artus® Borrelia LC PCR Kit

Handbook

Σ 24 (catalog no. 4551063)

Σ 96 (catalog no. 4551065)

Quantitative in vitro Diagnostics

For use with the

LightCycler® *1.1/1.2/1.5* and *LightCycler 2.0* Instrument

March 2015 – Version 1

CE

IVD

REF 4551063, 4551065

1050871

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R3 MAT 1050871

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artus Borrelia LC PCR Kit

For use with the *LightCycler 1.1/1.2/1.5* or *LightCycler 2.0* Instrument.

1. Contents

	Labelling and contents	Art. No. 4551063 24 reactions	Art. No. 4551065 96 reactions
Blue	*Borrelia LC Master*	2 x 12 rxns	8 x 12 rxns
Red	*Borrelia LC QS 1*¤ *3 x 10^4 cop/µl*	1 x 200 µl	1 x 200 µl
Red	*Borrelia LC QS 2*¤ *3 x 10^3 cop/µl*	1 x 200 µl	1 x 200 µl
Red	*Borrelia LC QS 3*¤ *3 x 10^2 cop/µl*	1 x 200 µl	1 x 200 µl
Red	*Borrelia LC QS 4*¤ *3 x 10^1 cop/µl*	1 x 200 µl	1 x 200 µl
Green	*Borrelia LC IC*¤	1 x 1,000 µl	2 x 1,000 µl
White	*Water* (*PCR grade*)	1 x 1,000 µl	1 x 1,000 µl

¤ *QS* = *Quantitation Standard*
IC = *Internal Control*

2. Storage

The components of the *artus* Borrelia LC PCR Kit should be stored at –30°C to –15°C and are stable until the expiry date stated on the label. Repeated thawing and freezing (> 2 x) 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 +4°C should not exceed a period of five hours.

3. Additionally Required Materials and Devices

- Disposable powder-free gloves
- DNA isolation kit (see **8.1 DNA Isolation**)
- Pipettes (adjustable)
- Sterile pipette tips with filters
- Vortex mixer
- Desktop centrifuge with rotor for 2 ml reaction tubes
- *Color Compensation Set* (Roche Diagnostics, Cat. No. 2 158 850) for the installation of a *Crosstalk Color Compensation* file for the *LightCycler 1.1/1.2/1.5*
- *LightCycler Multicolor Demo Set* (Cat. Nr. 03 624 854 001) for the *LightCycler 2.0* Instrument
- *LightCycler* Capillaries (20 µl)
- *LightCycler* Cooling Block
- *LightCycler 1.1/1.2/1.5* (Software Version 3.5) or *LightCycler 2.0* (Software Version 4.0) Instrument
- *LightCycler* Capping Tool

4. General Precautions

The user should always pay attention to the following:

- Use sterile pipette 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 on ice or in the *LightCycler* Cooling Block.

5. Pathogen Information

The *Borrelia burgdorferi* bacterium has a world-wide distribution, is spread by ticks and leads to Lyme Disease (Lyme-Borreliose). Early stages of the disease are known to resolve spontaneously, but chronic infections also occur. Within a few days to weeks following infection a skin rash (Erythema migrans) appears and spreads out from a central core. In secondary phases, the skin, the central and peripheral nervous systems, the heart as well as the musculoskeletal system are primarily affected. Chronic forms of the disease are characterized by acrodermatitis chronica atrophicans and rheumatory problems in the form of joint inflammations.

6. Principle of Real-Time PCR

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 re-open the reaction tubes after the PCR run (Mackay, 2004).

7. Product Description

The *artus* Borrelia LC PCR Kit constitutes a ready-to-use system for the detection of *Borrelia* DNA using polymerase chain reaction (PCR) in the *LightCycler* Instrument. The *Borrelia LC Master* contains reagents and enzymes for the specific amplification of a 102 bp region of the *Borrelia* genome, and for the direct detection of the specific amplicon with the *LightCycler 1.1/1.2/1.5* or *LightCycler 2.0*. In addition, the *artus* Borrelia LC PCR Kit contains a second heterologous amplification system to identify possible PCR inhibition.

PCR product	Selection of the fluorescence channels	
	LightCycler 1.1/1.2/1.5 Instrument	*LightCycler 2.0* Instrument
Borrelia	F1	530
Borrelia LC IC	F2	610

The amplification and detection of this *Internal Control* (*IC*) do not reduce the detection limit of the analytical *Borrelia* PCR (see **11.1 Analytical Sensitivity**). External positive controls (*Borrelia LC QS 1 - 4*) are supplied which allow the determination of the pathogen load. For further information, please refer to section **8.3 Quantitation**.

8. Protocol

8.1 DNA Isolation

Various manufacturers offer DNA isolation kits. Sample amounts for the DNA isolation procedure depend on the protocol used. Please carry out the DNA isolation according to the manufacturer's instructions. The following isolation kit is recommended:

Sample Material	Nucleic Acid Isolation Kit	Catalogue Number	Manufacturer	Carrier RNA
Skin biopsies, synovial fluid, CSF, blood, ticks, culture	QIAamp® DNA Mini Kit (50)	51 304	QIAGEN	not included

- The use of **carrier RNA** is critical for the extraction efficiency and, consequently, for DNA/RNA yield. If the selected isolation kit does not contain carrier RNA, please note that the addition of carrier (RNA-Homopolymer Poly(A), Amersham Biosciences, Cat. No. 27-4110-01) is strongly recommended for the extraction of nucleic acids from cell free body fluids and material low in DNA/RNA content (e.g. CSF). Please proceed as follows in these cases:
  a) Resuspend the lyophilised carrier RNA using the elution buffer (do not use lysis buffer) of the extraction kit (e.g. AE buffer 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 adequate to your needs and store them at -20°C. Avoid repeated thawing (> 2 x) of a carrier RNA aliquot.

b) Use 1 µg carrier RNA per 100 µl lysis buffer. For instance, if the extraction protocol suggests 200 µl lysis buffer, please add 2 µl carrier RNA (1 µg/µl) directly into the lysis buffer. Before beginning of each extraction, a mixture of lysis buffer and carrier RNA (and *Internal Control*, where applicable, see **8.2 Internal Control**) should be prepared freshly according to the following pipetting scheme:

Number of samples	1	12
Lysis buffer	e.g. 200 µl	e.g. 2,400 µl
Carrier RNA (1 µg/µl)	2 µl	24 µl
Total Volume	**202 µl**	**2,424 µl**
Volume per extraction	**200 µl**	**each 200 µl**

c) Please use the freshly prepared mixture of lysis buffer and carrier RNA instantly for extraction. Storage of the mixture is not possible.

- It is recommended to elute the DNA in 50 µl elution buffer to get the highest sensitivity of the *artus* Borrelia LC PCR Kit.
- When using isolation protocols with **ethanol**-containing washing buffers, please 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* Borrelia LC PCR Kit should not be used with **phenol**-based isolation methods.

Important: The *Internal Control* of the *artus* Borrelia LC PCR Kit can be used directly in the isolation procedure (see **8.2 Internal Control**).

8.2 Internal Control

An *Internal Control* (*Borrelia LC IC*) is supplied. This allows the user **both to control the DNA isolation procedure and to check for possible PCR inhibition** (see Fig. 1). 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 50 µl AE buffer. Hence, 5 µl of the *Internal Control* should be added initially. The quantity of *Internal Control* used depends **only** on the elution volume. The *Internal Control* and carrier RNA (see **8.1 DNA Isolation**) 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 please 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 in the fridge for only a few hours may lead to *Internal Control* failure and a reduced extraction efficiency). Please do **not** add the *Internal Control* and the carrier RNA to the sample material directly.

The *Internal Control* can optionally be used **exclusively to check for possible PCR inhibition** (see Fig. 2). For this application, add 0.5 µl of the *Internal Control* per reaction directly to 15 µl *Borrelia LC Master*. For each PCR reaction use 15 µl of the Master Mix produced as described above* and add 5 µl of the purified sample. If you are preparing a PCR run for several samples please increase the volume of the *Borrelia LC Master* and the *Internal Control* according to the number of samples (see **8.4 Preparing the PCR**).

8.3 Quantitation

The enclosed *Quantitation Standards* (*Borrelia LC QS 1 - 4*) are treated as previously purified samples and the same volume is used (5 µl). To generate a standard curve on the *LightCycler* Instrument, all four *Quantitation Standards* should be used as follows:

* 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.

***LightCycler 1.1/1.2/1.5* Instrument**

Define the *Borrelia LC QS 1 - 4* in the *Sample Loading Screen* as standards with the specified concentrations (see *LightCycler Operator's Manual*, Version 3.5, Chapter B, 2.4. Sample Data Entry).

***LightCycler 2.0* Instrument**

In order to define the standards, please activate the function *Analysis Type* in the menu of the window *Samples* and select *Absolute Quantification*. You can now define the *Borrelia LC QS 1 - 4* as standards and enter the corresponding concentrations for each standard (see *LightCycler Operator's Manual*, Version 4.0, Chapter 2.2 Entering Sample Information). Make sure that the function *Enable Controls* is **not** activated. Otherwise the selection of analysis options for the data analysis is restricted (see **9.2 Data Analysis of the PCR Data on the *LightCycler 2.0* Instrument**).

The standard curve generated as 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 (see *LightCycler Operator's Manual*, Version 3.5, Chapter B, 4.2.5. Quantitation with an External Standard Curve or Version 4.0, Chapter 4.2.2 Saving a Standard Curve). However, this quantitation method may lead to deviations in the results due to variability between different PCR runs.

Attention: 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:

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

Please note that 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).

Important: A guideline for the quantitative analysis of *artus* systems on the *LightCycler 1.1/1.2/1.5* or *LightCycler 2.0* Instrument is provided at **www.qiagen.com/Products/ByLabFocus/MDX** (**Technical Note for quantitation on the *LightCycler 1.1/1.2/1.5* or *LightCycler 2.0* Instrument**).

8.4 Preparing the PCR

Make sure that the Cooling Block as well as the capillary adapters (accessories of the *LightCycler* Instrument) are pre-cooled to +4°C. Place the desired number of *LightCycler* capillaries into the adapters of the Cooling Block. Please 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 supplied *Quantitation Standards* (*Borrelia LC QS 1 - 4*) for each PCR run. Before each use, all reagents need to be thawed completely, mixed (by repeated up and down pipetting or by quick vortexing) and centrifuged briefly.

If you want to use the *Internal Control* **to monitor the DNA isolation procedure and to check for possible PCR inhibition,** it has already been added to the isolation (see **8.2 Internal Control**). In this case, please use the following pipetting scheme (for a schematic overview see Fig. 1):

	Number of samples	**1**	**12**
1. Preparation of Master Mix	*Borrelia LC Master*	15 µl	180 µl
	Borrelia LC IC	0 µl	0 µl
	Total Volume	**15 µl**	**180 µl**
2. Preparation of PCR assay	Master Mix	15 µl	15 µl each
	Sample	5 µl	5 µl each
	Total Volume	**20 µl**	**20 µl each**

If you want to use the *Internal Control* **exclusively to check for PCR inhibition**, it must be added directly to the *Borrelia LC Master*. In this case, please use the following pipetting scheme (for a schematic overview see Fig. 2):

	Number of samples	1	12
1. Preparation of Master Mix	*Borrelia LC Master*	15 µl	180 µl
	Borrelia LC IC	0.5 µl	6 µl
	Total Volume	**15.5 µl***	**186 µl**
2. Preparation of PCR assay	Master Mix	15 µl	15 µl each
	Sample	5 µl	5 µl each
	Total Volume	**20 µl**	**20 µl each**

Pipette 15 µl of the Master Mix into the plastic reservoir of each capillary. Then add 5 µl of the eluted sample DNA. Correspondingly, 5 µl of at least one of the *Quantitation Standards* (*Borrelia LC QS 1 - 4*) must be used as a positive control and 5 µl of water (*Water*, *PCR grade*) as a negative control. Close the capillaries. To transfer the mixture from the plastic reservoir into the capillary, centrifuge the adapters containing the capillaries in a desktop centrifuge for ten seconds at a maximum of 400 x g (2,000 rpm).

* 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.

Addition of the *Internal Control* to the Purification Procedure

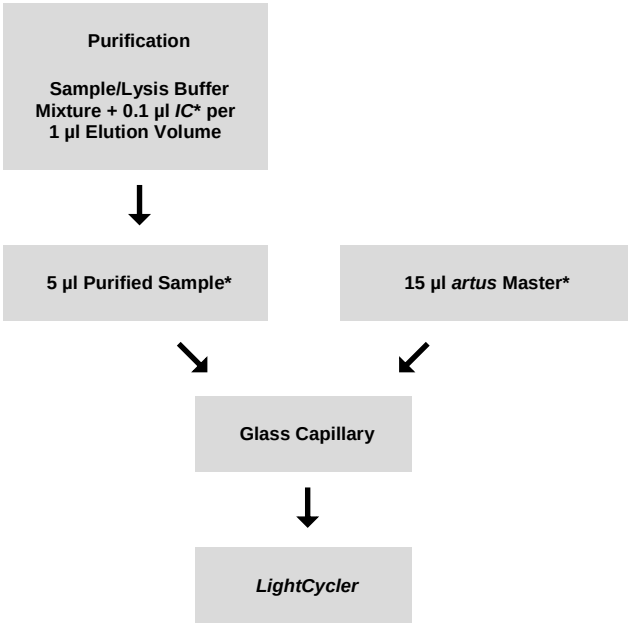


Fig. 1: Schematic workflow for the control of both the purification procedure and PCR inhibition.

* Please make sure that the solutions are thawed completely, mixed well and centrifuged briefly.

Addition of the *Internal Control* into the *artus* Master

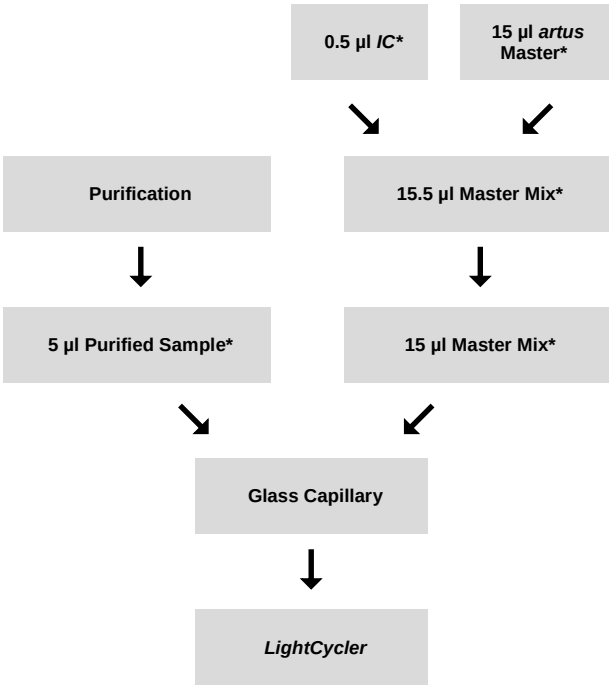


Fig. 2: Schematic workflow for the control of PCR inhibition.

*Please make sure that the solutions are thawed completely, mixed well and centrifuged briefly.

8.5 Programming of the *LightCycler* Instruments

8.5.1 Programming the *LightCycler 1.1/1.2/1.5* Instrument

For the detection of *Borrelia* DNA, create a temperature profile on your *LightCycler 1.1/1.2/1.5* Instrument according to the following three steps (see Fig. 3 - 5).

A.	Initial Activation of the Hot Start Enzyme	Fig. 3
B.	Amplification of the DNA	Fig. 4
C.	Cooling	Fig. 5

Pay particular attention to the settings for *Analysis Mode*, *Cycle Program Data* and *Temperature Targets*. In the illustrations these settings are framed in bold black. Please find further information on programming the *LightCycler 1.1/1.2/1.5* Instrument in the *LightCycler Operator's Manual*.

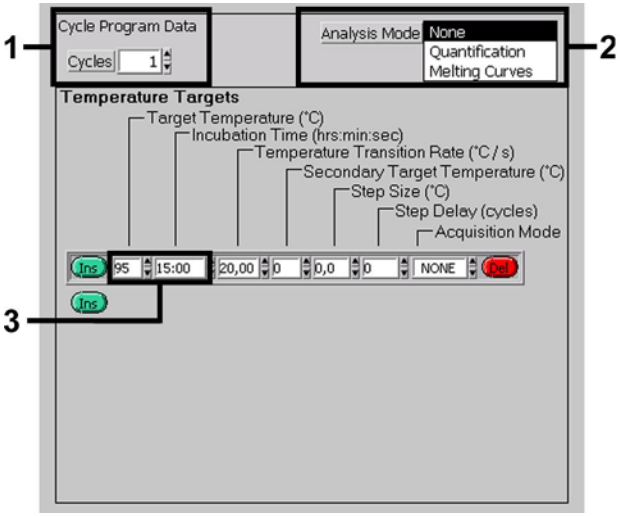


Fig. 3: Initial Activation of the Hot Start Enzyme

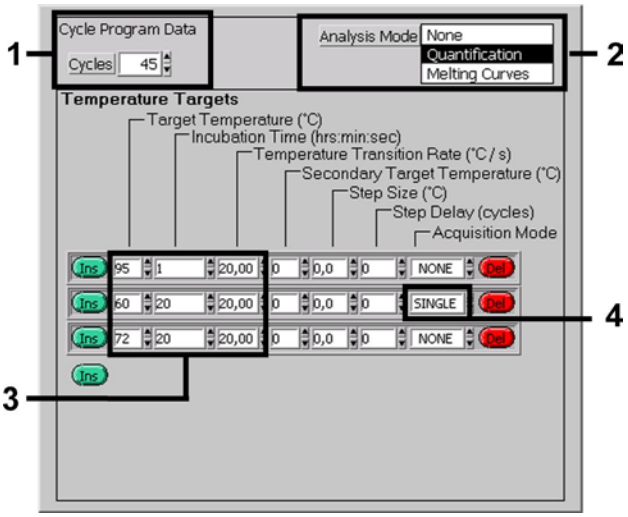


Fig. 4: Amplification of the DNA.

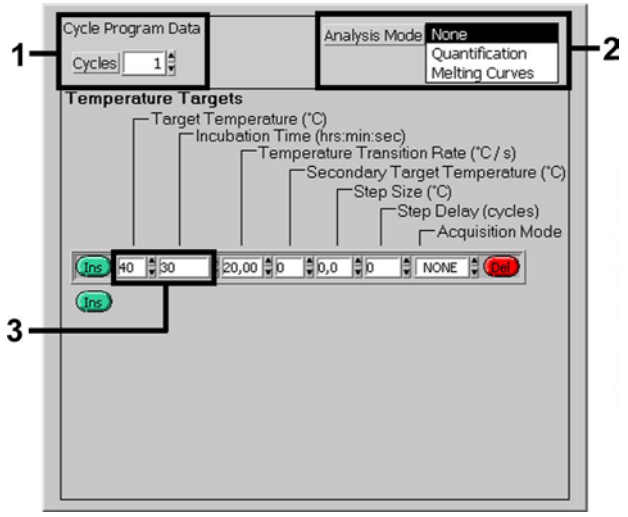


Fig. 5: Cooling.

8.5.2 Programming the *LightCycler 2.0* Instrument

To program a PCR run with *LightCycler 2.0* Instrument please activate the option *New* in the main menu and select *LightCycler Experiment*.

Subsequently, for the detection of *Borrelia* DNA, create a temperature profile on your *LightCycler 2.0* Instrument according to the following three steps (see Fig. 6 - 8).

A.	Initial Activation of the Hot Start Enzyme	Fig. 6
B.	Amplification of the DNA	Fig. 7
C.	Cooling	Fig. 8

Pay particular attention to the settings framed in bold black in the following illustrations. Please find further information on programming the *LightCycler 2.0* Instrument in the *LightCycler Operator's Manual*.

Make sure that you first enter the number of capillaries prepared for this PCR run (*Max. Seek Pos.*, see Fig. 6).

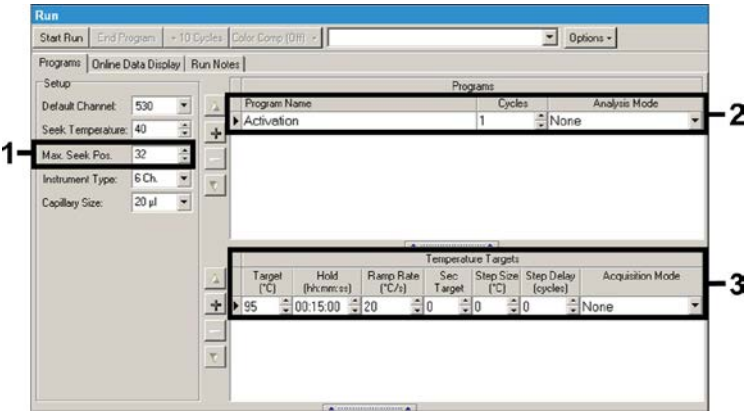


Fig. 6: Initial Activation of the Hot Start Enzyme.

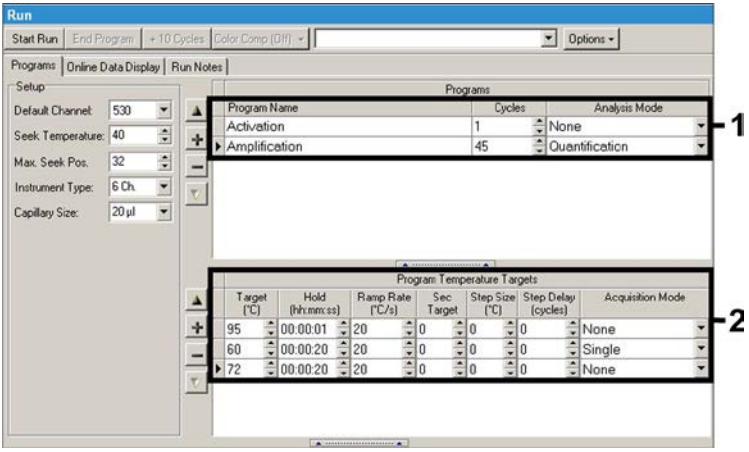


Fig. 7: Amplification of the DNA.

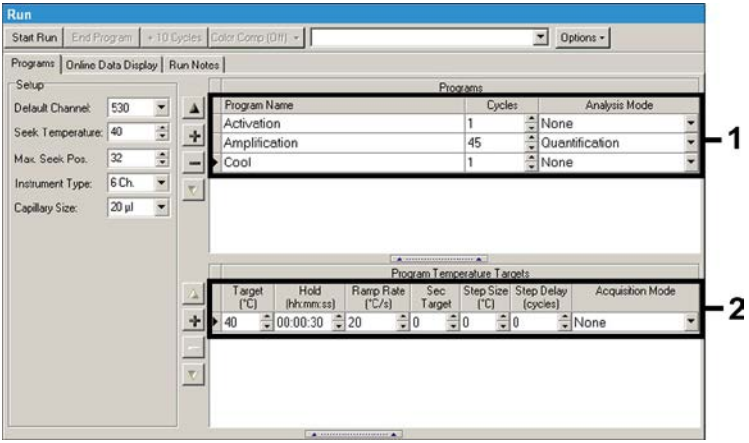


Fig. 8: Cooling.

To enter the sample specifications, please activate the button *Samples*.

- In the window *Capillary View* first enter the total number of planned PCR preparations for the PCR run (*Sample Count*).
- Then, you can assign names to the samples under *Sample Name*.
- Also select under *Selected Channels* the fluorescence channels 530 for the detection of the analytical *Borrelia* PCR and 610 for the detection of the *Internal Control* PCR.
- To define the standards and to assign the corresponding concentrations, please select the option *Absolute Quantification* under *Analysis Type* (see **8.3 Quantitation**).
- Make sure that the function *Enable Controls* is **not** activated. Otherwise the selection of analysis options for the data analysis is restricted (the mode *Fit Points* is not available, see **9.2 Data Analysis of the PCR Data on the *LightCycler 2.0* Instrument**). Under *Target Name* you can assign the target sequences to be detected (*Borrelia* or *Internal Control*) in the selected fluorescence channels 530 and 610. The completion of the column *Target Name* can be facilitated with the function *Auto Copy.* To define the *Target Name* helps to get a better overview, but it is not strictly required for data analysis.
- To generate a standard curve for data analysis, the *Quantitation Standards* should be defined with their corresponding concentrations. Therefore, please select *Standard* under *Sample Type* and enter the corresponding concentration for each standard under *Concentration*.
- The programmed temperature profile can be stored on the computer's hard drive, to make use of it again for further runs. For this purpose, activate the function *Save As* under the menu *File*, upon which a new window appears. Please select under *Templates and Macros* the submenu *Run Templates* and save the data under an appropriate name.
- In order to start the PCR run, change to the field *Run* and activate the function *Start Run* (see Fig. 9). The PCR program will start after entering the location, where the data should be saved.

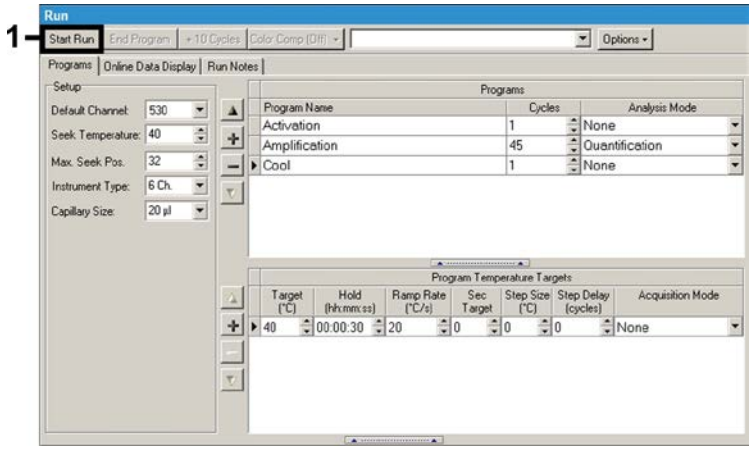


Fig. 9: Start of the PCR run.

9. Data Analysis

9.1 Data Analysis of the PCR Data on the *LightCycler 1.1/1.2/1.5* Instrument

For the analysis of the PCR data collected with the *LightCycler 1.1/1.2/1.5* Instrument, we recommend the use of the *LightCycler* Software Version 3.5.

In multicolor analyses interferences occur between fluorimeter channels. The *LightCycler 1.1/1.2/1.5* Instrument's software contains a file termed *Color Compensation File*, which compensates for these interferences. Open this file before, during or after the PCR run by activating the *Choose CCC File* or the *Select CC Data* button. If no *Color Compensation File* is installed, generate the file according to the instructions in the *LightCycler Operator's Manual*. After the *Color Compensation File* has been activated, separate signals appear in fluorimeter channels F1, F2 and F3. For analysis of the PCR results gained with the *artus* Borrelia LC PCR Kit please select fluorescence display options F1 for the analytical *Borrelia* PCR and F2 for the *Internal Control* PCR. For the analysis of quantitative runs, please follow the instructions given in **8.3 Quantitation** and in the **Technical Note for quantitation on the**

***LightCycler 1.1/1.2/1.5* or *LightCycler 2.0* Instrument at www.qiagen.com/Products/ByLabFocus/MDX**.

The following results are possible:

1. A signal is detected in fluorimeter channel F1.

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

   In this case, the detection of a signal in the F2 channel is dispensable, since high initial concentrations of *Borrelia* DNA (positive signal in the F1 channel) can lead to a reduced or absent fluorescence signal of the *Internal Control* in the F2 channel (competition).

2. In fluorimeter channel F1 no signal is detected. At the same time, a signal from the *Internal Control* appears in the F2 channel.

   In the sample no *Borrelia* DNA is detectable. It can be considered negative.

   In the case of a negative *Borrelia* PCR the detected signal of the *Internal Control* rules out the possibility of PCR inhibition.

3. No signal is detected in the F1 or F2 channels.

   No result can be concluded.

   Information regarding error sources and their solution can be found in **10. Troubleshooting**.

Examples of positive and negative PCR reactions are given in Fig. 10 and Fig. 11.

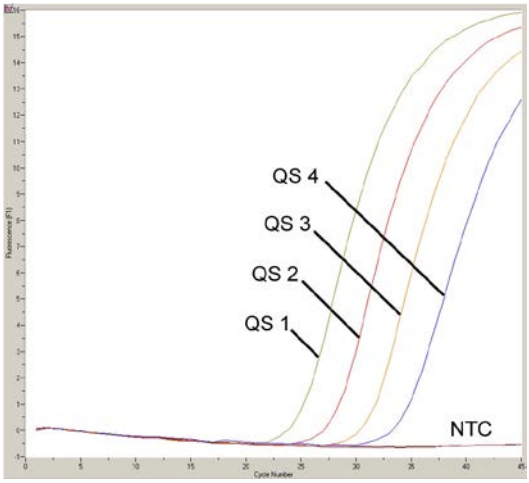


Fig. 10: Detection of the *Quantitation Standards* (*Borrelia LC QS 1 - 4*) in fluorimeter channel F1 of the *LightCycler 1.1/1.2/1.5*. NTC: non-template control (negative control).

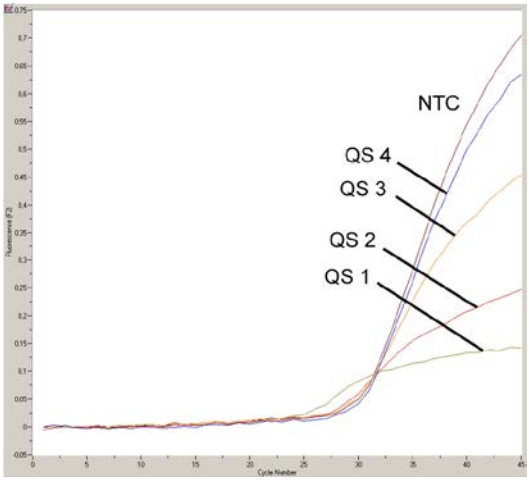


Fig. 11: Detection of the *Internal Control* (*IC*) in fluorimeter channel F2 of the *LightCycler 1.1/1.2/1.5* with simultaneous amplification of *Quantitation Standards* (*Borrelia LC QS 1 - 4*). NTC: non-template control (negative control).

9.2 Data Analysis of the PCR Data on the *LightCycler 2.0* Instrument

For the analysis of the PCR data collected with the *LightCycler 2.0* Instrument please use the *LightCycler* Software Version 4.0. Please consider the instructions given in the *LightCycler 2.0 Instrument Operator's Manual Version 4.0*.

For the analysis of PCR data please proceed as follows (see Fig. 12):

- Activate the function *Analysis* in the menu strip and select the option *Absolute Quantification*. As a matter of principle, all amplification data generated with the *artus* LC PCR Kit should be analyzed with this function.
- The *LightCycler* Software Version 4.0 contains a file termed *Color Compensation File*, which compensates multicolor analyses interferences between fluorescence channels. Open this file during or after the PCR run by activating the *Color Comp* (*On/Off*) and then the *Select Color Compensation* button (see Fig. 12). If no *Color Compensation File* is installed, generate the file according to the instructions given in the *LightCycler Operator's Manual*.
- After the *Color Compensation File* has been activated, separate signals appear in the fluorescence channels. For analysis of the PCR results gained with the *artus* Borrelia LC PCR Kit please select fluorescence display options 530 for the analytical *Borrelia* PCR and 610 for the *Internal Control* PCR.

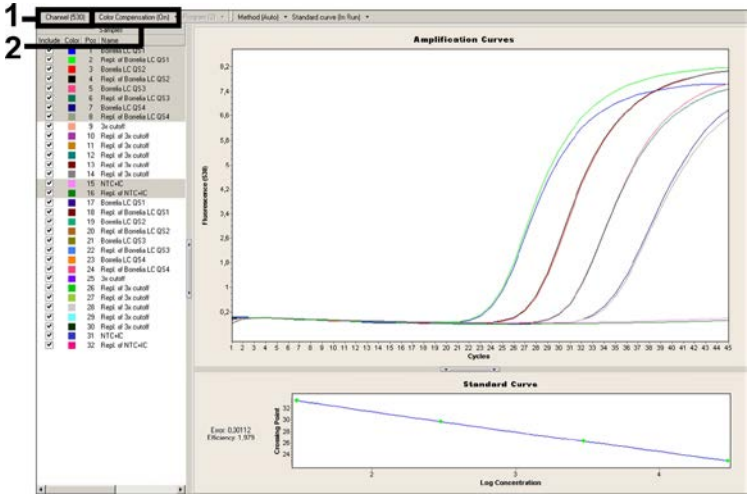


Fig. 12: Activation of the *Color Compensation File* and selection of the fluorescence channel.

For the analysis of quantitative runs, please follow the instructions given in **8.3 Quantitation** and in the **Technical Note for quantitation on the *LightCycler 1.1/1.2/1.5* or *LightCycler 2.0* Instrument** at **www.qiagen.com/Products/ByLabFocus/MDX**.

Once the setting of analysis options is completed, following results are possible:

4. A signal is detected in fluorescence channel 530.

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

   In this case, the detection of a signal in the 610 channel is dispensable, since high initial concentrations of *Borrelia* DNA (positive signal in the 530 channel) can lead to a reduced or absent fluorescence signal of the *Internal Control* in the 610 channel (competition).

5. In fluorescence channel 530 no signal is detected. At the same time, a signal from the *Internal Control* appears in 610 channel.

In the sample no *Borrelia* DNA is detectable. It can be considered negative.

In the case of a negative Borrelia PCR the detected signal of the *Internal Control* rules out the possibility of PCR inhibition.

6. No signal is detected in the 530 or 610 channels.

   No diagnosis can be concluded.

   Information regarding error sources and their solution can be found in **10. Troubleshooting**.

Examples of positive and negative PCR reactions are given in Fig. 13 and Fig. 14.

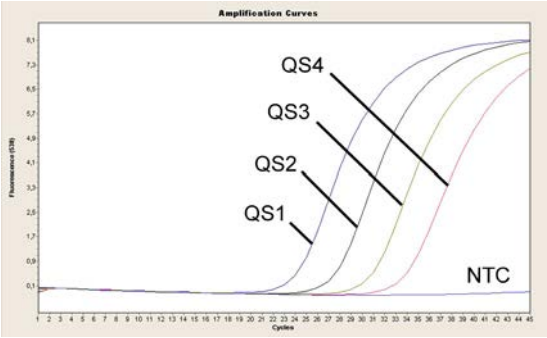


Fig. 13: Detection of the *Quantitation Standards* (*Borrelia LC QS 1 - 4*) in fluorescence channel 530 of the *LightCycler 2.0* Instrument. NTC: non-template control (negative control).

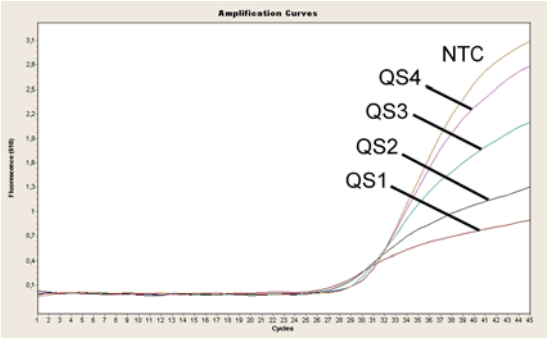


Fig. 14: Detection of the *Internal Control* (*IC*) in fluorescence channel 610 of the *LightCycler 2.0* Instrument with simultaneous amplification of *Quantitation Standards* (*Borrelia LC QS 1 - 4*). NTC: non-template control (negative control).

10. Troubleshooting

No signal with positive controls (*Borrelia LC QS 1 - 4*) in fluorescence channel F1 or 530:

- The selected fluorescence channel for PCR data analysis does not comply with the protocol.
  - → For data analysis select the fluorescence channel F1 or 530 for the analytical *Borrelia* PCR and the fluorescence channel F2 or 610 for the *Internal Control* PCR.
- Incorrect programming of the temperature profile of the *LightCycler 1.1/1.2/1.5* or *LightCycler 2.0* Instrument.
  - → Compare the temperature profile with the protocol (see **8.5 Programming of the *LightCycler* Instruments**).
- Incorrect configuration of the PCR reaction.
  - → Check your work steps by means of the pipetting scheme (see **8.4 Preparing the PCR**) and repeat the PCR, if necessary.
- The storage conditions for one or more kit components did not comply with the instructions given in **2. Storage** or the *artus* Borrelia LC PCR Kit had expired.
  - → Please 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 F2 or 610 and simultaneous absence of a signal in channel F1 or 530:

- The PCR conditions do not comply with the protocol.
  - → Check the PCR conditions (see above) and repeat the PCR with corrected settings, if necessary.
- The PCR was inhibited.
  - → Make sure that you use a recommended isolation method (see **8.1 DNA Isolation**) and stick closely to 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 **8.1 DNA Isolation**).

- 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 **8.1 DNA Isolation**) and stick closely to the manufacturer's instructions.
- The storage conditions for one or more kit components did not comply with the instructions given in **2. Storage** or the *artus* Borrelia LC PCR Kit had expired.
  - → Please 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 F1 or 530 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.
  - → Strictly pipette the positive controls at last.
  - → Make sure that work space and instruments are decontaminated at regular intervals.
- A 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, please contact our Technical Service.

11. Specifications

11.1 Analytical Sensitivity

11.1.1 *LightCycler 1.1/1.2/1.5* Instrument

To determine the analytical sensitivity of the *artus* Borrelia LC PCR Kit, a dilution series of genomic *B. burgdorferi* DNA (DSMZ 4681-30) has been set up from 36.5 to nominal 0.0115 *Borrelia* copies/µl and analyzed on the *LightCycler 1.1/1.2/1.5* Instrument in combination with the *artus* Borrelia LC PCR Kit. Testing was carried out on three different days on eight replicates. The results were determined by a probit analysis. A graphical illustration of the probit analysis is shown in Fig. 15. The analytical detection limit of the *artus* Borrelia LC PCR Kit in combination with the *LightCycler 1.1/1.2/1.5* Instrument is consistently 3.34 copies/µl (p = 0.05). This means that there is a 95 % probability that 3.34 copies/µl will be detected.

Probit analysis: *Borrelia* (*LightCycler 1.1/1.2/1.5*)

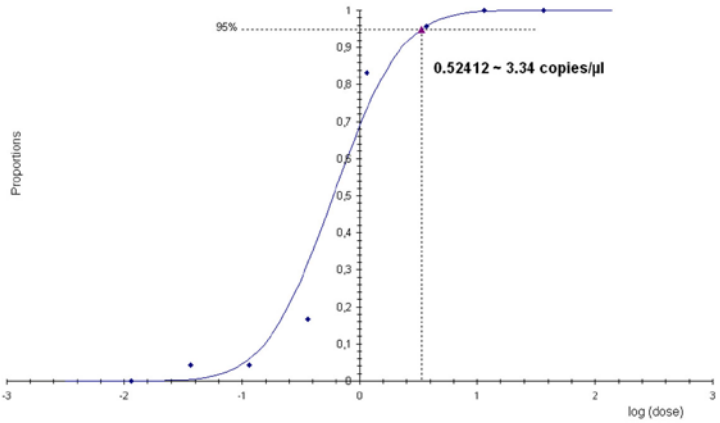


Fig. 15: Analytical sensitivity of the *artus* Borrelia LC PCR Kit on the *LightCycler 1.1/1.2/1.5* Instrument.

11.1.2 *LightCycler 2.0* Instrument

To determine the analytical sensitivity of the *artus* Borrelia LC PCR Kit, a dilution series of genomic *B. burgdorferi* DNA (DSMZ 4681-30) has been set

up from 36.5 to nominal 0.0115 *Borrelia* copies/µl and analyzed on the *LightCycler 2.0* Instrument in combination with the *artus* Borrelia LC PCR Kit. Testing was carried out on three different days on eight replicates. The results were determined by a probit analysis. A graphical illustration of the probit analysis is shown in Fig. 16. The analytical detection limit of the *artus* Borrelia LC PCR Kit in combination with the *LightCycler 2.0* Instrument is consistently 2.76 copies/µl (p = 0.05). This means that there is a 95 % probability that 2.76 copies/µl will be detected.

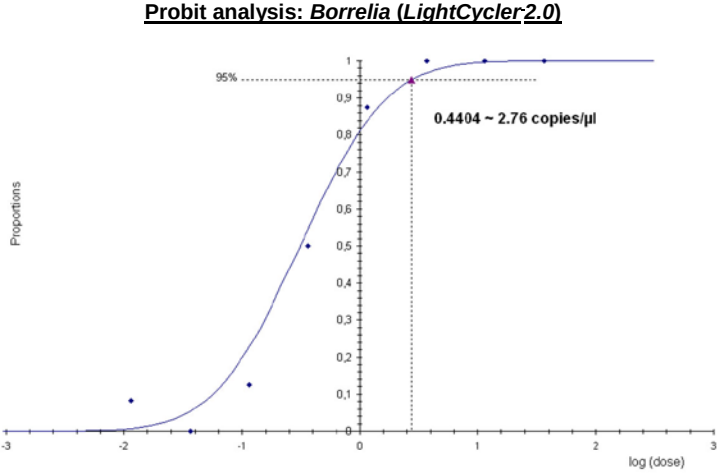


Fig. 16: Analytical sensitivity of the *artus* Borrelia LC PCR Kit on the *LightCycler 2.0* Instrument.

11.2 Specificity

The specificity of the *artus* Borrelia LC 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 in gene banks published sequences by sequence comparison analysis. The detectability of all *Borrelia* species has thus been controlled (pathogen of Lyme disease and pathogen of recurrent fever).

Moreover, the specificity was validated with 32 different *Borrelia* negative cerebrospinal fluid samples. These did not generate any signals with the *Borrelia* specific primers and probes, which are included in the *Borrelia LC Master.*

The following species have been additionally confirmed by a PCR run on a *LightCycler* Instrument (see Table 1).

Table 1: Testing of the specificity of relevant *Borrelia* species.

***Borrelia* species**	**Source**	***Borrelia* (F1 or 530)**	***Internal Control* (F2 or 610)**
B. burgdorferi	DSMZ¤	+	+
B. garinii	DSMZ¤	+	+
B. afzelii	DSMZ¤	+	+
*B. valaisiana**	INSTAND¤	+	+
B. hermsii	QIAGEN¤	+	+

¤ DSMZ: German Collection of Microorganisms and Cell Cultures, Braunschweig
INSTAND: INSTAND e. V., Düsseldorf
QIAGEN: QIAGEN GmbH, Hilden

To determine the specificity of the *artus* Borrelia LC PCR Kit the control group listed in the following table (see Table 2) has been tested for cross-reactivity. None of the tested pathogens has been reactive.

* Evidence for pathogenicity of *B. valaisiana* is provided by several studies (see **15. References**).

Table 2: Testing the specificity of the kit with potentially cross-reactive pathogens.

Control group	*Borrelia* (F1 or 530)	*Internal Control* (F2 or 610)
Treponema phagedenis	–	+
Chlamydia pneumoniae	–	+
Chlamydia trachomatis	–	+
Mycoplasma pneumoniae	–	+
Bordetella pertussis	–	+
Escherichia coli K12	–	+
Salmonella typhi	–	+
Pseudomonas aeruginosa	–	+
Legionella pneumophila	–	+
Legionella longbeachae	–	+
Bacillus anthracis	–	+
Staphylococcus aureus	–	+
Candida albicans	–	+
Aspergillus fumigatus	–	+
Human genomic DNA	–	+

11.3 Precision

The precision data of the *artus* Borrelia LC PCR Kit have been collected by means of the *LightCycler 1.1/1.2/1.5* Instrument 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* Borrelia LC PCR Kit have been collected using a dilution of *B. burgdorferi* genomic DNA (DSMZ 4681-30) with a concentration of 36.5 copies/µl. Testing was performed with eight replicates. The precision

data were calculated on basis of the Ct values of the amplification curves (Ct: threshold cycle, see Table 3). Based on these results, the overall statistical spread of any given sample with the mentioned concentration is 0.94 % (Ct), for the detection of the *Internal Control* 1.68 % (Ct). These values are based on the totality of all single values of the determined variabilities.

Table 3: Precision data on basis of the Ct values.

	Standard deviation	Variance	Coefficient of variation [%]
Intra-assay variability: *Borrelia* gDNA (36.5 copies/µl)	0.20	0.04	0.59
Intra-assay variability: *Internal Control*	0.09	0.01	0.29
Inter-assay variability: *Borrelia* gDNA (36,5 cop/µl)	0.23	0.06	0.69
Inter-assay variability: *Internal Control*	0.39	0.15	1.28
Inter-batch variability: *Borrelia* gDNA (36.5 copies/µl)	0.31	0.10	0.92
Inter-batch variability: *Internal Control*	0.62	0.38	2.04
Total variance: *Borrelia* gDNA (36.5 copies/µl)	0.32	0.10	0.94
Total variance: *Internal Control*	0.51	0.26	1.68

11.4 Robustness

The verification of the robustness allows the determination of the total failure rate of the *artus* Borrelia LC PCR Kit. 32 *Borrelia* negative samples of cerebrospinal fluid were spiked with 10 copies/µl elution volume of *Borrelia* control DNA (threefold concentration of the analytical sensitivity limit). After extraction using the QIAamp DNA Mini Kit (see **8.1 DNA Isolation**) these samples were analyzed with the *artus* Borrelia LC PCR Kit. For all *Borrelia* samples the failure rate was 0 %. In addition, the robustness of the *Internal Control* was assessed by purification and analysis of 32 *Borrelia* negative cerebrospinal fluid samples. The total failure rate was 0 %. Inhibitions were

not observed. Thus, the robustness of the *artus* Borrelia LC PCR Kit is ≥ 99 %.

11.5 Reproducibility

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

11.6 Diagnostic Evaluation

Currently, the *artus* Borrelia LC PCR Kit is undergoing a series of evaluation studies.

12. Product Use 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.

13. Warnings and Precautions

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

14. Quality Control

In accordance with QIAGEN's ISO 9001 and ISO 13485-certified Total Quality Management System, each lot of *artus* Borrelia LC PCR Kit is tested against predetermined specifications to ensure consistent product quality.

15. References

(1) Mackay IM. Real-time PCR in the microbiology laboratory. Clin. Microbiol. Infect. 2004; 10 (3): 190 - 212.

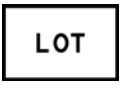
(2) Rijpkema SG, Tazelaar DJ, Molkenboer MJ, Noordhoek GT, Plantinga G, Schouls LM, Schellekens JF. Detection of *Borrelia afzelii*, *Borrelia burgdorferi* sensu stricto, *Borrelia garinii* and group VS116 by PCR in skin biopsies of patients with erythema migrans and acrodermatitis chronica atrophicans. Clin Microbiol Infect., 1997; 3 (1): 109 - 116.

(3) Ryffel K et al. OspA heterogeneity of *Borrelia valaisiana* confirmed by phenotypic and genotypic analyses. BMC Infect Dis., 2003; 3 (1): 14.

(4) Ryffel K et al. Scored antibody reactivity determined by immunoblotting shows an association between clinical manifestations and presence of *Borrelia burgdorferi* sensu stricto, *B. garinii*, *B. afzelii*, and *B. valaisiana* in humans. J Clin Microbiology, 1999; 37 (12): 4086 - 4092.

16. Explanation of Symbols

Use by



Batch code

Manufacturer

Catalogue number

Material number

Handbook

In vitro diagnostic medical device

Ethanol

Global Trade Item Number

<N>

Contains sufficient for <N> tests

Temperature limitation

QS *Quantitation Standard*

IC *Internal Control*

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artus Borrelia LC PCR Kit

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The QIAamp DNA Mini Kit is intended for general laboratory use. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of a disease.

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