October 2015

artus® M. tuberculosis RG PCR Kit Handbook

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Version 1

Quantitative in vitro diagnostics

For use with Rotor-Gene® Q instruments

IVD

CE

REF 4555263 (24 reactions)
4555265 (96 reactions)

QIAGEN GmbH
QIAGEN Strasse 1
40724 Hilden
GERMANY

R5 MAT 1046960EN

Sample to Insight

QIAGEN

Contents

Intended Use

The *artus* M. tuberculosis RG PCR Kit is an in vitro nucleic acid amplification test for the detection of all members of the *M. tuberculosis* complex (*M. tuberculosis*, *M. africanum*, *M. bovis*, *M. bovis* BCG, *M. microti*, *M. pinnipedii*) in human sputum, BAL, bronchial secretion, CSF, stomach fluid or peritoneal punction samples. This diagnostic test kit utilizes the polymerase chain reaction (PCR) and is configured for use with Rotor-Gene Q instruments.

Summary and Explanation

Tuberculosis (TB) is still one of the most important infectious diseases worldwide. Some two billion people, one-third of the world's population, are infected with *Mycobacterium tuberculosis*, the causative agent of TB. The incidence of TB worldwide is about 8 million and about 3 million people die each year. TB is a reemerging disease in industrialized nations, mainly due to the immigration of infected people and the development of drug resistant TB. Homeless people, drug users and immunocompromised persons are affected disproportionately by the disease.

TB is a chronic, cyclic disease, mainly affecting the lung and the associated lymph nodes. However, depending on the immune status of the patient, the *M. tuberculosis* bacteria can also colonize other organs. TB is primarily transmitted from person to person via aerosols. Only people with active disease are contagious. Especially in immunosuppressed people, *M. tuberculosis* bacteria can be reactivated (recrudescent) even years after the initial infection.

Principle of the Procedure

Pathogen diagnosis by the polymerase chain reaction (PCR) is based on the amplification of specific regions of the pathogen genome. In real time PCR the amplified product is detected via fluorescent dyes. These are usually linked to oligonucleotide probes which bind specifically to the amplified product. Monitoring the fluorescence intensities during the PCR run (i.e. in real time) allows the detection and quantitation of the accumulating product without having to reopen the reaction tubes after the PCR run (1).

The *artus* M. tuberculosis RG PCR Kit constitutes a ready-to-use system for the detection of all members of the *M. tuberculosis* complex (*M. tuberculosis*, *M. africanum*, *M. bovis*, *M. bovis* BCG, *M. microti*, *M. pinnipedii*) using polymerase chain reaction (PCR) on Rotor Gene Q instruments. The M. tuberculosis RG Master contains reagents and enzymes for the specific amplification of a 159 bp region of the mycobacterial genome, and for the direct detection of the specific amplicon

in fluorescence channel **Cycling Green** of the Rotor-Gene Q MDx, Rotor-Gene Q, or Rotor-Gene 6000, or **Cycling A.FAM** of the Rotor-Gene 3000.

In addition, the *artus* M. tuberculosis RG PCR Kit contains a second heterologous amplification system to identify possible PCR inhibition. This is detected as an internal control (IC) in fluorescence channel **Cycling Yellow** of the Rotor Gene Q MDx, Rotor-Gene Q, or Rotor-Gene 6000, or **Cycling A.JOE** of the Rotor-Gene 3000. The amplification and detection of this internal control do not reduce the detection limit of the analytical *M. tuberculosis* complex PCR (see "Analytical sensitivity," page 25). External positive controls (M. tuberculosis RG/TM QS 1–4) are supplied which allow the determination of the pathogen load. For further information, refer to "Quantitation," page 12.

Materials Provided

Kit contents

***artus* M. tuberculosis RG PCR Kit**				
Catalog number			**4555263**	**4555265**
Number of reactions			**24**	**96**
Cap color	**Reagent name**	**Symbol**	**Amount**	**Amount**
Blue	M. tuberculosis RG Master		2 x 12 reactions	8 x 12 reactions
Yellow	M. tuberculosis RG Mg-Sol*	Mg-Sol	1 x 400 µl	1 x 400 µl
Red	M. tuberculosis RG/TM QS† 1 (3×10^4 copy/µl)	QS	1 x 200 µl	1 x 200 µl
Red	M. tuberculosis RG/TM QS 2 (3×10^3 copy/µl)	QS	1 x 200 µl	1 x 200 µl
Red	M. tuberculosis RG/TM QS 3 (3×10^2 copy/µl)	QS	1 x 200 µl	1 x 200 µl
Red	M. tuberculosis RG/TM QS 4 (3×10^1 copy/µl)	QS	1 x 200 µl	1 x 200 µl
Green	M. tuberculosis RG IC‡	IC	1 x 1000 µl	2 x 1000 µl
White	Water (PCR grade)		1 x 1000 µl	1 x 1000 µl

* Mg-Sol: Magnesium solution.

† QS: Quantitation Standard

‡: IC: Internal Control

Materials Required but Not Provided

Important: Make sure that the instruments used in these procedures have been checked and calibrated according to the manufacturer's recommendations.

- Disposable powder-free gloves
- QIAamp® DNA Mini Kit (QIAGEN, cat. no. 51304)
- Lysozyme mix (see page 10)
- Pipets (adjustable)
- Sterile pipet tips with filters
- Vortex mixer
- Heating block or thermomixer capable of heating from 37°C to 95°C
- Benchtop centrifuge with rotor for 2 ml reaction tubes
- Rotor-Gene Q MDx, Rotor-Gene Q or Rotor-Gene instrument with fluorescence channels for **Cycling Green** and **Cycling Yellow** or with fluorescence channels for **Cycling A.FAM** and **Cycling A.JOE**
- Rotor-Gene Q MDx/Rotor-Gene Q software version 1.7.94 or higher (Rotor-Gene 6000 software version 1.7.65, 1.7.87, 1.7.94; Rotor-Gene 3000 software version 6.0.23)
- Strip Tubes and Caps, 0.1 ml, for use with 72-well rotor (cat. no. 981103 or 981106)
- Alternatively: PCR Tubes, 0.2 ml, for use with 36-well rotor (cat. no. 981005 or 981008)
- Cooling block (Loading Block 72 x 0.1 ml Tubes, cat. no. 9018901, or Loading Block 96 x 0.2 ml Tubes, cat. no. 9018905)

Warnings and Precautions

The user should always pay attention to the following:

- Use sterile pipet tips with filters.
- Store and extract positive material (specimens, controls and amplicons) separately from all other reagents and add it to the reaction mix in a spatially separated facility.
- Thaw all components thoroughly at room temperature before starting an assay.
- When thawed, mix the components and centrifuge briefly.
- Work quickly and keep components on ice or in the cooling block (72/96-well loading block).

Warnings

For safety information of the *artus* M. tuberculosis RG PCR Kit, please consult the appropriate safety data sheets (SDSs). The SDSs are available online in convenient and compact PDF format at **www.qiagen.com/safety**.

Reagent Storage and Handling

The components of the *artus* M. tuberculosis RG PCR Kit should be stored at –15 to –30°C and are stable until the expiry date stated on the label. Repeated thawing and freezing (> 2x) should be avoided, as this may reduce the sensitivity. If the reagents are to be used only intermittently, they should be frozen in aliquots. Storage at 2–8°C should not exceed a period of 5 hours.

Procedure

Important points before starting

- The use of carrier RNA is critical for the extraction efficiency and, consequently, for DNA/RNA yield. The addition of carrier (RNA Homopolymer Poly[rA]; not included in the QIAamp DNA Mini Kit) is strongly recommended for the extraction of nucleic acids from cell free body fluids and material low in DNA/RNA content (e.g., CSF).
- Resuspend the lyophilized carrier RNA (RNA Homopolymer Poly[rA], not included in the QIAamp DNA Mini Kit) using the elution buffer (do not use lysis buffer) of the extraction kit (Buffer AE of the QIAamp DNA Mini Kit) and prepare a dilution with a concentration of 1 µg/µl. Divide this carrier RNA solution in a number of aliquots sufficient for your needs and store them at –15°C to –30°C. Avoid repeated thawing (> 2x) of a carrier RNA aliquot.
- Use 1 µg carrier RNA per 100 µl lysis buffer. For example, if the extraction protocol suggests 200 µl lysis buffer, add 2 µl carrier RNA (1 µg/µl) directly into the lysis buffer (Buffer AL of the QIAamp DNA Mini Kit). Before beginning each extraction, a mixture of lysis buffer, carrier RNA and internal control (see "Internal Control," page 12) should be freshly prepared according to the following pipetting scheme:

	Number of samples	
Reagent	**1**	**12**
Buffer AL (lysis buffer)	e.g., 200 µl	e.g., 2400 µl
Carrier RNA (1µg/µl)	2 µl	24 µl
Internal control	10 µl	120 µl
Total volume	**212 µl**	**2544 µl**
Volume per extraction	**200 µl**	**each 200 µl**

- Use the freshly prepared mixture of lysis buffer, internal control and carrier RNA **instantly** for extraction. Storage of the mixture is **not** possible.
- The *artus* M. tuberculosis RG PCR Kit should not be used with phenol-based isolation methods.
- **Important**: The internal control of the *artus* M. tuberculosis RG PCR Kit is used directly in the isolation procedure (see "Internal Control", page 12).

DNA isolation

Before the DNA isolation, large sample volumes or strongly acidic samples must first be concentrated or neutralized, respectively. For the analysis of sputum, we recommend a NALC-NaOH decontamination; stomach fluid should be neutralized with phosphate buffer. After a final centrifugation, the bacteria pellet can be used for the following DNA isolation.

The QIAamp DNA Mini Kit (cat. no. 51304) is validated for mycobacterial DNA purification from human sputum, BAL, bronchial secretion, CSF, stomach fluid or peritoneal punction for use with the *artus* M. tuberculosis RG PCR Kit.

To ensure an effective and contamination-free lysis of the mycobacteria, carry out the DNA purification according to the following steps, which differ from the protocols in the *QIAamp DNA Mini and Blood Mini Handbook*.

Important: All pipetting steps before the incubation at 95°C have to be performed in a class II safety cabinet, since the samples are potentially infectious.

1. Transfer between 250 µl and 500 µl of the NALC-NaOH-decontaminated sample into a 1.5 ml screw-cap tube.
   - The use of screw-cap tubes is absolutely essential.
   - The screw-cap tubes must always be locked tightly.
2. Centrifuge for 10 minutes at 17,000 x *g* (13,000 rpm) in a desktop centrifuge.
3. Carefully discard the supernatant by pipetting.
   - Do not touch the inside of the tube lid. If you do so, change the potentially contaminated glove immediately.
4. Add 180 µl lysozyme mix (20 mg/ml lysozyme; 20 mM Tris-HCl (pH 8.0); 2 mM EDTA; 1.2% Triton™) and resuspend the pellet by pipetting up and down.
5. Incubate for at least 1 hour at 37°C in a heating block or thermomixer.
   - The use of a water bath is not recommended.
6. Centrifuge briefly to remove drops from the inside of the lid.
   - After each incubation step, centrifuge the tube briefly to remove drops from the inside of the lid.

7. Add 20 µl Proteinase K and 200 µl AL buffer with carrier RNA and IC (see above and "Internal Control," page 12).
   - Do not touch the inside of the tube lid. If you do so, change the potentially contaminated glove immediately.
8. Mix well by vortexing.
9. Incubate for 30 minutes at 56°C in a heating block or thermomixer.
   - The use of a water bath is not recommended.
10. Centrifuge briefly to remove drops from the inside of the lid.
    - After each incubation step centrifuge the tube briefly to remove drops from the inside of the lid.
11. Incubate for 15 minutes at 95°C.
    Important: Incubation time should not be exceeded as this may cause DNA degradation.
12. **Note**: Only after completion of the incubation at 95°C are the samples no longer infectious. Cool the sample to room temperature.
    - Make sure the samples cool down to room temperature after the 95°C heating step, since otherwise the risk of aerosol-mediated contamination after opening the tube is extremely high.
13. Centrifuge briefly to remove drops from the inside of the lid.

Follow the "Protocol: DNA Purification from Tissues" in the *QIAamp DNA Mini and Blood Mini Handbook* (Third Edition, June 2012) starting with the addition of ethanol at step 6, and perform the final DNA elution using 100 µl Buffer AE.

- Make sure that you do not wet the rim of a QIAamp spin column.
- Do not touch the lid of a QIAamp spin column on the inside. If so, change the potentially contaminated glove immediately.
- Do not use the same pipet tip for different samples, not even to apply the washing buffers AW1 and AW2 or the elution buffer AE. This avoids cross-contamination between samples and the contamination of a buffer.
- Use each 2 ml collection tube only once. If you run out of collection tubes you may also use 2 ml microcentrifuge tubes, the lids of which have to be removed before use.
- We strongly recommend performing the recommended centrifugation step 10 in the protocol to remove any residual ethanol. We recommend increasing the time of this centrifugation to 3 minutes.

Internal Control

An internal control (M. tuberculosis RG IC) is supplied. This allows the user both to control the DNA isolation procedure and to check for possible PCR inhibition. For this application, add the internal control to the isolation at a ratio of 0.1 µl per 1 µl elution volume. For example, using the QIAamp DNA Mini Kit, the DNA is eluted in 100 µl Buffer AE. Therefore, 10 µl of the internal control should be added initially. The volume of the internal control is dependent on the elution volume. The use of 10 µl is **only valid** for an elution volume of 100 µl (0.1 µl per 1 µl elution volume).

Note: The internal control and carrier RNA (see "DNA isolation," page 10) should be added only to the mixture of lysis buffer and sample material or directly to the lysis buffer.

The internal control must not be added to the sample material directly. If added to the lysis buffer, note that the mixture of internal control and lysis buffer/carrier RNA has to be prepared freshly and used instantly. Storage of the mixture at room temperature or at 4°C for only a few hours may lead to internal control failure and reduced extraction efficiency.

Note: Do not add the internal control and the carrier RNA to the sample material directly.

Quantitation

To generate a standard curve on Rotor-Gene Q instruments, all 4 quantitation standards should be used and defined in the **Edit Samples** dialog box as standards with the specified concentrations (refer to the applicable instrument user manual).

The standard curve generated as described above can also be used for subsequent runs, provided that at least one standard of **one** given concentration is used in the current run. For this purpose, the previously generated standard curve needs to be imported (refer to the applicable instrument user manual). However, this quantitation method may lead to deviations in the results due to variability between different PCR runs.

To ensure accurate quantitation, it is highly recommended to add the internal control to M. tuberculosis RG Master and M. tuberculosis RG Mg-Sol used for the quantitation standards. For this application, add the internal control directly to the M. tuberculosis RG Master and M. tuberculosis RG Mg-Sol, as described in step 2 of the protocol (page 13), and use this master mix for each quantitation standard (M. tuberculosis RG/TM QS 1–4).

The quantitation standards are defined as copies/µl. The following equation has to be applied to convert the values determined using the standard curve into copies/ml of sample material:

$$\text{Results (copies/ml)} = \frac{\text{Result (copies/µl)} \times \text{Elution volume (µl)}}{\text{Sample volume (ml)}}$$

As a matter of principle the initial sample volume should be entered in the equation above. This has to be considered when the sample volume has been changed prior to the nucleic acid extraction (e.g., narrowing the volume by centrifugation or increase of volume by replenishment to the volume required for the isolation).

PCR on Rotor-Gene Q instruments

- Take time to familiarize yourself with the Rotor-Gene Q instrument before starting the protocol. Refer to the instrument user manual.
- Make sure that at least one quantitation standard as well as one negative control (Water, PCR grade) are included per PCR run. To generate a standard curve, use all 4 quantitation standards supplied (M. tuberculosis RG/TM QS 1–4) for each PCR run.
- Make sure that the cooling block (accessory of the Rotor-Gene Q instrument) is precooled to 2–8°C.
- Before each use, all reagents need to be thawed completely, mixed (by repeated up and down pipetting or by quick vortexing) and centrifuged briefly.

1. Place the desired number of PCR tubes into the adapters of the cooling block.
2. Prepare a master mix according to the following table:

	Number of samples	
	1	**12**
M. tuberculosis RG Master	13 µl	156 µl
M. tuberculosis RG Mg-Sol	2 µl	24 µl
Total volume	**15 µl**	**180 µl**

3. Pipet 15 µl of the master mix into each PCR tube. Then add 10 µl of the eluted sample DNA (see the table below).

   Correspondingly, 10 µl of at least one of the quantitation standards (M. tuberculosis RG QS 1–4) must be used as a positive control and 10 µl of water (Water, PCR grade) as a negative control.

	Number of samples	
	1	**12**
Master mix	15 µl	15 µl each
Sample	10 µl	10 µl each
Total volume	**25 µl**	**25 µl each**

4. Close the PCR tubes.
5. Make sure that the locking ring (accessory of the Rotor-Gene instrument) is placed on top of the rotor to prevent accidental opening of the tubes during the run.
6. For detection of all members of the *M. tuberculosis* complex, create a temperature profile according to the following steps.

Setting the general assay parameters	**Figures 1, 2 and 3**
Initial activation of the hot start enzyme	**Figure 4**
Amplification of the DNA	**Figure 5**
Adjusting the fluorescence channel sensitivity	**Figure 6**
Starting the run	**Figure 7**

All specifications refer to the Rotor-Gene Q MDx/Rotor-Gene Q software version 1.7.94, Rotor Gene 6000 software versions 1.7.65, 1.7.87, 1.7.94, and Rotor-Gene 3000 software version 6.0.23. Please find further information on programming Rotor-Gene instruments in the applicable user manual.

In the illustrations, these settings are framed in bold black. Illustrations are included for Rotor-Gene Q instruments. Where different values are required for the Rotor-Gene 3000, these differences are described in the text.

7. First, open the **New Run Wizard** dialog box (Figure 1). Check the **Locking Ring Attached** box and click **Next**.

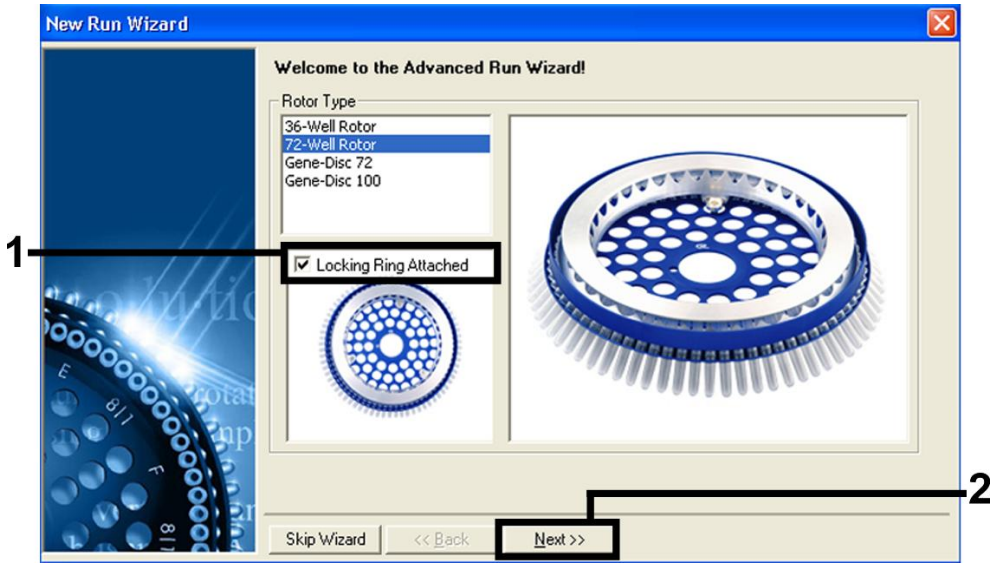

Figure 1. The New Run Wizard dialog box.

8. Select **25** for the PCR reaction volume and click **Next** (Figure 2).

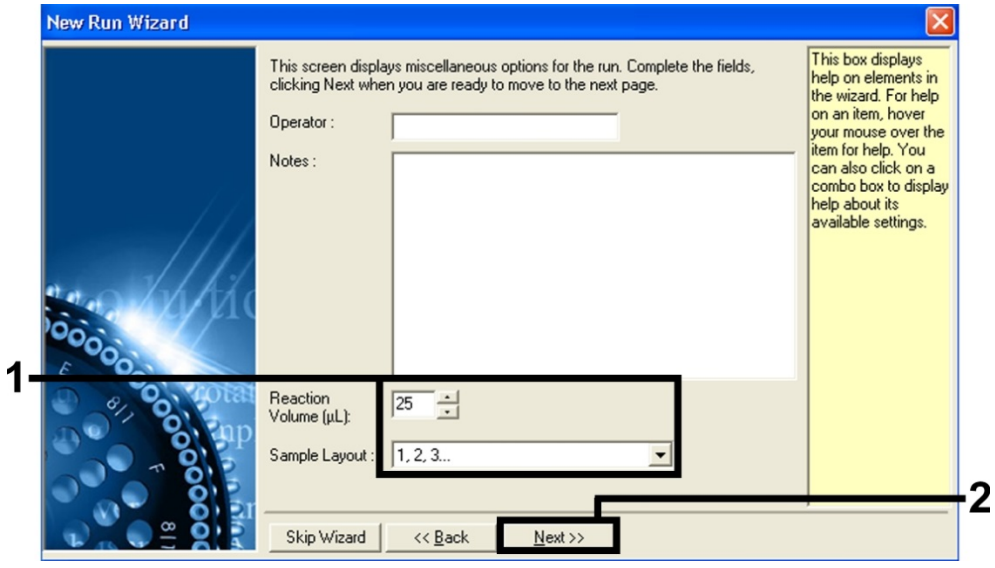

Figure 2. Setting the general assay parameters.

9. Click the **Edit Profile** button in the next **New Run Wizard** dialog box (Figure 3) and program the temperature profile as shown in Figures 4 and 5.

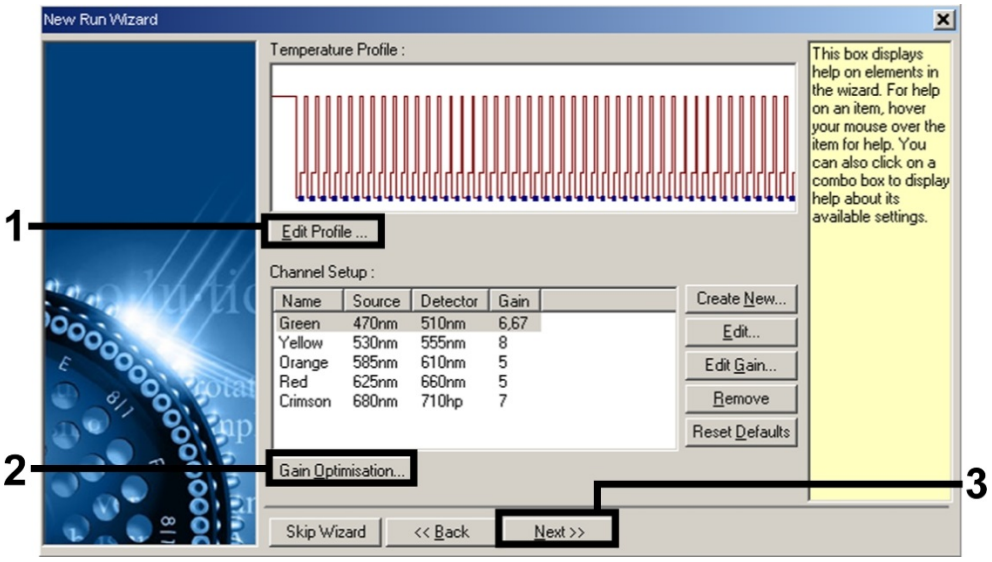

Figure 3. Editing the profile.

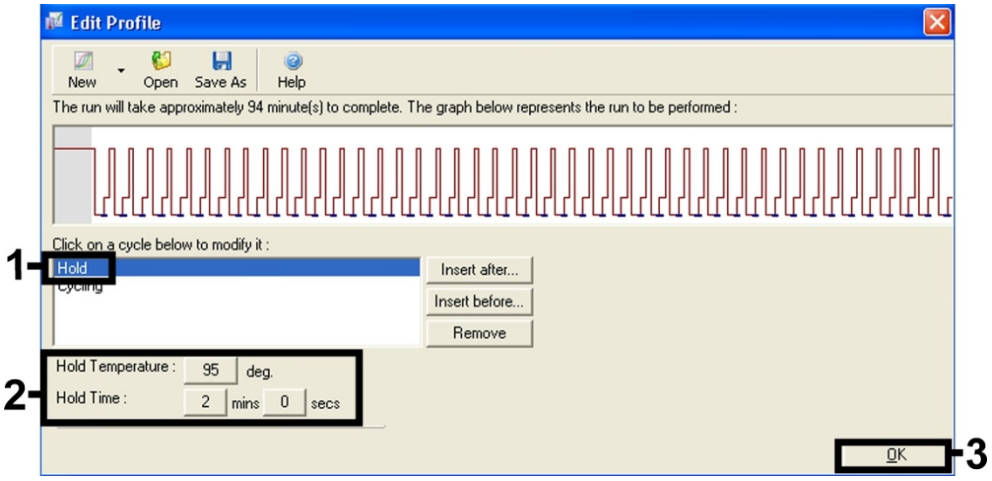

Figure 4. Initial activation of the hot-start enzyme.

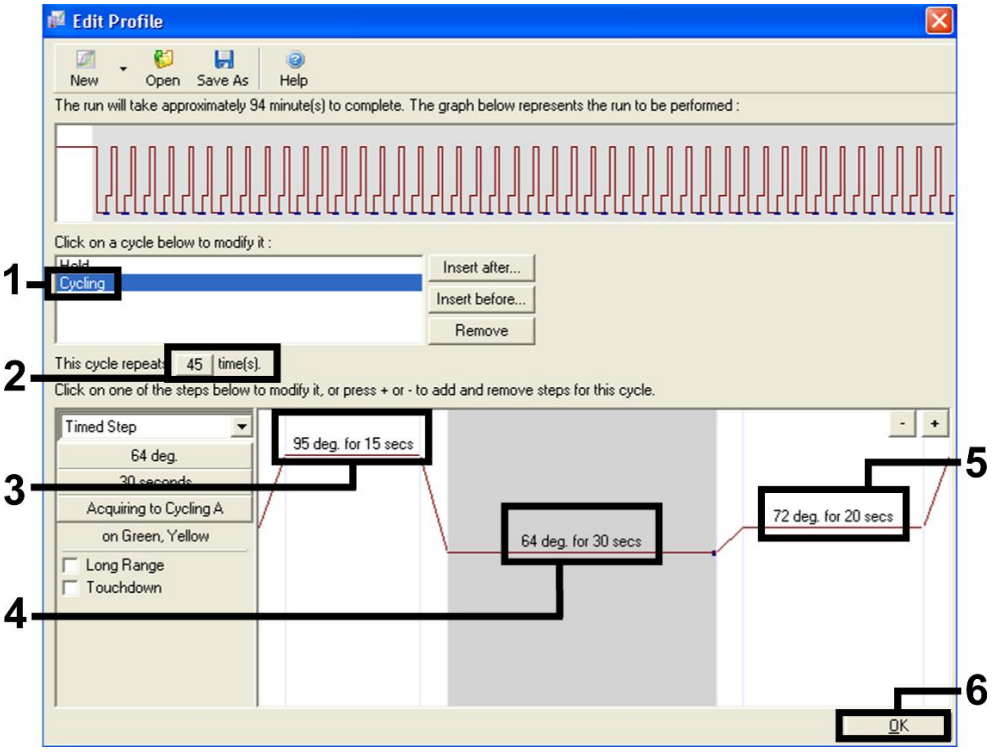

Figure 5. Amplification of the DNA.

Note: On the Rotor-Gene 3000, the software will define the fluorescence dyes as **FAM/Sybr®, JOE**.

10. The detection range of the fluorescence channels has to be determined according to the fluorescence intensities in the PCR tubes. Click **Gain Optimisation** in the **New Run Wizard** dialog box (see Figure 3) to open the **Auto-Gain Optimisation Setup** dialog box.

11. Set the calibration temperature to **64** to match the annealing temperature of the amplification program (Figure 6).

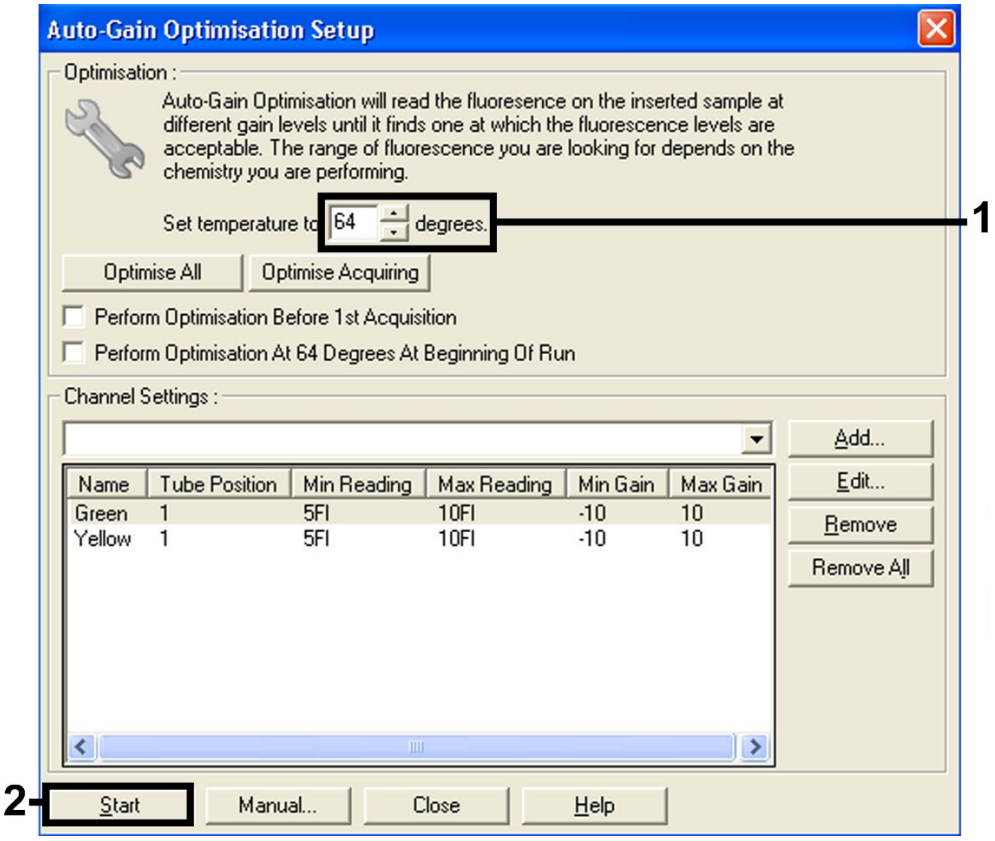

Figure 6. Adjusting the fluorescence channel sensitivity.

Note: On the Rotor-Gene 3000, the software will define the fluorescence dyes as **FAM/Sybr** and **JOE**.

12. The gain values determined by the channel calibration are saved automatically and are listed in the last menu window of the programming procedure (Figure 7). Click **Start Run**.

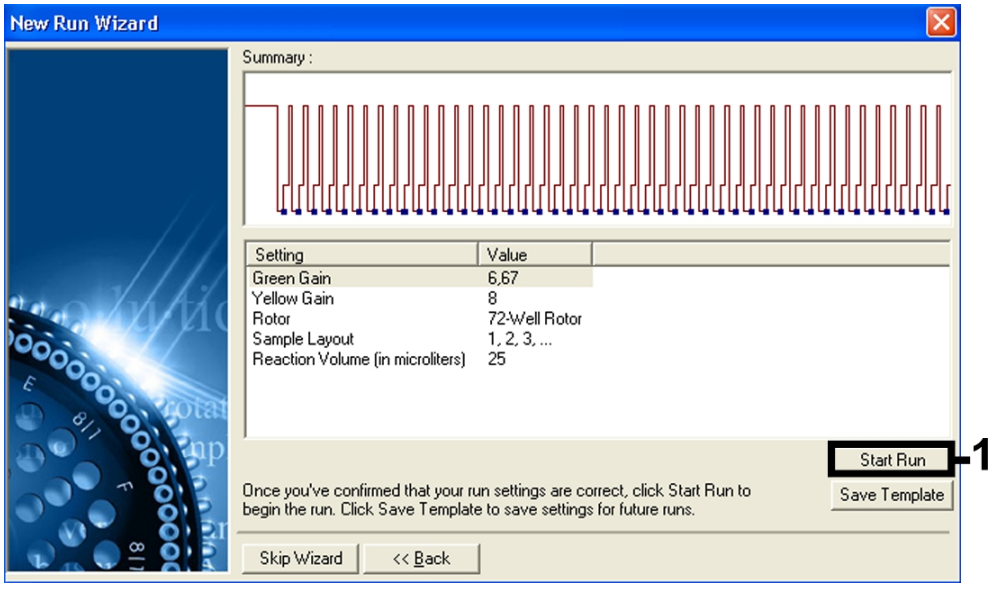

Figure 7. Starting the run.

Note: On the Rotor-Gene 3000, the software will define the fluorescence dyes as **FAM/Sybr** and **JOE**.

13. After the run is finished, analyze the data according to "Interpretation of Results," page 20.

Interpretation of Results

Examples of positive and negative PCR reactions are given in Figure 8 and Figure 9.

The following results are possible:

- A signal is detected in fluorescence channel **Cycling Green**.

  The result of the analysis is positive. The sample contains DNA of one or more members of the *M. tuberculosis* complex.

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

  Note: On the Rotor-Gene 3000, the relevant channels are **Cycling A.FAM** for the positive signal and **Cycling A.JOE** for the internal control.

- In fluorescence channel **Cycling Green,** no signal is detected. At the same time, a signal from the internal control appears in the **Cycling Yellow** channel.

  In the sample, no DNA of members of the *M. tuberculosis* complex is detectable. It can be considered negative.

  In the case of a negative *M. tuberculosis* complex PCR, the detected signal of the IC rules out the possibility of PCR inhibition.

  Note: On the Rotor-Gene 3000, the relevant channels are **Cycling A.JOE** for the internal control and lack of a signal for **Cycling A.FAM**.

- No signal is detected in the **Cycling Green** or in the **Cycling Yellow** channels.

  No result can be concluded.

  Note: On the Rotor-Gene 3000, the relevant channels are **Cycling A.FAM** and **Cycling A.JOE**.

  Information regarding error sources and their solution can be found in “Troubleshooting,” page 22.

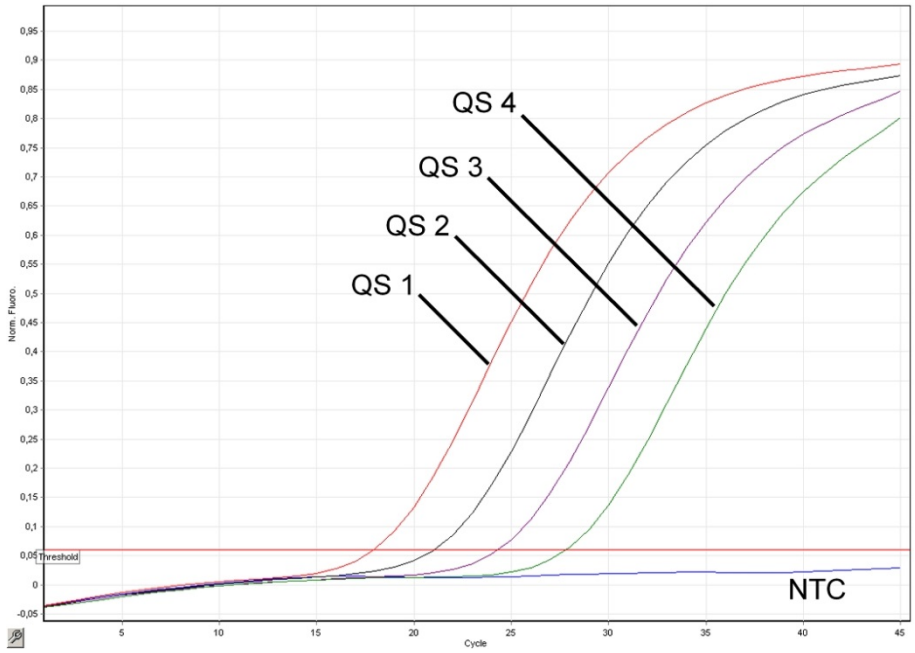

Figure 8. Detection of the quantitation standards (M. tuberculosis RG/TM QS 1–4) in fluorescence channel Cycling Green. NTC: non-template control (negative control).

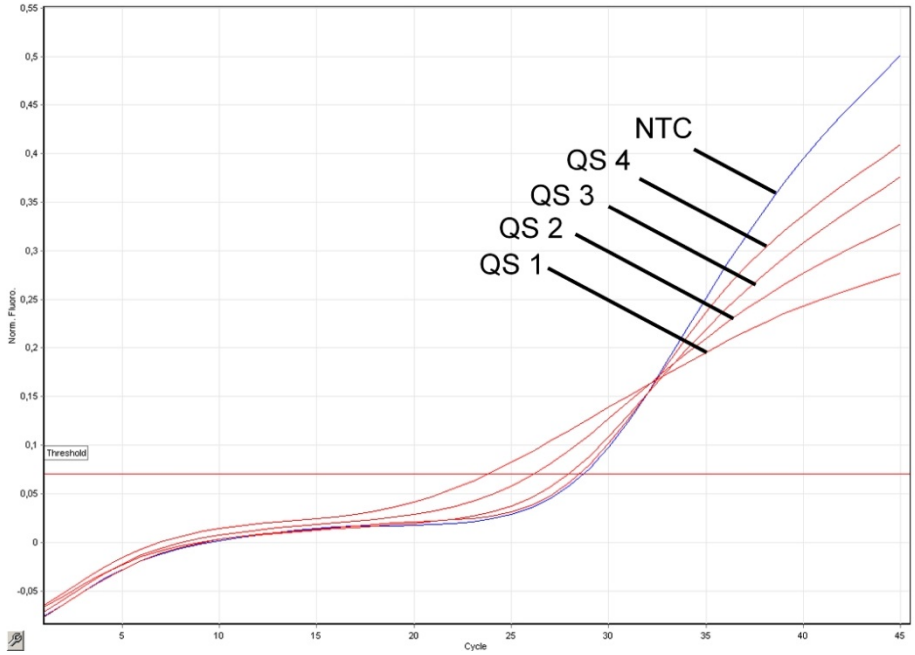

Figure 9. Detection of the internal control in fluorescence channel Cycling Yellow with simultaneous amplification of the quantitation standards (M. tuberculosis RG/TM QS 1–4). NTC: non-template control (negative control).

Troubleshooting

This troubleshooting guide may be helpful in solving any problems that may arise.

		Comments and suggestions
No signal with positive controls (M. tuberculosis RG/TM QS 1–4) in fluorescence channel Cycling Green or Cycling A.FAM		
a)	The selected fluorescence channel for PCR data analysis does not comply with the protocol	For data analysis, select the fluorescence channel **Cycling Green** or **Cycling A.FAM** for the analytical *M. tuberculosis* complex PCR and the fluorescence channel **Cycling Yellow** or **Cycling A.JOE** for the internal control PCR.
b)	Incorrect programming of the temperature profile of the Rotor-Gene instrument	Compare the temperature profile with the protocol (see "PCR on Rotor-Gene Q instruments," page 13).
c)	Incorrect configuration of the PCR reaction	Check your work steps by means of the pipetting scheme (see "PCR on Rotor-Gene Q instruments," page 13) and repeat the PCR, if necessary.
d)	The storage conditions for one or more kit components did not comply with the instructions	Check the storage conditions (see "Reagent Storage and Handling," page 8) and the expiration date (see the kit label) of the reagents and use a new kit, if necessary.
e)	The *artus* M. tuberculosis RG PCR Kit has expired.	Check the storage conditions (see "Reagent Storage and Handling," page 8) and the expiration date (see the kit label) of the reagents and use a new kit, if necessary.
Weak or no signal of the internal control in fluorescence channel Cycling Yellow or Cycling A.JOE and simultaneous absence of a signal in channel Cycling Green or Cycling A.FAM for the specific *M. tuberculosis* complex PCR		
a)	The PCR conditions do not comply with the protocol	Check the PCR conditions (see "No signal with positive controls [M. tuberculosis RG QS 1–4] in fluorescence channel Cycling Green or Cycling A.FAM," above) and repeat the PCR with corrected settings, if necessary.

		Comments and suggestions
b)	The PCR was inhibited	Make sure that you use a recommended isolation method (see "DNA isolation," page 10) and closely follow the manufacturer's instructions. Make sure that during the DNA isolation the recommended additional centrifugation step has been carried out before the elution in order to remove any residual ethanol (see "DNA isolation," page 10).
c)	DNA was lost during extraction	If the internal control had been added to the extraction, an absent signal of the internal control can indicate the loss of DNA during the extraction. Make sure that you use a recommended isolation method (see "DNA isolation," page 10) and closely follow the manufacturer's instructions.
d)	The storage conditions for one or more kit components did not comply with the instructions	Check the storage conditions (see "Reagent Storage and Handling," page 8) and the expiration date (see the kit label) of the reagents and use a new kit, if necessary.
e)	The *artus* M. tuberculosis RG PCR Kit has expired	Check the storage conditions (see "Reagent Storage and Handling," page 8) and the expiration date (see the kit label) of the reagents and use a new kit, if necessary.

Signals with the negative controls in fluorescence channel Cycling Green or Cycling A.FAM of the analytical PCR

a)	Contamination occurred during preparation of the PCR	Repeat the PCR with new reagents in replicates. If possible, close the PCR tubes directly after addition of the sample to be tested. Make sure to pipet the positive controls last. Make sure that work space and instruments are decontaminated at regular intervals.

		Comments and suggestions
b)	Contamination occurred during extraction.	Repeat the extraction and PCR of the sample to be tested using new reagents. Make sure that work space and instruments are decontaminated at regular intervals.

If you have any further questions or if you encounter problems, contact QIAGEN Technical Services.

Quality Control

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

Limitations

- All reagents may exclusively be used in in vitro diagnostics.
- The product is to be used by personnel specially instructed and trained in the in vitro diagnostics procedures only.
- Strict compliance with the user manual is required for optimal PCR results.
- Attention should be paid to expiration dates printed on the box and labels of all components. Do not use expired components.
- Although rare, mutations within the highly conserved regions of the bacterial genome covered by the kit's primers and/or probe may result in underquantitation or failure to detect the presence of the bacteria in these cases. Validity and performance of the assay design are evaluated at regular intervals.

Performance Characteristics

Analytical sensitivity

To determine the analytical sensitivity of the *artus* M. tuberculosis RG PCR Kit, a standard dilution series was set up from 10 to nominal 0.003 and from 10 to nominal 0.05 *M. tuberculosis* genome equivalents/µl and analyzed on Rotor-Gene 6000 and Rotor-Gene 3000, respectively, in combination with the *artus* M. tuberculosis RG PCR Kit. Testing was carried out on 3 different days on 8 replicates. The results were determined by a probit analysis. A graphical illustration of the probit analysis on Rotor-Gene 6000 is shown in Figure 10. The analytical detection limit of the *artus* M. tuberculosis RG PCR Kit in combination with Rotor-Gene Q MDx/Q/6000 and Rotor-Gene 3000 is 0.23 copies/µl (p = 0.05) and 0.9 copies/µl (p = 0.05), respectively. This means that there is a 95% probability that 0.23 copies/µl or 0.9 copies/µl will be detected.

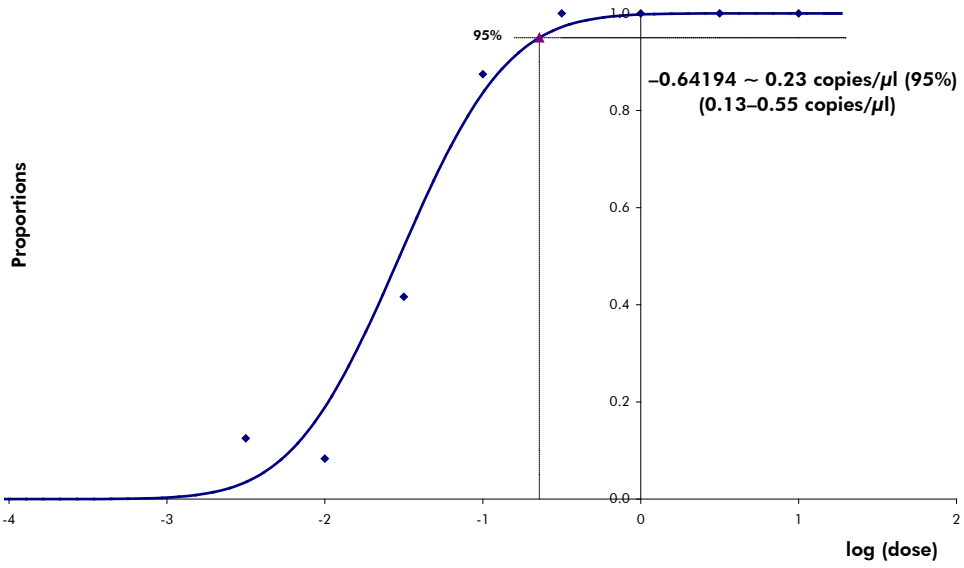

Figure 10. Probit analysis: *M. tuberculosis* (Rotor-Gene 6000). Analytical sensitivity of the *artus* M. tuberculosis RG PCR Kit on Rotor-Gene 6000.

Specificity

The specificity of the *artus* M. tuberculosis RG PCR Kit is first and foremost ensured by the selection of the primers and probes, as well as the selection of stringent reaction conditions. The primers and probes were checked for possible homologies to all sequences published in gene banks by sequence comparison analysis. The detectability of all members of the *M. tuberculosis* complex has thus been ensured.

Moreover, the specificity was validated with 90 different *M. tuberculosis* complex-negative samples (30 sputum, 30 BAL and 30 bronchial secretion samples). These did not generate any signals with the *M. tuberculosis* complex specific primers and probes, which are included in the M. tuberculosis RG Master.

To determine the specificity of the *artus* M. tuberculosis RG PCR Kit, the control group listed in Table 1 were tested for cross-reactivity. None of the tested pathogens were reactive.

Table 1. Testing the specificity of the kit with potentially cross-reactive pathogens

Control group	***M. tuberculosis*** **(Cycling Green or Cycling A.FAM)**	**Internal control (Cycling Yellow or Cycling A.JOE)**
Actinomyces israelii	–	+
Aeromonas hydrophila	–	+
Bordetella pertussis	–	+
Candida albicans	–	+
Chlamydia trachomatis	–	+
Chlamydia pneumoniae	–	+
Citrobacter freundii	–	+
Corynebacterium diphtheriae	–	+
Corynebacterium jeikeium	–	+
Cryptococcus neoformans	–	+
Eikenella corrodens	–	+
Enterobacter aerogenes	–	+
Enterobacter cloacae	–	+

Control group	*M. tuberculosis* (Cycling Green or Cycling A.FAM)	Internal control (Cycling Yellow or Cycling A.JOE)
Enterococcus faecalis	–	+
Enterococcus faecium	–	+
Escherichia coli	–	+
Fusobacterium nucleatum ssp. *polymorphum*	–	+
Haemophilus influenzae	–	+
Haemophilus parainfluenzae	–	+
Klebsiella pneumoniae	–	+
Lactobacillus acidophilus	–	+
Mycobacterium avium ssp. *avium*	–	+
Mycobacterium celatum	–	+
Mycobacterium chelonae	–	+
Mycobacterium fortuitum	–	+
Mycobacterium gordonae	–	+
Mycobacterium intracellulare	–	+
Mycobacterium kansasii	–	+
Mycobacterium lentiflavum	–	+
Mycobacterium malmoense	–	+
Mycobacterium marinum	–	+
Mycobacterium scrofulaceum	–	+
Mycobacterium szulgai	–	+
Mycobacterium ulcerans	–	+
Mycobacterium xenopi	–	+
Neisseria gonorrhoeae	–	+
Neisseria meningitidis	–	+
Nocardia asteroides	–	+

Control group	*M. tuberculosis* (Cycling Green or Cycling A.FAM)	Internal control (Cycling Yellow or Cycling A.JOE)
Nocardia brasiliensis	–	+
Nocardia farcinia	–	+
Nocardia otitidiscaviarum	–	+
Peptostreptococcus productus	–	+
Porphyromonas gingivalis	–	+
Prevotella denticola	–	+
Propionibacterium acnes	–	+
Pseudomonas aeruginosa	–	+
Salmonella enteritidis	–	+
Salmonella typhi	–	+
Staphylococcus aureus	–	+
Staphylococcus epidermidis	–	+
Streptococcus agalactiae	–	+
Streptococcus pyogenes	–	+
Streptococcus mutans	–	+
Streptococcus pneumoniae	–	+
Streptomyces venezuelae	–	+
Veillonella parvula	–	+
Xanthomonas maltophilia	–	+

Precision

The precision data of the *artus* M. tuberculosis RG PCR Kit have been collected by means of Rotor-Gene instruments and allow the determination of the total variance of the assay. The total variance consists of the intra-assay variability (variability of multiple results of samples of the same concentration within one experiment), the inter-assay variability (variability of multiple results of the assay generated on different instruments of the same type by different operators within one

laboratory) and the inter-batch variability (variability of multiple results of the assay using various batches). The data obtained were used to determine the standard deviation, the variance and the coefficient of variation for the pathogen-specific and the internal control PCR.

Precision data of the *artus* M. tuberculosis RG PCR Kit were collected using the quantitation standard of the lowest concentration (QS 4; 30 copies/µl). Testing was performed with 8 replicates. The precision data were calculated on basis of the C_T values of the amplification curves (C_T: threshold cycle, see Table 2). In addition, precision data for quantitative results in copies/µl were determined using the corresponding C_T values (see Table 3). Based on these results, the overall statistical spread of any given sample with the mentioned concentration is 1.26% (C_T) or 14.64% (copies/µl), and for the detection of the IC 1.57% (C_T). These values are based on the totality of all single values of the determined variabilities.

Table 2. Precision data on basis of the C_T values

	Standard deviation	Variance	Coefficient of variation (%)
Intra-assay variability: M. tuberculosis RG/TM QS 4	0.10	0.01	0.32
Intra-assay variability: Internal Control	0.13	0.02	0.45
Inter-assay variability: M. tuberculosis RG/TM QS 4	0.24	0.06	0.78
Inter-assay variability: Internal Control	0.29	0.08	0.95
Inter-batch variability: M. tuberculosis RG/TM QS 4	0.39	0.15	1.28
Inter-batch variability: Internal Control	0.66	0.43	2.16
Total variance: M. tuberculosis RG/TM QS 4	0.38	0.15	1.26
Total variance: Internal Control	0.48	0.23	1.57

Table 3. Precision data on basis of the quantitative results (in copies/µl)

	Standard deviation	Variance	Coefficient of variation (%)
Intra-assay variability: M. tuberculosis RG/TM QS 4	1.97	3.90	6.56
Inter-assay variability: M. tuberculosis RG/TM QS 4	3.93	15.43	13.00
Inter-batch variability: M. tuberculosis RG/TM QS 4	5.51	30.41	18.09
Total variance: M. tuberculosis RG/TM QS 4	4.44	19.69	14.64

Robustness

The verification of the robustness allows the determination of the total failure rate of the *artus* M. tuberculosis RG PCR Kit. A total of 30 *M. tuberculosis* complex-negative samples of each sputum, BAL and bronchial secretion were spiked with 3 copies/µl elution volume of *M. tuberculosis* control DNA (approximately 3-fold concentration of the analytical sensitivity limit). After extraction using the QIAamp DNA Mini Kit (see "DNA isolation," page 10), these samples were analyzed with the *artus* M. tuberculosis RG PCR Kit. For all *M. tuberculosis* samples, the failure rate was 0%. In addition, the robustness of the IC was assessed by purification and analysis of *M. tuberculosis* complex-negative sputum, BAL and bronchial secretion samples (30 each). The total failure rate was 0%. Inhibitions were not observed. Thus, the robustness of the *artus* M. tuberculosis RG PCR Kit is ≥ 99%.

Reproducibility

Reproducibility data permit a regular performance assessment of the *artus* M. tuberculosis RG PCR Kit as well as an efficiency comparison with other products. These data are obtained by the participation in established proficiency programs.

References

1. Mackay I.M. (2004) Real-time PCR in the microbiology laboratory. Clin. Microbiol. Infect. **10**, 190.

Symbols

Symbol	Symbol definition
	Use by
	Batch code
	Manufacturer
	Catalog number
	Material number
	CE mark for European conformity
	In vitro diagnostic medical device
	Contains reagents sufficient for <N> tests

Symbol	Symbol definition
COMP	Components
CONT	Contains
NUM	Number
GTIN	Global Trade Item Number
	Temperature limitation
QS	Quantitation Standard
IC	Internal Control
Mg-Sol	Magnesium solution

Ordering Information

Product	Contents	Cat. no.
artus M. tuberculosis RG PCR Kit (24)	For 24 reactions: Master, Mg Solution, 4 Quantitation Standards, Internal Control, Water (PCR grade)	4555263
artus M. tuberculosis RG PCR Kit (96)	For 96 reactions: Master, Mg Solution, 4 Quantitation Standards, Internal Control, Water (PCR grade)	4555265
QIAamp DNA Mini Kit — for isolation of genomic, mitochondrial, bacterial, parasite or viral DNA		
QIAamp DNA Mini Kit (50)	For 50 DNA preps: 50 QIAamp Mini Spin Columns, QIAGEN Proteinase K, Reagents, Buffers, Collection Tubes (2 ml)	51304
Rotor-Gene Q MDx and accessories		
Rotor-Gene Q MDx 5plex Platform	Real-time PCR cycler with 5 channels (green, yellow, orange, red, crimson), laptop computer, software, accessories: includes 1-year warranty on parts and labor, installation and training not included	9002022
Rotor-Gene Q MDx 5plex System	Real-time PCR cycler with 5 channels (green, yellow, orange, red, crimson), laptop computer, software, accessories: includes 1-year warranty on parts and labor, installation and training	9002023
Rotor-Gene Q MDx 5plex HRM Platform	Real-time PCR cycler and High Resolution Melt analyzer with 5 channels (green, yellow, orange, red, crimson) plus HRM channel, laptop computer, software, accessories: includes 1-year warranty on parts and labor, installation and training not included	9002032
Rotor-Gene Q MDx 5plex HRM System	Real-time PCR cycler and High Resolution Melt analyzer with 5 channels (green, yellow, orange, red, crimson) plus HRM channel, laptop computer, software, accessories: includes 1-year warranty on parts and labor, installation and training	9002033
Rotor-Gene Q MDx 6plex Platform	Real-time PCR instrument with 6 channels (blue, green, yellow, orange, red, crimson), including laptop computer, software, accessories: includes 1-year warranty on parts and labor, installation and training not included	9002042

Rotor-Gene Q MDx 6plex System	Real-time PCR instrument with 6 channels (blue, green, yellow, orange, red, crimson), including laptop computer, software, accessories: includes 1-year warranty on parts and labor, installation and training	9002043
Rotor-Gene Q MDx 2plex Platform	Real-time PCR cycler with 2 channels (green, yellow), laptop computer, software, accessories: includes 1-year warranty on parts and labor, installation and training not included	9002002
Rotor-Gene Q MDx 2plex System	Real-time PCR cycler with 2 channels (green, yellow), laptop computer, software, accessories: includes 1-year warranty on parts and labor, installation and training	9002003
Rotor-Gene Q MDx 2plex HRM Platform	Real-time PCR cycler and High Resolution Melt analyzer with 2 channels (green, yellow) plus HRM channel, laptop computer, software, accessories: includes 1-year warranty on parts and labor, installation and training not included	9002012
Rotor-Gene Q MDx 2plex HRM System	Real-time PCR cycler and High Resolution Melt analyzer with 2 channels (green, yellow) plus HRM channel, laptop computer, software, accessories: includes 1-year warranty on parts and labor, installation and training	9002013
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
Loading Block 96 x 0.2 ml Tubes	Aluminum block for manual reaction set-up in a standard 8 x 12 array using 96 x 0.2 ml tubes	9018905
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
PCR Tubes, 0.2 ml (1000)	1000 thin-walled tubes for 1000 reactions	981005
PCR Tubes, 0.2 ml (10000)	10 x 1000 thin-walled tubes for 1000 reactions	981008

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