January 2013

artus® Aspergillus diff. RG PCR Handbook

Σ 24

For amplification and differentiation of *Aspergillus* spp. DNA

For research use only. Not for use in diagnostic procedures.

For use with Rotor-Gene® Q instruments

REF 4640203

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Contents

Intended Use

The *artus* Aspergillus diff. RG PCR Kit is intended for research use only. Not for use in diagnostic procedures. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of a disease.

The *artus* Aspergillus diff. RG PCR Kit is an in vitro nucleic acid amplification test for the detection and differentiation of *Aspergillus fumigatus/terreus/flavus* DNA in whole blood. This kit utilizes the polymerase chain reaction (PCR) and is configured for use with Rotor-Gene Q Instruments.

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

Although rare, mutations within the highly conserved regions of the *Aspergillus fumigatus/terreus/flavus* genome covered by the kit's primers and/or probe may result in failure to detect the presence of the fungi DNA in these cases.

Summary and Explanation

The *artus* Aspergillus diff. RG PCR Kit constitutes a ready-to-use system for the detection and differentiation of *Aspergillus fumigatus/terreus/flavus* DNA using polymerase chain reaction (PCR) on Rotor-Gene Q Instruments. The *artus* Aspergillus RG Master contains reagents and enzymes for the specific amplification of 110 bp regions of the *A. fumigatus/terreus/flavus* genomes, and for the direct detection of the specific amplicon in fluorescence channel Cycling A Green/Orange/Crimson of the Rotor-Gene Q.

In addition, the *artus* Aspergillus diff. 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 A Yellow of the Rotor-Gene Q.

The detection limit of the analytical *A. fumigatus/terreus/flavus* PCR is not reduced. An external positive control (Aspergillus diff. RG Control) allows the control of the 3 specific PCR reactions.

Pathogen information

Aspergillus fumigatus/terreus/flavus are widespread filamentous fungi species of the genus *Aspergillus* and may be pathogenic to humans. They can be found in BAL (broncheoalveolar lavage), blood, and nasopharyngeal swabs of infected persons.

The pathogens can be transmitted as conidia or filamentous fragments incorporated with aerosols, food, transplant, skin lesions, contaminated needles, or other ways.

Principle of the Procedure

Pathogen detection 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 that bind specifically to the amplified product. Monitoring the fluorescence intensities during the PCR run allows the detection and quantitation of the accumulating product without having to re-open the reaction tubes after the PCR run.*

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

Materials Provided

Kit contents

***artus* Aspergillus diff. RG PCR Kit**			**(24)**
Catalog no.			**4640203**
Number of reactions			**24**
Blue	Aspergillus diff. RG Master	RG MASTER	2 x 190 µl
Yellow	Aspergillus diff. RG Mg-Sol*	RG MG-SOL	200 µl
Red	Aspergillus diff. RG Control†	RG CONTROL +	200 µl
Green	Aspergillus diff. RG IC‡	RG IC	1000 µl
White	Water (PCR grade)		1000 µl
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* Magnesium solution.

† Includes 3 PCR targets (plasmid clones), each specific for one of the 3 *Aspergillus* species.

‡ 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.

Reagents

- DNA isolation kit (see "DNA isolation", page 10)

Consumables

- Sterile pipet tips with filters
- Strip Tubes and Caps, 0.1 ml, for use with 72-well rotor (cat. no. 981103 or 981106)

Equipment

- Pipets (adjustable)*
- Vortex mixer*
- Benchtop centrifuge* with rotor for 2 ml reaction tubes
- Rotor-Gene Q, or Rotor-Gene 6000 Instrument* with fluorescence channels for Cycling A Green, Cycling A Yellow, Cycling A Orange, and Cycling A Crimson
- Rotor-Gene software version 1.7.94 or higher
- 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)

* Please ensure that all instruments have regularly been checked and calibrated according to the manufacturer's recommendations.

Warnings and Precautions

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.

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

24-hour emergency information

Emergency medical information in English, French, and German can be obtained 24 hours a day from:

Poison Information Center Mainz, Germany

Tel: +49-6131-19240

General precautions

The user should always pay attention to the following:

- Use sterile pipet tips with filters.
- Store and extract positive materials (specimens, positive controls, and amplicons) separately from all other reagents, and add them to the reaction mix in a spatially separated facility.
- Thaw all components thoroughly at room temperature (15–25°C) before starting an assay.
- When thawed, mix the components (by pipetting repeatedly up and down or by pulse vortexing) and centrifuge briefly.
- Work quickly and keep components on ice or in the cooling block (72/96-well loading block).

Reagent Storage and Handling

The components of the *artus* Aspergillus diff. RG PCR Kit should be stored at –20°C and are stable until the expiration date stated on the label. Repeated thawing and freezing (>2 x) should be avoided, as this may reduce assay 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.

Specimen Handling and Storage

Note: All samples have to be treated as potentially infectious material.

Note: As *Aspergillus* species are ubiquitous and widespread, all preparations and handlings have to be done carefully under almost sterile conditions in

order to avoid contamination of the samples and subsequent generation of false positive results.

Note: Current practices refer to whole blood as the most suitable sample material for sensitive detection of *Aspergillus fumigatus/terreus/flavus*. Other sample materials may work as well but were not tested with the *artus* Aspergillus diff. RG PCR Kit.

In a study, the *artus* Aspergillus diff. RG PCR Kit has been tested with DNA from whole blood samples. Other sample matrices were not tested. Please use only the recommended nucleic acid isolation kit (see "DNA isolation", page 10) for sample preparation.

Specimen collection

A volume of 5–10 ml EDTA blood should be withdrawn. Tubes should be mixed overhead directly after sample collection (8 x, do not agitate).

Note: Samples containing other anticoagulants, such as heparin, must not be used.

Sample storage

Whole blood should be separated into plasma and cellular components by centrifugation for 20 minutes at 800–1600 x *g* within 6 hours. The isolated plasma has to be transferred into sterile polypropylene tubes. The sensitivity of the assay can be reduced if you freeze the samples as a matter of routine or store them for a longer period of time.

Sample transport

Sample material should be transported in a shatterproof transport container as a matter of principle. Thus, a potential danger of infection due to a leakage of sample can be avoided. The samples should be transported following the local and national instructions for the transport of pathogen material.*

The samples should be shipped within 6 hours. It is not recommended to store the samples where they have been collected. It is possible to ship the samples by mail, following the legal instructions for the transport of pathogen material. We recommend the sample transport with a courier. The blood samples should be shipped cooled (2–8°C) and the separated plasma deep frozen (–15 to –30°C).

Interfering substances

Elevated levels of human genomic DNA (150 ng/µl) do not influence the performance/sensitivity of the amplification system.

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

Procedure

DNA isolation

The QIAamp® UCP PurePathogen Blood Kit from QIAGEN is recommended for fungal DNA purification from whole blood for use with the *artus* Aspergillus diff. RG PCR Kit. Carry out the fungal DNA purification according to the instructions in the extraction kit handbook.

Table 1. Purification kit to be used with the *artus* Aspergillus diff. RG PCR Kit

Sample material	Sample size	Nucleic acid isolation kit	Catalog number (QIAGEN)
Whole blood	≥1000 µl	QIAamp UCP PurePathogen Blood Kit	50112

Note: Carrier RNA is not required for extraction of nucleic acids from whole blood using the QIAamp UCP PurePathogen Blood Kit. Other, cell-free sample matrices, such as BAL, require a different sample preparation method, which may require carrier RNA for efficient nucleic acid yield.

Internal control

Internal control (IC): An internal control (Aspergillus diff. RG IC) is supplied. This allows the user 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 UCP PurePathogen Blood Kit, if the DNA is finally eluted in 100 µl Buffer AVE, 10 µl of the internal control should be added initially. The quantity of internal control used depends only on the elution volume.

Note: The internal control must not be added to the sample material directly. Instead it should be added to the mixture of lysis buffer and sample material or directly to the lysis buffer.

If added to the lysis buffer note that the mixture of internal control and lysis buffer has to be prepared freshly and used immediately (storage of the mixture at room temperature or in the refrigerator for only a few hours may lead to internal control failure, which could incorrectly be interpreted as an indication of reduced extraction efficiency).

The internal control can optionally be used exclusively to check for possible PCR inhibition. For this application, add the internal control directly to the

Aspergillus diff. RG Master and Aspergillus diff. Mg-Sol, as described in step 2b of the protocol (page 13).

Note: If the internal control of the *artus* Aspergillus diff. RG PCR Kit is applied directly in the isolation procedure, make sure to include one negative sample or water in the isolation procedure. The corresponding IC signal is the basis for the evaluation of the isolation success.

Note: To ensure the full extraction of all pathogen DNA from samples of suspected fungal infections, a treatment with glass beads is necessary as described in the *QIAamp UCP PurePathogen Blood Handbook*. A glass bead (pre)treatment is recommended for all possible sample matrices as well in order to ensure quantitative disintegration of the fungal cell wall.

Protocol: PCR

Important points before starting

- Take time to familiarize yourself with the Rotor-Gene Q instrument before starting the protocol. See the instrument user manual.
- Make sure that at least one positive control as well as one negative control (Water, PCR grade) is included per PCR run.

Things to do before starting

- 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.

Procedure

1. **Place the desired number of PCR tubes into the adapters of the cooling block.**
2. **If you are using the internal control to monitor the DNA isolation procedure and to check for possible PCR inhibition, follow step 2a. If you are using the internal control exclusively to check for PCR inhibition, follow step 2b.**

   Note: It is highly recommended to add the internal control to Aspergillus diff. RG Master and Aspergillus diff Mg-Sol used for the quantitation standards. For the quantitation standards, add the internal control directly to the Aspergillus diff. RG Master and Aspergillus diff. RG Mg-Sol, as described in step 2b of the protocol, and use this master mix for each quantitation standard.

2a. **The internal control has already been added to the isolation (see "Internal control", page 10). In this case, prepare a master mix according to Table 2.**

   The reaction mix typically contains all of the components needed for PCR except the sample.

Table 2. Preparation of master mix (internal control used to monitor DNA isolation and check for PCR inhibition)

Number of samples	1	12
Aspergillus diff. RG Master	14.5 µl	174 µl
Aspergillus diff. RG Mg-Sol	0.5 µl	6 µl
Aspergillus diff. RG IC	0 µl	0 µl
Total volume	**15 µl**	**180 µl**

2b. The internal control must be added directly to the mixture of Aspergillus diff. RG Master and Aspergillus diff. RG Mg-Sol. In this case, prepare a master mix according to Table 3.

The reaction mix typically contains all of the components needed for PCR except the sample.

Table 3. Preparation of master mix (internal control used exclusively to check for PCR inhibition)

Number of samples	1	12
Aspergillus diff. RG Master	14.5 µl	174 µl
Aspergillus diff. RG Mg-Sol	0.5 µl	6 µl
Aspergillus diff. RG IC	1 µl	12 µl
Total volume	**16 µl***	**192 µl***

* The volume increase caused by adding the internal control is neglected when preparing the PCR assay. The sensitivity of the detection system is not impaired.

3. Pipet 15 µl of the master mix into each PCR tube. Then add 10 µl of the eluted sample DNA (see Table 4). Correspondingly, 10 µl must be used as a positive control and 10 µl of water (Water, PCR grade) as a negative control.

Table 4. Preparation of PCR assay

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. Make sure that the locking ring (accessory of the Rotor-Gene Q instrument) is placed on top of the rotor to prevent accidental opening of the tubes during the run.**
5. **For the detection of *Aspergillus fumigatus/terreus/flavus* DNA, create a temperature profile according to the following steps.**

Setting the general assay parameters	**Figures 1, 2, 3**
Initial activation of the hot-start enzyme	**Figure 4**
Amplification of the DNA (touchdown PCR)	**Figure 5**
Adjusting the fluorescence channel sensitivity	**Figures 6–11**
Starting the run	**Figure 12**

All specifications refer to the Rotor-Gene Q software version 1.7.94. Please find further information on programming Rotor-Gene Instruments in the instrument user manual. In the illustrations these settings are framed in bold black. Illustrations are included for Rotor-Gene Q Instruments.

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

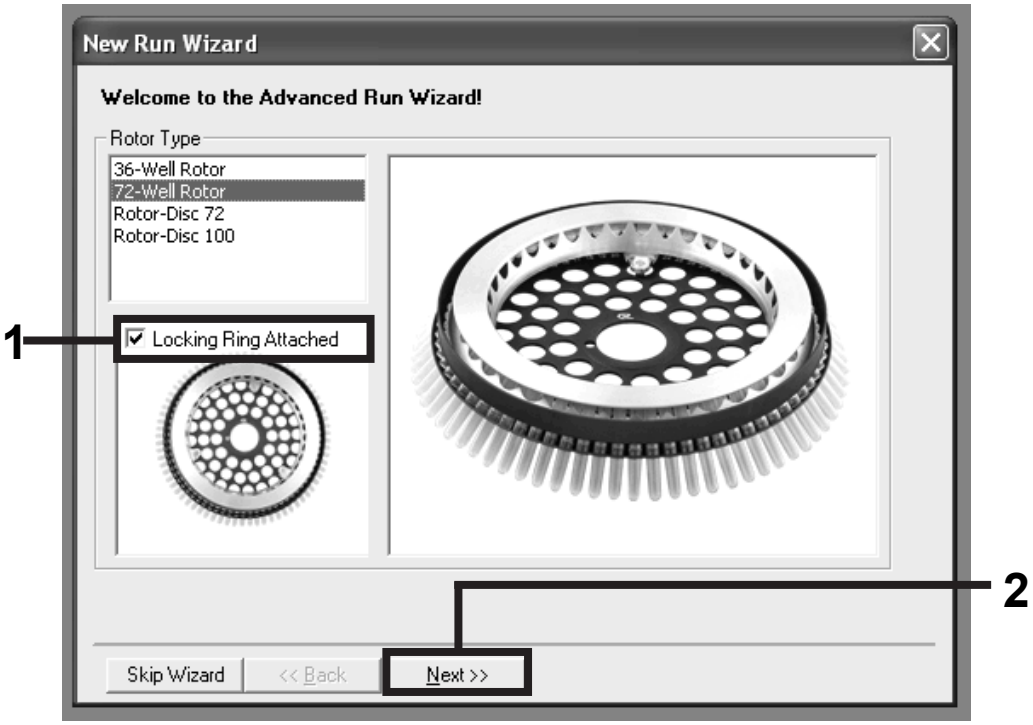

Figure 1. The "New Run Wizard" dialog box.

7. **Select *25* for the reaction volume and click "Next" (Figure 2).**

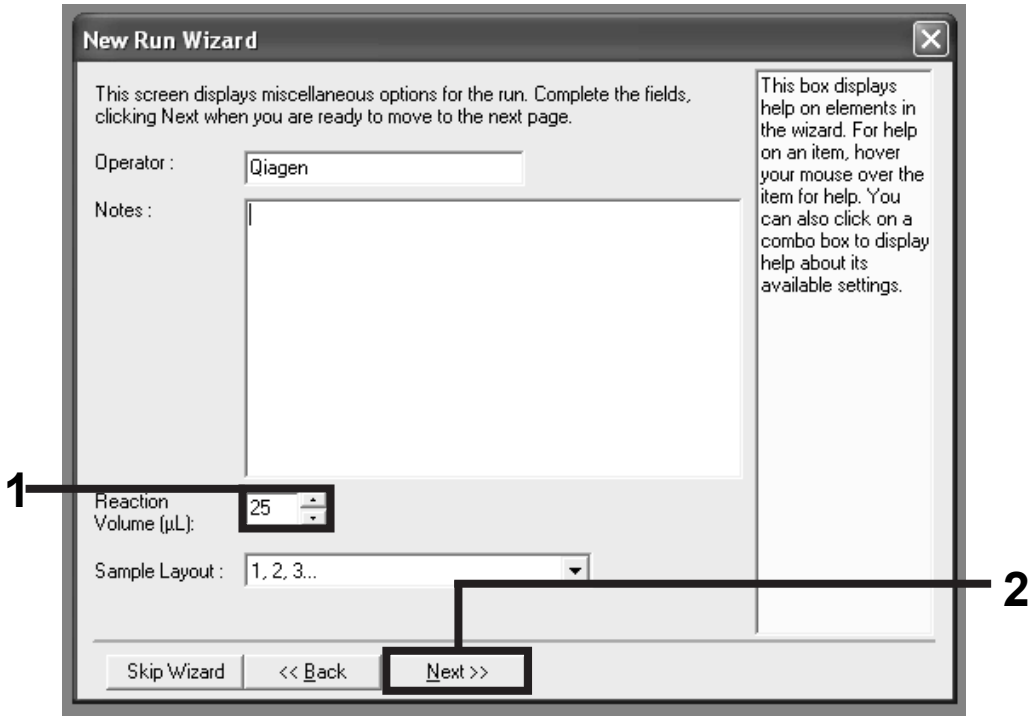

Figure 2. Setting the general assay parameters.

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

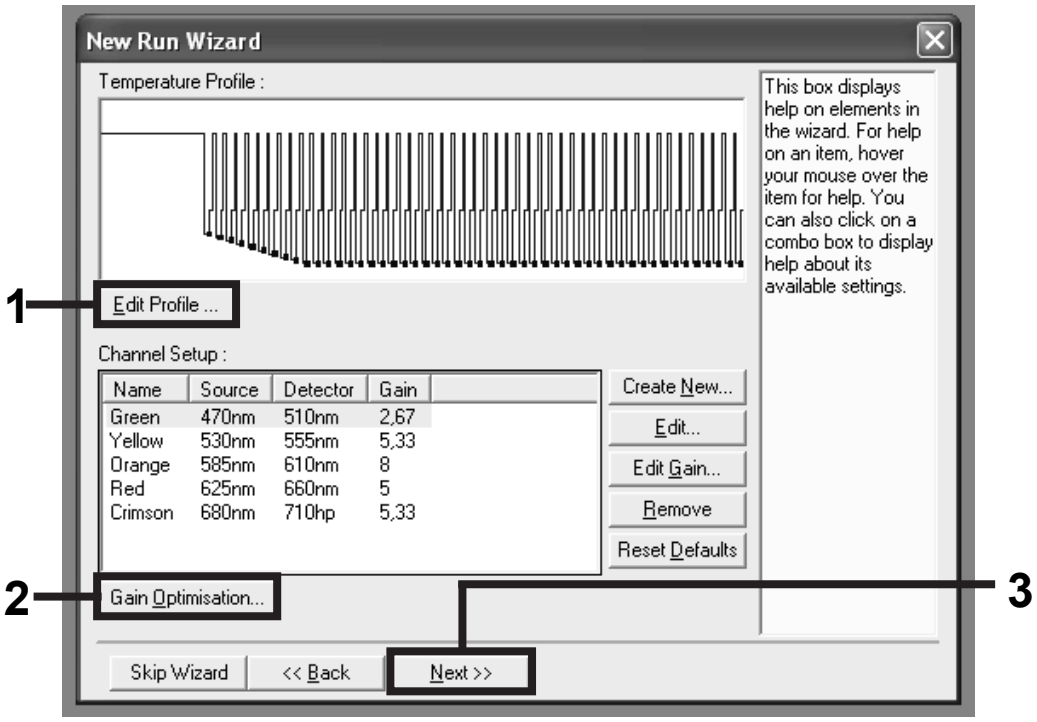

Figure 3. Editing the profile.

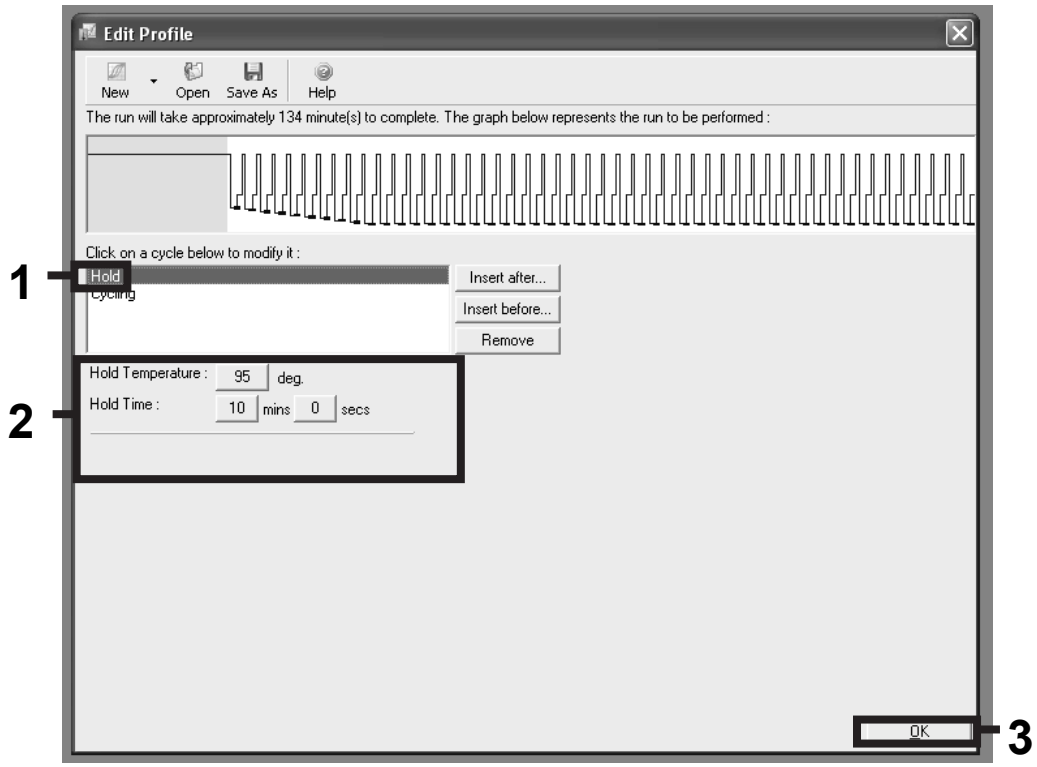

Figure 4. Initial activation of the hot-start enzyme.

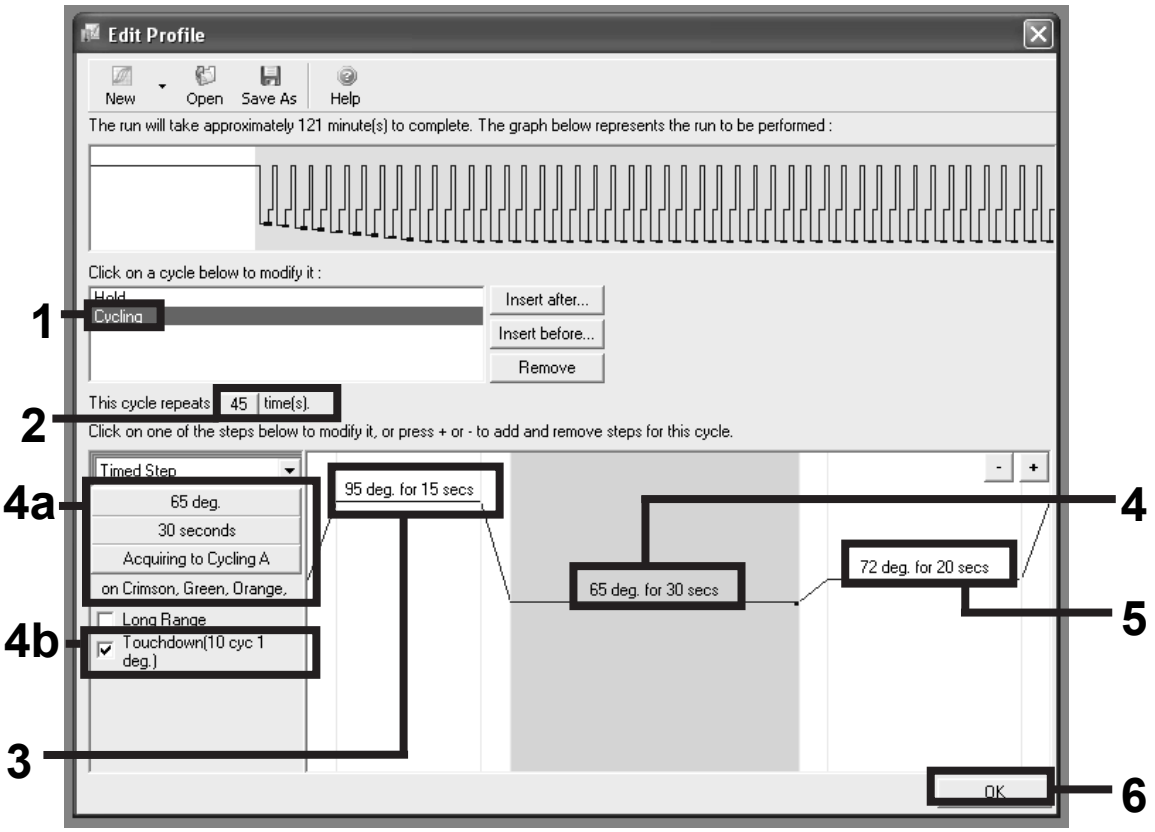

Figure 5. Amplification of the DNA. Set Acquiring to Cycling A on Crimson, Green, Orange, Yellow in the Annealing step. Make sure to activate the touchdown function for 10 cycles in the Annealing step.

9. **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. Set the calibration temperature to *65* degrees to match the annealing temperature of the amplification program (Figure 6). Click "Edit" to set the gain optimization of each channel (Figure 6).**

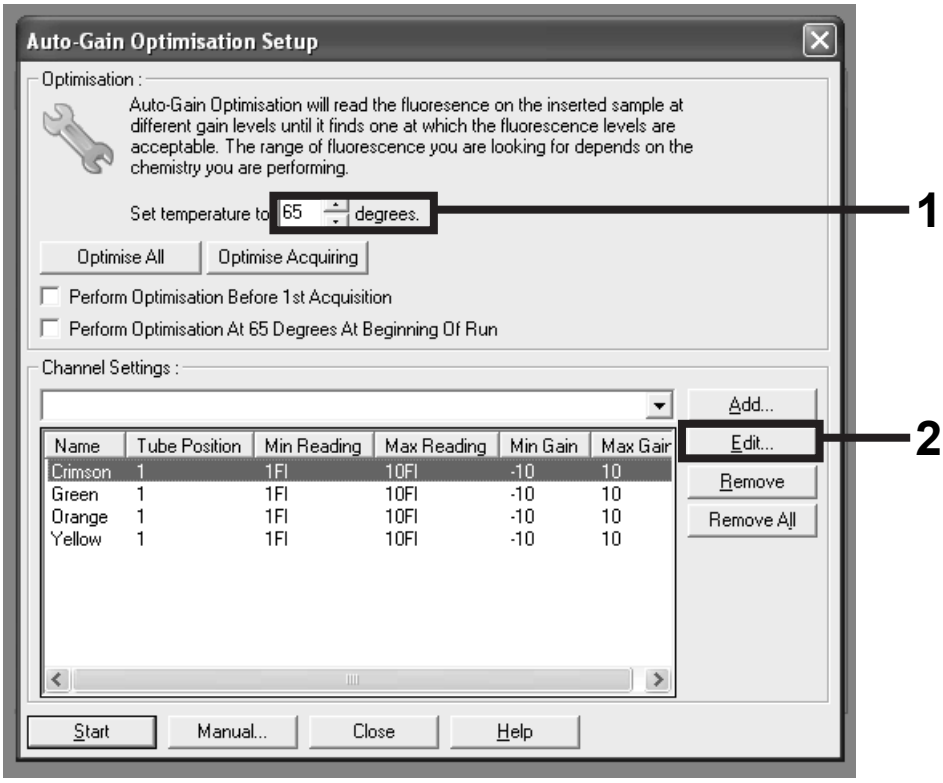

Figure 6. Adjusting the fluorescence channel sensitivity.

10. **Set "Target Sample Range" for the gain optimization of all channels used (Cycling A Crimson, Cycling A Green, Cycling A Orange, and Cycling A Yellow) from *1* FI up to *10* FI. (Figures 7–10).**

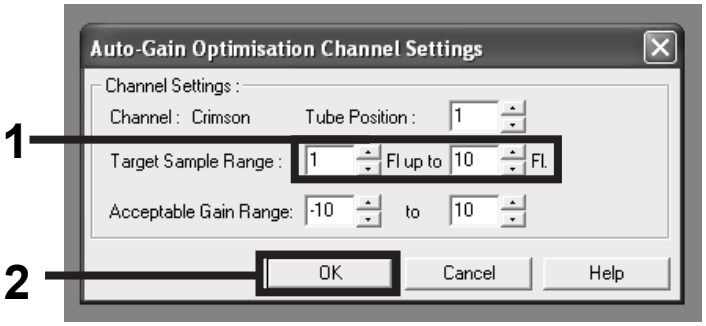

Figure 7. Adjusting the gain optimization range for channel Cycling A Crimson.

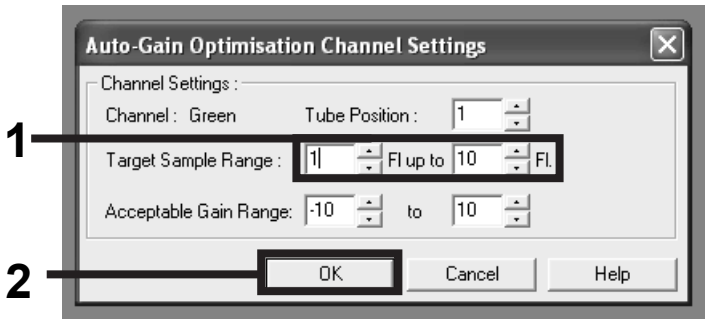

Figure 8. Adjusting the gain optimization range for channel Cycling A Green.

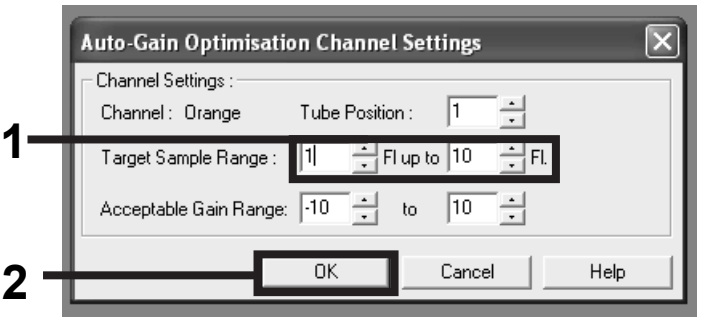

Figure 9. Adjusting the gain optimization range for channel Cycling A Orange.

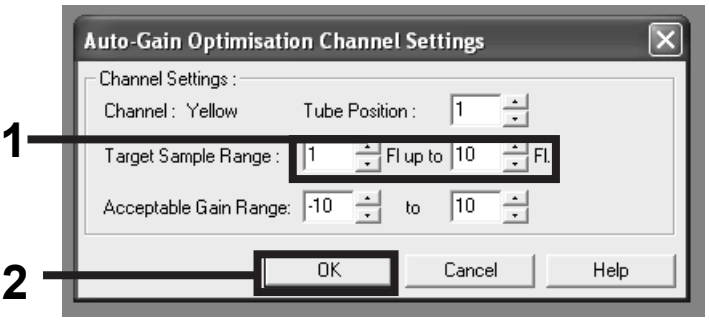

Figure 10. Adjusting the gain optimization range for channel Cycling A Yellow.

11. Click "Start" in the "Auto-Gain Optimisation Setup" dialog box to perform the gain optimization (Figure 11).

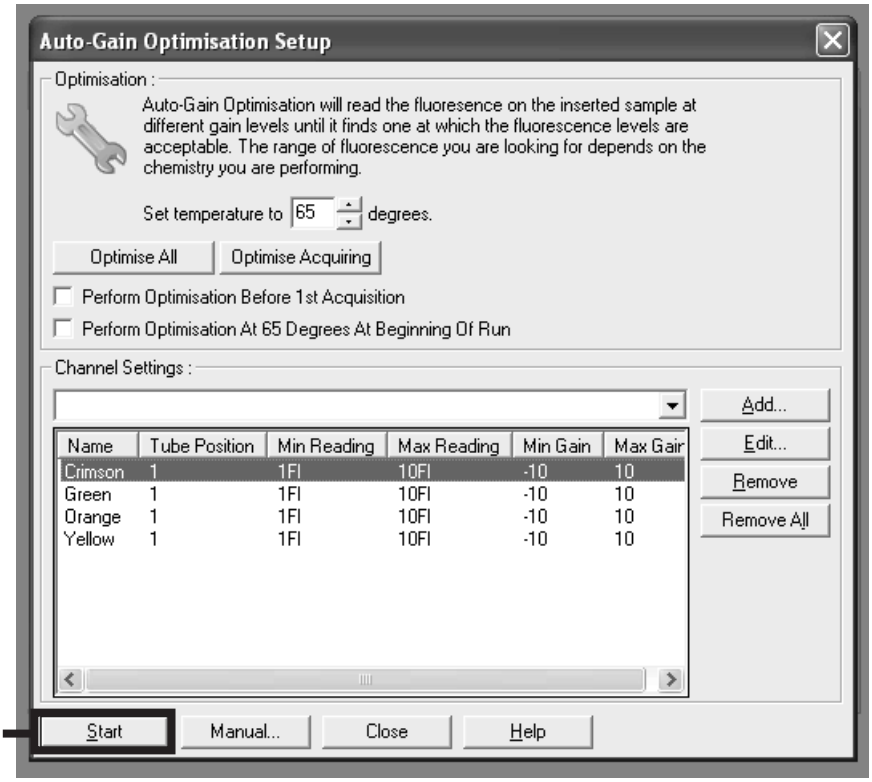

Figure 11. Gain optimization.

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 12). Click "Start Run".**

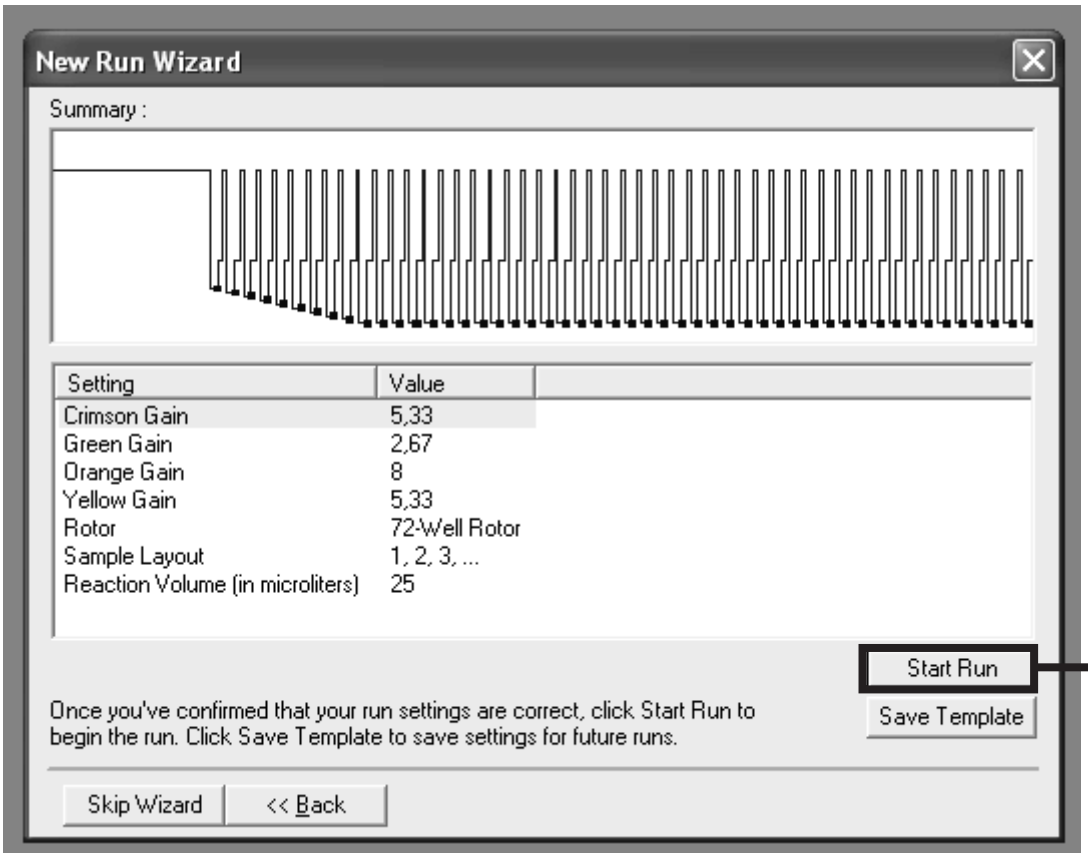

Figure 12. Starting the run.

Interpretation of Results

Data analysis

Analysis of the run data is carried out using software version 1.7.94 or higher of the Rotor-Gene Q instrument. The settings are shown in Table 5.

Table 5. Analysis settings

Channel	Dynamic tube	Ignore 1st	Pathogen
Cycling A Green	ON	–	*A. fumigatus* DNA
Cycling A Orange	ON	10	*A. terreus* DNA
Cycling A Crimson	ON	–	*A. flavus* DNA
Cycling A Yellow IC	ON	–	Internal control

Setting the threshold

The optimal threshold settings in a given combination of Rotor-Gene Q instrument and an *artus* real-time assay should be set empirically by testing each individual combination, since it is a relative value depending on the individual combination of the considered diagnostic workflow (sample preparation and the real-time PCR kit). As a starting point the threshold can be set at a preliminary value of 0.05 for the analysis of the first experiment but should be fine-tuned in a comparative analysis of the next 3 to 5 runs of the *artus* Aspergillus diff. RG PCR Kit. When analyzing these runs the threshold should be manually set just above the background signal of all negative controls and negative samples. The highest threshold value calculated from these experiments will most likely work for the majority of future runs, but the user should nevertheless review the generated threshold value at regular intervals. It will usually be in the range of 0.04–0.13 and should be rounded to no more than 3 decimal places.

The following sections describe the analysis for all 3 specific pathogens, with examples.

Note: These examples represent signals obtained from the positive control.

Signals derived from positive samples may look different, with higher or lower fluorescence intensities and C_T values.

Analysis: Cycling A Green (*A. fumigatus*)

A signal is detected in fluorescence channel Cycling A Green.
The result of the analysis is positive: the sample contains *Aspergillus fumigatus* DNA.

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

In fluorescence channel Cycling A Green no signal is detected. At the same time, a signal from the internal control appears in the Cycling A Yellow channel.
In the sample no *Aspergillus fumigatus* DNA is detectable. It can be considered negative.

In the case of a negative *A. fumigatus* PCR, the detected signal of the internal control rules out the possibility of PCR inhibition (see Figure 13).

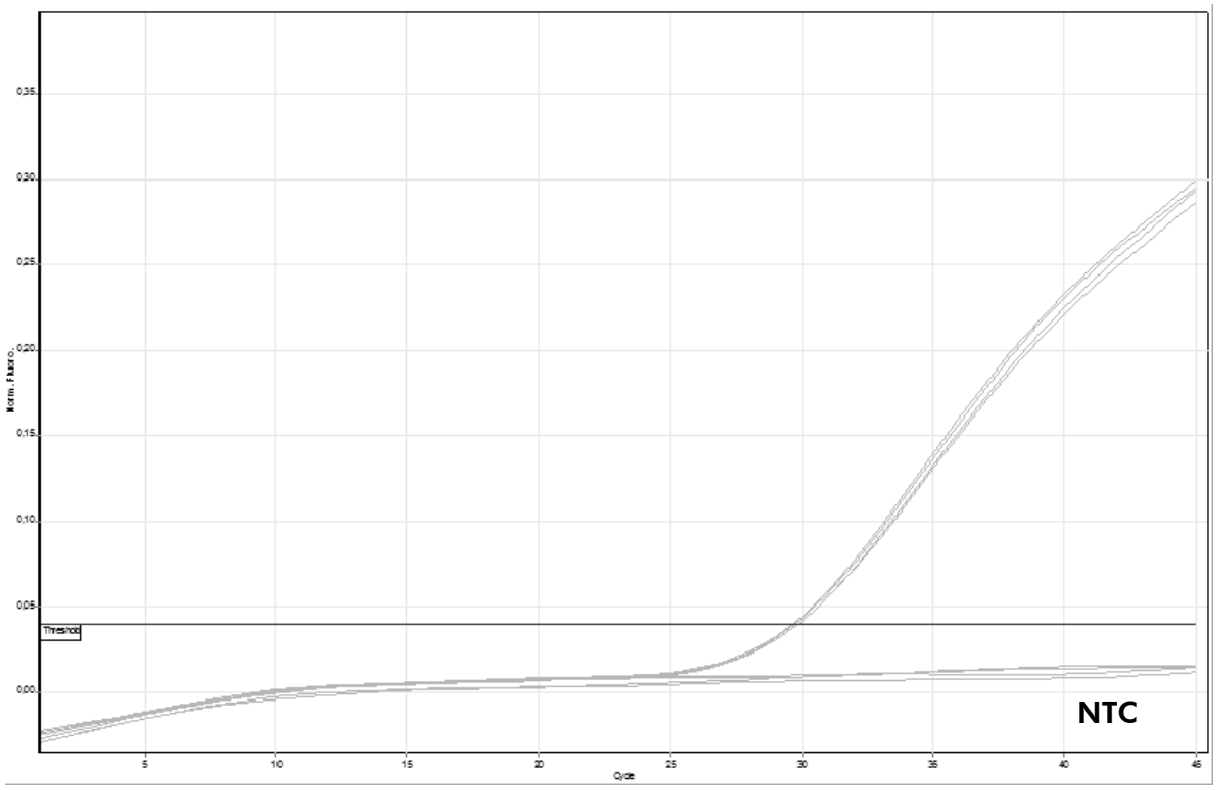

Figure 13. Example of detection of the positive control Cycling A Green (*A. fumigatus*).
NTC: No template control (Aspergillus fum/terr/fla negative control).

Analysis: Cycling A Orange (*A. terreus*)

A signal is detected in fluorescence channel Cycling A Orange.
The result of the analysis is positive: the sample contains *Aspergillus terreus* DNA.

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

In fluorescence channel Cycling A Orange no signal is detected. At the same time, a signal from the internal control appears in the Cycling A Yellow channel.
In the sample no *Aspergillus terreus* DNA is detectable. It can be considered negative.

In the case of a negative *A. terreus* PCR, the detected signal of the internal control rules out the possibility of PCR inhibition (see Figure 14).

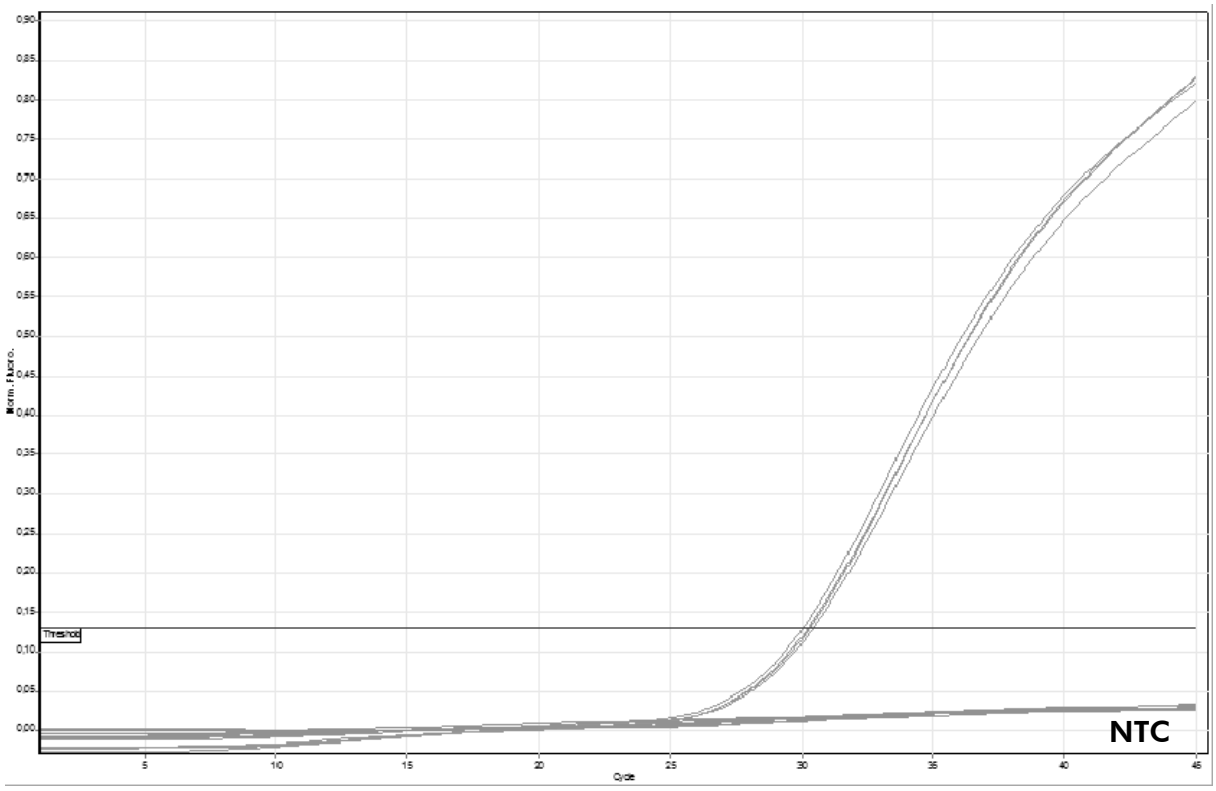

Figure 14. Example of detection of the positive control Cycling A Orange (*A. terreus*).
NTC: No template control (*Aspergillus fum/terr/fla* negative control).

Analysis: Cycling A Crimson (*A. flavus*)

A signal is detected in fluorescence channel Cycling A Crimson.
The result of the analysis is positive: the sample contains *Aspergillus flavus* DNA.

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

In fluorescence channel Cycling A Crimson no signal is detected. At the same time, a signal from the internal control appears in the Cycling A Yellow channel.
In the sample no *Aspergillus flavus* DNA is detectable. It can be considered negative.

In the case of a negative *A. flavus* PCR, the detected signal of the internal control rules out the possibility of PCR inhibition (see Figure 15).

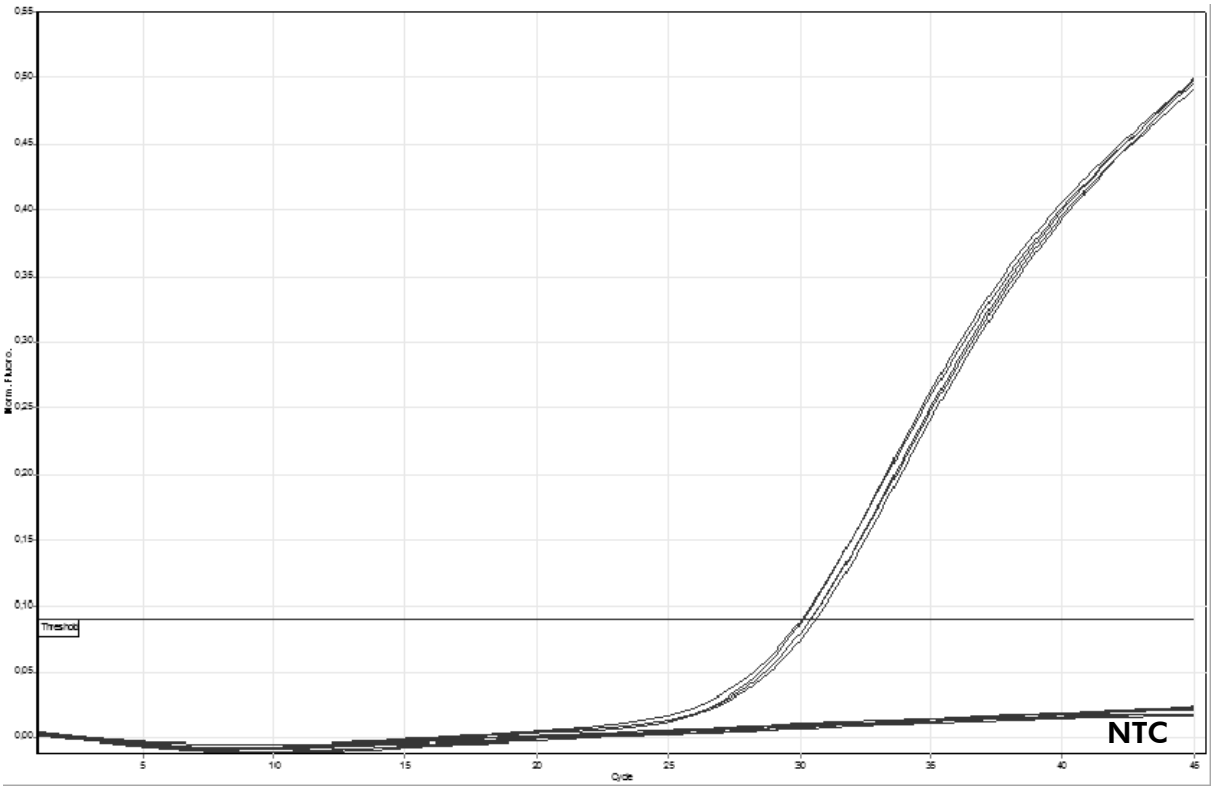

Figure 15. Example of detection of the positive control Cycling A Crimson (*A. flavus*).
NTC: No template control (*Aspergillus fum/terr/fla* negative control).

Analysis: Cycling A Yellow (internal control)

Figure 16 shows an example of normal detection of the internal control.

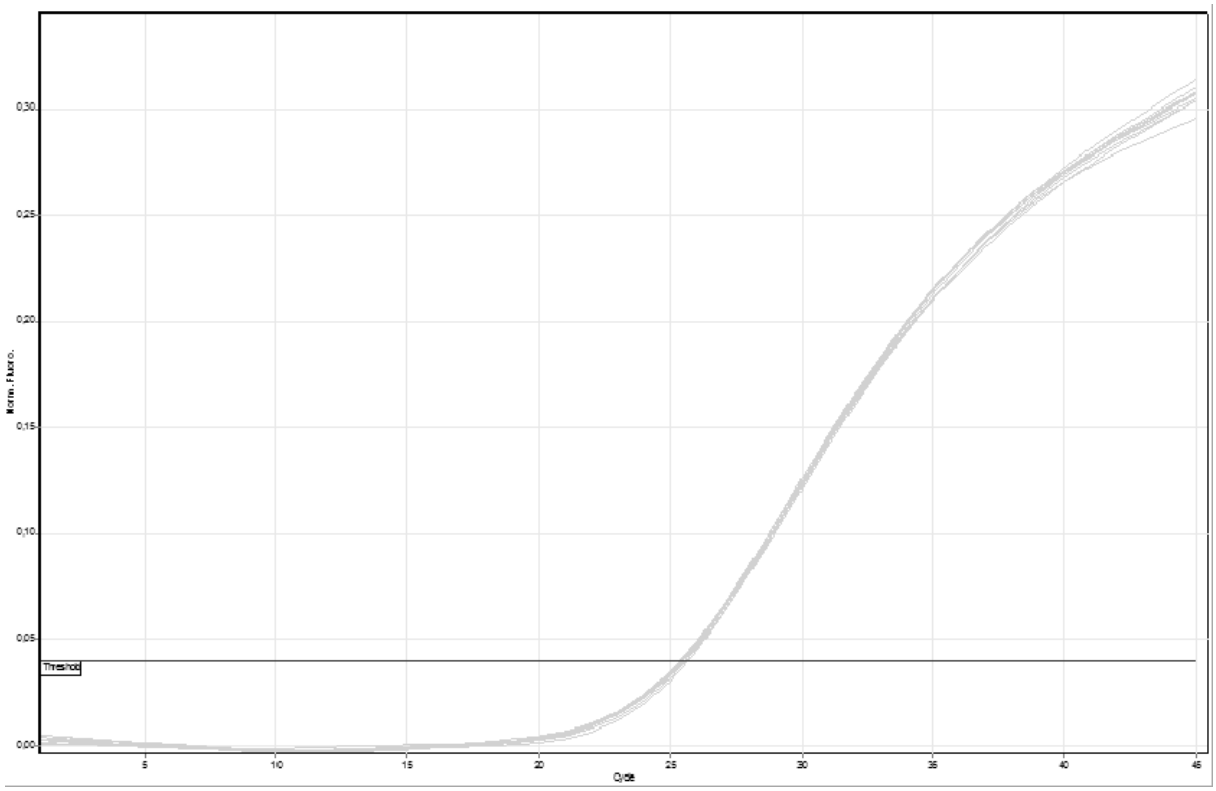

Figure 16. Example of detection of the internal control Cycling A Yellow.

Due to inhibitory effects of the sample eluate, the IC signal could be decreased.

No signal is detected in the Cycling A Green/Orange/Crimson or in the Cycling Yellow channels.
No result can be concluded.

Information regarding error sources and their solution can be found in “Troubleshooting guide”, page 28.

Analysis overview

Table 6 summarizes the various results that could be obtained with the *artus* Aspergillus diff. RG PCR Kit.

Table 6. Overview of results obtained with the *artus* Aspergillus diff. RG PCR Kit

Channel				
Cycling A Green	**Cycling A Orange**	**Cycling A Crimson**	**Cycling A Yellow IC**	**Result**
+	–*	–*	+ (–)†	*Aspergillus fumigatus* DNA detected
–*	+	–*	+ (–)†	*Aspergillus terreus* DNA detected
–*	–*	+	+ (–)†	*Aspergillus flavus* DNA detected
–	–	–	+	No specific DNA detected
–	–	–	–	Result inconclusive

* Multiple positive signals are possible.

† Strong positive signal in specific channel or inhibitory substances could minimize signal of IC.

Troubleshooting guide

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

	Comments and suggestions
No signal with positive controls (*Aspergillus fumigatus/terreus/flavus*) in fluorescence channel Cycling A Green/Orange/Crimson	
a) The selected fluorescence channel for PCR data analysis does not comply with the protocol	For data analysis select the fluorescence channel Cycling A Green/Orange/Crimson or Cycling for the analytical Aspergillus diff. PCR and the fluorescence channel Cycling A Yellow 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 "Protocol: PCR", page 12.
c) Incorrect configuration of the PCR	Check your work steps by means of the pipetting scheme, and repeat the PCR, if necessary. See "Protocol: PCR", page 12.
d) The storage conditions for one or more kit components did not comply with the instructions given in "Reagent Storage and Handling" (page 8)	Check the storage conditions and the expiration date (see the kit label) of the reagents and use a new kit, if necessary.
e) The *artus* Aspergillus diff. RG PCR Kit has expired	Check the storage conditions and the expiration date (see the kit label) of the reagents and use a new kit, if necessary.

	Comments and suggestions
Weak or no signal of the internal control of a negative sample subjected to purification using the QIAamp UCP PurePathogen Blood Kit in fluorescence channel Cycling A Yellow and simultaneous absence of a signal in channel Cycling A Green/Orange/Crimson	
a) The PCR conditions do not comply with the protocol	Check the PCR conditions (see above) and repeat the PCR with corrected settings, if necessary.
b) The PCR was inhibited	Make sure that you use the recommended isolation method and closely follow the manufacturer's instructions.
c) DNA was lost during extraction	If the internal control was 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 the 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 given in "Reagent Storage and Handling" (page 8)	Check the storage conditions and the expiration date (see the kit label) of the reagents and use a new kit, if necessary.
e) The *artus* Aspergillus diff. RG PCR Kit has expired	Check the storage conditions and the expiration date (see the kit label) of the reagents and use a new kit, if necessary.
Signals with the negative controls in fluorescence channel Cycling A Green/Orange/Crimson 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.

Quality Control

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

Specifications

Analytical sensitivity

The analytical sensitivity (without sample preparation) of the *artus* Aspergillus diff. RG PCR Kit was determined by probit analysis with a probability of 95% for positive samples. Analytical sensitivity for all 3 targets was ≤10 copies/µl.

Specificity

The analytical specificity of the *artus* Aspergillus diff. 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 relevant strains has thus been ensured.

A potential cross-reactivity of the *artus* Aspergillus diff. RG PCR Kit was tested using the control group listed in Table 7. None of the tested pathogens was reactive. No cross-reactivity appeared with mixed infections.

Table 7. Testing the specificity of the *artus* Aspergillus diff. RG PCR Kit with potentially cross-reactive pathogens

Control group	*Aspergillus* sp. (Cycling A Green/Orange/Crimson)	Internal control (Cycling A Yellow)
Aspergillus niger	–	+
Aspergillus ustus	–	+
Aspergillus versicolor	–	+
Candida albicans	–	+
Candida glabrata	–	+
Candida kefyr	–	+
Candida tropicalis	–	+
Panicillium chrysogenum	–	+
Saccharomyces cervisiae	–	+
Controls	–	+
Aspergillus fumigatus Control	+ (green)	+
Aspergillus terreus Control	+ (orange)	+
Aspergillus flavus Control	+ (crimson)	+
No template control	–	+

References

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

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

Symbols

The following symbols may appear on the packaging and labeling:

 <N>	Contains reagents sufficient for <N> tests
	Use by
	Catalog number
	Lot number
	Material number
	Components
	Contains
	Number
	Temperature limitation
	Manufacturer
	Consult instructions for use

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 Aspergillus diff. RG PCR Kit (24)	For 24 reactions: Master, Magnesium Solution, Positive Control, Internal Control, Water (PCR grade)	4640203
QIAamp UCP PurePathogen Blood Kit — for simultaneous purification of bacterial, fungal, viral, and free-circulating nucleic acids from whole blood		
QIAamp UCP PurePathogen Blood Kit (10)	For 10 preps, includes: QIAamp Mini columns, QIAGEN proteinase K, tube extenders (20 ml), buffers, VacConnectors, pathogen lysis tubes, and collection tubes (2 ml)	50112
Rotor-Gene Q — for outstanding performance in real-time PCR		
Rotor-Gene Q 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	9001570
Rotor-Gene Q 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	9001640
Rotor-Gene Q 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	9001580

Product	Contents	Cat. no.
Rotor-Gene Q 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	9001650
Rotor-Gene Q 6plex Platform	Real-time PCR instrument with 6 channels (blue, green, yellow, orange, red, crimson), including laptop comptuer, software, accessories: includes 1-year warranty on parts and labor, installation and training not included	9001590
Rotor-Gene Q 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	9001660
Rotor-Gene Q accessories		
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 10,000 reactions	981008

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

Notes

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Trademarks: QIAGEN®, QIAamp®, *artus*®, HRM®, Rotor-Gene® (QIAGEN Group).

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1074318 01/2013

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