2.xml<br>APS_artus_InflAB_400_Indep_V2.xml<br>APS_artus_InflH1_400_Indep_V2.xml |
| Default assay control set (ACS) | ACS_Complex_Influenza_V1.xml |
| Elution volume | 60 µl |
| QIAsymphony software version | 4.0 or higher |
| QIAsymphony SP/AS configuration profile | Default profile 1 |
| Reaction mix volume | 15 µl |
| Template volume | 5 µl |
| Number of reactions | 72[†] (including both assay controls, therefore 33 samples maximum per run) |
| Rotor-Gene AssayManager software | 1.0.x, where x = 4 or higher |
| Epsilon plug-in | 1.0.x, where x = 1 or higher |
| Assay Profiles version | 1.0.x, where x = 3 or higher for Influenza AB Assay Profile<br>1.0.x, where x = 3 or higher for Influenza H1 AssayProfile |
| Runtime on QIAsymphony SP/AS module | Approximately 320 minutes for 72 reactions |
| Runtime on Rotor-Gene Q instrument | Approximately 75 minutes |

\* This workflow comprises four assay parameter sets, allowing the user to run influenza AB and influenza H1 assays in one run in an integrated workflow or independent workflow.

† Ensure that the limit of 71 reactions (33 samples per assay and five output positions for controls) and 1 assay rack adapter is not exceeded. Avoid extended incubation time (>30 minutes) between completion of the assay setup run and transfer to the Rotor-Gene Q instrument.

# Procedure

## General information

The QIAsymphony SP and QIAsymphony AS are to be used with an integrated workflow meaning that the sample preparation is immediately proceeded by assay setup within one single QS SP/AS run.

The integrated workflow tests samples for influenza A, influenza B, and influenza H1 detection. Thus, RNA extraction and assay setup with AB Master and H1 Master are executed within one QS-SP/AS run. When retests are necessary (see "Interpretation of Results", page 68), the workflow can be re-extracted and tested using the integrated workflow or independent depending on the different scenario (see Figure 20 on page 74 for more details about retests).
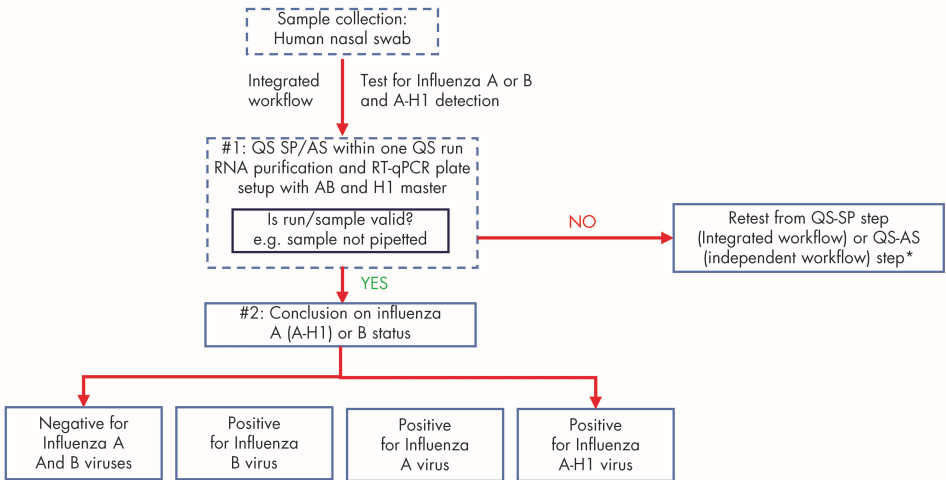


**\*** Refer to Interpretation of Results section.

**Figure 3: Integrated workflow scenario.**

# Controls

Controls are not infectious. However, all reagents should be handled in accordance with safe laboratory procedures (see "Warnings and Precautions", page 14).

## Positive controls

The influenza positive controls (i.e. Influenza Control plus Influenza B Control used with AB Master and Influenza H1 Control used with H1 Master) supplied with the *artus* Infl. A/B/H1 QS-RGQ Kit monitor the efficiency of the downstream assay. These positive controls are loaded onto QIAsymphony AS. (See "Load reagents and controls on QIAsymphony AS", page 39 for further details on loading positive controls).

## Negative control

The influenza negative control supplied with the *artus* Infl. A/B/H1 QS-RGQ Kit is loaded onto QIAsymphony AS to monitor the PCR for contamination. The negative control is referred as NTC in the QIAsymphony software.

## Internal control

Using the QIAsymphony DSP Virus/Pathogen Midi Kit in combination with the *artus* Infl. A/B/H1 QS-RGQ Kit requires introduction of the internal control (Influenza IC) into the purification procedure to monitor the efficiency of sample preparation and the downstream assay. The internal control is loaded onto QIAsymphony SP.

The internal control (Influenza IC), supplied with the *artus* Infl. A/B/H1 QS-RGQ Kit, must be added to the carrier RNA (CARRIER)–Buffer AVE (AVE) mixture. The total volume of the internal control–carrier RNA (CARRIER)–Buffer AVE (AVE) mixture is 120 µl per sample.

To prepare the carrier RNA (CARRIER)–Buffer AVE (AVE) mixture, add 1350 µl Buffer AVE (AVE), supplied with the QIAsymphony DSP Virus/Pathogen Mini Kit, to resuspend the lyophilized carrier RNA (CARRIER). Invert the tube 20 times to mix.

**Important**: For IC, a maximum of 2 freeze-thaw cycles is allowed as more than this may reduce assay sensitivity. Storage at 2 to 8°C should not exceed 5 hours.

**Note**: The concentration of the Carrier RNA-Buffer AVE mixture is 1μg/μl.

For internal control (IC) calculation, the "IC Calculator" within the QIAsymphony Management Console (QMC) should be used.

Table 3 represents the preparation of internal control per 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. Reaction mixes for the *artus* Infl. A/B/H1 QS-RGQ Kit**

| Component | Reactions | |
|---|---|---|
| | Volume (μl) for n≤13 in Sarstedt tubes* | Volume (μl) for n>13 in BD tubes† |
| Stock carrier RNA (CARRIER) | (n + 3) × 3 | (n + 5) × 3 |
| Internal Control (Influenza Internal Control) | (n + 3) × 9 | (n + 5) × 9 |
| Buffer AVE | (n + 3) × 108 | (n + 5) × 108 |
| Final volume per sample (excluding dead volume) | 120 | 120 |
| Total volume for n samples | (n + 3) × 120 | (n + 5) × 120 |

\* Microtubes 2.0 ml Type I (Sarstedt, cat. no. 72.694). If preparing the internal control (IC) as a stock solution in a larger tube, multiply the total volume of each component by the number of IC tubes used. Internal control mixture corresponding to n samples plus 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 2.0 ml microtubes, 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 (Corning, cat. no. 352051. BD was the previous supplier of this tube; Corning, Inc. is the new supplier). Internal control mixture corresponding to n samples plus 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).

Calculation of mixture by IC Calculator

1. Open the QMC.
2. Select the IC Calculator icon.
3. Select "**Complex_Influenza_V1**" from the ACS drop-down list.
4. Enter the required number of samples.
5. Select the labware used for the IC.
6. Select "**Internal Control/Eluate**" and "**0.1 µl**" for internal control mode.
7. Press "**Calculate**" to start calculation of IC 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.

> **Important**: Do not add the internal control and the carrier RNA to the sample material directly.

## Assay control sets and assay parameter sets

Assay control sets are the combination of an extraction 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 the integrated run on the QIAsymphony SP/AS, both assay parameter sets "**APS_artus InflAB_400_V2.xml**" and "**APS_artus InflH1_400_V2.xml**", are already linked to the upfront assay control set, "**ACS_Complex_Influenza_V1.xml**", specifying the associated sample purification process.

For the independent run on the QIAsymphony AS, the assay parameter sets are "**APS_artus_InflAB_400_Indep_V2.xml**" and "**APS_artus_InflH1_400_Indep_V2.xml**".

Each assay parameter set described above is associated to a respective assay definition ("**AD_artus_InflAB_400_V2.xml**" and "**AD_artus_InflH1_400_V2.xml**") for integrated and independent runs, allowing the user to run Influenza AB and Influenza H1 assays in one run in the integrated workflow or two separate runs in the independent workflow.

## Reagent preparation

### Reagent Cartridge

Each time before using the reagent cartridge (RC), make sure that Buffers QSL2 and QSB1 in the RC do not contain a precipitate. If precipitate has formed, perform the following procedure to dissolve the precipitate.

1. If the reagent cartridge has not been pierced, remove the troughs containing Buffers QSL2 and QSB1 from the RC. If the reagent cartridge has been pierced, make sure that the troughs are sealed with Reuse Seal Strips.

2. Place the unopened troughs or entire reagent cartridge sealed with Reuse Seal Strips in a water bath for 30 minutes at 37°C with occasional shaking to dissolve precipitate.

3. Remove the unopened troughs or reagent cartridge from the water bath.

4. As applicable, replace the troughs in the correct positions of the reagent cartridge.

5. Allow the reagents to cool down to room temperature (15–25°C).

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

### Magnetic beads

Resuspend the magnetic beads in the reagent cartridge before each use.

1. Remove the magnetic bead trough from the reagent cartridge. If the reagent cartridge is already opened, make sure the magnetic-particle trough is sealed with a Reuse Seal Strip.
2. Vortex the magnetic bead trough for 3 minutes.
3. Replace the magnetic bead trough in the reagent cartridge.

### Buffer ATL

- Check whether a precipitate has formed in Buffer ATL. If necessary, dissolve by heating the buffer at 70°C with gentle agitation in a water bath. Aspirate bubbles from the surface.
- Avoid vigorous shaking of Buffer ATL bottle. Otherwise foam may be generated, which can lead to liquid-level detection problems.

## Important points before starting

- Before beginning the procedure, read "Warnings and Precautions", page 14.
- Take time to familiarize yourself with the Rotor-Gene Q MDx 5plex HRM before starting the protocol. See the instrument user manual.
- Ensure that you are familiar with operating QIAsymphony RGQ System (cat.no. 9001850). Refer to the user manual supplied for operating instructions. The add-on training for the QIAsymphony RGQ System workflow is mandatory to operate the system. The training can either be provided by QIAGEN Field Service or by already trained persons in the laboratory. If you have not received this add-on training, please contact QIAGEN Technical Service.
- Download the Application Package from "Protocol Files" on the "Resources" tab of the *artus* Infl. A/B/H1 QS-RGQ Kit web catalog.

- Refer to the applicable user manual for more information on instrument operating conditions such as temperature or humidity.

- Make sure that all necessary protocol files given in "Assay control sets and assay parameter sets", page 23 are installed on QIAsymphony SP/AS.

- Proceed continuously from one part of the workflow to the next.

- Do not use an Elution Microtubes CL rack that has already been used for another QIAsymphony SP run. Do not enter a rack ID manually.

- Work quickly and keep PCR reagents on ice or in the cooling block before loading.

- The reagent volumes are optimized for a maximum of 3 batches of 24 reactions or 1 batch of 72 reactions per kit per run.

- Eluates from the sample preparation and all components of the *artus* Infl. A/B/H1 QS-RGQ Kit are stable onboard the instrument for the time required for sample purification and assay setup for 72 assay reactions. The time includes 30 minutes for the transfer from the QIAsymphony AS to the Rotor-Gene Q MDx 5plex HRM instrument.

## Integrated workflow

### Things to do before starting

- Before each use, thaw the components of the artus Infl. A/B/H1 QS-RGQ Kit required for that run thoroughly at room temperature (15–25°C). Mix all components by pulse vortexing followed by centrifuging for at least 3 seconds. Make sure that no foam or bubbles are present in the reagent tubes.

  **Important:** The reagent volumes are optimized for 3 x 24 reactions per kit. Fewer or more samples can be run, but sub-optimal usage of the reagents will occur due to the calculated dead volume required for the QIAsymphony.

- Make sure the required adapters are precooled to 2–8°C.

- Prepare all required reagents. Prepare mixtures containing carrier RNA and internal controls just before starting. For more information, see "Procedure, Internal control" on page 21.

- 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 perform maintenance regularly to minimize the risk of cross-contamination.

- Make sure 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".

## Protocol for integrated assay setup on the QIAsymphony SP/AS

**Power ON QIAsymphony**

1. Close all drawers as well as the QIAsymphony SP and QIAsymphony AS hoods.

2. Power ON QIAsymphony SP/AS and wait until the "Sample Preparation" screen appears and the initialization procedure has finished.

   **Note**: The power switch is located at the bottom-left corner of the QIAsymphony SP

3. Log in to the QIAsymphony SP/AS.

**Load the "Waste" drawer on QIAsymphony SP**

1. Open the "Waste" drawer.

2. Install an empty liquid waste bottle.

   **Important**: Make sure the lid is removed from the liquid waste container.

3. Insert the tip chute.

   **Note:** Different tip chutes must be used depending on if QIAsymphony SP and QIAsymphony AS are on a benchtop or a QIAsymphony Cabinet SP/AS.

4. Insert the tip park station.

5. Insert the empty unit boxes into the "Waste" drawer for the plastic waste generated during the protocol run (see Table 4).

   **Important**: Make sure that there is at least one empty unit box in slot 4 (slot closest to you).

   **Important**: Make sure that the covers of the unit boxes are removed before loading the unit boxes into the "Waste" drawer. If using 8-Rod Cover boxes for collection plastic waste, make sure that the box spacer has been removed.

6. Install an empty tip disposal bag.

   **Note**: The presence of a tip disposal bag is not checked by the instrument. Make sure that the tip disposal bag is properly attached before starting a run. For more information on installing a tip disposal bag, refer to the applicable user manuals.

   **Note**: The empty tip disposal bag is installed below the waste drawer for benchtop operation or in the waste bin for QIAsymphony Cabinet SP/AS operation.

7. Close the "Waste" drawer and perform an inventory scan of the "Waste" drawer

**Table 4. Required plasticware for various sample batch sizes**

|  | 1 batch, 21 samples | 1 batch, 33 samples |
|---|---|---|
| Empty unit boxes | 2 | 4 |

**Load the "Eluate" drawer on QIAsymphony SP**

1. Place the Elution Microtubes Rack QS cooling adapter onto the transfer frame.

   **Note**: Do not forget the transfer frame otherwise the elution rack will not be transferred to the QIAsymphony AS and the run will be stopped.

2. Open the "Eluate" drawer.

3. Place the assembly of the cooling adapter and transfer frame onto elution slot 1 of the "Eluate" drawer.

4. Remove the bottom of the Elution Microtubes CL rack by twisting the rack until the bottom comes out.

   **Recommendation**: Leave the lid of the Elution Microtubes CL rack on the rack until it is placed on the rack adapter.

5. Press "**Elution Slot 1**" on the touchscreen. The Eluate drawer/Elution Slot/change Rack1 screen appears.

   **Note**: Make sure to use the elution slot 1 to permit the use of elution rack cooling.

6. Scan the bar code on the Elution Microtubes CL rack using the handheld bar code scanner.

   **Important**: Do not enter a rack ID manually.

7. Place the Elution Microtubes CL rack into the assembly of the adapter and transfer frame on "Slot 1" in "Eluate" drawer. Make sure that the orientation of the rack in the adapter is correct (i.e., skewed edge of the rack points to the right side of the adapter placed on elution slot 1).

8. Remove the lid of the Elution Microtubes CL rack.

9. Close the "Eluate" drawer.

10. Press "**OK**". QIAsymphony SP performed an inventory scan of the "Eluate" drawer. Wait until the inventory scan is complete.

**Load the "Reagents and Consumables" drawer on QIAsymphony SP**

1. Open the "Reagents and Consumables" drawer.

2. Place the reagent cartridge (RC) in the gray reagent cartridge holder. Make sure that all reagent troughs are completely inserted; if not, press reagent troughs into position.

3. Resuspend the magnetic beads by vortexing the bead trough for 3 minutes before proceeding. See "Reagent preparation", page 24 for additional instructions.

4. Remove the lid or Reuse Seal Strips from the magnetic bead trough.

5. Open the enzyme tubes in the enzyme rack and place the enzyme tube lids onto the cap holders on the grey reagent cartridge holder.

6. Make sure the enzyme tubes do not contain air bubbles. If air bubbles are present, aspirate the bubbles from the surface.

7. Mount the enzyme rack (ER) on the reagent cartridge (RC).

   **Note**: If using the reagent cartridge for the first time, mount the piercing lid on top of the reagent cartridge and gently click it into place (noticeable click on left and right sides). If using a partially used reagent cartridge, remove the reuse seal strips from the trough.

   **Important**: Make sure to place the piercing lid onto the reagent cartridge in the correct orientation.

8. Load the required reagent cartridge(s) onto position RC 1 and/or RC 2. One new reagent cartridge (RC) is sufficient for up to 48 samples.

9. Press the "**R+C**" button on the touchscreen.

10. Press the "**Bottle ID**" button.

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

    The following table describes the plastic ware loaded on QIAsymphony SP for an integrated QIAsymphony RGQ System run with the *artus* Infl. A/B/H1 QS-RGQ Kit.

**Table 5. Required plasticware for various sample batch sizes**

|  | 1 batch, 21 samples | 1 batch, 33 samples |
|---|---|---|
| Disposable filter-tips, 200 µl*† | 21 | 33‡ |
| Disposable filter-tips, 1500 µl*† | 75 | 132 |
| Sample prep cartridges | 18 | 30 |
| 8-rod covers | 3 | 5 |

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

† The number of required filter-tips includes filter-tips for one inventory scan per reagent cartridge.

‡ Do not load more than 33 samples as five output positions will be needed for the controls.

> **Note**: The number of filter-tips given may differ from the numbers displayed on the touchscreen depending on the settings.

12. Open the bottle of Buffer ATL and make sure that it does not contain a precipitate. If Buffer ATL contains a precipitate, follow the instructions on "Reagent preparation", page 24.

13. Place the bottle of Buffer ATL into position B1 (next to the reagent cartridge slot 1)

    **Note**: Do not vigorously shake the buffer bottle otherwise foam may be generated. This can lead to liquid-level detection problems.

14. Load sufficient racks of disposable 200 µl filter-tips in tip rack holder positions 1–4 (see Table 5, page 31). Make sure to click all racks into place, otherwise, this may result in detection problem during the inventory scan.

    **Note**: There are 32 filter-tips per tip rack.

15. Load sufficient racks of 1500 µl disposable filter-tips in tip rack holder positions 5–18 (see Table 5, page 31). Make sure to click all racks into place otherwise, this may result in detection problem during the inventory scan.

    **Note**: There are 32 filter-tips per tip rack.

    **Recommendation**: Load more than the required number of filter-tips of each size so that sufficient filter-tips are available for automated error handling.

16. Remove the covers of the unit boxes to be loaded.

17. Load the required Sample Prep Cartridges in unit box holder positions 1–3 (see Table 5, page 31) in "Reagents and Consumables" drawer.

    **Note**: There are 28 sample prep cartridges per unit box.

    **Important**: Plastic consumables may shift during transit or storage and prevent the run to start. Check that all plastics are aligned properly inside the unit box before loading on the QIAsymphony SP otherwise, this may result in detection problem during the inventory scan.

18. Load the required 8-Rod Covers in unit box holder position 4 (see Table 5, page 31) in the "Reagents and Consumables" drawer.

    **Note**: There are twelve 8-Rod Covers per unit box.

    **Important**: Plastic consumables may shift during transit or storage and prevent the run to start. Check that all plastics are aligned properly inside the unit box before loading on the QIAsymphony SP, otherwise, this may result in detection problem during the inventory scan.

19. Press "**OK**" in the consumables screen.

20. Close the "Reagents and Consumables" drawer and perform an inventory scan.

### Load the "Sample" drawer on QIAsymphony SP

Do not load more than 33 samples in one run in the tube carriers on the QIAsymphony SP, so that the capacity of the Rotor-Gene Q MDx 5plex HRM rotor is not exceeded. The 2 positive controls for influenza A/B assay and the single positive control for influenza H1 assay plus the negative control (NTC) for each assay will be loaded on the QIAsymphony AS. A run of 71 reactions (33 samples) will require the preparation of 2 tube carriers.

**Important**: Consider all specimens as potentially infectious. See "Warnings and Precautions", page 14, for additional instructions.

1. Vortex vigorously at a maximum speed for 10–12 seconds and centrifuge for 10 seconds for the primary sample.

   **Note**: Ensure that no foam or bubbles are present in the samples.

2. Carefully open the primary sample tube and place a minimum of 500 µl in a microtube 2.0 ml Type I (Sarstedt tube, cat. no. 72.694) for the QIAsymphony SP sample preparation.

   **Important**: Make sure that a minimum of 500 µl are transferred in the bottom of the tube and centrifuge samples twice each time for 5 seconds to remove the drops and avoid a pipetting problem.

3. Place the sample tube in a Tube Insert 3B.

4. Place the Tube Insert into the tube carrier in the order in which samples will be placed onto the Elution Microtubes CL rack.

   **Note**: If samples are bar coded, orient samples in the tube carrier so that the bar codes face the bar code reader within the 'Sample' drawer at the left side of the QIAsymphony SP.

5. Make sure that sample tubes are correctly loaded and clicked into the tube carrier and the caps are removed from the tube.

6. Load tube carriers in "Sample" drawer slots 1–4. Place the sample carrier on its corresponding loading slot on the drawer lid and wait until the LED light is blinking green before inserting the whole sample carrier into the slot at a constant speed. The LED light changes to orange when the tube carrier is loaded correctly

   **Note:** First load the sample tube carrier containing the samples into slot 1. Do not load more than 33 samples, as output positions will be required for both assays and loading of controls on the QIAsymphony AS.

**Define the QIAsymphony SP batch for integrated run**

1. Press the "**Integrated Run**" tab on the touchscreen.
2. Press "**Define run**".
3. Select "**SP Batch 1**".
4. Press "**Edit samples**".
5. Make sure that the correct labware is assigned to the samples. If necessary, correct the labware assignment.
6. Press "**ID/Type**".
7. Select the sample position and press "**Sample ID**".
8. Press the text field and enter the Sample ID, then press "**OK**"

   Note: Ensure the correct Sample ID is entered to avoid loss of sample traceability.
9. Repeat the procedure for all batches and samples for the integrated QIAsymphony RGQ System run.

### Define the assay

1. Press the corresponding "**SP Batch**" button.

2. Press "**Define assays**".

3. Select the samples to be processed with the assay.

4. Select the Assay Parameter Set under the "**_artus_ QS-RGQ**" category:

   - **_artus_ InflAB_400_V2** (with Influenza AB Master)
   - **_artus_ InflH1_400_V2** (with Influenza H1 Master in case of retest; Refer to "Procedure-General information"; pages 20 for details.)

5. Press "**OK**".

6. Repeat the procedure for all batches and samples to be run.

### Define the QIAsymphony AS batch

1. Select all batches that should be processed in one integrated QIAsymphony RGQ System run.

2. Press "**Create AS batch**"

   **Note**: All QIAsymphony SP batches assigned to the same QIAsymphony AS batch will be processed in the same assay setup procedure for the integrated QIAsymphony RGQ System run.

3. Press "**OK**" to queue the run.

Load the "the IC mixture and define the internal control

1. Prepare the internal control (see "Internal control", page 21, for instructions).

   **Note**: Microtubes 2.0 ml Type I (Sarstedt, cat. no. 72.694) or tubes 14ml (Corning, cat. no. 352051) can be used for the IC mixture.

   **Note**: For certain liquid levels in unlabeled 14 ml tubes (see "Consumables and reagents for QIAsymphony SP"), scan errors can occur due to the clear liquid and tube. To avoid this, attach a blank label to the tube or mark the tube area facing the barcode scanner with a permanent marker.

2. Place the tube in a tube carrier.

   **Note**: use Tube Insert 3B when using microtubes 2.0ml.

3. Insert the tube carrier in slot "A" of the "Sample" drawer and wait until the LED light is blinking green then insert the whole sample carrier into the slot at a constant speed. The LED light changes to orange when the tube carrier is loaded correctly.

4. Press the "**Define ICs**" button.

5. Select the position(s) of the IC mixture.

6. Select the corresponding IC "**Complex_Influenza_V1**" from the "**Required**" folder.

7. Make sure that the correct labware is assigned. If not, assign the correct labware by pressing "**IC Tubes**".

8. Press "**OK**".

### Start the QIAsymphony SP run

The position of samples on the assay rack can be displayed before the start of the run. After creation of the AS batch, press the assays drawer button (**A**) on the touchscreen and select the respective **Assay** slot. The sample type of each position will be display (**Type**) if the toggle button **Sample** is pressed.

1. Press the "**Run**" button to start the run. A message will appear.
2. Read and confirm the message. All processing steps are fully automated.

   **Recommendation**: Wait beside the QIAsymphony SP until the liquid level detection of the IC tube(s) is complete. When complete, the QIAsymphony SP carrier status changes to "**running**"

   **Important**: 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".

   **Note**: Until the reagents are loaded, it is possible to continuously load samples and add the samples to this run or to a new QIAsymphony RGQ run.

### Load consumables on QIAsymphony AS

1. Install an empty tip disposal bag below the "Waste" drawer for benchtop operation or in the waste bin for QIAsymphony SP/AS Cabinet operation.
2. Open the "Eluate and Reagents" drawer and the "Assays" drawer of QIAsymphony AS.
3. Open the hood and insert the tip chute inside the instrument

   **Note**: The type of tip chute installed depends on if QIAsymphony SP and QIAsymphony AS are on a benchtop or a QIAsymphony Cabinet SP/AS.
4. Close hood and a message will appear.
5. Read and confirm the message.

**Load assay rack on QIAsymphony AS**

1. Open the "Eluate and Reagents" drawer and the "Assays" drawer of the QIAsymphony AS.

2. Press the yellow "**Assay**" panel on the touchscreen.

3. Fill the required number of 0.1 ml Strip tubes (4 tubes = 1 segment) in a pre-cooled RG Strip Tubes 72 QS cooling adapter as indicated on the touchscreen.

   **Important**: Load complete strip tubes. Do not break segment.

4. Load RG Strip Tubes 72 QS cooling adapter with the strip tubes in slot 5 of the "Assays" drawer.

5. 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.

6. Press "**Load**".

**Load filter-tips on QIAsymphony AS**

1. Load at least the number of filter-tips provided in the "**Assay Setup | Loading Information**" screen.

   **Important**: Start loading tip racks from the positions at the back near the cooling adapter. 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.

   **Recommendation**: Load more than the required number of filter-tips of each size so that sufficient filter-tips are available for automated handling and to prevent errors.

## Load reagents and controls on QIAsymphony AS

**Important**: Before each use, thaw the components of the artus Infl. A/B/H1 QS-RGQ Kit required for that run thoroughly at room temperature (15–25°C). Mix all components by pulse vortexing followed by centrifuging for at least 3 seconds. Makes sure that no foam or bubbles are present in the reagent tubes.

The reagent volumes are optimized for 3 x 24 reactions per kit. Fewer or more sample can be run, but sub-optimal usage of reagent will occur due to the calculated dead volume required for the QIAsymphony.

1. Press the yellow "Reagent" panel on the touchscreen.

    Prepare the applicable precooled cooling adapter reagent holder as displayed on the touchscreen .

    **Important**: do not include lids on tubes.

2. Select the tube positions on the touchscreen and load an empty tube(s) for the master mix.

3. Place the required reagents tubes (i.e. Master, Negative [NTC], Positive Control[s], and Mg-Sol) in the corresponding positions as indicated on the touchscreen, otherwise the run will be invalid.

    **Note**: One tube each of the Influenza Master and Mg-Sol is sufficient for 24 reactions, including QIAsymphony SP eluates and controls. QIAsymphony AS will display a warning message and pause if insufficient reagent volume is loaded (where >100 µl is requested).

    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 QIAsymphony AS batch is defined and queued.

4. Press the "Scan Kit Barcode" button on the touchscreen and press the light-blue kit bar code line.

   **Note**: If the kit bar code is not scanned at this step, Rotor-Gene AssayManager will reject the QIAsymphony AS result file during import if Rotor-Gene AssayManager is configured to require the material number, valid expiry date, or lot number (see "Settings" in the Rotor-Gene AssayManager Core Application User Manual).

5. Press the text field and, using the handheld bar code scanner, scan the kit bar code on the upper side of the artus Infl. A/B/H1 QS-RGQ Kit.

6. Load the prepared cooling adapter reagent holder onto slot 3 of the "Eluate and Reagents" drawer.

7. Press the "Load" button.

8. Close the "Eluate and Reagents" and "Assays" drawers.

**Start the QIAsymphony AS run**

1. Press "**Scan**" to enter the scan dialog.

2. Press "**Scan**" to perform an inventory scan of all QIAsymphony AS components.

   **Recommendation**: Wait beside the instrument until the scan is completed. 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. After the scan is finished, the calculated integrated run time is shown on the **Integrated Run Overview** screen.

3. After sample preparation on QIAsymphony SP is complete, assay setup starts automatically and the eluate racks are transferred to QIAsymphony AS. Check the end time of the QIAsymphony AS batch so that you know when to remove the assay rack.

   **Important**: Make sure to transfer the assay rack to Rotor-Gene Q MDx 5plex HRM within 30 minutes of the run finishing on QIAsymphony AS. The maximum time permitted from the end of the run on QIAsymphony AS until the start of the run on Rotor-Gene Q MDx 5plex HRM is 30 minutes.

**Remove the assay rack from QIAsymphony AS**

1. After the QIAsymphony AS run is finished, open the "Assays" and the "Eluate and Reagents" drawers.

2. Switch to the assay setup user interface.

3. The message "Run is completed" appears.

4. Press "**OK**".

5. Remove the adapter with the strip tubes and close the tubes with the appropriate strip tube caps.

   **Recommendation**: Mark the strip tube caps to ensure correct positioning in the rotor of the Rotor-Gene Q MDx 5plex HRM. Do not mark the tubes within the optical path otherwise fluorescence will not be detected correctly.

   **Important**: Use a cooled transport frame to avoid contamination. Do not leave the reaction mixes exposed to light during transfer from QIAsymphony to Rotor-Gene Q MDx 5plex HRM as incorrect results may be obtained.
   **Note**: Check the tubes with Positive Control(s) and the negative control (NTC) and visually confirm similar liquid levels in the tubes to show that the transfers were performed correctly.

6. Press slot "**5 Assay**".

7. Press the "**Remove**" button.

   **Note**: Keep the strip tubes with PCR reactions in the cooled adapter on ice and load the Rotor-Gene Q MDx 5plex HRM instrument within the shown remaining timeframe.

8. Remove the cooling adapter reagent holder and discard the reagents according to your local safety regulations.

9. Press slot "**3 Reagent**".

10. Press the "**Remove**" button.

11. Close the "Assays" and the "Eluate and Reagents" drawers.

12. Press "**Scan**" to enter the scan dialog.

13. Press "**Scan**" to perform an inventory scan for adapters on the left and right drawers. This option is typically preselected.

14. Press the "**Integrated Batch**" button (green) to remove the integrated run and a message will appear.

15. Read and confirm the message.

16. The final QIAsymphony 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.

    **Note**: If multiple batches on the QIAsymphony AS are configured in an integrated run, check the tip disposal bag for remaining capacity, and reload the QIAsymphony AS drawers, starting at step on page 37.

**Transfer result file from QIAsymphony AS**

The result files must be downloaded and transferred to Rotor-Gene Q MDx 5plex HRM using either QIAsymphony Management Console (QMC) or a USB flash drive. Use either "Procedure to transfer result file using the USB stick," or "Procedure to transfer result file using the QMC," as follows to transfer the result files.

Procedure to transfer result file using the USB stick

1. Insert the USB stick.

2. Select "**Tools**".

3. Select "**File Transfer**".

4. Select "**Result Files**" and the "cycler file" in the "**Save to USB Stick**" column.

5. Press the "**Transfer**" button. A message appears.

6. Read and confirm the message.

7. After successful transfer, press "**OK**" and remove the USB stick.

8. Proceed to "Procedure for RT-qPCR on the Rotor-Gene Q MDx 5plex HRM System", page 53.

Procedure to transfer result file using the QMC

1. Log in to the instrument.

2. Select the **File Transfer** icon.

3. Choose file format **Result File AS**.

4. Select the result file with the correct time stamp and batch ID from the list of **Remote Site** files (right column).

5. Transfer result file to the **Local Site**.

   **Note**: The file is saved under the path defined in **Tools, Options, File Transfer**, in the folder **\log\Results\AS**

6. If multiple batches for QIAsymphony AS are configured in an integrated run, proceed to "Load consumables on QIAsymphony AS," page 37, to reload QIAsymphony AS for a second assay setup.

7. Once the second assay is set up or if a second assay is not required, proceed to "Procedure for RT-qPCR on the Rotor-Gene Q MDx 5plex HRM System," page 53

**Remove the eluate rack**

**Note**: The removal of the eluate rack can be done during the Rotor-Gene Q MDx 5plex HRM PCR run. Upon completion of a QIAsymphony SP/AS integrated run the eluate rack must be removed in the software as well as physically from the module.

1. Open the "**Eluate**" drawer.

2. Select "**Elution Slot 1**" on the touchscreen.

3. Press "**Remove**" and "**Yes**".

4. Close the Elution Microtubes CL rack with the lid.

5. If a QIAsymphony SP/AS integrated run has been performed, unload the rack from the adapter.

6. Close the "**Eluate**" drawer.

7. Press "**OK**". Wait until the scan has finished.

8. The Elution Microtubes CL rack can be stored at 2–8°C for up to 48 hours or at –20°C for up to 7 days.

**Remove the sample carrier**

This procedure must be ignored if a QIAsymphony AS independent run has been performed. If a QIAsymphony SP/AS integrated run has been performed, the removal of the samples can be performed during the Rotor-Gene Q MDx 5plex HRM PCR run.

1. Open the "**Sample**" drawer.

2. Remove the sample carriers and samples from QIAsymphony SP. Discard samples according to your local safety regulations. Procedure for an independent QIAsymphony AS run.

## Independent workflow

The QS-AS independent run is performed where repeat testing is required (see Interpretation of Results, page 68).

### Things to do before starting

- Before each use, thaw the components of the *artus* Infl. A/B/H1 QS-RGQ Kit required for that run thoroughly at room temperature (15–25°C). Mix all components or by pulse vortexing followed by centrifuging for at least 3 seconds. Makes sure that no foam or bubbles are present in the reagent tubes.

    **Important**: The reagent volumes are optimized for 3 x 24 reactions per kit. Fewer or more samples can be run, but sub-optimal usage of reagents will occur due to the calculated dead volume required for the QIAsymphony.

- Refer to the applicable user manual for more information on instrument operating conditions such as temperature or humidity.

- Refer to the applicable user manual for more information on instrument operating conditions, such as temperature.

- Make sure the required adapters are precooled to 2–8°C.

- If necessary, thaw the Elution Microtubes CL rack to test in this run at room temperature.

    **Note**: The Elution Microtubes CL rack can be stored at 2-8°C for up to 48 hours or at −20°C for up to 7 days.

- Before starting an independent 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 AS*, and *QIAsymphony Management Console User Manual* supplied.

- Perform maintenance regularly to minimize the risk of cross-contamination.

- Make sure that the 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".

- Make sure all necessary protocol files are installed on QIAsymphony AS:
  - Assay parameter set for independent workflow:
    - "**APS_artus_InflAB_400_Indep_V2.xml**" using Influenza AB Master
    - "**APS_artus_InflH1_400_Indep_V2.xml**" using Influenza H1 Master
  - Assay definition:
    - "**AD_artus_InflAB_400_V2.xml**"
    - "**AD_artus_InflH1_400_V2.xml**"

Protocol for independent assay setup on the QIAsymphony AS

1. Close all drawers as well as the QIAsymphony AS hood.

2. Power on QIAsymphony AS and wait until the "Sample Preparation" screen appears and the initialization procedure has finished (around 15 minutes).

   **Note**: The power switch is located at the bottom-left corner of the QIAsymphony SP.

3. Log in to the QIAsymphony AS.

   **Note**: When the QIAsymphony SP is not connected with the QIAsymphony AS, select "**Transfer**" in "**Tools**" tab to upload the "**Rack File**", if necessary.

4. Install an empty tip disposal bag into the QIAsymphony AS.

5. Open the "**Eluate and Reagents**" drawer and the "**Assays**" drawer of QIAsymphony AS.

6. Open the hood and insert the tip chute inside the instrument.

   **Note**: The type of tip chute installed depends on if QIAsymphony SP and QIAsymphony AS are on a benchtop or a QIAsymphony Cabinet SP/AS.

7. Close all drawers and the hood and a message will appear.

8. Read and confirm message.

### Loading information

1. Switch to the assay setup user interface.

2. Press "**New**" to start the assay definition process. The screens on the touchscreen guide the user through the assay definition process in a step-by-step fashion.

3. Press the yellow "**Sample**" panel on the touchscreen.

4. Press "**Rack ID**".

5. Using the hand-held bar code scanner, scan the bar code on the Elution Microtubes CL rack.

   **Note**: Do not manually enter a rack ID.

6. Press "Next".

7. Check sample rack type for sample and select "**Deep Well QIA# 19588\*EMTR**" if necessary.

8. Select the appropriate assay parameter set to use in the run under the "***artus* QS-RGQ**" category:

   ○ "**APS_artus_InflH1_400_Indep_V2**" using with Influenza H1 Master

   ○ "**APS_artus_InflAB_400_Indep_V2**" using with Influenza AB Master

   **Important**: Ensure the appropriate assay parameter set is selected as it is linked to the assay profile. The selection of an incorrect assay parameter set will lead to the failure of the run on Rotor-Gene Q MDx 5plex HRM.

9. Press "**Next**".

10. Select the samples to test in the run.

11. Press "**Assign**" to assign the assay parameter set to the appropriate sample.

12. Queue the assay run by pressing "**Queue**".

### Load assay rack on the QIAsymphony AS

1. Open the "**Eluate and Reagents**" drawer and the "**Assays**" drawer of the QIAsymphony AS.

2. Press the yellow "**Assay**" panel on the touchscreen.

3. Fill the required number of 0.1 ml Strip tubes (4 tubes = 1 segment) in a pre-cooled RG Strip Tubes 72 QS cooling adapter as indicated on the touchscreen.

   **Important**: Load complete strip tubes. Do not break segment.

4. Load RG Strip Tubes 72 QS cooling adapter with the strip tubes in slot 5 of the "**Assays**" drawer.

5. 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.

6. Press "**Load**".

### Load filter-tips on the QIAsymphony AS

1. Load at least the number of filter-tips provided in the "**Assay Setup | Loading Information**" screen.

   **Important**: Start loading tip racks from the positions at the back near the cooling adapter. 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.

   **Recommendation**: Load more than the required number of filter-tips of each size so that sufficient filter-tips are available for automated error handling.

### Load reagents and controls on the QIAsymphony AS

**Important**: Before each use, thaw the components of the *artus* Infl. A/B/H1 QS-RGQ Kit required for that run thoroughly at room temperature (15-25°C). Mix all components by pulse vortexing followed by centrifuging for at least 3 seconds. Makes sure that no foam or bubbles are present in the reagent tubes.

**Important**: The reagents volumes are optimized for 3 x 24 reactions per kit. Fewer or more samples can be run, but sub-optimal usage of the reagents will occur due to the calculated dead volume required for the QIAsymphony.

1. Press the yellow "**Reagent**" panel on the touchscreen.
2. Prepare the applicable precooled cooling adapter reagent holder as displayed on the touchscreen.

   **Important**: Do not include lids on tubes.
3. Select the tube positions on the touchscreen, load an empty tube(s) for the master mix.
4. Place the required reagents tubes (i.e. Master, Negative [NTC], Positive Control(s), and Mg-Sol) in the corresponding positions as indicated on the touchscreen.

   **Note**: One tube each of the Influenza Master and Mg-Sol is sufficient for 24 reactions including controls.

   **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 QIAsymphony AS batch is defined and queued.
5. Press the "**Scan Kit Barcode**" button on the touchscreen and press the light-blue kit bar code line.

   **Important**: If the kit bar code is not scanned at this step, Rotor-Gene AssayManager will reject the QIAsymphony AS result file during import.
6. Press the text field and, using the handheld bar code scanner, scan the kit bar code on the upper side of the *artus* Infl. A/B/H1 QS-RGQ Kit.
7. Load the prepared cooling adapter reagent holder onto slot of the "Eluate and Reagents" drawer.
8. Press "**Load**".

## Load samples on the QIAsymphony AS

**Important**: Before each use, thaw the Elution Microtubes CL rack to test in this run at room temperature if necessary.

1. Load the Elution Microtubes rack QS cooling adapter manually.

   **Note**: Do not place the Elution Microtubes Rack QS cooling adapter onto the transfer frame.

2. Press the blue "Sample" panel on the touchscreen.

3. Remove the lid of the Elution Microtubes CL rack.

   **Note**: If not already completed, remove the bottom of the Elution Microtubes CL rack by twisting the rack until the bottom comes out.

4. Carefully open the caps of the Elution Microtubes CL rack previously scanned to avoid cross-contamination.

5. Load the Elution Microtubes CL rack previously scanned on the cooling rack adapter.

6. Press "Load".

7. Close the "Eluate and Reagents" and "Assays" drawers.

## Start the QIAsymphony AS run

1. Press "**Scan**" to enter the scan dialog

2. Press "**Scan**" to perform an inventory scan of all QIAsymphony AS components

   **Recommendation**: **Wait** beside the instrument until the scan is completed. 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. After the scan is finished, the calculated run time is shown on the **touchscreen**.

3. Check the end time of the QIAsymphony AS batch to remove the assay rack.

   **Important**: Make sure you transfer the assay rack to Rotor-Gene Q MDx 5plex HRM within 30 minutes of the run finishing on QIAsymphony AS. The maximum time permitted from the end of the run on QIAsymphony AS until the start of the run on Rotor-Gene Q MDx 5plex HRM is 30 minutes.

**Remove the assay rack from QIAsymphony AS**

1. After the QIAsymphony AS run is finished, open the "**Assays**" and the "**Eluate and Reagents**" drawers.

2. Switch to the assay setup user interface.

3. The message "**Run is completed**" will appear.

4. Press "**OK**".

5. Remove the adapter with the strip tubes and close the tubes with the appropriate strip tube caps.

   **Recommendation**: Mark the strip tube caps to ensure correct positioning in the rotor of the Rotor-Gene Q MDx 5plex HRM. Do not mark the tubes within the optical path otherwise fluorescence will not be detected correctly.

   **Important**: Ensure strip tubes are correctly capped to avoid evaporation during qPCR.

   **Important**: Use a cooled transport frame to avoid contamination. Do not leave the reaction mixes exposed to light during transfer from QIAsymphony to Rotor-Gene Q MDx 5plex HRM as incorrect results may be obtained.

   **Note**: Check the tubes with Positive Control(s) and the negative control (NTC) and visually confirm similar liquid levels in the tubes to show that the transfers were performed correctly.

6. Press slot "**Assay**".

7. Press the "**Remove**" button.

   **Note**: Keep the strip tubes with PCR reactions in the cooled adapter on ice and load the Rotor-Gene Q MDx 5plex HRM instrument within the shown remaining timeframe.

8. Remove the cooling adapter reagent holder and discard the reagents according to your local safety regulations.

9. Press "**Reagent**" panel.

10. Press the "**Remove**" button.

11. Close the "**Assays**" and the "**Eluate and Reagents**" drawers.

12. Press "**Scan**" to enter the scan dialog.

13. Press "**Scan**" to perform an inventory scan for adapters on the left and right drawers This option is typically preselected.

14. A message will then appear.

15. Read and confirm the message.

16. The final QIAsymphony 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.

17. Transfer the result file from the QIAsymphony AS (see "Transfer result file from QIAsymphony AS"; page 42).

18. Once the second assay is set up or if a second assay is not required, proceed to "Procedure for RT-qPCR on the Rotor-Gene Q MDx 5plex HRM System," page 53.

# Procedure for RT-qPCR on the Rotor-Gene Q MDx 5plex HRM System

## Important points before starting

- To avoid exceeding the recommended standing time of the PCR reaction, proceed with PCR on the Rotor-Gene Q MDx 5plex HRM before starting QIAsymphony post-run maintenance activities, such as removing the eluate rack and sample carrier. For a description of maintenance activities, see "Maintenance", page 67.

- Take time to familiarize yourself with the Rotor-Gene Q MDx 5plex HRM instrument before starting the protocol. See the instrument module user manual.

- The *artus* Infl. A/B/H1 QS-RGQ Kit must be run on the Rotor-Gene Q MDx 5plex HRM instrument using automated interpretation of results with Rotor-Gene AssayManager. The cycling parameters are locked for the run.

- After installing the plug-in and importing the assay profile (see "Things to do before starting", below), the 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.

## Things to do before starting

- For automated interpretation of results using the *artus* Infl. A/B/H1 QS-RGQ Kit with Rotor-Gene AssayManager, the latest Epsilon Plug-in v1.0.x or higher must be installed to your Rotor-Gene AssayManager. For a detailed description refer to "Installing Plug-ins" in the *Rotor-Gene AssayManager Core Application User Manual* supplied).

- To use the specific assay profiles of the *artus* Infl. A/B/H1 QS-RGQ Kit, four files must be imported to Rotor-Gene AssayManager:

  - **AP_artus_Infl.AB_CE_V1_0_x.iap** (where x = 3 or higher) for AB influenza detection within a QIAsymphony integrated run (QS-SP and QS-AS performed within one single run)

  - **AP_artus_Infl.AS_AB_CE_V1_0_x.iap** (where x = 3 or higher) for AB influenza detection within a QIAsymphony <u>independent run</u> (QS-AS run only)

- o **AP_artus_Infl.H1_CE_V1_0_x.iap** (where x = 3 or higher) for influenza H1 detection within a QIAsymphony integrated run (QS-SP and QS-AS performed within one single run)
- o **AP_artus_Infl.AS_H1_CE_V1_0_x.iap** (where x = 3 or higher) for influenza H1 detection within the independent run (QS-AS run only)

- To import the assay profile into Rotor-Gene AssayManager, navigate to the "**Configuration Environment**" and change to the "**Assay Profile**" tab. Click on "**Import**" and select the appropriate file in the open file dialog. Click on "**Open**", and the assay profile is loaded and added to the list of available assay profiles. For repeat runs import the above operations to import the second Assay Profile for independent workflow.

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

- After installing the applicable plug-in and importing the applicable assay profile, Rotor-Gene AssayManager can use the information provided in the QIAsymphony AS result file to set up a run for real-time PCR amplification and subsequent automated interpretation of results.

## Setup Rotor-Gene Q MDx 5plex HRM

1. Place a 72-well rotor on to the rotor holder and press so that a click is heard.
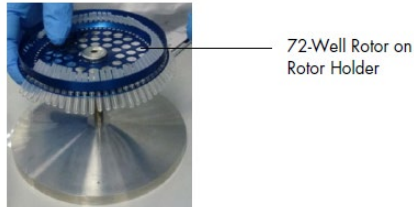


**Figure 4: The 72-well rotor on the rotor holder**

2. Place the strip tubes from the QIAsymphony AS in the 72 well rotor.

   **Important**: Make sure to transfer the strip tubes to Rotor-Gene Q MDx 5plex HRM in the correct orientation, starting at position 1 so that the position indices of the cooling adapter and the 72-Well Rotor match.

3. Visually confirm that samples were transferred correctly by checking the volume is the same in all strip tubes. Controls are in the last strip tube position per master mix of the Rotor-Gene Q MDx 5plex HRM run.

4. Use empty capped strip tubes to fill all unused positions.

   **Note**: Using empty strip tubes will guarantee an optimal temperature distribution in Rotor-Gene Q MDx 5plex HRM run.

5. Attach the locking ring.

6. Load the Rotor-Gene Q MDx 5plex HRM with the rotor and locking ring (there will be a noticeable click when this is placed correctly).

Set-up the Rotor-Gene Q MDx 5plex HRM run

1. If using a USB stick for data transfer directly from the QIAsymphony SP/AS, unzip the result file from the QIAsymphony 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. Files must be unzipped to be imported into Rotor-Gene AssayManager.

2. Paste the Result Files previously unzipped under
   **Rotor-Gene Assay Manager\Import\QIAsymphony Worklist**

   **Important**: Make sure to paste the correct QIAsymphony worklist otherwise sample traceability will be lost.

3. Start the Rotor-Gene AssayManager.

4. Log in to the closed mode.
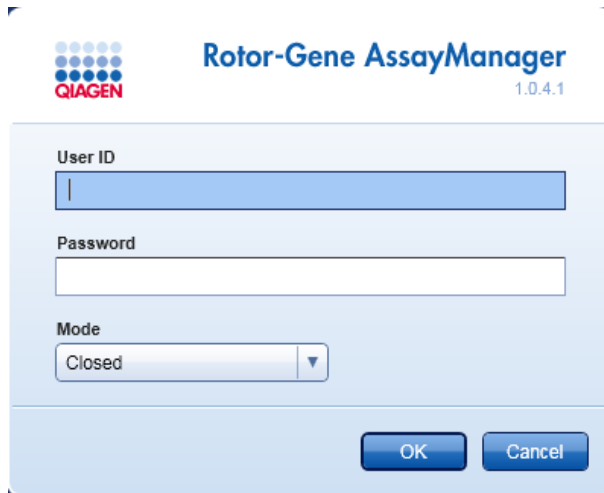


**Figure 5: Log to the closed mode**

5. Check that the Rotor-Gene Q MDx 5plex HRM instrument has been correctly detected by the software.

Figure 6: Verification of the Rotor-Gene Q MDX 5plex HRM connection

6. Select the "Setup" environment, if not already preselected.

7. Import the QIAsymphony AS result file at the bottom of the screen. Select the source "QIAsymphony" as "Import type".

8. Click on "Import".
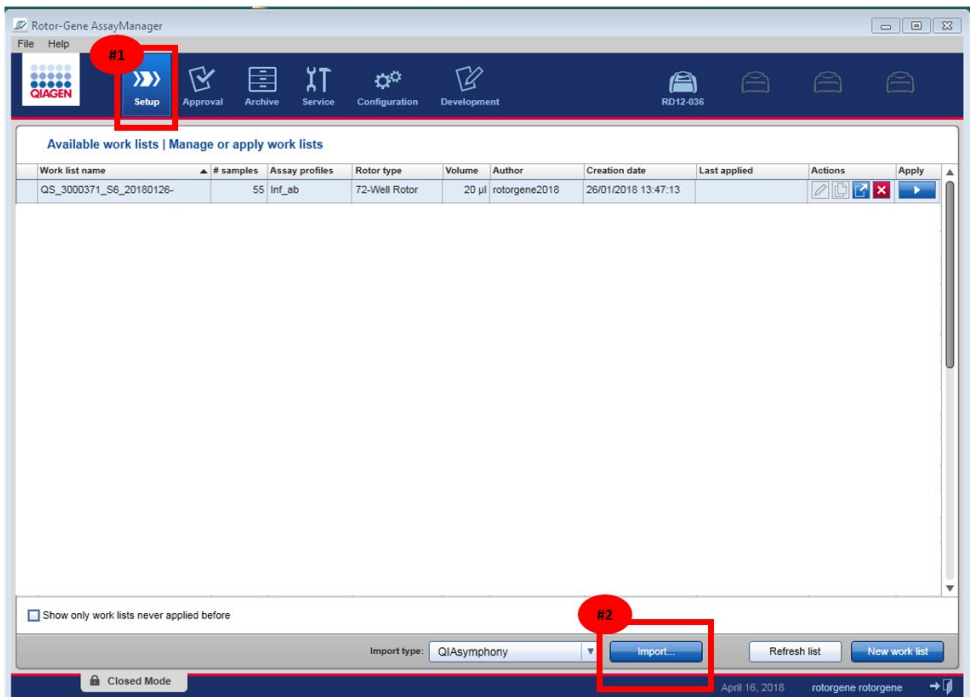


Figure 7: Import the QIAsymphony AS result file

9. In the "Select file" dialog, open the corresponding QIAsymphony AS result file and click "Open".
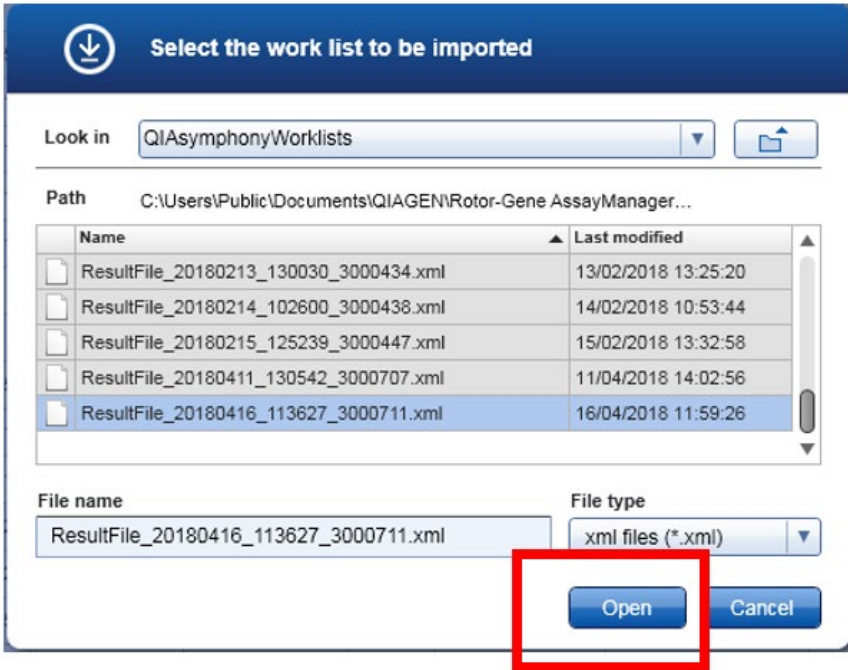


**Figure 8: Open the QIAsymphony AS result file**

10. Read and confirm the message.

11. After successful import, select the corresponding work list from the work list manager list and click the Apply button.
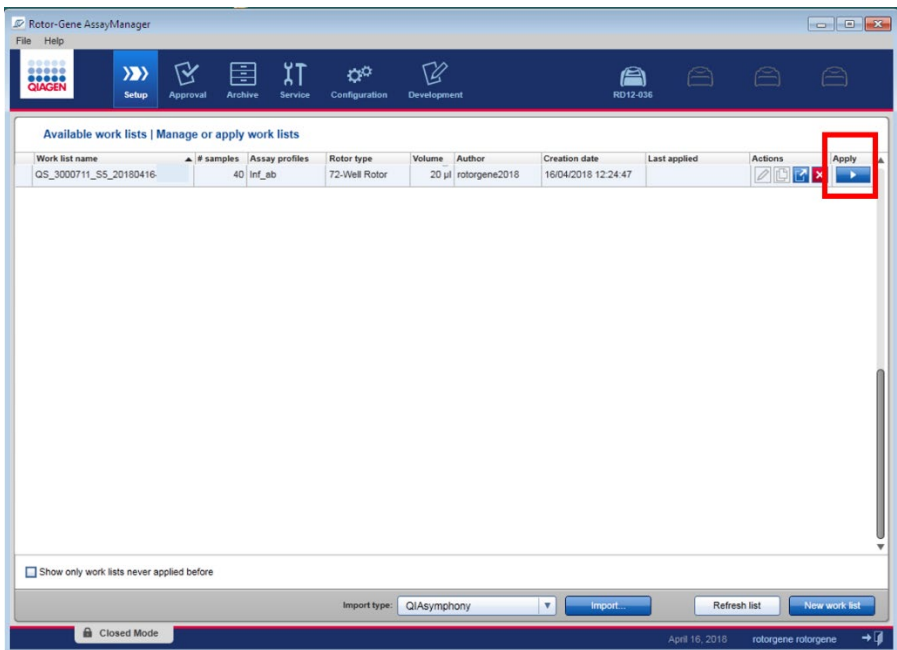
Figure 9: Apply the QIAsymphony worklist

12. Enter an experiment name.

13. Select the cycler to be used in the "**Cycler selection**" dialog.

14. Make sure the locking ring is correctly attached and confirm on the screen that the locking ring is attached.
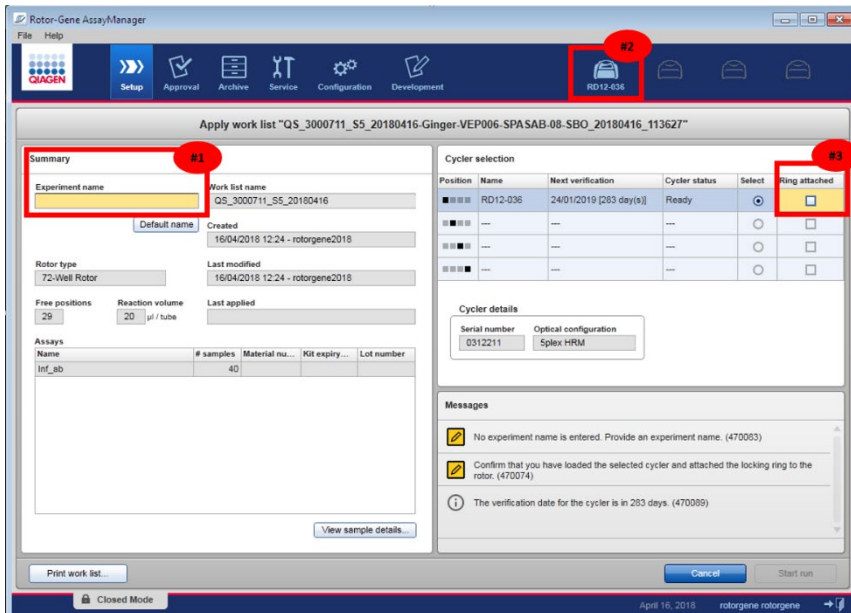
**Figure 10: Experiment name, cycler selection and locking ring attached**

15. Close the Rotor-Gene Q MDx 5plex HRM instrument lid.

16. Click the "**Start run**" button. The RT-qPCR should start.

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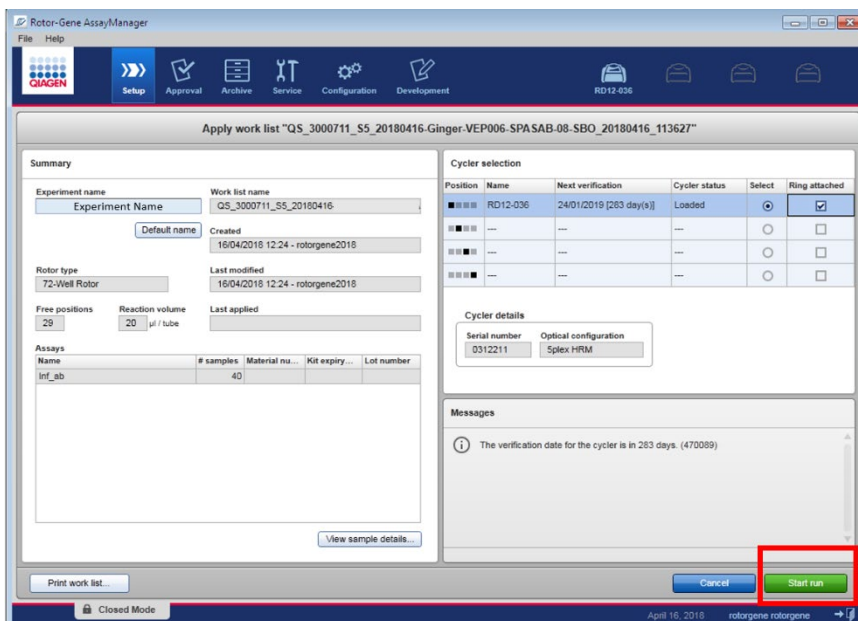
Figure 11: Start run

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

17. Perform the QIAsymphony post-run maintenance activities, such as removing the eluate rack and sample carrier. For a description of maintenance activities, see "Maintenance", page 67.

Finish the Rotor-Gene Q MDx 5plex HRM run

1. When the run is finished, click "**Finish run**".
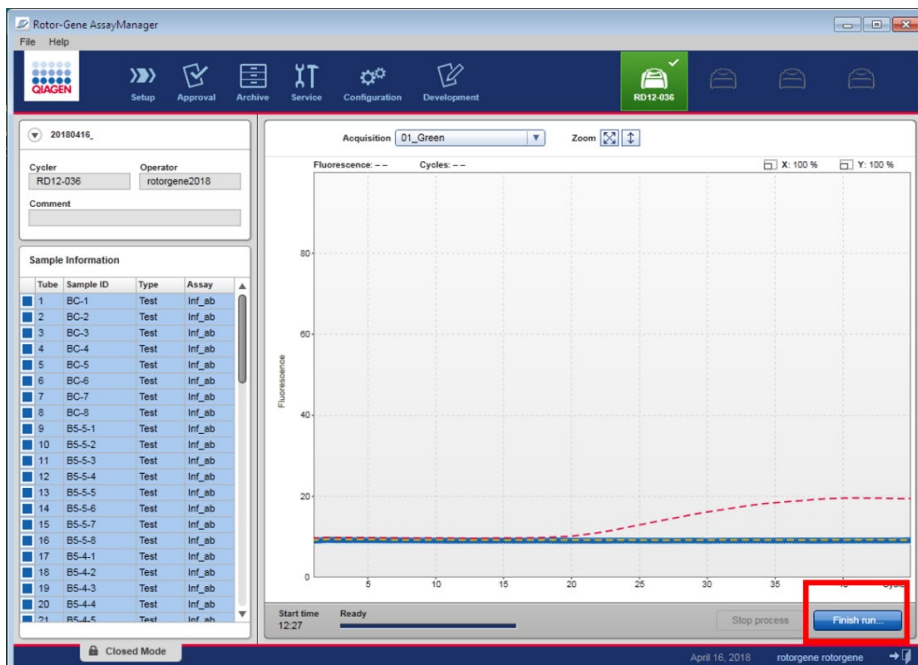


Figure 12: Finish run

For users logged in with the Operator role: Click "**Release**". For users logged in with the Approver role: Click "**Release and go to approval**".

**Note**: The general functionality of the **Approval** environment is described in *Rotor-Gene AssayManager Plug-in Epsilon Online User Manual*. After a run has finished and the cycler has been released, the experiment will be stored in the internal database. The analysis of the acquired data is performed automatically depending on the plug-in corresponding to the assay profile and the rules and parameter values defined by the assay profile.

**Note**: The user role "**Approver**" is required to approve a run. If the run was released with the "**Release and go to approval**" option, the run is automatically open in the "**Approval**" environment and the approval can be performed.

2. If the run was released with the "**Release**" option, select the "**Approval**" environment.
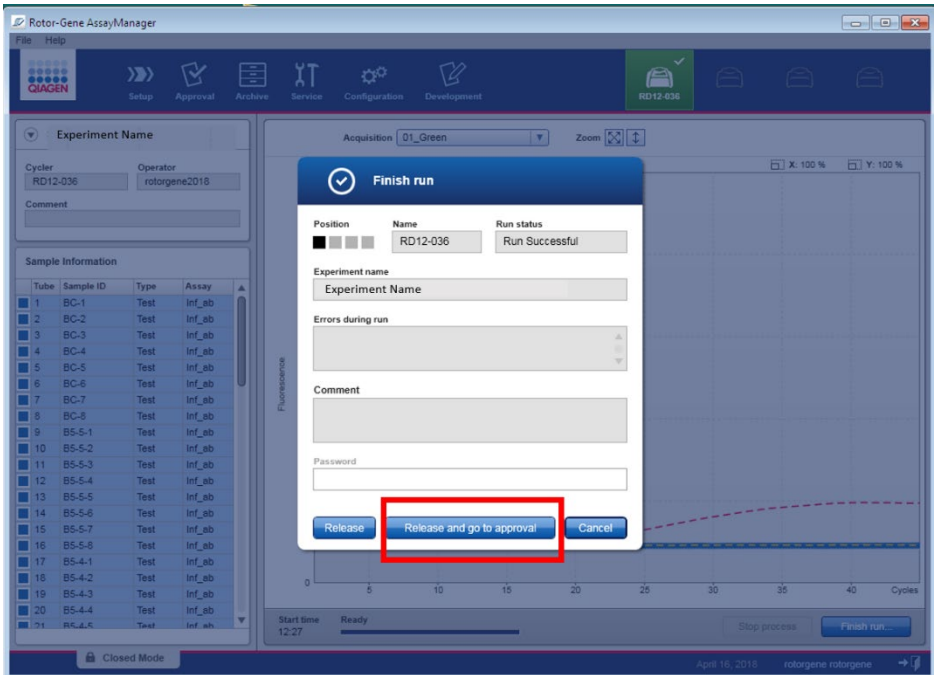


Figure 13: Release and for to approval

3. Press "**Apply filter**" (or choose own filter options beforehand).

4. Select experiment.

Click "**Start approval**". In the Epsilon Plug-in, a specific result for each target is determined. The results for controls in this plug-in do not have to be approved because tailored analysis parameters and rules are applied to the raw data of the controls. Each lot of controls is tested against predetermined specifications to ensure consistent product quality. Therefore, only the approval buttons for test samples are activated.

5. Click "**OK**" when prompted.
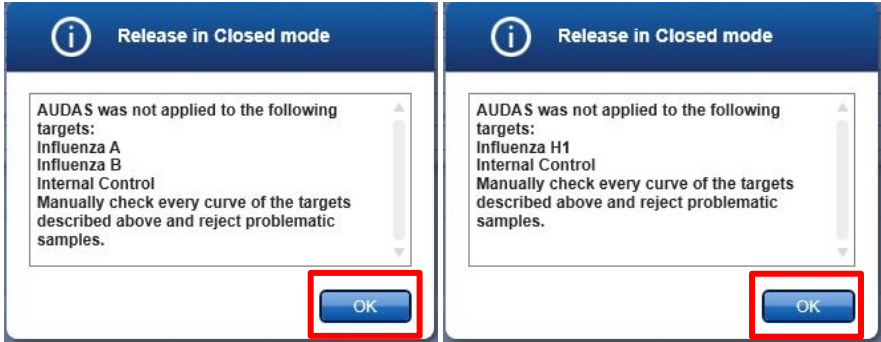


Figure 14: Release in Closed Mode

6. 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**: All samples are initially set to "Undefined" status before selecting either "Approved" or "Rejected". A run cannot be completely released until approval status has been changed to "Approved" or "Rejected".
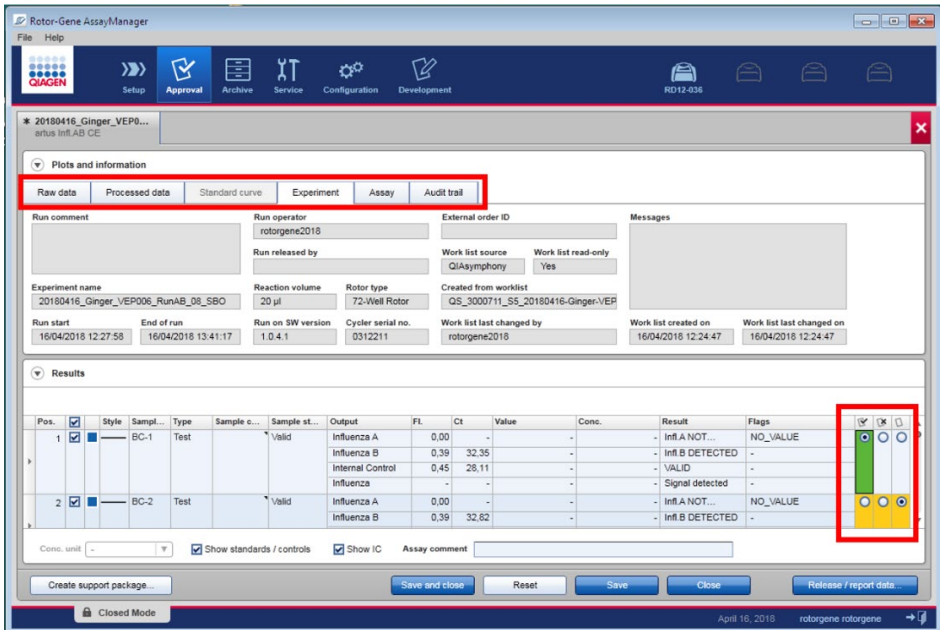
Figure 15: Approval status

**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.

**Note**: It is possible to check the plots information such as curve profile.

**Optional**: Enter assay comments or sample comments.

7. Click "**Release / report data**".
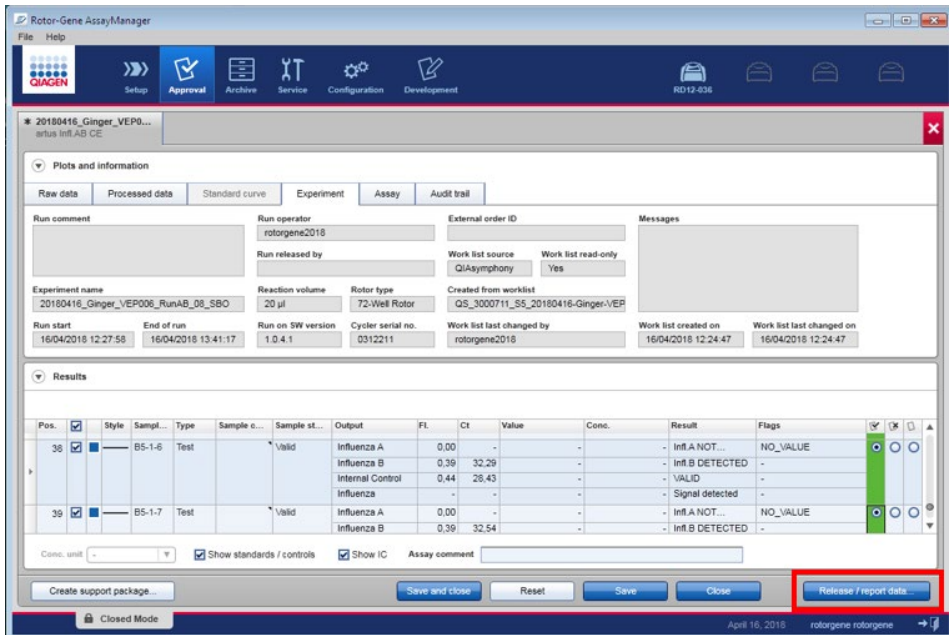
Figure 16: Release report

8. Click "**OK**". The report will be generated and stored automatically.

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

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

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## Maintenance

### Regular maintenance

When all QIAsymphony AS batches have finished, perform the regular maintenance as described in the *QIAsymphony SP/AS User Manual — General Description*. This can be conducted during the Rotor-Gene Q MDx 5plex HRM instrument run.

**Note**: This procedure can be performed at any time before the start of the next integrated run as part of a regular maintenance schedule, according to local regulations or priorities.

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

# Interpretation of Results

## Data analysis

The analysis of the *artus* Infl. A/B/H1 QS-RGQ Kit results is performed automatically for each control and sample by the Rotor-Gene AssayManager software (V1.0.4 or higher) associated to the Epsilon CE plug-in (V1.0.1 or higher) and the dedicated influenza assay profile.

To determine assay validity, the assay profiles analyze the run controls (see Table 6a and Table 6b on page 68–73 for information):

- Influenza A positive control (PC A), influenza B positive control B (PC B) and no template sample (NTC) for assay profiles dedicated to influenza A and B detection
- Influenza H1 positive control (PC H1) and NTC for assay profiles dedicated to influenza H1 detection

Validity for each control is based on compliance of Ct values with pre-defined specifications.

**Note**: if at least one external control is invalid, the result obtained for all test samples are considered as invalid.

The results of the test samples are automatically analyzed (see Table 6a and Table 6b for information) and set by Rotor-Gene AssayManager. The results must be approved by the user logged in with an "Approver" role. Samples results to be approved have three approval buttons ("**Undefined**", "**Accepted**", and "**Rejected**") at the end of the dedicated row. These buttons are used to interactively accept or reject the samples results (at the end of the experiment, the samples have the status "**Undefined**"). For further information, please refer to the *Rotor-Gene AssayManager Epsilon Plug-in User Manual*.

**Table 6a. Summary of results interpretation – controls analysis for Positive Control A (PC A), Positive Control B (PC B), Positive Control H1 (PC H1), and the no template control (NTC)**

| Controls | Influenza A/ Influenza A/H1 | Influenza B | Internal Control | Interpretation |
|---|---|---|---|---|
| | valid | valid | valid | Control valid |
| | INVALID | INVALID | INVALID: "UNEXPECTED_VALUE" | Control invalid. Retest the complete workflow (i.e., from QIAsymphony SP to RT-qPCR step) |
| PC A | INVALID | INVALID: "UNEXPECTED_VALUE" | INVALID | Control invalid. Retest the complete workflow (i.e., from QIAsymphony SP to RT-qPCR step) |
| | INVALID: "NO_VALUE" | INVALID | INVALID | Control invalid. Retest from QIAsymphony AS to RT-qPCR on Rotor-Gene Q MDx 5plex HRM |
| | INVALID: "ABOVE_ACCEPTED RANGE" | INVALID | INVALID | Control invalid. Retest from QIAsymphony AS to RT-qPCR on Rotor-Gene Q MDx 5plex HRM |
| | valid | valid | valid | Control valid |
| | INVALID "UNEXPECTED_VALUE" | INVALID | INVALID | Control invalid. Retest the complete workflow (i.e., from QIAsymphony SP to RT-qPCR step) |
| PC B | INVALID | INVALID | INVALID: "UNEXPECTED_VALUE" | Control invalid. Retest the complete workflow (i.e., from QIAsymphony SP to RT-qPCR step) |
| | INVALID | INVALID: "NO_VALUE" | INVALID | Control invalid. Retest from QIAsymphony AS to RT-qPCR on Rotor-Gene Q MDx 5plex HRM |
| | INVALID | INVALID: "ABOVE_ACCEPTED_RANGE" | INVALID | Control invalid. Retest from QIAsymphony AS to RT-qPCR on Rotor-Gene Q MDx 5plex HRM |

Cont.

**Table 6a. continued**

| Controls | Influenza A/Influenza A/H1 | Influenza B | Internal Control | Interpretation |
|---|---|---|---|---|
| PC H1 | valid | Not analyzed in Yellow channel | valid | Control valid |
| | INVALID | Not analyzed in Yellow channel | INVALID: "UNEXPECTED_ VALUE" | Control invalid. Retest the complete workflow (i.e., from QIAsymphony SP to RT-qPCR step) |
| | INVALID: "NO_VALUE" | Not analyzed in Yellow channel | INVALID | Control invalid. Retest from QIAsymphony AS to RT-qPCR on Rotor-Gene Q MDx 5plex HRM |
| | INVALID: "ABOVE_ACCEPTE D_RANGE" | Not analyzed in Yellow channel | INVALID | Control invalid. Retest from QIAsymphony AS to RT-qPCR on Rotor-Gene Q MDx 5plex HRM |
| NTC | valid | valid | valid | Control valid |
| | INVALID: "UNEXPECTED_ VALUE" | INVALID | INVALID | Control invalid. Retest the complete workflow (i.e., from QIAsymphony SP to RT-qPCR step) |
| | INVALID | INVALID: "UNEXPECTED_ VALUE" | INVALID | Control invalid. Retest the complete workflow (i.e., from QIAsymphony SP to RT-qPCR step) |
| | INVALID | INVALID | INVALID: "UNEXPECTED_ VALUE" | Control invalid. Retest the complete workflow (i.e., from QIAsymphony SP to RT-qPCR step) |
| | INVALID: "UNEXPECTED_ VALUE" | INVALID: "UNEXPECTED_ VALUE" | INVALID: "UNEXPECTED_ VALUE" | Control invalid. Retest the complete workflow (i.e., from QIAsymphony SP to RT-qPCR step) |

**Table 6b. Summary of results interpretation – test sample analysis for influenza A, B, and H1**

| Result per target | Influenza A/Influenza A/H1 | Influenza B | Internal Control | Interpretation |
|---|---|---|---|---|
| Influenza A | Infl.A Detected | Infl.B Not Detected: "NO_VALUE" | IC Detected/ IC Not Detected "NO_VALUE"* | "Infl. A Detected" |
| | Infl.A Not Detected "NO_VALUE" | Infl.B Not Detected "NO_VALUE" | IC detected | "Infl. A Not Detected" |
| | INVALID "NO_VALUE" | INVALID" NO_VALUE" | INVALID "NO_VALUE" or signal "ABOVE_ACCEPTED_RANGE" | No result can be concluded (Extraction or RT-qPCR issues). Retest the complete workflow |
| Influenza B | Infl.A Not Detected "NO_VALUE" | Infl.B Detected | IC Detected/ IC Not Detected "NO_VALUE"* | "Infl. B Detected" |
| | Infl.A Not Detected "NO_VALUE" | Infl.B Not Detected "NO_VALUE" | IC Detected | "Infl. B Not Detected" |
| | INVALID "NO_VALUE" | INVALID "NO_VALUE" | INVALID "NO_VALUE" or signal "ABOVE_ACCEPTED_RANGE" | No result can be concluded (Extraction or RT-qPCR issues). Retest the complete workflow |
| Influenza H1 | Infl.H1 Detected | Not analyzed in Yellow channel | IC Detected / IC Not Detected "NO_VALUE"* | "Infl. H1 Detected" |
| | Infl.H1 Not Detected "NO_VALUE" | Not analyzed in Yellow channel | IC Detected | "Infl. H1 Not Detected" |
| | INVALID "NO_VALUE" | Not analyzed in Yellow channel | INVALID "NO_VALUE" or signal ABOVE_ACCEPTED_RANGE" | No result can be concluded (Extraction or RT-qPCR issues). Retest the complete workflow |

* The detection of Internal Control is dispensable in case of signal detected in Influenza A, Influenza H1 or Influenza B. The Internal Control signal can be lost due to competition with Influenza RNA (high initial concentration of Influenza RNA).

**Note**: In some cases, the competitive IC PCR may reduce the signal of the analytical influenza PCR.

If an invalid result is obtained, a retest should be performed (see Figure 20 on page 74).

Information regarding error sources and their solution can be found in "", page 96.

Curve examples of detected or not detected signals are given for influenza A/B viruses in Figure 17 and Figure 18 for influenza H1 virus in Figure 19.
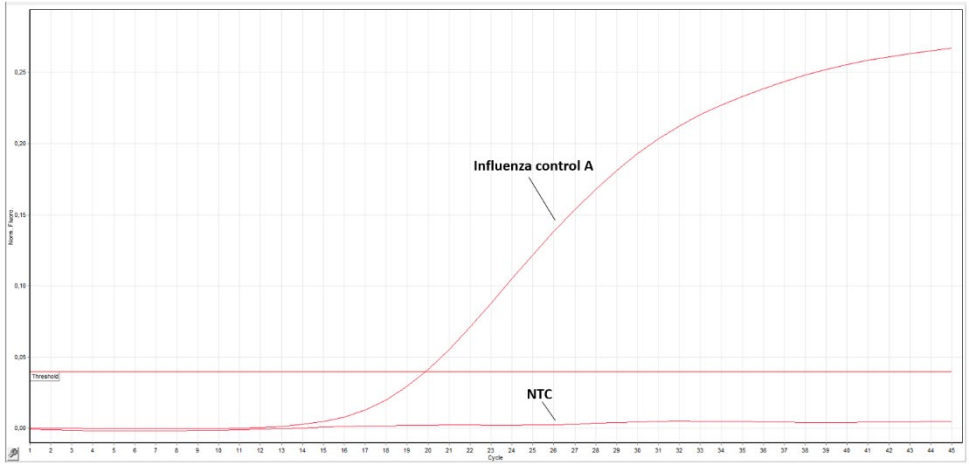
**Figure 17: Detection of the Influenza A Control in fluorescence channel Cycling Green. NTC: No template control (negative control).**
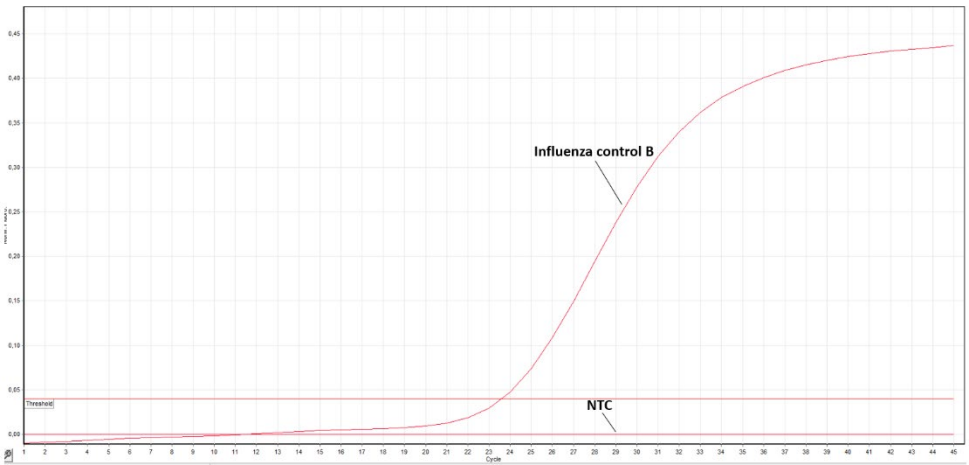


**Figure 18: Detection of the Influenza B Control in fluorescence channel Cycling Yellow. NTC: No template control (negative control).**
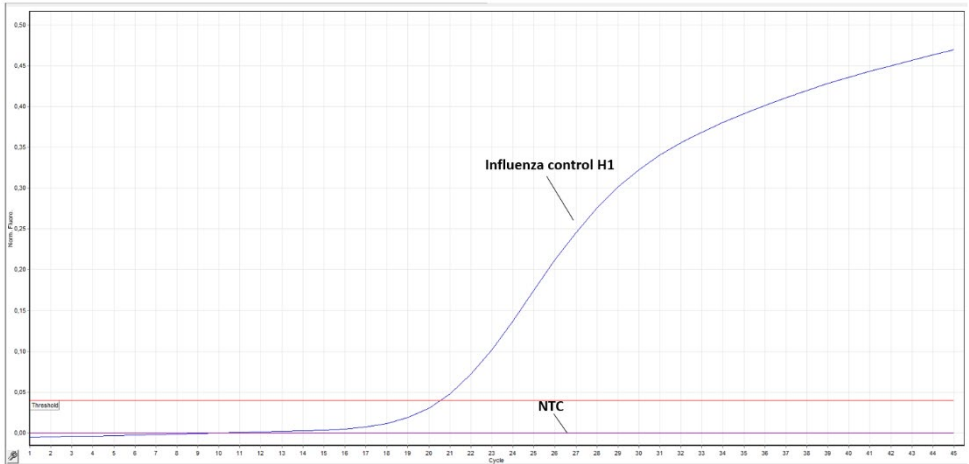
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**Figure 19: Detection of the Influenza H1 Control in fluorescence channel Cycling Green. NTC: No template control (negative control).**

### Assay status is FAILED for RT-qPCR run due to one or more invalid control(s)

If the control failure results from a fluorescence signal detected in the wrong channel (e.g., a signal detected in yellow channel for PC A, see Table 6), which will be flagged "UNEXPECTED_VALUE", the entire run including all tested samples should be retested and the entire workflow repeated (i.e. RNA extraction on QIAsymphony Sample preparation module, RT-qPCR plate preparation on QIAsymphony Assay Setup, and RT-qPCR run on Rotor-Gene Q MDx 5plex HRM Instrument).

All control failure(s) identified by another flag (such as "NO_VALUE" if the expected signal is not detected or "ABOVE_ACCEPTED_RANGE", if the signal detected is above the specification) will require the repeat of the workflow from the QIAsymphony Assay Setup to RT-qPCR run on Rotor-Gene Q MDx 5plex HRM Instrument.

## Assay status is SUCCESSFUL however, if one or several samples are displayed as INVALID

If the assay is valid but one or several samples are invalid, the relevant sample(s) should be retested after investigating the type of failure (see "Flags", page 78, and Table 8). Depending on the failure type, the plate eluate obtained at the first run could be reused (if other samples in the plate are valid indicating the extraction step is valid). A workflow for the retest procedure is shown in Figure 20, page 74.
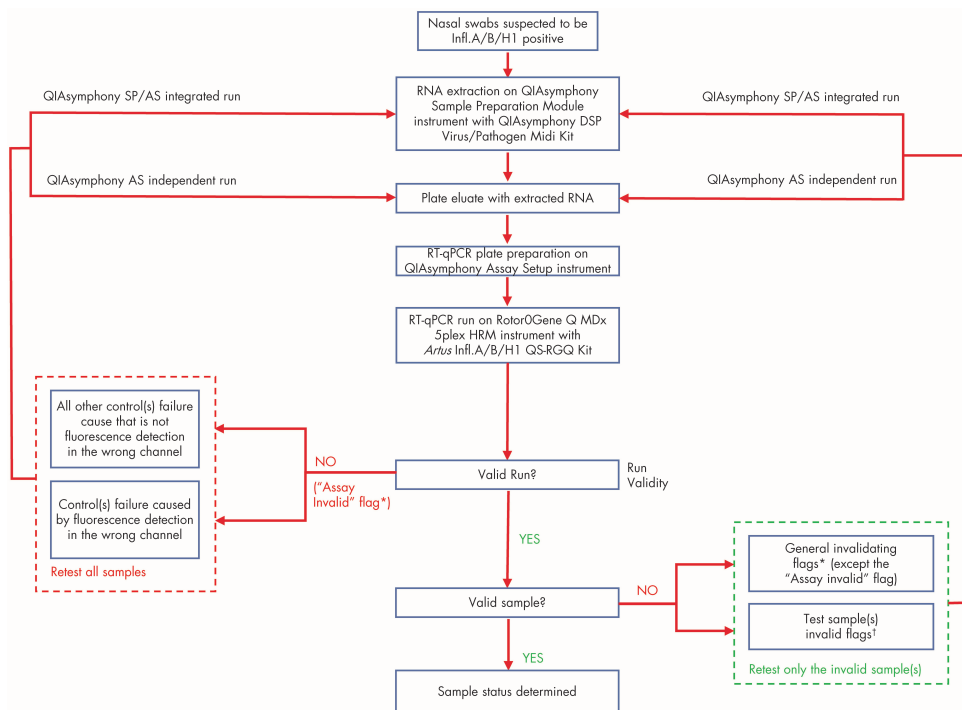


\* see Table 9 on page 80.
† see Table 8 on page 79).

**Figure 20. Retest workflow for artus Infl. A/B/H1 QS-RGQ Kit.**

## Result display

### Targets and combined targets

The result for each reaction of the *artus* Infl. A/B/H1 QS-RGQ Kit is displayed under the following target and combined target names.

<u>For AB assay profiles:</u>

- Influenza A: a signal should be detected in green channel for positive control PC A and for samples positive for influenza A.
- Influenza B: a signal should be detected in yellow channel for positive control PC B and for samples positive for influenza B.
- Internal control: A signal should be detected in orange channel for IC amplification only for negative samples. This signal may be not detected for positive samples in case of competition between IC and influenza RNA template. Thus, the absence of IC signal does not invalidate positive samples.

  **Note**: Controls do not contain the IC template and should not give any signal in orange channel.

<u>For H1 assay profiles:</u>

- Influenza H1: a signal should be detected in green channel for positive control PC H1 and for samples positive for influenza H1.
- Internal control: A signal should be detected in orange channel for IC amplification only for negative samples. This signal may be not detected for positive samples in case of competition between IC and influenza RNA template. Thus, the absence of IC signal does not invalidate positive samples.

  **Note**: Controls do not contain the IC template and should not give any signal in orange channel.

## Overall sample results

The conclusion of the analysis for each control and sample is displayed in the "Result" column of the report. For Infl.A/H1 status, refer to Figure 21a, Figure 21b, and Figure 21c.

Table 7. Results and actions for controls

| Sample result | Sample type | Description | Action |
|---|---|---|---|
| Valid | PC A, PC B, PC H1, NTC, test sample | Control or test sample is valid | - |
| Invalid | PC A, PC B, PC H1, NTC | Control is invalid | Repeat the entire run. |
| Invalid | Test sample | Test sample is invalid | Launch a new run to repeat the invalid sample (refer to Figure 21). |
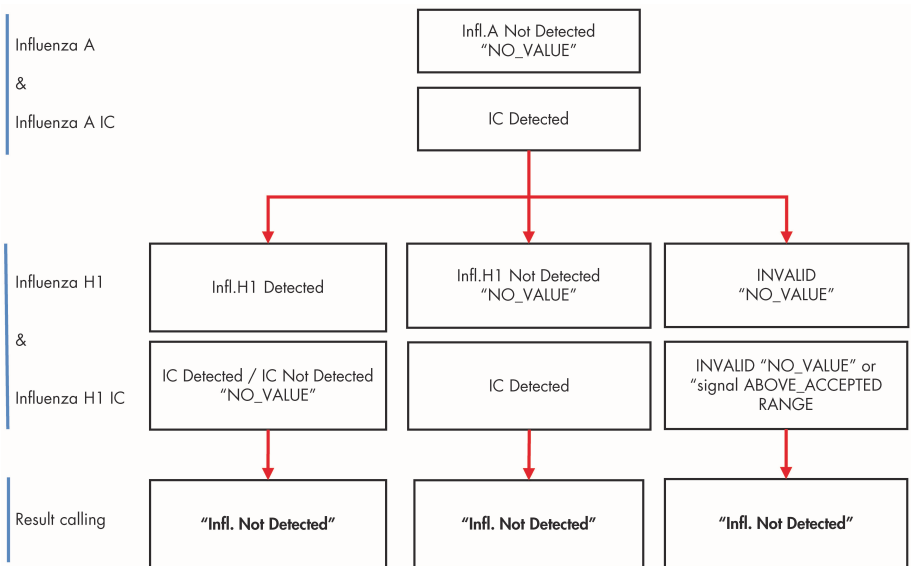


Figure 21a: Results and actions for test samples - combined rules for influenza A/H1 status: "Infl.A Not Detected"
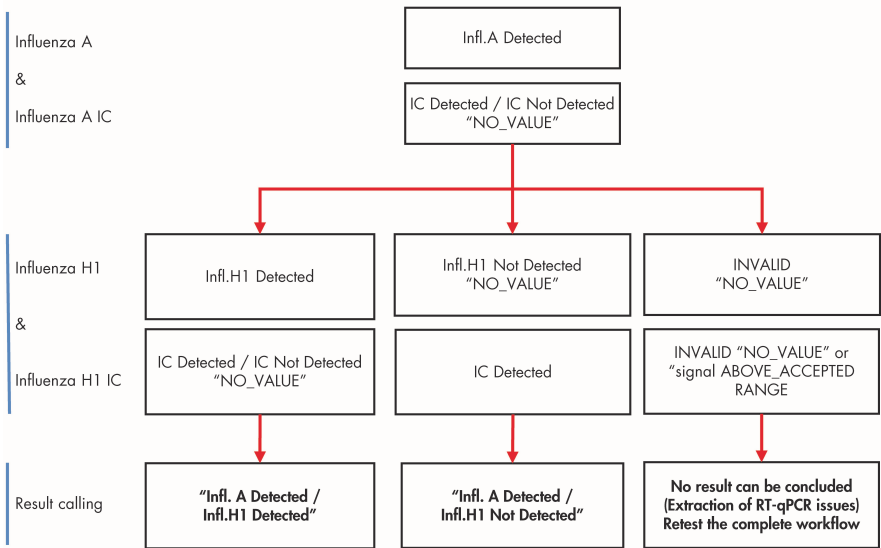
**Figure 22b: Results and actions for test samples - combined rules for influenza A/H1 status: "Infl.A Detected"**
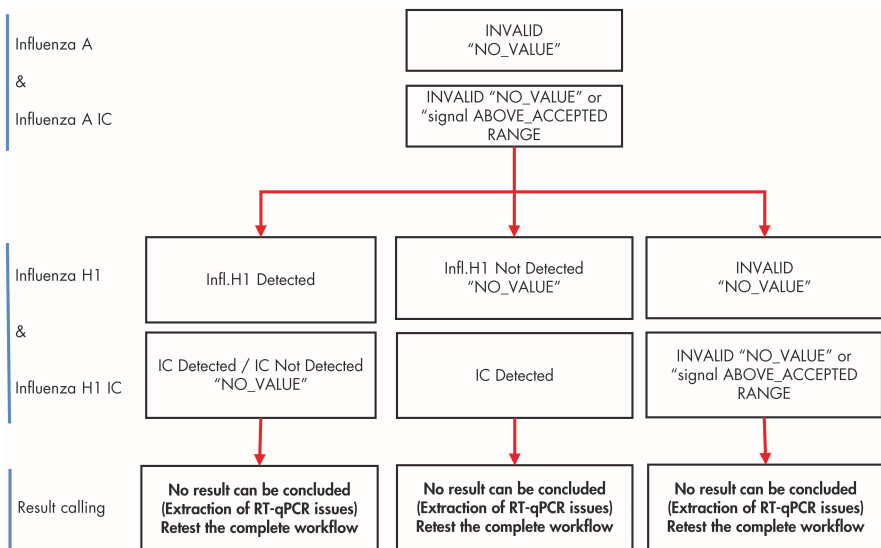


**Figure 23c: Results and actions for test samples - combined rules for influenza A/H1 status: "Infl.A Invalid"**

## Flags

Two types of flags can be displayed to give additional information on the sample results:

- **Warning flags** highlight unproblematic anomalies and do not invalidate results.
- **Invalidating flags** invalidate results and give the failure cause.

The automated analysis of the *artus* Infl. A/B/H1 QS-RGQ Kit assay may provide the following **assay-specific** (Table 8, page 79) and **general** flags (Table 9, page 80).

**Note**: For general flags included in the Epsilon Plug-in, also refer to the *Rotor-Gene AssayManager Epsilon Plug-in User Manual*.

**Table 8. Assay specific flags**

| Assay-specific flag | Sample type | Target | Description | Action |
|---|---|---|---|---|
| **Assay specific flag for controls** | | | | |
| ABOVE_ACCEPTED_RANGE | PC A, PC B, PC H1 | Influenza A, influenza B, influenza H1 | The Ct value result is above the accepted range. | Repeat the workflow from independent QIAsymphony AS to RT-qPCR. |
| NO_VALUE | PC A, PC B, PC H1 | Influenza A, influenza B, influenza H1 | No Ct value is detected in the specific target. | Repeat the workflow from independent QIAsymphony AS to RT-qPCR. |
| UNEXPECTED_VALUE | PC A, PC B, PC H1 | Influenza A, influenza B, influenza H1 | The target had a value but it is not expected to have one. | Repeat the workflow from independent QIAsymphony SP to RT-qPCR. |
| **Assay specific flags for test samples** | | | | |
| NO_VALUE | Test sample | Influenza A and influenza B and Internal control or influenza H1 and Internal control | No Ct value is detected. | Repeat the samples from QIAsymphony sample preparation to RT-qPCR. |
| ABOVE_ACCEPTED_RANGE | Test sample | Internal control | The Ct value result is above the accepted range. | Repeat the sample from QIAsymphony Sample preparation to RT-qPCR if no value is obtained in influenza A or influenza B or influenza H1. |

**Table 9. General flags**

| General Flag | Behavior | Description | Action |
|---|---|---|---|
| ASSAY_INVALID° | Invalid | The assay is invalid because at least one external control is invalid. | Repeat the workflow from independent QIAsymphony AS to RT-qPCR. |
| IC_NO_SIGNAL° | Invalid | No internal control signal detected. | Repeat the sample from QIAsymphony Sample preparation to RT-qPCR. |
| LOW_FLUORESCENCE_CHANGE* | Warning | The percentage fluorescence change for this sample relative to the sample tube with the largest fluorescence change is lower than a defined limit. | None |
| MULTIPLE_THRESHOLD_CROSSING* | Invalid | The amplification curve crosses the threshold more than once. An unambiguous Ct-value cannot be determined. | Repeat the sample from QIAsymphony Sample preparation to RT-qPCR or repeat the run from independent QIAsymphony AS if the flag is associated to a control. |
| OTHER_TARGET_INVALID° | Invalid | Another target for the same sample is invalid. | Repeat the sample from QIAsymphony Sample preparation to RT-qPCR on Rotor-Gene Q MDx 5plex HRM. |
| UPSTREAM | Variable | Sample status was set to "Invalid" or "Unclear" by an upstream process (e.g., QIAsymphony). **Note**: For sample that are flagged as unclear, the behavior of Rotor-Gene AssayManager is defined in the "Configuration" environment of the AssayManager software. "Invalid" flags from upstream processes always result in an invalid corresponding sample in Rotor-Gene AssayManager. | Unclear samples can be interpreted. For invalid samples, Repeat the sample from QIAsymphony Sample preparation to RT-qPCR on Rotor-Gene Q MDx 5plex HRM. |

\* Flags linked to a core analysis °Flags denote assay and sample analysis.

# Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of *artus* Infl. A/B/H1 QS-RGQ Kit is tested against predetermined specifications to ensure consistent product quality.

# Limitations

- The product is to be used by personnel specially instructed and trained in the in vitro diagnostics procedures only.
- The *artus* Infl. A/B/H1 QS-RGQ Kit is to be used by laboratory professionals trained in the use of the QIAsymphony RGQ system, Rotor-Gene Q MDx 5plex HRM and Rotor-Gene AssayManager software.
- Any diagnostic results that are generated must be interpreted in conjunction with other clinical or laboratory findings.
- Strict compliance with the user manual(s) is required for optimal RT-qPCR results.
- Attention should be paid to expiration dates printed on the box and labels of all components. Do not use expired components.
- Improper collection, storage, or transport may lead to false negative results.
- Inhibitors present in the sample and/or errors in following the assay procedure may lead to false-negative result.
- Although rare, mutations within the highly conserved regions of the influenza A, B, or A/H1N1 genome covered by the kit's primers and/or probe may result in failure to detect the presence of the influenza A, B, or A/H1N1 in these cases. Validity and performance of the assay design are revised at regular intervals.

# Performance Characteristics

## Limit of blank

The limit of blank (LOB) is defined as the highest measurement result that is likely to be observed for a blank sample. In the case of the *artus* Infl. A/B/H1 QS-RGQ Kit, an appropriate parameter to analyze for the LOB is the cycle threshold (Ct) value in the Test Channel. The Ct value of negative samples should remain above a threshold value (45) to generate a result "not detected".

Only negative patient specimens (i.e., human nasal swabs) were used to determine the LOB of each influenza assay (i.e., with AB and H1 Master). RNA purification was performed with two QIAsymphony Sample Preparation (QS-SP) modules using a single batch of QIAsymphony DSP Virus/Pathogen Midi Kit. The RT-qPCR plates were set-up with a single QS-AS module using two reagent lots of *artus* Infl. A/B/H1 QS-RGQ Kit. A separate LOB was established for influenza A and influenza B on 61 measurements across two reagent lots. For influenza A-H1, the LOB was established on 67 measurements across 2 reagent lots.

**Table 10. Limit of blank (LOB) results**

| Influenza target | Number of measurements | LOB value |
|---|---|---|
| Influenza A | 61 | 45 |
| Influenza B | 61 | 45 |
| Influenza A-H1 | 67 | 45 |

## Limit of detection

The limit of detection (LOD) is defined as the lowest amount of analyte in a sample that can be detected with a probability of 95%. For *artus* Infl. A/B/H1 QS-RGQ Kit, the LOD was established for the three targeted analytes in the kit (i.e., influenza A, influenza B and influenza A/H1pdm2009). The LOD was determined in 50% tissue culture infective dose ($TCID_{50}$)/ml.

### Influenza A assay limit of detection

For this assay, the LOD was determined using a statistical method (unilateral test). Influenza A/H3N2 (A/Marseille/90454111/2011) and influenza A/H1N1 (A/Marseille/4590681204/2014 [season 2013-2014]) strains were diluted at the LOD and tested in 66 to 72 replicates with three pilot batches. The sample extractions were performed in the QIAsymphony Sample preparation module using one batch of QIAsymphony DSP Virus/Pathogen Midi Kit. The RT-qPCR plate preparation was performed in the QIAsymphony Assay Setup module.

### Influenza B and H1pdm2009 assays limit of detection

Two viral strain were used, i.e. influenza B/Yamagata (B/Marseille/74506131/2013) and influenza A/H1N1 (A/Marseille/4590681204/2014 [season 2013-2014]). For each viral strain, five dilutions points were prepared. For each dilution point, 60 measurements were generated across 17 days. One LOD was determined for each of these strains. The sample extractions were performed in the QIAsymphony Sample preparation module using one batch of QIAsymphony DSP Virus/Pathogen Midi Kit. The RT-qPCR plate preparation was performed in the QIAsymphony Assay Setup module and using two batches of *artus* Infl. A/B/H1 QS-RGQ Kit. A probit regression was performed to determine the LOD for each strain.

**Table 11. Limit of detection (LOD) in 50% tissue culture infective dose (TCID$_{50}$/ml)**

| Master | Influenza virus strain | LOD in TCID$_{50}$/ml |
|--------|------------------------|------------------------|
|        | A/H3N2                 | $3.82 \times 10^{-2}$  |
| AB     | A/H1N1                 | $1.75 \times 10^{-2}$  |
|        | B                      | $2.16 \times 10^{-2}$  |
| H1     | A/H1N1                 | $3.97 \times 10^{-4}$  |

## Cross-reactivity and reactivity

The analytical cross-reactivity of the *artus* Infl. A/B/H1 QS-RGQ Kit was evaluated by testing a panel of 40 commonly co-infected pathogens (Table 12) at clinically relevant concentrations. The analytical reactivity of the *artus* Infl. A/B/H1 QS-RGQ Kit was assessed against 11 influenza A strains and 9 influenza B strains (Table 13).

This study demonstrated no evidence of cross-reactivity and confirmed the reactivity for all 20 Influenza strains tested.

**Table 12. Pathogens tested in the cross-reactivity study**

| Pathogen | Influenza A | Influenza B | Influenza H1 |
|---|---|---|---|
| Respiratory syncytial virus (RSV) A | - | - | - |
| RSV B | - | - | - |
| Parainfluenza-1 | - | - | - |
| Parainfluenza-2 | - | - | - |
| Parainfluenza-3 | - | - | - |
| Parainfluenza-4 | - | - | - |
| Human metapneumovirus (hMPV) | - | - | - |
| Coxsackievirus (CVA21) | - | - | - |
| Coxsackievirus (CVB4) | - | - | - |
| Echovirus | - | - | - |
| Rhinovirus | - | - | - |
| Adenovirus A | - | - | - |
| Adenovirus E | - | - | - |
| Coronavirus 229E | - | - | - |
| Coronavirus OC43 | - | - | - |
| Enterovirus 71 | - | - | - |
| *Bordetella pertussis* | - | - | - |
| *Bordetella parapertussis* | - | - | - |
| *Mycobacterium pneumoniae* | - | - | - |
| *Chlamydophilia pneumoniae* | - | - | - |
| *Legionella pneumophila* | - | - | - |
| *Mycobacterium tuberculosis* (MTB) | - | - | - |
| Methicillin-resistant *Staphylococcus aureus* (MRSA) | - | - | - |
| Methicillin-sensitive *Staphylococcus aureus* (MSSA) | - | - | - |
| Mumps virus | - | - | - |
| Adenovirus type 1 | - | - | - |
| Cytomegalovirus | - | - | - |
| Epstein Barr Virus | - | - | - |
| Measles virus | - | - | - |

Cont.

**Table 12. continued**

| Pathogen | Influenza A | Influenza B | Influenza H1 |
|---|---|---|---|
| *Escherichia coli* | - | - | - |
| *Neisseria* species | - | - | - |
| *Staphylococcus epidermidis* | - | - | - |
| *Streptococcus pneumonia* | - | - | - |
| *Pseudomonas aeruginosa* | - | - | - |
| *Streptococcus pyogenes* | - | - | - |
| *Streptococcus salivarius* | - | - | - |
| *Haemophilus influenza* | - | - | - |
| *Lactobacillus sp.* | - | - | - |
| *Corynebacterium sp.* | - | - | Not tested |
| *Neisseria meningitidis* | - | - | - |

**Table 13. Influenza strains tested in the cross-reactivity study**

| Influenza strain | Influenza A | Influenza B | Influenza H1 |
|---|---|---|---|
| Infl. A H3N2, A/Hong Kong/8/68 | + | - | - |
| Infl. A H3N2, A/Victoria/3/75 | + | - | - |
| Infl. A H1N1, A/WS/22 | + | - | - |
| Infl. A H1N1, A/PR/8/34 | + | - | - |
| Infl. A H1N1, A/Denver/1/75 | + | - | - |
| Infl. A/Marseille/90454111/2011 (H3N2 season 2011) | + | - | - |
| Infl. A/Marseille/4590681204/2014 (H1N1 season 2013-2014) | + | - | + |
| Infl. Virus Infectious NIB-26 (H3N2) | + | - | - |
| Infl. Virus Infectious NYMC X-161B (A/Wisconsin/67/2005) | + | - | - |
| Infl. Virus Infectious A/Hong Kong/4801/2014 (H3N2) | + | - | - |
| Infl. Virus Infectious A/California/7/2009 (H1N1) | + | - | + |
| Infl. B, B/Lee/40 | - | + | - |

Cont.

**Table 13. continued**

| Influenza strain | Influenza A | Influenza B | Influenza H1 |
|---|---|---|---|
| Infl. B, B/Taiwan/2/62 | - | + | - |
| Infl. B, B/Maryland/1/59 | - | + | - |
| Infl. Virus Infectious B/Brisbane/60/2008 (Victoria) | - | + | Not tested |
| Infl. Virus Infectious B/ Malaysia/2506/2004 (Victoria) | - | + | Not tested |
| Infl. Virus Infectious B/Victoria/504/00 | - | + | Not tested |
| Infl. B/Marseille/74506131/2013 (Yamataga) | - | + | Not tested |
| Influenza Virus Infectious B/Phuket/3073/2013 (Yamataga) | - | + | Not tested |
| Infl. Virus Infectious B/Utha/9/2014 (Yamataga) | - | + | Not tested |

## Carryover and cross-contamination

The study was conducted on contrived samples (i.e., influenza strain* diluted in virocult for the positive sample and virocult only for negative sample). The positive sample was tested at the highest viral load found in the intended population as exceptional and rare cases, i.e., $1 \times 10^{10}$cp/ml (1 –3).

Cross-contamination was defined as the amount of analyte carried over between adjacent wells during automated runs and was calculated as:

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

* The cross-contamination and carryover were assessed by testing the Influenza A/Marseille/58863121/2012 (H1N1) strain with Influenza AB and H1 Master.

The positive samples were tested alternating with negative replicates over four consecutive runs. The cross-contamination rate was calculated (as above) and the results are shown in Table 14 on page 88. A final run testing only negative samples was performed after testing the 135 highly-positive influenza A-H1 samples (at $1 \times 10^{10}$ cp/ml), demonstrating no evidence of carryover (Table 15 on page 88).

**Table 14. Percentage cross-contamination rate**

| Influenza target | Sample | Frequency of contamination | Proportion of intra-run contamination rate (%) |
|---|---|---|---|
| Influenza AB Master | Negative | 0/136 | 0.00 |
| Influenza H1 Master | Negative | 0/136 | 0.00 |

**Table 15. Percentage carryover rate**

| Influenza target | Sample | Frequency of contamination | Intra-run contamination rate (%) |
|---|---|---|---|
| Influenza AB Master | Negative | 0/69 | 0.00 |
| Influenza H1 Master | Negative | 0/69 | 0.00 |

## Repeatability and reproducibility

Repeatability and reproducibility of the *artus* Infl. A/B/H1 QS-RGQ Kit were determined using single-site and a multi-site studies. These studies were performed on two viral strains, Infl. B/Yamagata (B/Marseille/74506131/2013) and Infl. A/H1N1 (A/Marseille/4590681204/ 2014 [season 2013-2014]). These strains were diluted to generate a low and a high concentration of the influenza virus. A negative sample was also included in the assay run.

In the single-site precision test, the source of variability was assessed on 12 non-consecutive working days using three different pilot batches of the *artus* Infl. A/B/H1 QS-RGQ Kit, one

QIAsymphony Sample Preparation (QS-SP) module, one QIAsymphony Assay Setup (QS-AS) module, and three Rotor-gene Q MDx instruments. Two identical runs per day were performed and three measurements per sample were obtained for each run.

The multi-site study was conducted at three different sites where one batch of *artus* Infl. A/B/H1 QS-RGQ Kit was used. At each site, one QS-SP module, one QS-AS module, and two Rotor-Gene Q instruments (one per master) were used. Five valid runs were performed per site with five measurements per sample per run. One run per day was performed at each site, with runs alternating between morning and afternoon.

For the single-site study, the sample status was compared between Rotor-Gene Q instruments and *artus* Infl. A/B/H1 QS-RGQ Kit batches. No Rotor-Gene Q instrument nor batch effect was observed except for assay A of master AB where a batch effect was observed when the sample was at a low concentration.

For multi-site study, the sample status was compared between QS-SP-AS modules and site. No QS-SP-AS modules and site effect was observed.

## Endogenous and exogenous interfering substances

The interfering substance study was conducted in accordance with CLSI guidelines EP07-A2 (3). Two viral strains, i.e., influenza B and influenza A, and one negative sample were used to test all assays in the *artus* Infl. A/B/H1 QS-RGQ Kit (A, B and IC assays of AB Master and H1 and IC assays of H1 Master). Two potential interfering endogenous substances and nine potential exogenous interfering substances were tested at or above concentrations likely to be found in patient samples (see Table 16). The non-interfering concentrations of the substances tested using the *artus* Infl. A/B/H1 QS-RGQ Kit are presented in Table 16.

**Table 16. Non-interfering concentrations of tested endogenous and exogenous substances**

| Tested substances | | Active substance | Non-interfering concentration |
|---|---|---|---|
| Endogenous substances | Mucin | Mucin protein | 5 mg/ml |
| | Blood | Blood | 5% (v/v) |
| Exogenous substances | Aturgyl® | Oxymetazoline | 5% (v/v) |
| | Neo-synephrine® | Phenylephrine | 5% (v/v) |
| | Avamys® | Fluticasone | 5% (v/v) |
| | Zicam® | *Glaphimia glauca*, sulfur, *Luffa operculata* | 4% (v/v) |
| | Saline nasal spray | Sodium chloride with preservatives | 5% (v/v) |
| | Throat lozenges, oral anesthetic and analgesic | Menthol | 5 mg/ml |
| | Tamiflu® | Oseltamivir | 0.65 mg/ml |
| | Mupiderm® | Muciprocin | 5 mg/ml |
| | Antibacterial systemic | Tobramycin | 4 µg/ml |

## Eluate stability

The eluate (extracted RNA) stability was assessed using RNA extracted from five different contrived samples (influenza strains diluted in virocult). This included two positive samples for influenza A, two positive samples for influenza B and one negative sample. Positive samples were prepared using virus at high and low concentrations. Eluate plates were tested after storage at +4/+8°C and −30/−15°C using three artus Infl. A/B/H1 QS-RGQ Kit batches with influenza AB and H1 Masters.

A qualitative analysis was performed between tested time point 0 (TTP$_0$; date of extraction) and the time of the test condition (TTP$_n$). The study demonstrated that RNA extracted from influenza strains is stable at:

- +4 to +8°C for at least 48 hours
- −30 to −15°C for at least 7 days

## Performance characteristics evaluation

The evaluation of qualitative test performance characteristics for the *artus* Infl. A/B/H1 QS-RGQ Kit was designed following the recommendations given in EP12-A2, CLSI (4).

A total of 202 human nasal swab specimens were tested with the *artus* Infl. A/B/H1 QS-RGQ Kit and the results compared with those obtained previously using laboratory developed tests derived from published methods (5) at the University of Marseille, France. RNA was extracted on the QIAsymphony SP using a single batch of QIAsymphony DSP Virus/Pathogen Midi Kit. The RT-qPCR plates were set-up with a single QS-AS module using one reagent lot of the *artus* Infl. A/B/H1 QS-RGQ Kit. Discrepant results were resolved by testing samples with a third method, GeneXpert Xpert® Xpress Flu/RSV (Cepheid) according to protocols at the University of Marseille, France.

The percent positive agreement (PPA; Table 17, page 92), the percent negative agreement (PNA; Table 18, page 92) and the overall percent agreement (OPA; Table 19, page 92) were calculated. The results obtained showed the *artus* Infl. A/B/H1 QS-RGQ Kit agrees with the methods used for comparison at 97.5% for Influenza AB Master and at 100% for Influenza H1 Master (OPA).

**Table 17. Final percent positive agreement (PPA)**

| Percent agreement | Analysis | True positive | False negative | Estimate | 95% confidence interval |
|---|---|---|---|---|---|
| PPA | Influenza AB Master | 158 | 3* | 0.981 | 0.947, 0.996 |
| PPA | Influenza A target | 107 | 0 | 1.00 | 0.966, 1.000 |
| PPA | Influenza B target | 51 | 3* | 0.944 | 0.846, 0.988 |
| PPA | Influenza H1 Master | 57 | 0 | 1.00 | 0.937, 1.000 |

\* Discrepancies in results that could not be resolved as there was insufficient sample volumes available to re-test the sample using the third method.

**Table 18. Final percent negative agreement (PNA)**

| Percent agreement | Analysis | True negative | False positive | Estimate | 95% confidence interval |
|---|---|---|---|---|---|
| PNA | Influenza AB Master | 39 | 2 | 0.951 | 0.835; 0.994 |
| PNA | Influenza A target | 93 | 2 | 0,979 | 0.926; 0.997 |
| PNA | Influenza B target | 148 | 0 | 1.00 | 0.975; 1.000 |
| PNA | Influenza H1 Master | 54 | 0 | 1.00 | 0.934; 1.000 |

**Table 19. Final overall percent agreement (OPA)**

| Percent agreement | Analysis | True positive | True negative | n | Estimate | 95% confidence interval |
|---|---|---|---|---|---|---|
| OPA | Influenza AB Master | 158 | 39 | 202 | 0.975 | 0.943; 0.992 |
| OPA | Influenza A target | 107 | 93 | 202 | 0,990 | 0.965; 0.999 |
| OPA | Influenza B target | 51 | 148 | 202 | 0.985 | 0.957; 0.997 |
| OPA | Influenza H1 Master | 57 | 54 | 111 | 1.00 | 0.967; 1.000 |

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# References

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3. Clinical and Laboratory Standards Institute (CLSI) (2005) Interference Testing in Clinical Chemistry: Approved Guideline, 2nd ed. CLSI Document EP07-A2. Wayne, PA: Clinical and Laboratory Standards Institute (formerly NCCLS).

4. Clinical and Laboratory Standards Institute (CLSI) (2008) User Protocol for Evaluation of Qualitative Test Performances: Approved Guideline, 2nd ed. CLSI Document EP12-A2. Wayne, PA: Clinical and Laboratory Standards Institute (formerly NCCLS).

5. World Health Organization (July 2017). WHO information for the molecular detection of influenza viruses. Available at:
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# Symbols

The following symbols may appear on the packaging and labeling:

| Symbol | Symbol definition |
|--------|-------------------|
| ▽Σ < N> | Contains sufficient for <N> tests |
| ⧗ | Use by |
| IVD | In vitro diagnostic medical device |
| CE | CE mark for European conformity |
| REF | Catalog number |
| LOT | Lot number |
| MAT | Material number |
| COMP | Components |
| CONT | Contains |
| MASTER | Master mix |
| MG-SOL | Magnesium solution |

| Symbol | Symbol definition |
|--------|-------------------|
| **IC** | Internal control |
| **CONTROL +** | Positive Control |
| **CONTROL −** | No Template Control |
| **NUM** | Number |
| **Rn** | R is for revision of the Instructions for Use (Handbook) and n is the revision number |
| **GTIN** | Global Trade Item Number |
| (thermometer symbol) | Temperature limitation |
| (manufacturer symbol) | Manufacturer |
| (book symbol with i) | Consult instructions for use |
| (caution triangle symbol) | 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. |
|---------|----------|----------|
| ***artus* Infl. A/B/H1 QS-RGQ Kit** | | |
| *artus* Infl. A/B/H1 QS-RGQ Kit (72) | For 72 reactions: 2 Masters (Influenza AB and Influenza H1), Mg Solution, 3 Positive Controls (Influenza A, Influenza B and Influenza H1), 1 Internal Control, Water (PCR grade) | 4523366 |
| **QIAsymphony RGQ system and accessories** | | |
| QIAsymphony RGQ system | QIAsymphony SP, QIAsymphony AS, Rotor-Gene Q 5plex HRM; includes required accessories and consumables, installation, and training; includes 1-year warranty on parts and labor | 9001850 |
| QIAsymphony SP | QIAsymphony sample preparation module: includes 1-year warranty on parts and labor | 9001297 |
| QIAsymphony AS | QIAsymphony sample preparation module: includes 1-year warranty on parts and labor | 9001301 |
| **Adapters, consumables and reagents for QIAsymphony SP** | | |
| QIAsymphony DSP Virus/Pathogen Midi Kit | For 192 preps (200 µl each): includes 2 reagent cartridges and enzyme racks and accessories | 937055 |
| Cooling Adapter, EMT, v2, Qsym | Cooling adapter for EMT racks; for use with the QIAsymphony SP/AS instruments (software version 3.1 or higher) | 9020730 |
| Insert, 2.0 ml v2, samplecarr. (24), Qsym | Secondary tube adapter (for 2 ml screw-cap tubes, tube insert 3b) for use with the QIAsymphony SP tube carrier | 9242083 |

| Product | Contents | Cat. no. |
|---------|----------|----------|
| Buffer ATL (4 x 50 ml) | 4 x 50 ml lysis buffer for use in purification of nucleic acids using QIAsymphony DSP Virus/Pathogen Kits and the QIAsymphony DSP DNA Mini Kit | 939016 |
| Sample Prep Cartridges, 8-well (336) | 8-well sample prep cartridges for use with the QIAsymphony SP | 997002 |
| 8-Rod Covers (144) | 8-Rod Covers for use with the QIAsymphony SP | 997004 |
| Filter-Tips, 1500 µl (1024) | Disposable Filter-Tips, racked; (8 x 128). For use with the QIAsymphony SP/AS instruments | 997024 |
| Filter-Tips, 200 µl (1024) | Disposable Filter-Tips, racked; (8 x 128). For use with the QIAcube and the QIAsymphony SP/AS instruments | 990332 |
| Elution Microtubes CL (24 x 96) | Nonsterile polypropylene tubes (0.85 ml maximum capacity, less than 0.7 ml storage capacity, 0.4 ml elution capacity); 2304 in racks of 96; includes cap strips | 19588 |
| Tip disposal bags (15) | Tip disposal bags for use with the QIAsymphony SP/AS instruments | 9013395 |
| Cooling Adapter, Reagent Holder 1, Qsym | Adapter for holding 18 x 2 ml conical tubes, and 6 x 5 ml conical tubes; for use with the QIAsymphony AS (software version 3.1 or higher) | 9018090 |
| Cooling Adapter, RG Strip Tubes 72, Qsym | Adapter for holding 18 strips of 4 tubes; for use with the QIAsymphony AS (software version 3.1 or higher) | 9018092 |
| Strip Tubes and Caps, 0.1 ml (250) | 250 strips of 4 tubes and caps for 1000 reactions of 10–50 µl | 981103 |

| Product | Contents | Cat. no. |
|---------|----------|----------|
| Tubes, conical, 2 ml, Qsym AS (500) | Conical tubes (2 ml) for holding reagent; for use with the QIAsymphony AS | 997102 |
| Tubes, conical, 5 ml, Qsym AS (500) Microtube Cl rack that has already been used | Conical tubes (5 ml) for holding reagent; for use with the QIAsymphony AS | 997104 |
| Filter-Tips, 1500 μl (1024) | Disposable Filter-Tips, racked; (8 x 128). For use with the QIAsymphony SP/AS instruments | 997024 |
| Filter-Tips, 200 μl (1024) | Disposable Filter-Tips, racked; (8 x 128). For use with the QIAcube and the QIAsymphony SP/AS instruments | 990332 |
| Filter-Tips, 50 μl, Qsym AS (1024) | Sterile, Disposable Filter-Tips, racked; (8 x 128); for use with the QIAsymphony AS | 997120 |
| Tip disposal bags (15) | Tip disposal bags for use with the QIAsymphony SP/AS instruments | 9013395 |
| **Rotor-Gene Q MDx 5plex HRM System and accessories** | | |
| Rotor-Gene Q MDx 5plex HRM Platform | Real-time PCR cycler and HRM analyzer with 5 channels (green, yellow, orange, red, crimson) plus HRM channel, laptop computer, software, accessories: includes 1-year warranty on parts and labor, installation and training not included | 9002032 |
| Rotor-Gene Q MDx 5plex HRM System | Real-time PCR cycler and HRM analyzer with 5 channels (green, yellow, orange, red, crimson) plus HRM channel, laptop computer, software, accessories: includes 1-year warranty on parts and labor, installation and training | 9002033 |

| Product | Contents | Cat. no. |
|---------|----------|----------|
| 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 |
| Loading Block 72 x 0.1 ml Tubes | Aluminum block for manual reaction setup with a single-channel pipet in 72 x 0.1 ml tubes | 9018901 |
| 72-Well Rotor | For holding Strip Tubes and Caps 0.1 ml; requires Locking Ring 72 Well Rotor | 9018903 |
| Locking Ring 72-Well Rotor | For locking Strip Tubes and Caps, 0.1 ml in the 72-Well Rotor | 9018904 |
| Rotor Holder | Metal free-standing holder for assembling tubes and Rotor-Discs® into rotors | 9018908 |
| Strip Tubes and Caps, 0.1 ml (250) | 250 strips of 4 tubes and caps for 1000 reactions | 981103 |
| Strip Tubes and Caps, 0.1 ml (2500) | 10 x 250 strips of 4 tubes and caps for 10,000 reactions | 981106 |

# Document Revision History

| Date | Changes |
|------|---------|
| R2<br>12/2019 | • Addition of co-cycling of AB master and H1 master workflow<br><br>• Update of Assay Profile and Bioscripts with improvements in extraction methodology and update of PCA Target and IC specifications in AB Master and Internal control specification in H1 Master<br><br>• Update of LOB data points due to additional data from Assay Profile update<br><br>• Update of LOD claim for Infl. A/H1N1 in Master H1 due to additional data points post re-analysis with updated assay profile<br><br>• Update of pathogens in Cross-Reactivity data – inclusion of *Neisseria meningitides* in H1 master<br><br>• Update of Cross Contamination data with new extraction improvement<br><br>• Reagent Handling and Storage updated to include Master AB and H1 components<br><br>• Addition of results calling table for Infl. A/H1N1pdm2009<br><br>• Minor adjustment to the non-interfering concentrations of Avamys and Tamiflu |

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**Notes**