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artus® Malaria RG PCR Kit Handbook

24

Version 1
For use with
Rotor-Gene® Q MDx instruments

4601263

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1046977-EN

Sample to Insight

Contents

Intended Use

The *artus* Malaria RG PCR Kit is an in vitro nucleic acid amplification test for the detection and quantification of pathogenic *Plasmodium* spp. (malaria) DNA in human EDTA-blood and is configured for use with Rotor-Gene Q MDx Instruments.

The *artus* Malaria RG PCR Kit is intended for use in conjunction with clinical presentation and other laboratory markers (including thick and thin Giemsa-stain microscopy) for the diagnosis of malaria. The *artus* Malaria RG PCR Kit is not intended to be used as a screening test for malaria.

Summary and Explanation

The *artus* Malaria RG PCR Kit constitutes a ready-to-use system for the detection of *Plasmodium*-specific DNA using real-time PCR. This diagnostic test kit utilizes the polymerase chain reaction (PCR) and must be run on the Rotor-Gene Q MDx 5plex HRM instrument*. The *artus* Malaria RG PCR kit is intended for in vitro diagnostic use only by healthcare professionals in clinical laboratories.

The assay includes a heterologous amplification system (Internal Control) to identify possible PCR inhibition and to confirm the integrity of the kit reagents.

Pathogen information

Malaria is caused by pathogenic *Plasmodium* parasites, with 4 species (*Plasmodium falciparum, P. vivax, P. ovale* and *P. malariae*) causing disease in humans. Recently a fifth

* If applicable, the Rotor-Gene Q 5plex/5plex HRM instrument with a production date of May 2011, or later can be used. The production date can be obtained from the serial number on the back of the instrument. The serial number is in the format "mmyynnn" where "mm" indicates the production month in digits, "yy" indicates the last two digits of the production year, and "nnn" indicates the unique instrument identifier.

malarial parasite, *P. knowlesi*, has also been shown to cause human disease. All 5 parasites have different clinical manifestations in humans with *P. falciparum* being the most serious, and sometimes fatal, malarial parasite. Malaria is limited to tropical and sub-tropical regions with up to 500 million infections occurring annually and over 1 million deaths. Transmission requires a mosquito vector (*Anopheles*), which is found worldwide. Congenital transmission and infection via blood transfusion and contaminated syringes may also occur, albeit rarely (1, 2).

Following an initial attack of the liver, the parasites reach the blood stream. After infiltration of the parasites into erythrocytes, they commence proliferation. Eventually the erythrocytes burst and the released parasites can again infect erythrocytes. This cyclic course of disease causes most of the clinical symptoms seen with malaria, including headache, nausea and fever and more serious manifestations such as severe anemia, endothelial pathology and end organ failure.

The *artus* Malaria RG PCR Kit provides a fast determination from EDTA-blood of the 4 most common Plasmodium species affecting humans *Plasmodium* species affecting humans (*P. falciparum, P. vivax, P. ovale* and *P. malariae*).

Principle of the Procedure

The *artus* Malaria RG PCR Kit constitutes a ready-to-use system for the detection of *Plasmodium* DNA by polymerase chain reaction (PCR) using the Rotor-Gene Q MDx. The Malaria RG Master contains reagents and enzymes for the specific amplification of a 163 bp region of *Plasmodium* 18S rRNA, and for the direct detection of the specific amplicon in fluorescence channel Cycling A.Green of Rotor-Gene Q MDx.

In addition, the *artus* Malaria 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. The detection limit of the analytical *Plasmodium* PCR (see "Analytical sensitivity", page 33) is not reduced by inclusion of the internal control. External positive controls (Malaria RG QS 1-4) are supplied which allow the determination of the pathogen load. For further information, please refer to "Quantitation", page 31.

Materials Provided

Kit contents

***artus* Malaria RG PCR Kit**		**(24)**
Catalog number		**4601263**
Number of reactions		**24**
Blue	Malaria RG Master	2 x 12 reactions
Red	Malaria RG QS 1† 7×10^4 copies/µl	1 x 200 µl
Red	Malaria RG QS 2† 7×10^3 copies/µl	1 x 200 µl
Red	Malaria RG QS 3† 7×10^2 copies/µl	1 x 200 µl
Red	Malaria RG QS 4† 7×10^1 copies/µl	1 x 200 µl
Green	Malaria RG IC‡	1 x 1000 µl
White	Water (PCR grade)	1 x 1000 µl
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† QS: Quantitation standard.

‡ IC: Internal control.

Materials Required but Not Provided

Prior to use, ensure that instruments have been checked and calibrated according to the manufacturer's recommendations.

Reagents

- High Pure PCR Template Preparation Kit (Roche Life Science, cat. no. 11796828001); see "Sample preparation", page 13)

Consumables

- 0.1 ml Strip Tubes and Caps, for use with 72-well rotor (QIAGEN, cat. no. 981103 or 981106)
- Nuclease-free, low DNA-binding microcentrifuge tubes for preparing master mixes
- Nuclease-free pipet tips with aerosol barriers

Equipment

- Rotor-Gene Q MDx with software version 2.3 or later
- Cooling/Loading Block 72 x 0.1 ml Tubes, aluminum block for manual reaction setup
- Dedicated adjustable pipets for sample preparation
- Dedicated adjustable pipets for PCR master mix preparation
- Dedicated adjustable pipets for dispensing template DNA
- Vortex mixer
- Benchtop centrifuge with rotor for 2 ml reaction tubes

Warnings and Precautions

For in vitro diagnostic use.

Read all instructions carefully before using the test.

Warnings

When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles.

Precautions

- Use of this product is limited to personnel specially instructed and trained in the techniques of real-time PCR and in vitro diagnostic procedures.
- Specimens should always be treated as infectious and/or biohazardous in accordance with safe laboratory procedures.
- 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 pipet tips with aerosol barriers.
- 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.

- Store positive and/or potentially positive material separate from all other components of the kit.
- 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.
- Do not use components of the kit that have passed their expiration date.
- Discard sample and assay waste according to your local safety regulations.

Reagent Storage and Handling

Kit components

The *artus* Malaria RG PCR Kit is shipped on dry ice. The components of the kit should arrive frozen. If one or more components are not frozen upon receipt, or if tubes have been compromised during shipment, contact QIAGEN Technical Services for assistance. Upon receipt, store all kit components at –30°C to –15°C.

It is strongly recommended to use the product immediately after first thawing. Protect Malaria RG Master from light. The assay should be set up using a cooling block.

The *artus* Malaria RG PCR Kit includes:

- Master reagent (Malaria RG Master)
- Internal Control (Malaria RG IC)
- Four quantification standards (Malaria RG QS 1–4)
- PCR-grade water (H_2O)

The Malaria RG Master reagent contains all components (buffer, enzymes, primers and probes) for amplification and detection of *Plasmodium*-specific DNA and the internal control in a single reaction.

The quantification standards contain standardized concentrations of *Plasmodium*-specific DNA. These can be used individually as positive controls or together to generate a standard curve, which can be used to determine the pathogen load in the sample. The concentrations of the quantification standards are shown in Table 1.

Table 1. Concentration of *Plasmodium*-specific DNA in Quantification Standards

Quantification Standard	Concentration (copies/µl)
QS1	70,000
QS2	7000
QS3	700
QS4	70

Procedure

Specimen collection, storage and transport

Note: Current studies refer to EDTA-blood as the most suitable sample material. Therefore, we recommend the use of this material with the *artus* Malaria RG PCR Kit.

The internal validation of the *artus* Malaria RG PCR Kit has been performed using EDTA-samples. Other sample materials are not validated. Please use only recommended nucleic acid isolation kits (see "Sample preparation", page 13) for sample preparation.

The instructions regarding collection, transport and storage must be strictly observed.

Specimen collection

The use of commercially available EDTA tubes for blood sampling is essential. After blood draw, tubes must be inverted several times to prevent clogging. Taking blood between fever attacks may lead to notably smaller numbers of parasites per blood draw due to the cyclic nature of parasitemia. Therefore, blood must be taken from patients during a fever attack.

Sample storage

Note: The sensitivity of the assay can be reduced if the samples are frozen as a matter of routine or stored for a longer period of time than the recommended maximum of.<2 days at room temperature. Specimens not processed within 2 days must be stored at 2–8°C.

Sample transport

Sample material should be transported in a shatterproof transport container and according to the local and national instructions for the transport of pathogen material..*

Sample preparation

Plasmodium-specific target sequences are amplified from DNA. As assay performance is dependent on the quality of the template DNA, make sure to use a sample preparation kit that yields DNA suitable for use in downstream PCR.

The following isolation kit is recommended:

- High Pure PCR Template Preparation Kit (Roche Life Science, cat. no. 11796828001)

- Carrier RNA is not required.
- It is recommended to elute the DNA in 50 µl elution buffer to get the highest sensitivity of the *artus* Malaria RG PCR Kit.
- Blood collection tubes coated with anticoagulants may inhibit the PCR.

  These inhibitors will be eliminated by the use of the isolation kit listed above.
- Heparin blood should not be used.
- If isolation protocols include ethanol-containing washing buffers, carry out an additional centrifugation step (three minutes, 13,000 rpm) before the elution to remove any remaining ethanol. This prevents possible inhibition of PCR.
- The *artus* Malaria RG PCR Kit should not be used with phenol-based isolation methods.
- **Important**: The Internal Control of the *artus* Malaria RG PCR Kit can be used directly in the isolation procedure (see “Internal Control”, page 14).

* International Air Transport Association. Dangerous Goods Regulations.

Internal Control

An internal control (Malaria RG IC) is supplied. This allows the user both to monitor 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, if the DNA is eluted in 50 µl elution buffer, 5 µl of the internal control should be added. If a Proteinase K treatment is part of the extraction process, the internal control should be added after this treatment step.

Important: The internal control must not be added to the sample material directly.

Note: If the internal control is added to the lysis buffer, the mixture of internal control and lysis buffer must be freshly prepared and used instantly. Storage of the mixture at room temperature or at 2–8°C for only a few hours may lead to internal control failure and reduced extraction efficiency.

Optional: The internal control can be used exclusively to check for possible PCR inhibition. For this application, add 0.5 µl of the internal control per reaction directly to 15 µl Malaria RG Master. For each PCR reaction, use 15 µl of the master mix produced as described in Table 3 and add 5 µl of the purified sample. If you are preparing a PCR run for several samples, increase the volume of the Malaria RG Master and the internal control according to the number of samples (see "Detection of *Plasmodium*-specific DNA", page 15).

Quantification Standards

The enclosed quantification standards (Malaria RG QS 1–4) are treated as previously purified samples and the same volume is used (5 µl). To generate a standard curve on the Rotor-Gene Q MDx, all 4 quantification standards should be used and defined in the **Edit Samples** dialog box as standards with the specified concentrations (see the instrument user manual).

Detection of *Plasmodium*-specific DNA

Important points before starting

- Before beginning the procedure, read "Precautions", page 9.
- Take time to familiarize yourself with the Rotor-Gene Q MDx instrument before starting the protocol. Read the instrument user manual.
- Make sure that at least one positive control and one negative control (PCR-grade water) are included per PCR run.

Things to do before starting

- Make sure that the cooling block (accessory of the Rotor-Gene Q MDx) 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

Note: All specifications refer to the Rotor-Gene Q MDx software version 2.3. For further information on programming Rotor-Gene Q MDx instruments, see the applicable instrument user manual.

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: A master mix according to step 2b (that is, including the internal control) must be prepared to set up the reactions for the quantification standards and the NTC.

2a. The internal control has already been added to the isolation (see "Internal Control", page 14). 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)

Component	1 reaction	12 reactions
Malaria RG Master	15 µl	180 µl

2b. The internal control must be added directly to the mixture of Malaria RG Master. In this case, prepare a master mix according to Table 3.

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

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

Component	1 reaction	12 reactions
Malaria RG Master	15 µl	180 µl
Malaria RG IC*	0.5 µl	6 µl
Total volume	15.5 µl	186 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 as prepared in step 2a or 2b into each PCR tube. Then add 5 µl of the eluted sample DNA and mix well by pipetting repeatedly up and down. Correspondingly, add 5 µl of a positive control or quantification standard and 5 µl water (PCR-grade water) as a negative control.

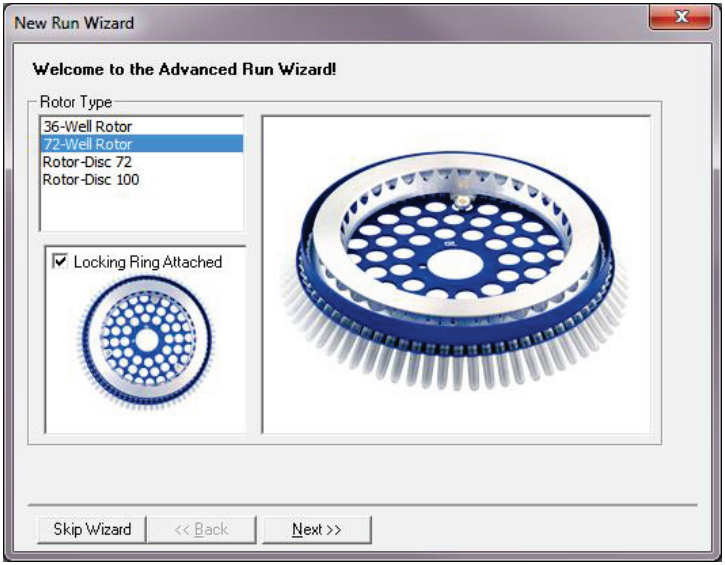
   Make sure to have at least one positive control and one negative control per run. For quantification, use all 4 quantification standards (QS 1–QS 4).

4. Close the PCR tubes. Make sure that the locking ring (accessory of the Rotor-Gene Q MDx instrument) is placed on top of the rotor.

5. Click **New** to start a new run.
   The **New Run** dialog box opens.

6. Click the **Advanced** tab, select **Empty Run** from the list and press **New**.

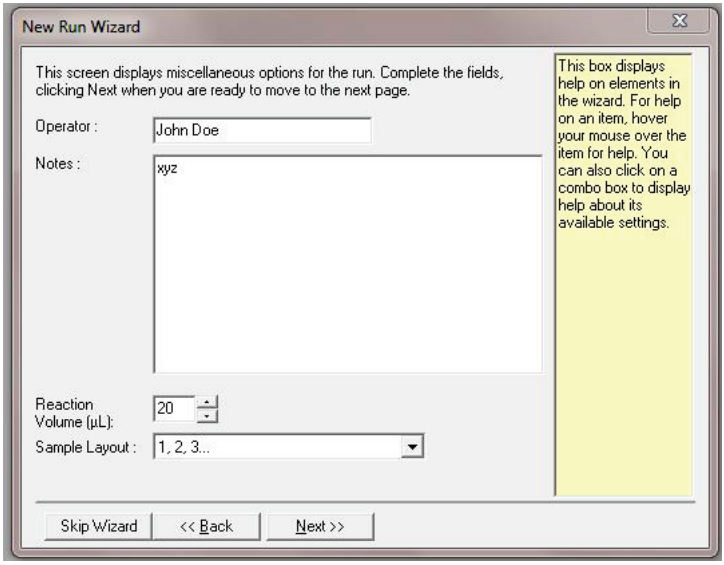
7. The **New Run Wizard** dialog box opens.
   Example:



8. In the **Rotor Type** panel, select **72-Well Rotor**, check the **Locking Ring Attached** box and click the **Next >>** button.

   The next screen of the **New Run Wizard** dialog box opens.

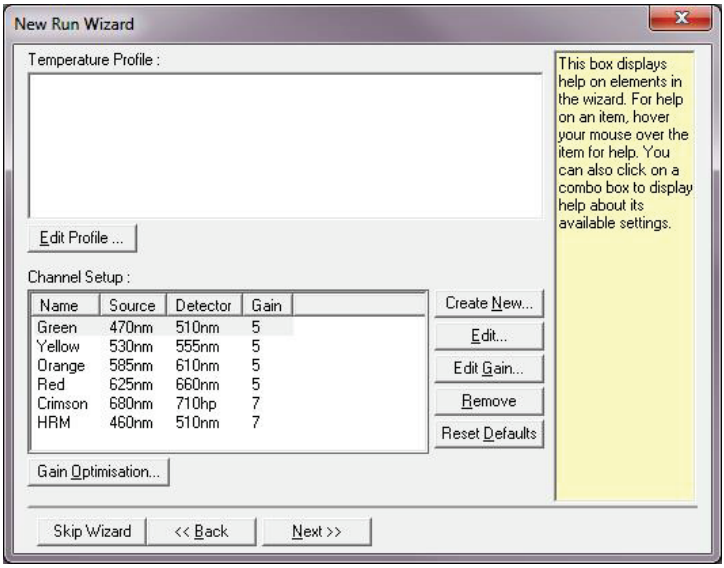
   Example:



9. In the **Reaction Volume (µL)** dialog field, enter **20** and click the **Next >>** button.

   The next screen of the **New Run Wizard** dialog box opens.

   Example:



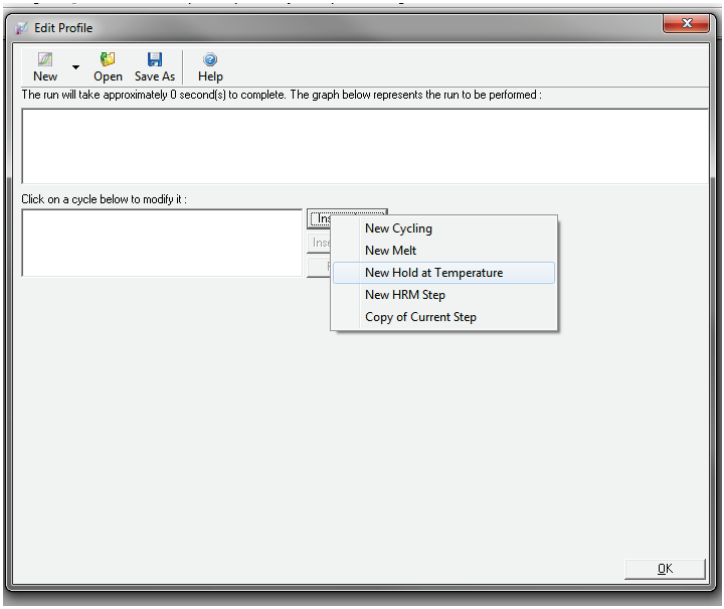
10. Click the **Edit Profile...** button.

    The **Edit Profile** dialog box opens.

11. In the **Click on a cycle below to modify it:** panel, click the **Insert after…** button.

    A list opens displaying multiple options.

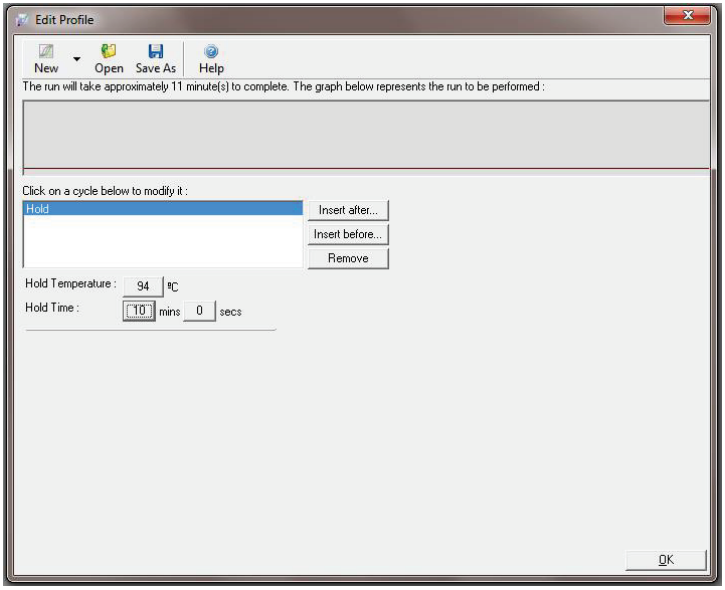
    Example:



12. Select **New Hold at Temperature** from the list.

    The hold parameters are displayed.

    Example:



13. Set the **Hold Temperature:** to **94°C** and the **Hold Time:** to **10 mins 0 secs**.

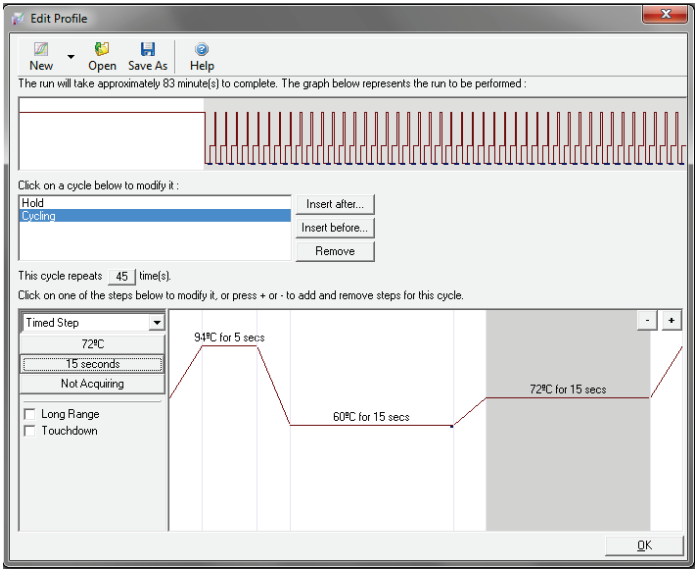
14. Select **Hold** in the **Click on a cycle below to modify it:** panel and click the **Insert after…** button.

    A list opens displaying multiple options.

15. Select **New Cycling** from the list.

    The cycle parameters are displayed.

    Example:



16. In the **This cycle repeats** field, enter **45 time(s)**.

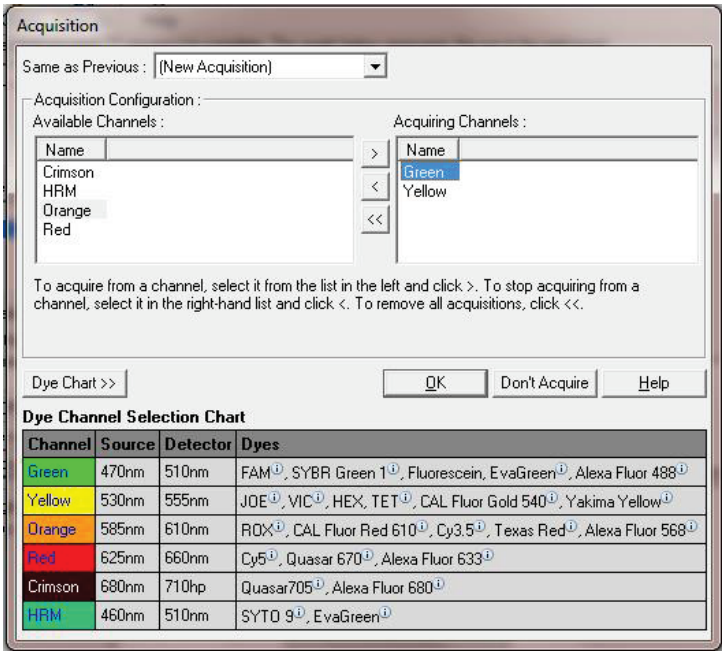
17. Using the software (refer to the instrument user manual), modify the steps of the program cycle to the following sequential parameters:

    - **94°C for 5 secs**
    - **60°C for 15 secs (acquisition step)**
    - **72°C for 15 secs**

18. Select the annealing step (the second step in the cycle – **60°C for 15 seconds**— and click the **Not Acquiring** button.

    The **Acquisition** dialog box opens.

    Example:



19. In the **Acquisition Configuration:** panel, select **Green** from the **Available Channels:** list. Click the **>** button to move **Green** to the **Acquiring Channels:** list.

20. In the **Acquisition Configuration:** panel, select **Yellow** from the **Available Channels:** list. Click the **>** button to move **Yellow** to the **Acquiring Channels:** list.

21. Click **OK**.

    The **Acquisition** dialog box closes, and the **Edit Profile** dialog box opens.

22. Select the **72°C** step, and click **Acquiring to Cycling B green**.

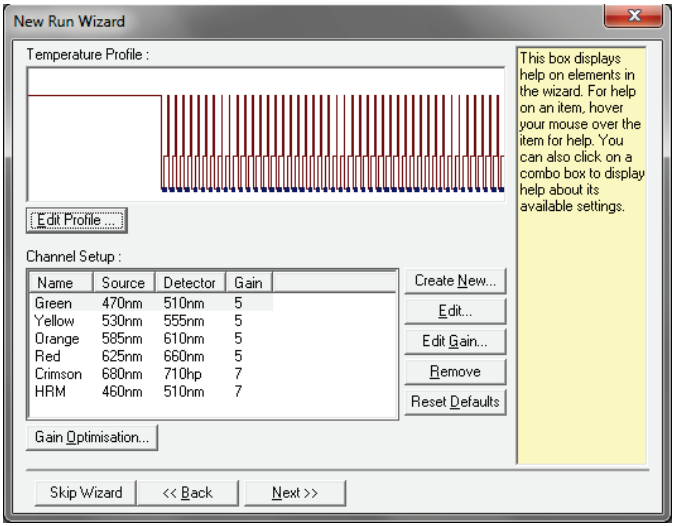
23. In the **Acquisition Configuration** panel, click **don't acquire**.

    The window closes. The run profile will only contain data acquired from the 60°C step (shown by the blue square in the scheme of the profile).

24. Click **OK**.

    The **New Run Wizard** dialog box opens.

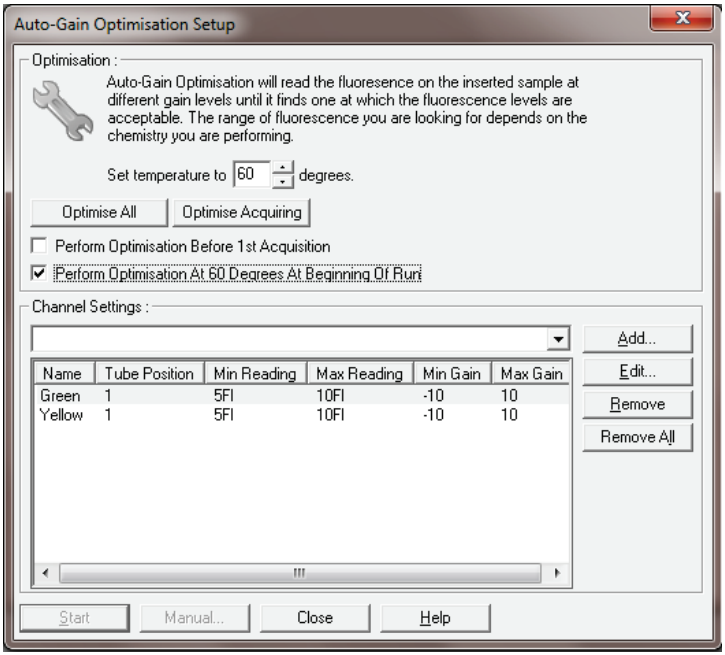
    Example:



25. Click **Gain Optimisation…**.

    The **Auto-Gain Optimisation Setup** dialog box opens.

    Example:



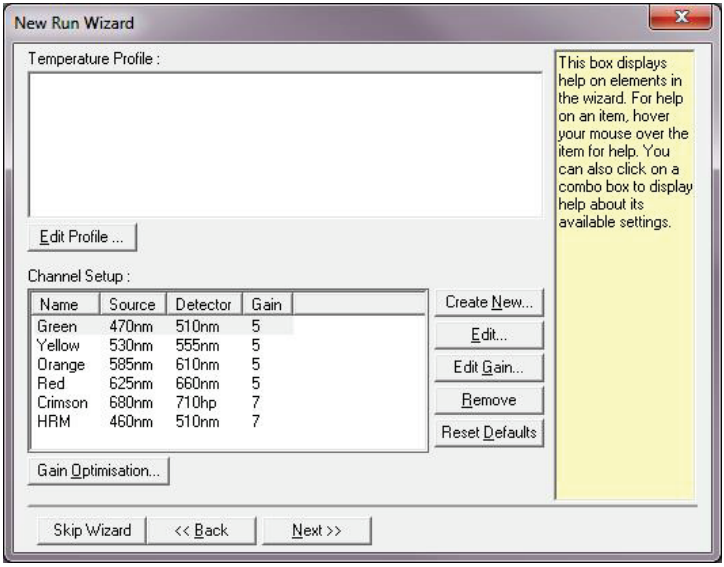
26. Click **Optimise Acquiring** and perform gain optimization using the values indicated above (i.e., the default values within the software).

27. In the **Set temperature to** dialog field, enter **60** to match the annealing temperature of the amplification program.

28. Check the box **Perform Optimisation At 60 Degrees At Beginning of Run**.

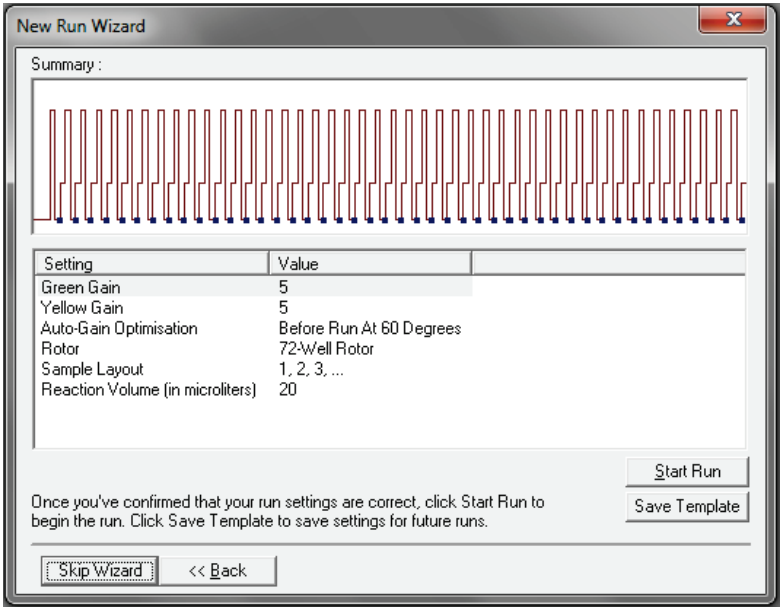
29. Click **Close** to return to the **New Run Wizard**.

The **New Run Wizard** dialog box opens.



30. Click **Next >>**.

The next screen of the **New Run Wizard** dialog box opens.



31. Click **Start Run**.

The PCR run will initiate and continue to completion.

32. After starting the run, import the sample information from the cycler file by clicking the **Open** button, or edit the samples manually.

33. Once the run is completed, unload the Rotor Gene Q MDx.

Discard all reagent aliquots and prepared reagents according to applicable local safety regulations.

34. Proceed to “Interpretation of Results,” page 28.

Interpretation of Results

Data analysis is performed with the Rotor-Gene Q MDx software according to the manufacturer's instructions in the instrument user manual.

The Rotor-Gene Q MDx run files should be analyzed using the same parameters for the *artus* Malaria RG PCR Kit assay to allow direct comparison between runs. The threshold should be set at a value of **0.04** for the analysis. If necessary, e.g., in cases where a high background signal is observed, the threshold can be adjusted to a value just above the background signal of the negative controls and negative samples. The **Dynamic Tube** setting should be applied.

The following results are possible:

1. A signal is detected in fluorescence channel Cycling A.Green.

   The result of the analysis is positive: The sample contains *Plasmodium* DNA.

   In this case, the detection of an internal control signal in the Cycling A.Yellow channel is dispensable, since high initial concentrations of *Plasmodium* 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).

2. 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.

   No *Plasmodium* DNA is detectable in the sample. It can be considered negative.

   In the case of a negative *Plasmodium* PCR, detection of signal from the internal control (if added to the extraction prior purification) confirms nucleic acid purification was performed correctly and that PCR inhibition was absent.

3. No signal is detected in the Cycling A.Green or in the Cycling A.Yellow channel.

   No result can be concluded. The sample is invalid and the purification and analysis should be repeated.

   Signal detection and conclusions are summarized in the following table:

Signal in channel Cycling A.Green	**Signal in channel Cycling A.Yellow**	**Result**	**Interpretation**
Yes	Yes*	Positive	The sample contains *Plasmodium* DNA
No	Yes†	Negative	No *Plasmodium* DNA is detected
No	No	Inconclusive	No result can be concluded

* Signal is dispensable since high initial concentrations of *Plasmodium* DNA can lead to reduced or absent fluorescent signal in the Cycling A.Yellow channel (competition).

† If the C_T value for the internal control of a negative sample is more than 3 cycles higher than the C_T value for the internal control of the NTC in the run ($C_{T\ IC\ Sample} - C_{T\ IC\ NTC} > 3$), then the sample should be treated as invalid. No result can be concluded.

Information regarding error sources and their solution can be found in "Troubleshooting Guide", page 42.

Examples of positive and negative PCR reactions are given in Figure 1 and Figure 2.

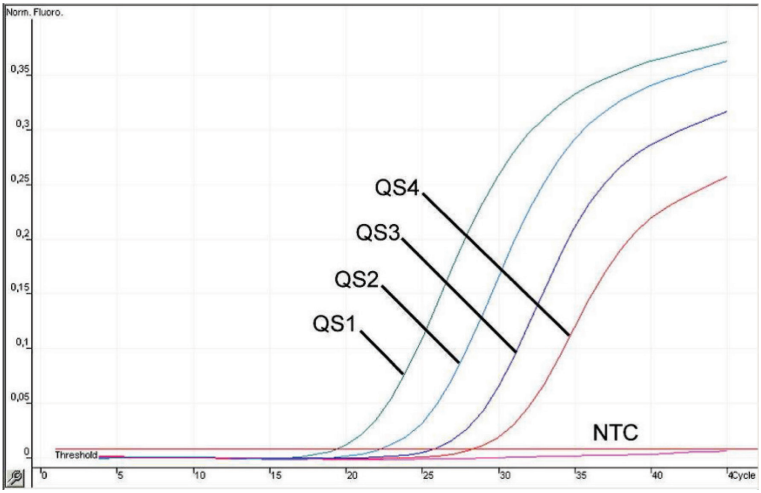


Figure 1. Detection of the Quantification Standards (Malaria RG QS 1–4) in fluorescence channel Cycling A.Green. NTC: non-template control (negative control).

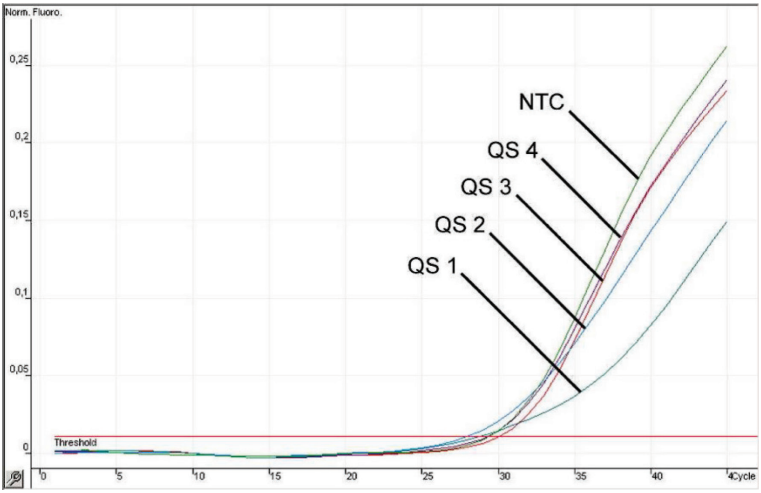


Figure 2. Detection of the internal control in fluorescence channel Cycling A.Yellow with simultaneous amplification of the quantification standards (Malaria RG QS 1–4). NTC: non-template control (negative control).

Quantitation

Important: The Quantification 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:

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

Note: As a matter of principle, the initial sample volume must 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).

Limitations

- Use of this product is limited to personnel specially instructed and trained in the techniques of real-time PCR and in vitro diagnostic procedures.
- Good laboratory practice is essential for proper performance of this assay.
- Take extreme care to preserve the purity of the components of the kit and reaction setups. Closely monitor all reagents for impurities and contamination. Discard any reagents suspected of contamination.
- Appropriate specimen collection, transport, storage and processing procedures are required for optimal performance of this assay.
- Do not use this assay directly on the specimen. Perform the applicable nucleic acid extraction procedures prior to using this assay.
- The presence of PCR inhibitors may cause false-negative or invalid results.
- Potential mutations within the target regions of the *Plasmodium* genome covered by the primers and/or probes used in the kit may result in failure to detect the presence of the pathogens.
- As with any diagnostic test, interpret the results obtained using the *artus* Malaria RG PCR Kit in consideration of all clinical and laboratory findings.
- The *artus* Malaria RG PCR Kit generates positive signals with other non-pathogenic *Plasmodium* species such as *P. berghei* from mice

Quality Control

Each lot of *artus* Malaria RG PCR Kit is tested against predetermined specifications to ensure consistent product quality.

Performance Characteristics

Analytical sensitivity

To determine the analytical sensitivity of the *artus* Malaria RG PCR Kit, a standard dilution series was set up from 758 to nominal 0.24 *Plasmodium* copy equivalents/µl and analyzed with the *artus* Malaria RG PCR Kit. Testing was carried out on 3 different days on 8 replicates. The results were determined by a probit analysis (see Figure 3). The analytical detection limit (limit of detection: LOD) of the *artus* Malaria RG PCR Kit is consistently 0.5 copies/µl (p = 0.05). This means that there is a 95 % probability that 0.5 copies/µl will be detected.

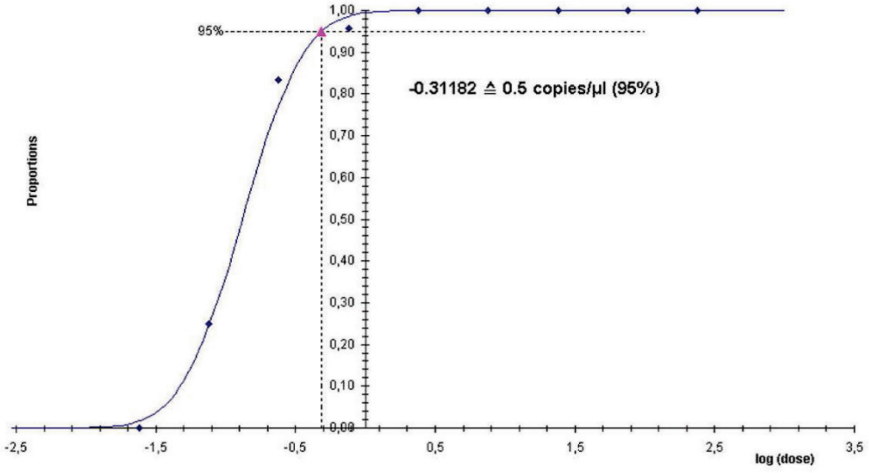


Figure 3. Analytical sensitivity of the *artus* Malaria RG PCR Kit.

Analytical specificity

The analytical specificity of the *artus* Malaria RG PCR Kit is ensured by careful selection of the oligonucleotides (primers and probes). The oligonucleotides are checked by sequence comparison analysis against publically available sequences to ensure that all relevant *Plasmodium* genotypes are detected. The detectability of all relevant *Plasmodium* species was ensured by a database alignment and by PCR with relevant *Plasmodium* species (Table 4).

Table 4. Specificity of *artus* Malaria RG PCR Kit with relevant *Plasmodium* species

Plasmodium species	Source	*Plasmodium* (Cycling A.Green	Internal control (Cycling A.Yellow)
P. falciparum	ATCC®* MRA-177	+	+
P. vivax	ATCC MRA-178	+	+
P. malariae	ATCC MRA 179	+	+
P. ovale	ATVV MRA-180	+	+

* ATCC: American Type Culture Collection.

Moreover, the specificity was validated with 30 different *Plasmodium*-negative human blood samples. These did not generate any signals with the *Plasmodium*-specific primers and probes, which are included in the Malaria RG Master.

To determine the specificity of the *artus* Malaria RG PCR Kit, the control group of microorganisms listed in Table 5 was tested for cross-reactivity.

Table 5. Testing the specificity of the *artus* malaria RG PCR Kit with potentially cross-reactive samples

Organism	*Plasmodium* (Cycling A.Green)	Internal Control (Cycling A.Yellow)
Trypanosoma cruzi	–	+
Trypanosoma brucei	–	+
Leishmania major	–	+
Leishmania brasiliensis	–	+
Leishmania mexicana mexicana	–	+
Leishmania donovani	–	+
Toxoplasma gondii	–	+
Theileria annulata	–	+
Babesia sp.	–	+

The *artus* Malaria RG PCR Kit did not cross-react with any of the specified organisms.

Precision

The precision data of the *artus* Malaria RG PCR Kit 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* Malaria RG PCR Kit have been collected using the Quantification Standard of the lowest concentration (QS 4; 70 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 6). In addition, precision data for quantitative results in copies/µl were determined using the corresponding C_T values (see Table 7). Based on these results, the overall statistical spread of any given sample with the mentioned concentration is 1.59 % (C_T) or 12.65 % (concentration), and for the detection of the Internal Control 1.99 % (C_T). These values are based on the totality of all single values of the determined variabilities.

Table 6. Precision data on the basis of C_T values

	Standard deviation	Variance	Coefficient of variation (%)
Intra-assay variability Malaria RG QS 4	0.50	0.26	1.67
Intra-assay variability Internal Control	0.37	0.14	1.34
Inter-assay variability Malaria RG QS 4	0.51	0.26	1.67
Inter-assay variability Internal Control	0.66	0.44	2.41
Inter-batch variability Malaria RG QS 4	0.42	0.17	1.39
Inter-batch variability Internal Control	0.47	0.22	1.70
Total variance Malaria RG QS 4	0.48	0.23	1.59
Total variance Internal Control	0.55	0.30	1.99

Table 7. Precision data on the basis of the quantitative results (in copies/µl)

	Standard deviation	Variance	Coefficient of variation (%)
Intra-assay variability Malaria RG QS 4	10.29	105.98	14.57
Inter-assay variability Malaria RG QS 4	9.24	85.31	13.09
Inter-lot variability Malaria RG QS 4	8.54	72.92	12.11
Total variance Malaria RG QS 4	8.93	79.66	12.65

Robustness

The verification of the robustness allows the determination of the total failure rate of the *artus* Malaria RG PCR Kit. A total of 30 *Plasmodium*-negative samples of blood were spiked with 1.5 copies/µl elution volume of *Plasmodium* control DNA (threefold concentration of the analytical sensitivity limit). After extraction using the High Pure PCR Template Preparation Kit (Roche Life Science) these samples were analyzed with the *artus* Malaria RG PCR Kit. For all *Plasmodium* samples, the failure rate was 0%. In addition, the robustness of the Internal Control was assessed by purification and analysis of 30 *Plasmodium*-negative blood samples. The total failure rate was 0%. Inhibitions were not observed. Thus, the robustness of the *artus* Malaria RG PCR Kit is ≥ 99%.

Reproducibility

Reproducibility data permit a regular performance assessment of the *artus* Malaria RG PCR Kit as well as a comparison of efficiency with other products. These data are obtained by performing evaluation studies (see "Diagnostic evaluation", page 38).

Diagnostic evaluation

The clinical performance of the *artus* Malaria RG PCR Kit was evaluated by testing clinical specimens and analyzing against the gold standard (microscopy). A total of 109 DNA samples obtained from whole blood collected from malaria infected patients as well as from 64 negative controls was tested with the *artus* Malaria RG PCR Kit and compared to microscopy results at an external laboratory. Table 8 shows the frequencies of the *artus* Malaria RG PCR Kit calls versus microscopy.

Table 8. *Plasmodium* spp. call agreement between *artus* Malaria RG PCR Kit result and microscopy; table of overall agreement

		Results of microscopy		
		***Plasmodium* spp. negative**	***Plasmodium* spp. positive**	**Total**
Results of *artus* Malaria RG PCR Kit testing	***Plasmodium* DNA negative**	58	3	**61**
	***Plasmodium* DNA positive**	6	106	**112**
Total		**64**	**109**	**n = 173**

Discrepant results that were negative by microscopy and tested positive with the artus Malaria RG PCR Kit (6) were evaluated with an independent PCR developed by the testing site. All 6 samples were confirmed to be positive with this PCR. Discrepant results that were positive by microscopy and tested negative with the artus Malaria RG PCR Kit (3) were obtained from patients with low parasitemia of 0.0001%-0.002% and were close to or below the LOD of this assay. The results analyzed in a categorical agreement analysis of PPA, NPA and OPA are presented in Table 9.

Table 9. *Plasmodium* spp. call agreement between *artus* Malaria RG PCR Kit result and microscopy

Measure of agreement	Frequency	Percent agreement	Clopper-Pearson (exact) binomial lower two-sided 90% confidence limit	Clopper-Pearson (exact) binomial upper two-sided 90% confidence limit
Overall percent agreement	164/173	94.80	91.10	97.26
Positive percent agreement	106/109	97.25	93.04	99.25
Negative percent agreement	58/64	90.63	82.33	95.84

References

1. Erdman, L.K. and Kain, K.C. (2008) Molecular diagnostic and surveillance tools for global malaria control. Travel Med. Infect. Dis. **6**, 82.

2. Tangpukdee, N., Duangdee, C., Wilairatana, P., and Krudsood, S. (2009) Malaria diagnosis: a brief review. Korean J. Parasitol. **47**, 93.

Symbols

The symbols in the following table are used in these instructions for use.

Symbol	Symbol definition
	Contains sufficient for 24 tests
	In vitro diagnostic medical device
	Catalog number
	Lot number
	Temperature limitation
	Manufacturer

Symbol	Symbol definition
	Use by
	Material number
	Global Trade Item Number
	Consult instructions for use

Troubleshooting Guide

Refer to this section for error handling and troubleshooting. If the recommended steps do not resolve the problem, contact QIAGEN Technical Services for assistance.

Possible problem or cause	Corrective action
No signal with positive controls (Malaria RG QS 1–4) in fluorescence channel Cycling A.Green	
The selected fluorescence channel for PCR data analysis does not comply with the protocol	For data analysis, select the fluorescence channel A.Green for the analytical *Plasmodium* PCR and the fluorescence channel A.Yellow for the internal control PCR.
Incorrect programming of the temperature profile of the Rotor-Gene Q MDx	Compare the temperature profile with the protocol (see the program settings for amplification, page 22).
Incorrect configuration of the PCR reaction	Check your work steps by means of the pipetting scheme (see "Detection of *Plasmodium-specific* DNA", page 15), and repeat the PCR, if necessary

Possible problem or cause	Corrective action
The storage conditions for one or more kit components did not comply with the instructions given in "Reagent Storage and Handling," page 10, or the *artus* Malaria RG PCR Kit had expired	Check the storage conditions and the expiration date (see the kit label) of the reagents and use a new kit, if necessary.
Weak or no signal of the internal control in fluorescence channel Cycling A.Yellow and simultaneous absence of a signal in channel Cycling A.Green	
The PCR conditions do not comply with the protocol	Check the PCR conditions and repeat the PCR with corrected settings, if necessary (see the program settings for amplification, page 22).
The PCR was inhibited	Make sure that you use a recommended isolation method (see "Sample preparation", page 13) and follow the manufacturer's instructions. Make sure that during the DNA isolation the recommended additional centrifugation step was carried out before elution to remove any residual ethanol (see "Sample preparation", page 13).

Possible problem or cause	Corrective action
DNA was lost during extraction	If the internal control was added to the extraction, absence of a signal from the internal control can indicate the loss of DNA during the extraction. Make sure that you use a recommended isolation method (see "Sample preparation", page 13) and follow the manufacturer's instructions.
The storage conditions for one or more kit components did not comply with the instructions given in "Reagent Storage and Handling," page 10, or the *artus* Malaria RG PCR Kit had 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 of the analytical PCR	
Contamination occurred during preparation of the PCR	Repeat the PCR with new reagents in replicates. If possible, close the PCR tubes immediately after addition of the sample to be tested. Observe strict procedure and pipette the positive controls last. Decontaminate work space and instruments at regular intervals.

Possible problem or cause	Corrective action
Contamination occurred during extraction	Repeat the extraction and PCR of the sample to be tested using new reagents. Decontaminate work space and instruments at regular intervals.

Ordering Information

Product	Contents	Cat. no.
artus Malaria RG PCR Kit (24)	For 24 reactions: Master, 4 Quantification Standards, Internal Control, H_2O (PCR grade)	4601263
Rotor-Gene Q MDx and accessories		
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 not included	9002033
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
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
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

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