artus® HBV LC PCR Kit

Handbook

Σ 24 (catalog no. 4506063)

Σ 96 (catalog no. 4506065)

Quantitative in vitro Diagnostics

For use with the

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

May 2011 – Version 1

CE 0197

IVD

REF 4506063, 4506065

HB 1046918EN

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R2 MAT 1046918EN

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artus® HBV LC PCR Kit

For use with the *LightCycler 1.1/1.2/1.5* or *LightCycler 2.0* Instrument for the quantitative detection of HBV DNA from EDTA plasma.

1. Contents

	Labelling and contents	Art. No. 4506063 24 reactions	Art. No. 4506065 96 reactions
Blue	*HBV LC Master*	2 x 12 rxns	8 x 12 rxns
Yellow	*HBV LC Mg-Sol*¤	1 x 400 µl	1 x 400 µl
Red	*HBV LC QS 1*¤ *1 x 10^5 IU/µl*	1 x 200 µl	1 x 200 µl
Red	*HBV LC QS 2*¤ *1 x 10^4 IU/µl*	1 x 200 µl	1 x 200 µl
Red	*HBV LC QS 3*¤ *1 x 10^3 IU/µl*	1 x 200 µl	1 x 200 µl
Red	*HBV LC QS 4*¤ *1 x 10^2 IU/µl*	1 x 200 µl	1 x 200 µl
Red	*HBV LC QS 5*¤ *1 x 10^1 IU/µl*	1 x 200 µl	1 x 200 µl
Green	*HBV 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*
Mg-Sol = *Magnesium Solution*

* The standard is a cloned PCR product, the concentration of which has been calibrated using the 1st International HBV standard (WHO).

2. Storage

The components of the *artus* HBV LC PCR Kit should be stored at –20°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 **8.2 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* or *LightCycler 2.0* Instrument
- *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

Hepatitis B virus (HBV) is mainly transmitted via blood or blood products. However, sexual, oral and perinatal infections are also possible. Following a general malaise, including appetite loss, vomiting and abdominal problems; about 10 – 20 % of patients develop fever, exanthema (skin rash) as well as rheumatoid joint and muscle problems. 2 – 14 days later jaundice develops which may be accompanied by itching. Fulminant hepatitis occurs in about 1 % of all infected patients and is frequently fatal. 5 – 10 % of hepatitis B patients develop chronic liver inflammation which can progress to cirrhosis of the liver or primary liver cell carcinoma.

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* HBV LC PCR Kit constitutes a ready-to-use system for the detection of hepatitis B virus DNA using polymerase chain reaction (PCR) in the *LightCycler* Instrument. The *HBV LC Master* contains reagents and enzymes for the specific amplification of a 134 bp region of the HBV genome, and for the direct detection of the specific amplicon with the *LightCycler 1.1/1.2/1.5* or

LightCycler 2.0 Instrument. In addition, the *artus* HBV 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
HBV	F2/Back-F1	640/Back 530
HBV LC IC	F3/Back-F1	705/Back 530

The amplification and detection of this *Internal Control* (*IC*) do not reduce the detection limit of the analytical HBV PCR (see **11.1 Analytical Sensitivity**). External positive controls (*HBV LC QS 1 – 5*) are supplied which allow the determination of the pathogen load. For further information, please refer to section **8.4 Quantitation**.

8. Protocol

8.1 Pre-analytics: Specimen Collection, Storage and Transport

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

Attention: Current studies refer to EDTA or citrate plasma as the most suitable sample materials for HBV detection. Therefore, we recommend the use of these materials with the *artus* HBV LC PCR Kit.

The internal validation of the *artus* HBV LC PCR Kit has been performed using human EDTA plasma samples. Other sample materials are not validated. Please use only recommended nucleic acid isolation kits (see **8.2 DNA Isolation**) for sample preparation.

Using certain sample materials, particular instructions regarding collection, transport and storage have to be strictly observed.

8.1.1 Specimen Collection

Each blood withdrawal causes an injury of blood vessels (arteries, veins, capillaries). Only innocuous and sterile material should be used. For blood withdrawal appropriate disposables are available. For the vein puncture, too fine capillary needles should not be employed. Venous blood withdrawal should be carried out on the appropriate parts of the elbow bend, the forearm or the back of the hand. Blood has to be withdrawn with standard specimen collection tubes (red cap, Sarstedt or equivalent tube of another manufacturer). 5 – 10 ml EDTA blood should be withdrawn. Tubes should be mixed overhead directly after sample collection (8 x, do not agitate).

Attention: Samples of heparinised humans must not be used (see **8.1.4 Interfering Substances**).

8.1.2 Sample Storage

Whole blood should be separated into plasma and cellular components by centrifugation for 20 minutes at 800 – 1,600 x g within six 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. Virus encapsulated DNA is stable for days if stored at +4°C, for weeks if stored at –20°C and even for months and years when stored at –70°C*.

8.1.3 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 six hours. It is not recommended to store the samples where they have been collected. It is possible to ship the

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* Arbeitskreis Blut, V17 (09.1997), Bundesgesundheitsblatt 11/1997, p. 452 - 456.

† International Air Transport Association. Dangerous Goods Regulations, 41st Edition, 2000.704.

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°C to +8°C) and the separated plasma deep frozen (–20°C).

8.1.4 Interfering Substances

Elevated levels of bilirubin (≤ 15 mg/dl) and lipids (≤ 800 mg/dl) and hemolytic samples do not influence the system. Heparin (≥ 10 IU/ml) affects the PCR. Samples, which have been collected in tubes containing heparin as an anticoagulant, should not to be used. Also, samples of heparinised patients must not be used.

8.2 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
EDTA plasma	QIAamp® DSP Virus Kit (50)	60 704	QIAGEN	included

- The use of **carrier RNA** is critical for the extraction efficiency and, consequently, for DNA/RNA yield. To increase the stability of the carrier RNA provided with the QIAamp DSP Virus Kit, we recommend to proceed according to the information about the reconstitution and storage of the carrier RNA given in the instruction manual ("Preparing reagents and buffers").
- 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.

Important: The *Internal Control* of the *artus* HBV LC PCR Kit can be added directly to the purification procedure. Please make sure to co-process a negative plasma sample in the purification. Its corresponding *Internal Control* signal serves as a basis for the assessment of the purification (see **8.3 Internal Control**).

8.3 Internal Control

An *Internal Control* (*HBV 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 DSP Virus Kit the DNA is eluted in 60 µl AVE buffer. Hence, 6 µl of the *Internal Control* should be added initially. If you elute e.g. in 20 µl, then use the corresponding volume of 2 µl. The quantity of *Internal Control* used depends **only** on the elution volume. The *Internal Control* and carrier RNA (see **8.2 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.

To consider a purification successful, the Ct value of the *Internal Control* of a negative plasma sample which has been processed through purification (QIAamp DSP Virus Kit) has to reach Ct = 32 ± 3 (method of analysis: second derivative maximum) using the *LightCycler* Instrument. The stated spreading is based on the variance of the instrument and the purification. A higher deviation points to a purification problem. In this case the purification has to be

checked and, if necessary, validated a second time. If you have any further questions or if you encounter problems, please contact our Technical Service.

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* and 3 µl *HBV LC Mg-Sol* per reaction directly to 12 µl *HBV 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 *HBV LC Master*, *HBV LC MG-Sol* and the *Internal Control* according to the number of samples (see **8.5 Preparing the PCR**).

8.4 Quantitation

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

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

Define the *HBV LC QS 1 – 5* 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 *HBV LC QS 1 – 5* 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.

* 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.

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 IU/µl. The following equation has to be applied to convert the values determined using the standard curve into IU/ml of sample material:

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

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**).

8.5 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* (*HBV LC QS 1 – 5*) 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 **8.3 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	*HBV LC Master*	12 µl	144 µl
	HBV LC Mg-Sol	3 µl	36 µl
	HBV 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 *HBV 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	*HBV LC Master*	12 µl	144 µl
	HBV LC Mg-Sol	3 µl	36 µl
	HBV 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**

* 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.

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* (*HBV LC QS 1 – 5*) 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).

Addition of the *Internal Control* to the Purification Procedure

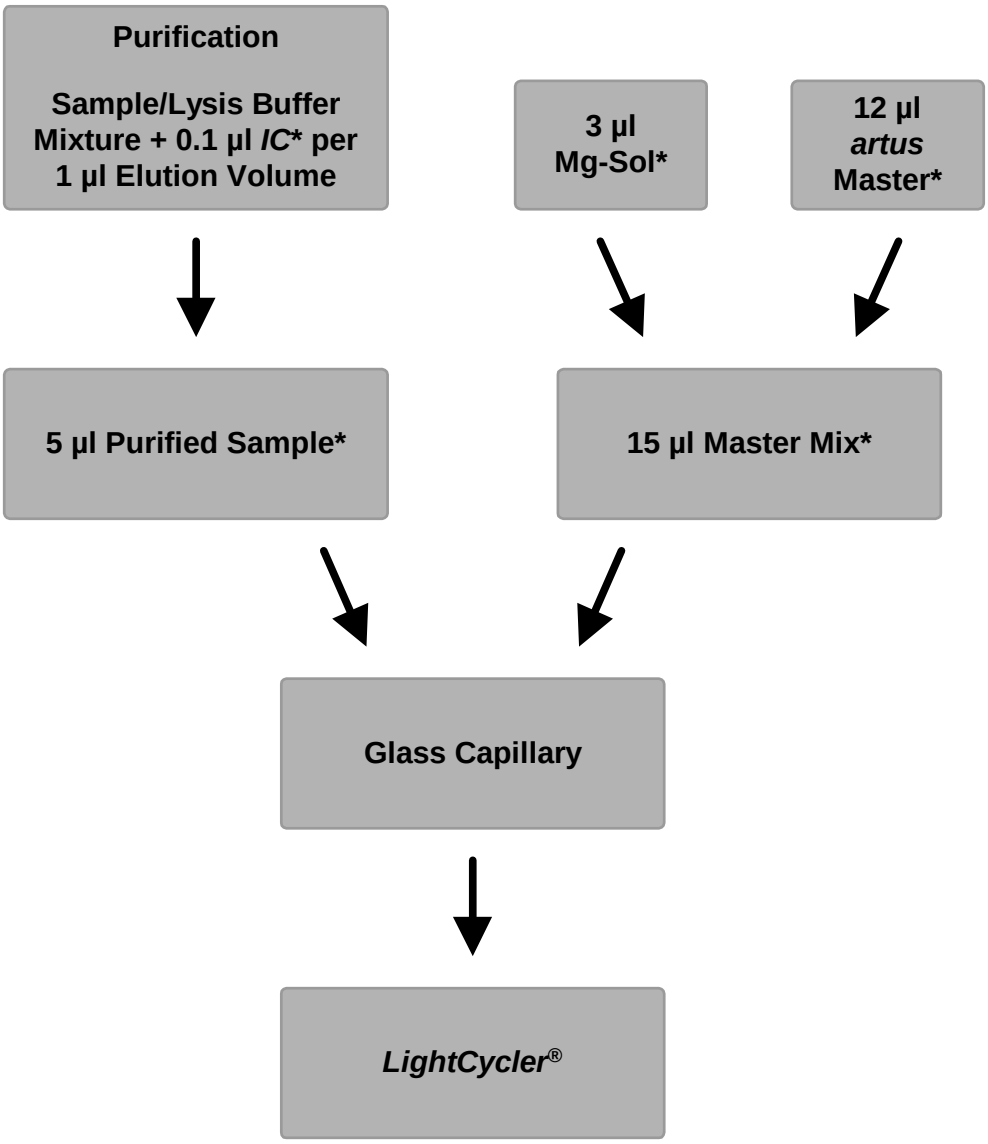

Fig. 1: Schematic workflow for the control of both the purification procedure and PCR inhibition.

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

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

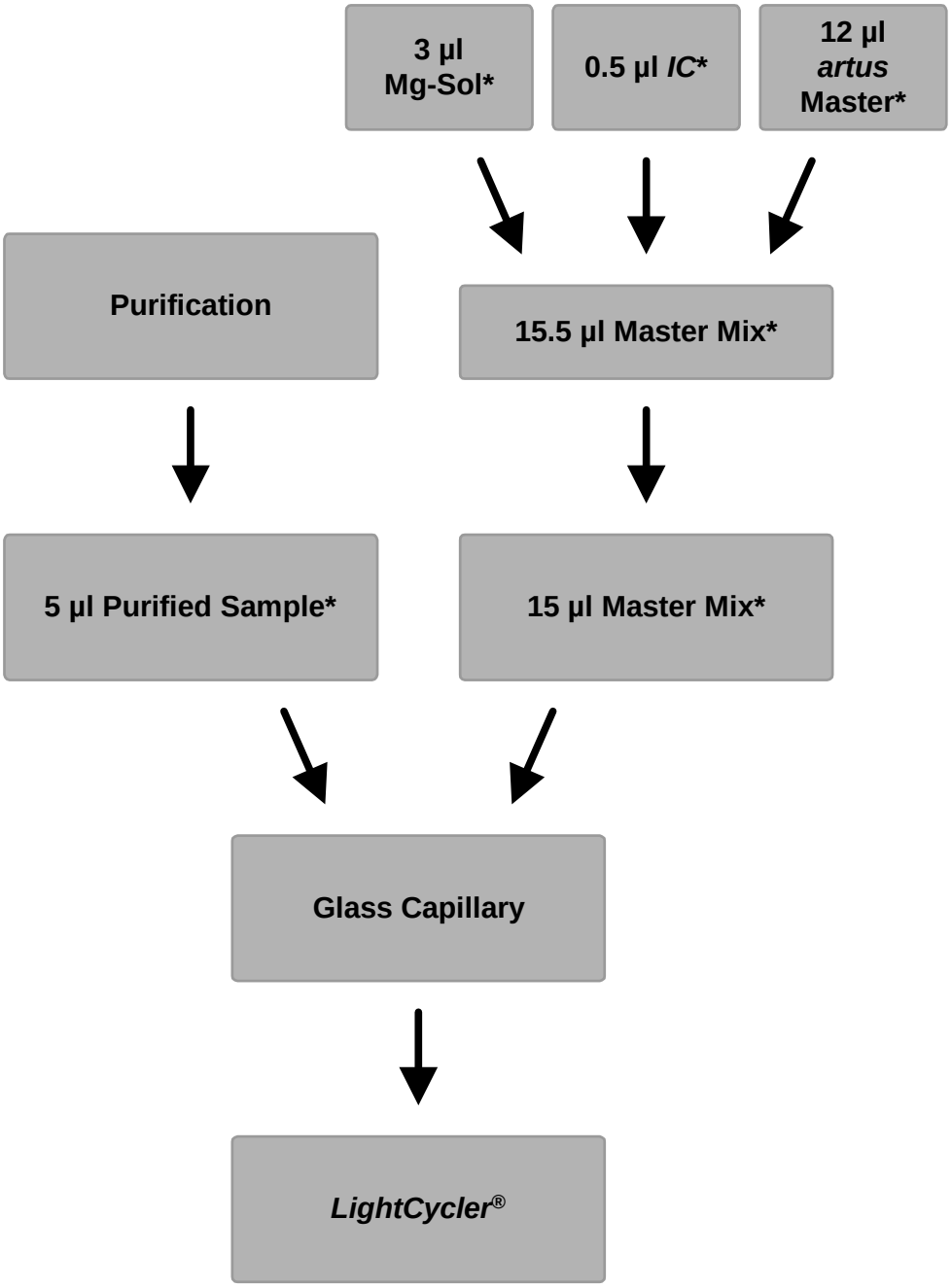

Fig. 2: Schematic workflow for the control of PCR inhibition.

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

8.6 Programming the *LightCycler* Instruments

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

For the detection of HBV 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*.

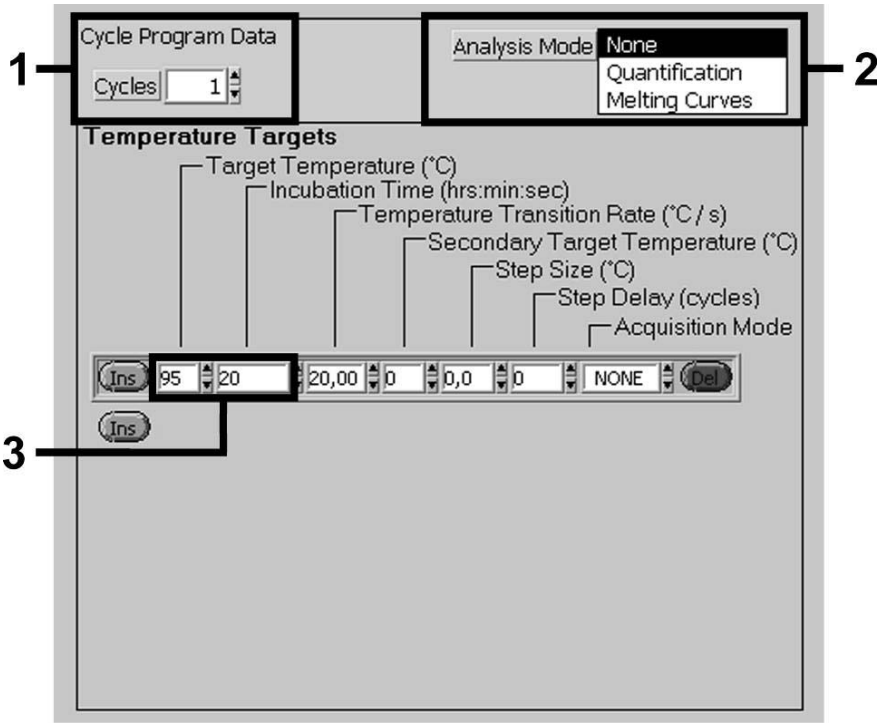

Fig. 3: Initial Activation of the Hot Start Enzyme.

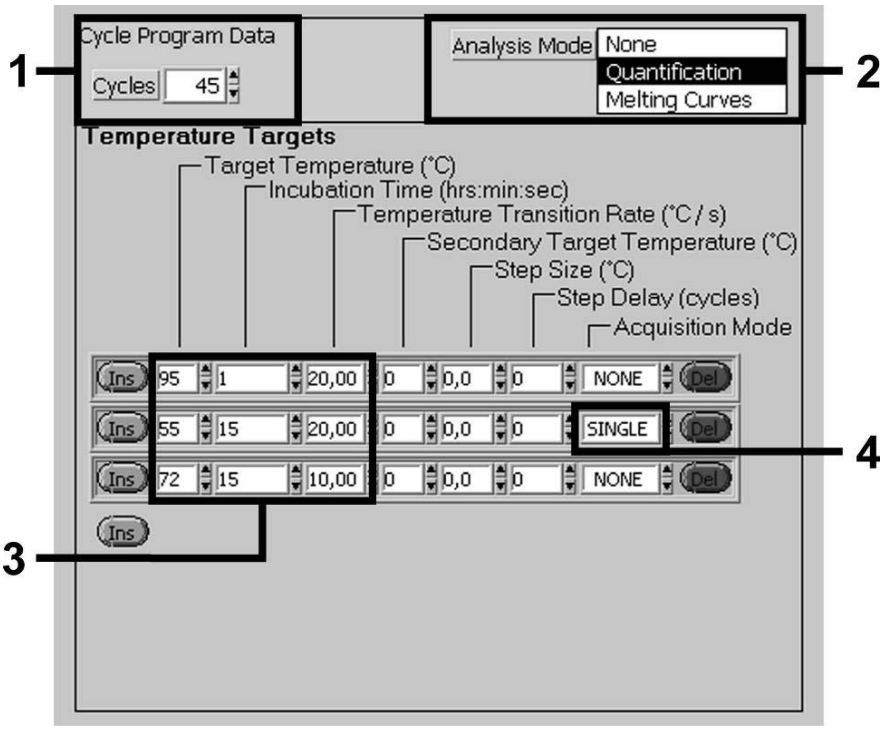

Fig. 4: Amplification of the DNA.

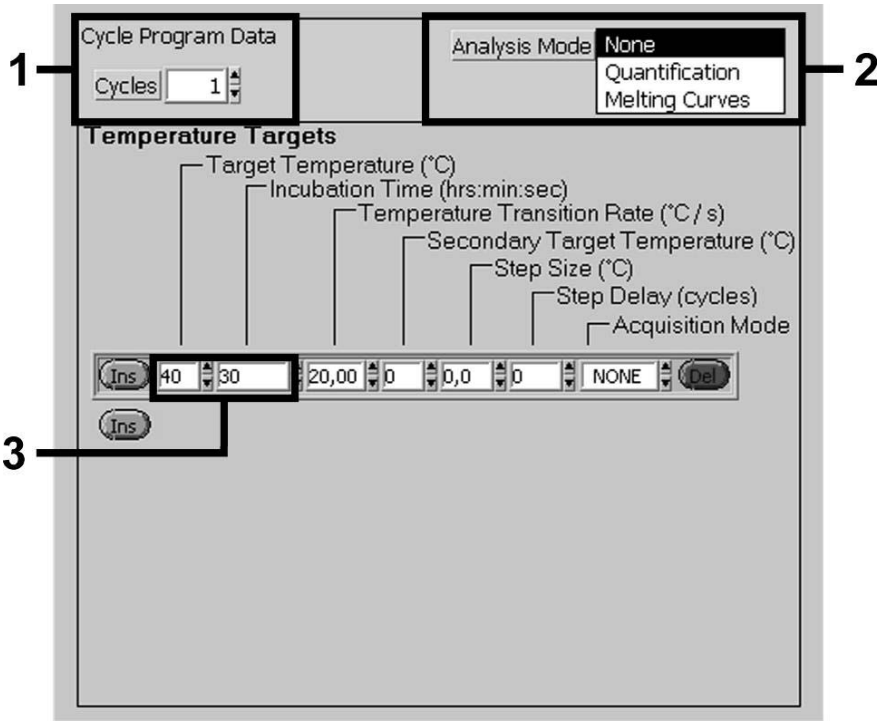

Fig. 5: Cooling.

8.6.2 Programming the *LightCycler 2.0* Instrument

To programme 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 HBV 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

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

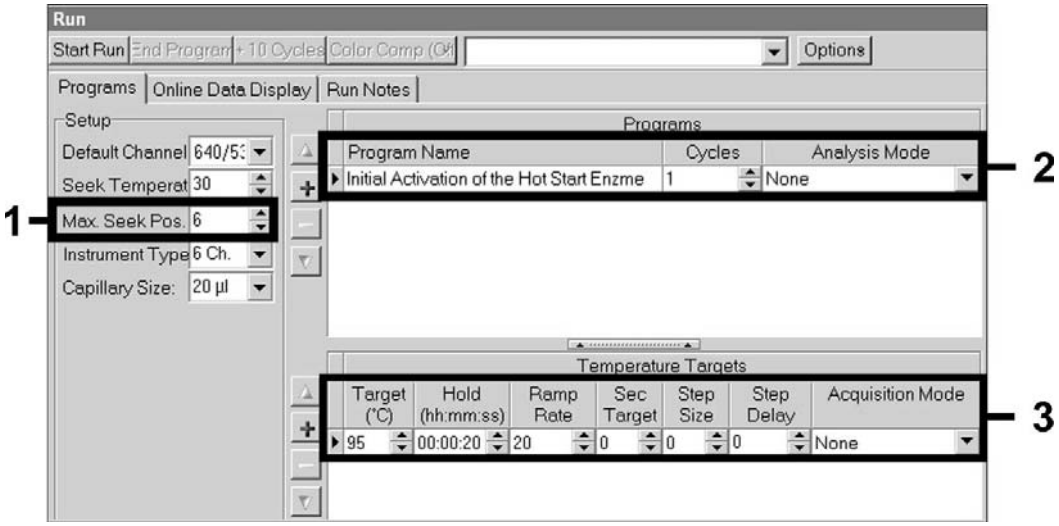

Fig. 6: Initial Activation of the Hot Start Enzyme.

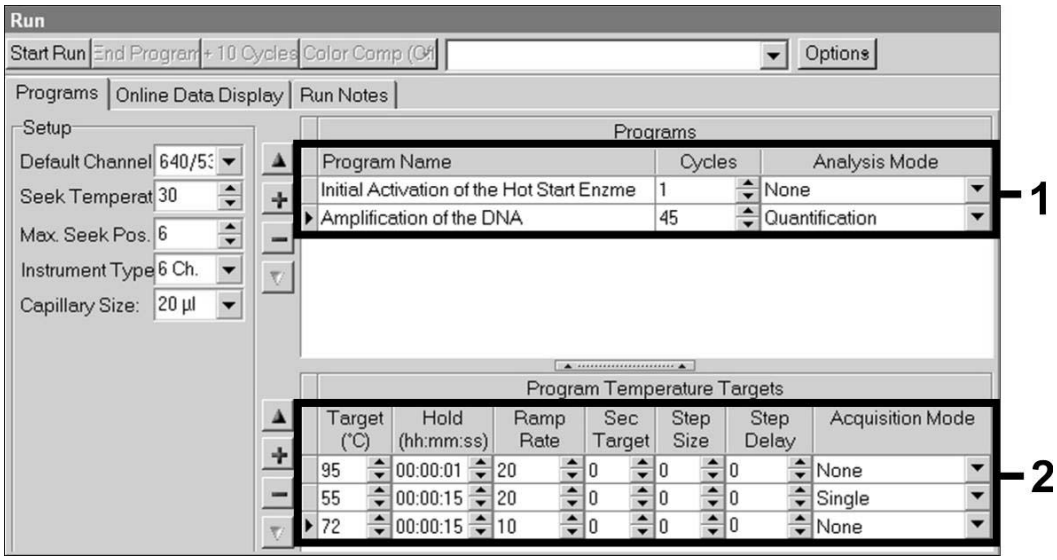

Fig. 7: Amplification of the DNA.

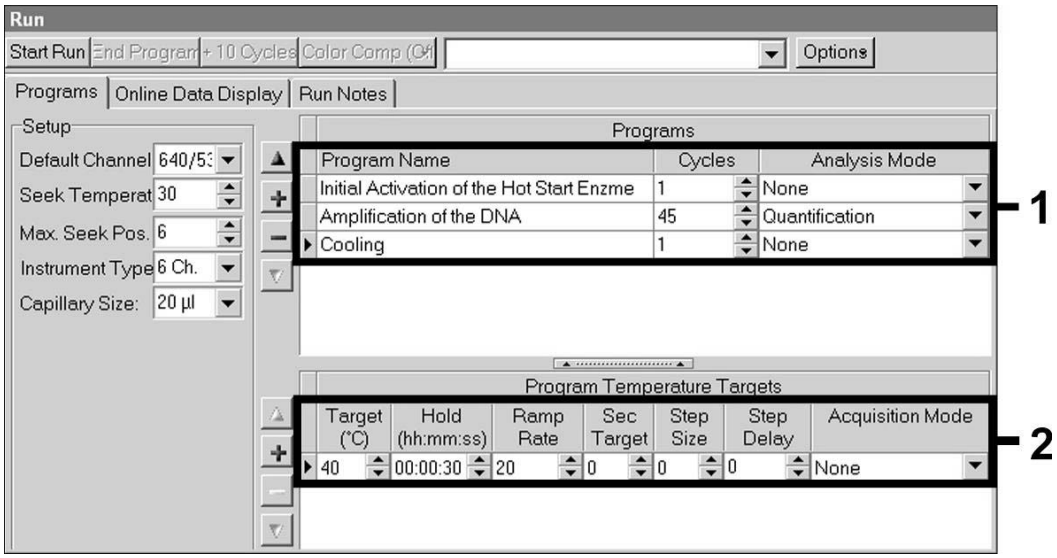

Fig. 8: Cooling.

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 640 for the detection of the analytical HBV PCR and 705 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 **8.4 Quantitation**).
- Make sure that the function *Enable Controls* is **not** activated. Otherwise the selection of analysis options for the data analysis is restricted (*Fit Points*). Under *Target Name* you can assign the target sequences to be detected (HBV or *Internal Control*) in the selected fluorescence channels 640 and 705. 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 programme will start after entering the location where the data should be saved.

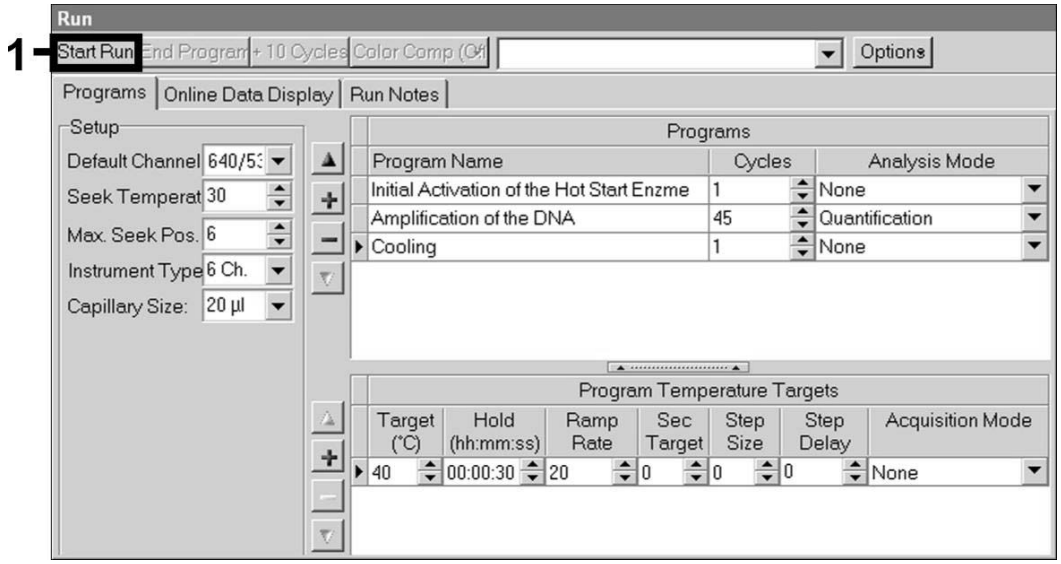

Fig. 9: Start of the PCR run.

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 multicolour 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* HBV LC PCR Kit please select fluorescence display options F2/Back-F1 for the analytical HBV PCR and F3/Back-F1 for the *Internal Control* PCR, respectively. For the analysis of quantitative runs, please follow the instructions given in **8.4 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 F2/Back-F1.

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

   In this case, the detection of a signal in the F3/Back-F1 channel is dispensable, since high initial concentrations of HBV DNA (positive signal in the F2/Back-F1 channel) can lead to a reduced or absent fluorescence signal of the *Internal Control* in the F3/Back-F1 channel (competition).

2. In fluorimeter channel F2/Back-F1 no signal is detected. At the same time, a signal from the *Internal Control* appears in the F3/Back-F1 channel.

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

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

3. No signal is detected in the F2/Back-F1 or in the F3/Back-F1 channel.

   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. 10 and Fig. 11.

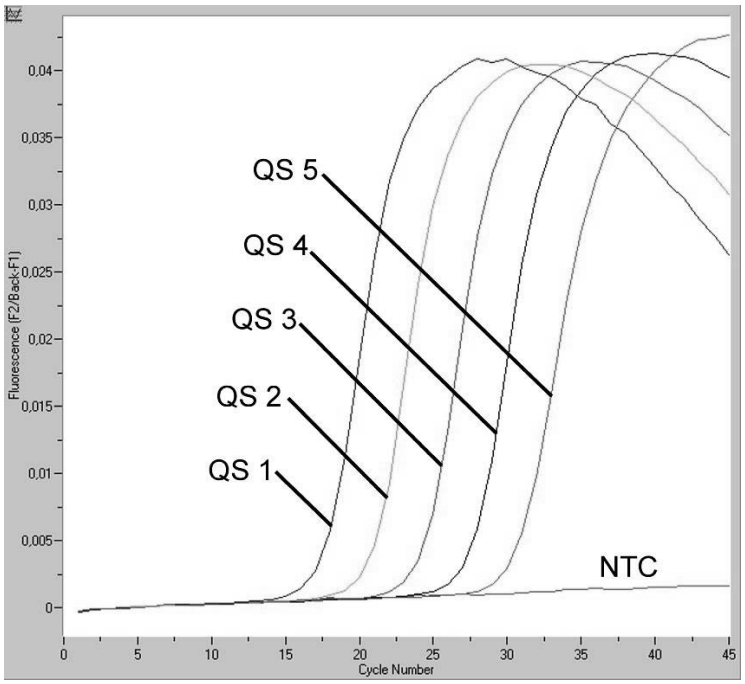

Fig. 10: Detection of the *Quantitation Standards* (*HBV LC QS 1 – 5*) in fluorimeter channel F2/Back-F1 of the *LightCycler 1.1/1.2/1.5* Instrument. NTC: non-template control (negative control).

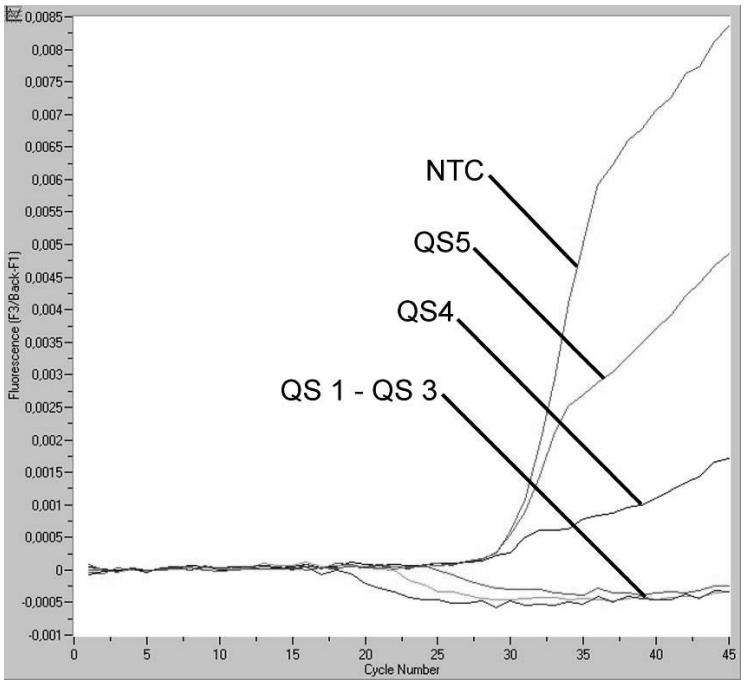

Fig. 11: Detection of the *Internal Control* (*IC*) in fluorimeter channel F3/Back-F1 of the *LightCycler 1.1/1.2/1.5* Instrument with simultaneous amplification of *Quantitation Standards* (*HBV LC QS 1 – 5*). NTC: non-template control (negative control).

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 analysed with this function.
- The *LightCycler* Software Version 4.0 contains a file termed *Color Compensation File*, which compensates multicolour 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* HBV LC PCR Kit please select fluorescence display options 640/Back 530 for the analytical HBV PCR and 705/Back 530 for the *Internal Control* PCR, respectively.

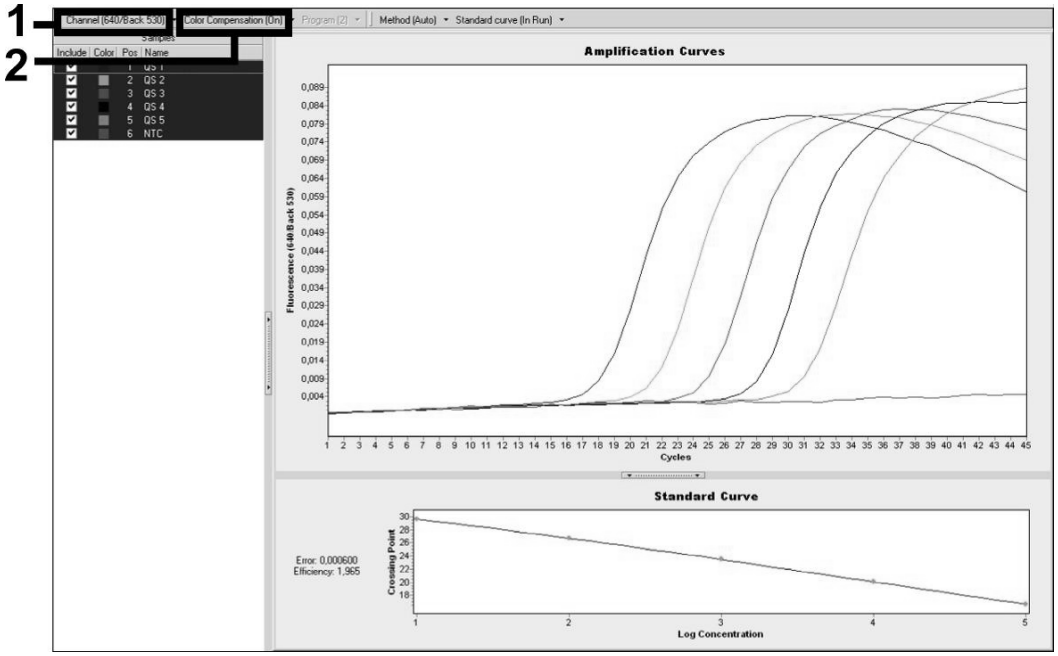

Fig. 12: Activation of the *Color Compensation File* and selection of the fluorescence channel.

For the analysis of quantitative runs, please follow the instructions given in **8.4 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, the following results are possible:

1. A signal is detected in fluorescence channel 640/Back 530.

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

   In this case, the detection of a signal in the 705/Back 530 channel is dispensable, since high initial concentrations of HBV DNA (positive signal in the 640/Back 530 channel) can lead to a reduced or absent fluorescence signal of the *Internal Control* in the 705/Back 530 channel (competition).

2. In fluorescence channel 640/Back 530 no signal is detected. At the same time, a signal from the *Internal Control* appears in the 705/Back 530 channel.

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

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

3. No signal is detected in the 640/Back 530 or in 705/Back 530 channel.

   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.

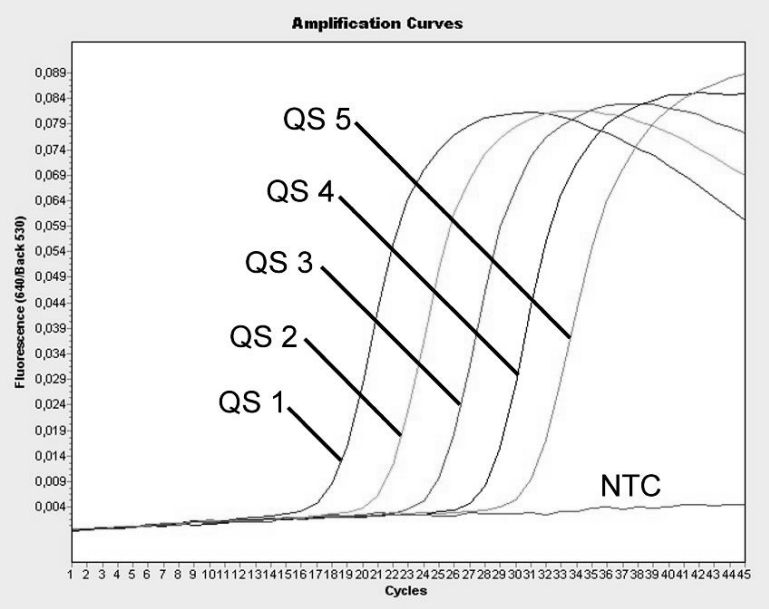

Fig. 13: Detection of the *Quantitation Standards* (*HBV LC QS 1 – 5*) in fluorescence channel 640/Back 530 of the *LightCycler 2.0* Instrument. NTC: non-template control (negative control).

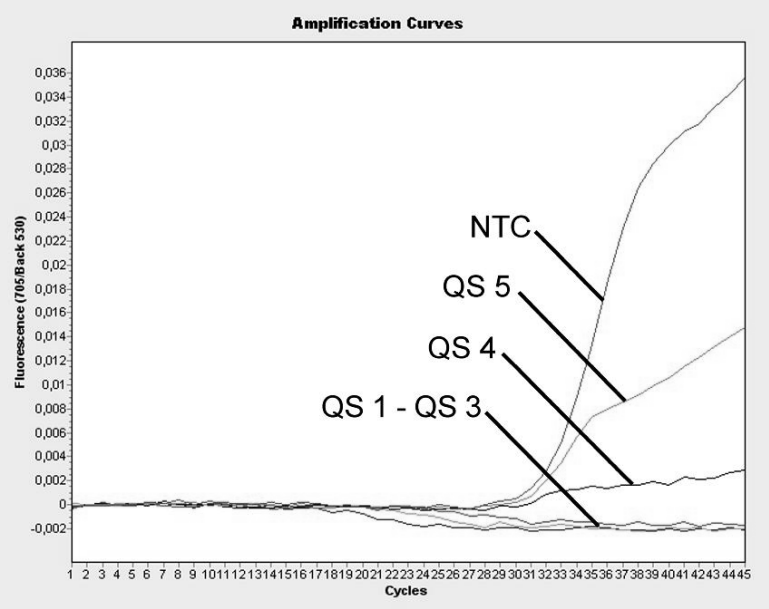

Fig. 14: Detection of the *Internal Control* (*IC*) in fluorescence channel 705/Back 530 of the *LightCycler 2.0* Instrument with simultaneous amplification of *Quantitation Standards* (*HBV LC QS 1 – 5*). NTC: non-template control (negative control).

10. Troubleshooting

No signal with positive controls (*HBV LC QS 1 – 5*) in fluorescence channel F2/Back-F1 or 640/Back 530:

- The selected fluorescence channel for PCR data analysis does not comply with the protocol.
  - → For data analysis select the fluorescence channel F2/Back-F1 or 640/Back 530 for the analytical HBV PCR and the fluorescence channel F3/Back-F1 or 705/Back 530 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 **8.6 Programming of the *LightCycler* Instruments**).
- Incorrect configuration of the PCR reaction.
  - → Check your work steps by means of the pipetting scheme (see **8.5 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* HBV 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* of a negative plasma sample which has been processed through purification (QIAamp DSP Virus Kit) in fluorescence channel F3/Back-F1 (deviation greater than Ct = 32 ± 3; method of analysis: Second Derivative Maximum) or 705/Back 530 (deviation greater than Ct = 32 ± 3; method of analysis: Auto) and simultaneous absence of a signal in channel F2/Back-F1 or 640/Back 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 **8.2 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 **8.2 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 **8.2 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* HBV 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 F2/Back-F1 or 640/Back 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 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

The analytical detection limit as well as the analytical detection limit in consideration of the purification (sensitivity limits) were assessed for the *artus* HBV LC PCR Kit. The analytical detection limit in consideration of the purification is determined using HBV positive clinical specimens in combination with a particular extraction method. In contrast, the analytical detection limit is determined without clinical specimens and independent from the selected extraction method, using a standard of known concentration.

To determine the **analytical sensitivity** of the *artus* HBV LC PCR Kit, a standard dilution series has been set up from 10 to nominal 0.001 HBV IU*/µl and analysed on the *LightCycler 1.1/1.2/1.5* Instrument in combination with the *artus* HBV LC PCR Kit. Testing was carried out on three different days on eight replicates. The results were determined by a probit analysis. The analytical detection limit of the *artus* HBV LC PCR Kit in combination with the *LightCycler 1.1/1.2/1.5* Instrument is 0.2 IU/µl (p = 0.05). This means that there is a 95 % probability that 0.2 IU/µl will be detected.

The **analytical sensitivity in consideration of the purification (QIAamp DSP Virus Kit)** of the *artus* HBV LC PCR Kit on the *LightCycler 1.1/1.2/1.5* Instrument was determined using a dilution series of the 1st International HBV standard (WHO) from 158 to nominal 0.5 HBV IU/ml spiked in clinical plasma specimens. These were subjected to DNA extraction using the QIAamp DSP Virus Kit (extraction volume: 0.5 ml, elution volume: 20 µl). Each of the six dilutions was analysed with the *artus* HBV LC PCR Kit 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

* The standard is a cloned PCR product, the concentration of which has been calibrated using the 1st International HBV standard (WHO).

detection limit in consideration of the purification of the *artus* HBV LC PCR Kit in combination with the *LightCycler 1.1/1.2/1.5* Instrument is 5.8 IU/ml (p = 0.05). This means that there is a 95 % probability that 5.8 IU/ml will be detected.

Probit analysis: Hepatitis B virus (*LightCycler 1.1/1.2/1.5*)

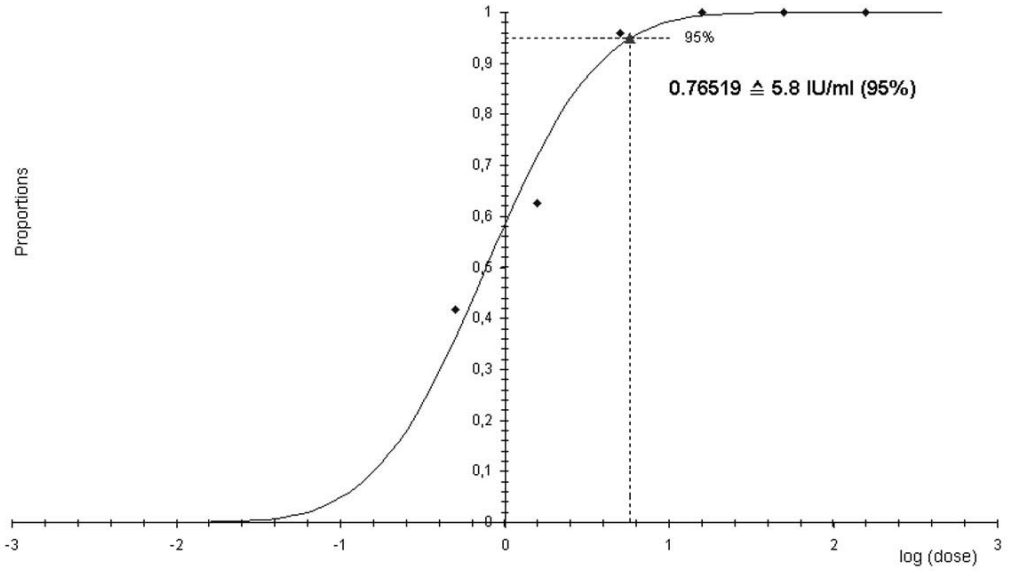

Fig. 15: Analytical sensitivity in consideration of the purification (QIAamp DSP Virus Kit) of the *artus* HBV LC PCR Kit on the *LightCycler 1.1/1.2/1.5* Instrument.

The **analytical sensitivity in consideration of the purification (QIAamp DSP Virus Kit)** of the *artus* HBV LC PCR Kit on the *LightCycler 2.0* Instrument was determined using a dilution series of the 1st International HBV standard (WHO) from 158 to nominal 0.5 HBV IU/ml spiked in clinical plasma specimens. These were subjected to DNA extraction using the QIAamp DSP Virus Kit (extraction volume: 0.5 ml, elution volume: 20 µl). Each of the six dilutions was analysed with the *artus* HBV LC PCR Kit 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 in consideration of the purification of the *artus* HBV LC PCR Kit in combination with the *LightCycler 2.0* Instrument is 6.0 IU/ml (p = 0.05). This means that there is a 95 % probability that 6.0 IU/ml will be detected.

Probit analysis: HBV (*LightCycler 2.0*)

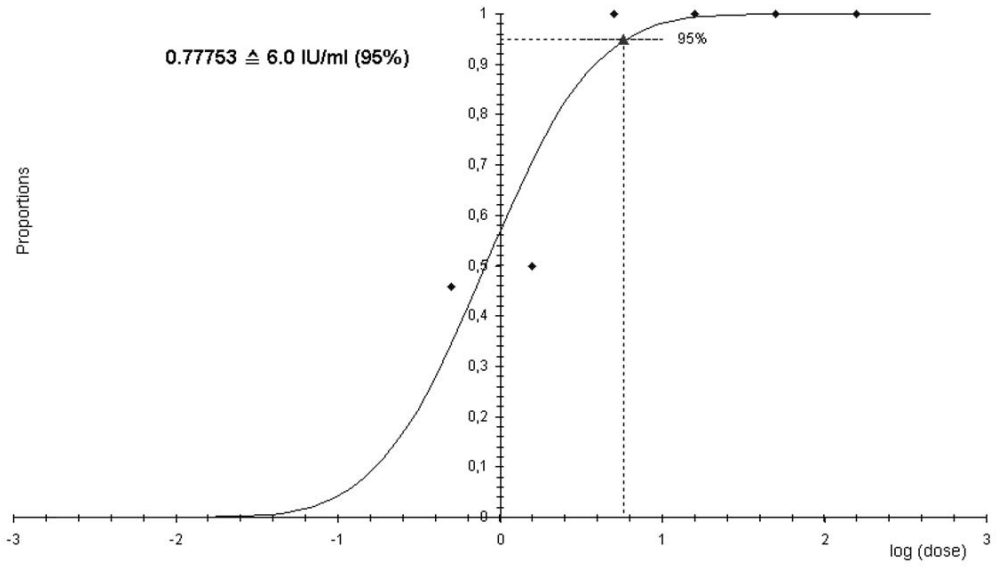

Fig. 16: Analytical sensitivity in consideration of the purification (QIAamp DSP Virus Kit) of the *artus* HBV LC PCR Kit on the *LightCycler 2.0* Instrument.

11.2 Specificity

The specificity of the *artus* HBV 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 relevant genotypes has thus been ensured by a database alignment and by a PCR run on a *LightCycler* Instrument with the following genotypes (see Table 1).

Table 1: Testing of the specificity of relevant genotypes.

Hepatitis B virus	Genotype	Source	HBV (F2/Back-F1 or 640/Back 530)	*Internal Control* (F3/Back-F1 or 705/Back 530)
Hepatitis B virus	A (USA)	teragenix¤	+	+
Hepatitis B virus	B (Indonesia)	teragenix¤	+	+
Hepatitis B virus	C (Indonesia)	teragenix¤	+	+
Hepatitis B virus	C (Venezuela)	teragenix¤	+	+
Hepatitis B virus	D (USA)	teragenix¤	+	+
Hepatitis B virus	E (Cote D' Ivoire)	teragenix¤	+	+
Hepatitis B virus	F (Venezuela)	teragenix¤	+	+
Hepatitis B virus	G (USA)	teragenix¤	+	+

¤teragenix corporation, Florida, USA

For further specificity testing HBV strains with known sequence differences in the pre-core region of the HBV genome (HBV Pre-Core Mutant Panel, Teragenix, Florida, USA) have been used. All nine pre-core mutant strains of this panel could be detected using the *artus* HBV LC PCR Kit.

Moreover, the specificity was validated with 100 different HBV negative plasma samples. These did not generate any signals with the HBV specific primers and probes, which are included in the *HBV LC Master*.

A potential cross-reactivity of the *artus* HBV LC PCR Kit was tested using the control group listed in the following table (see Table 2). None of the tested pathogens has been reactive. No cross-reactivities appeared with mixed infections.

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

Control group	HBV (F2/Back-F1 or 640/Back 530)	*Internal Control* (F3/Back-F1 or 705/Back 530)
Human herpesvirus 1 (Herpes simplex virus 1)	–	+
Human herpesvirus 2 (Herpes simplex virus 2)	–	+
Human herpesvirus 3 (Varicella-zoster virus)	–	+
Human herpesvirus 5 (Cytomegalovirus)	–	+
Human immunodeficiency virus 1	–	+
Hepatitis A virus	–	+
Hepatitis C virus	–	+

11.3 Linear Range

The linear range (analytical measurement) of the *artus* HBV LC PCR Kit was determined by analysing a dilution series of a HBV quantitation standard ranging from 1×10^{8} IU/µl to 1×10^{-2} IU/µl. The dilution series have been calibrated against the WHO 1st International HBV DNA Standard.

Each dilution has been tested in replicates (n = 8) using the *artus* HBV LC PCR Kit on a *LightCycler* instrument.

The linear range of the *artus* HBV LC PCR Kit has been determined to cover concentrations from 0.5 IU/µl to at least 1×10^{8} IU/µl (see Fig. 17).

Under the assumption that the QIAamp DSP Virus Kit is used for DNA extraction the *artus* HBV LC PCR Kit covers a linear range from 20 IU/ml to at least 4×10^{9} IU/ml.

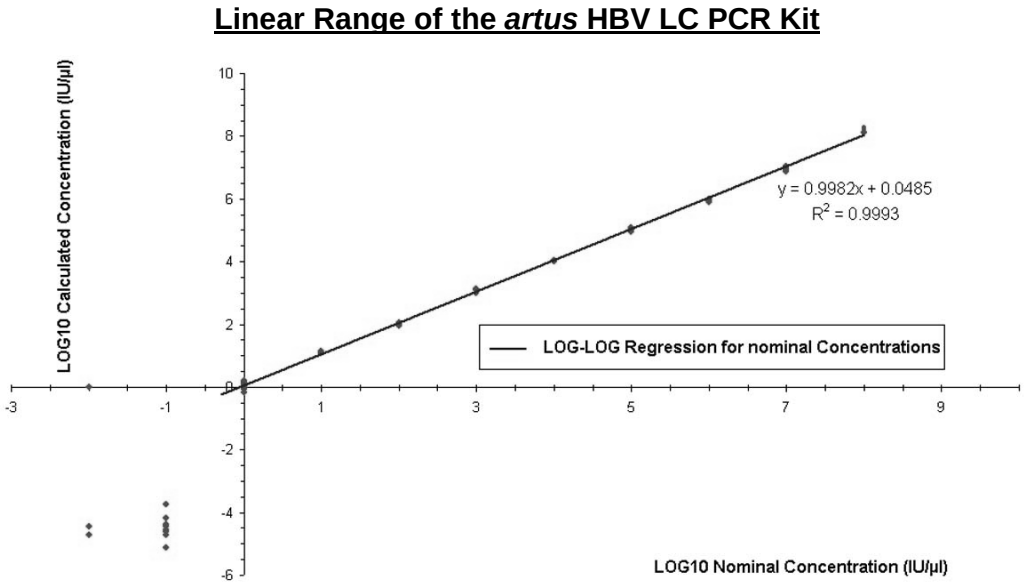

Fig. 17: Calculation of the linear range. The straight line was determined by a linear regression of the $\log_{10}$ calculated concentrations with the $\log_{10}$ nominal concentrations. The equation of the regression line is included in the figure.

11.4 Precision

The precision data of the *artus* HBV 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* HBV LC PCR Kit have been collected using the *Quantitation Standard* of the lowest concentration (*QS 5*; 10 IU/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). In addition, precision data for quantitative results in IU/µl were

determined using the corresponding Ct values (see Table 4). Based on these results, the overall statistical spread of any given sample with the mentioned concentration is 0.96 % (Ct) or 8.03 % (conc.), for the detection of the *Internal Control* 0.97 % (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: *HBV LC QS 5*	0.16	0.03	0.52
Intra-assay variability: *Internal Control*	0.08	0.01	0.28
Inter-assay variability: *HBV LC QS 5*	0.27	0.08	0.89
Inter-assay variability: *Internal Control*	0.15	0.02	0.53
Inter-batch variability: *HBV LC QS 5*	0.32	0.10	1.02
Inter-batch variability: *Internal Control*	0.30	0.09	1.03
Total variance: *HBV LC QS 5*	0.30	0.09	0.96
Total variance: *Internal Control*	0.28	0.08	0.97

Table 4: Precision data on basis of the quantitative results (in IU/µl).

	Standard deviation	Variance	Coefficient of variation [%]
Intra-assay variability: *HBV LC QS 5*	1.07	1.15	10.66
Inter-assay variability: *HBV LC QS 5*	0.80	0.64	7.99
Inter-batch variability: *HBV LC QS 5*	0.85	0.72	8.46
Total variance: *HBV LC QS 5*	0.81	0.65	8.03

11.5 Robustness

The verification of the robustness allows the determination of the total failure rate of the *artus* HBV LC PCR Kit. 100 HBV negative samples of plasma were spiked with 0.6 IU/µl elution volume of HBV control DNA (threefold concentration of the analytical sensitivity limit). After extraction using the QIAamp DSP Virus Kit (see **8.2 DNA Isolation**) these samples were analysed with the *artus* HBV LC PCR Kit. For all HBV samples the failure rate was 0 %. In addition, the robustness of the *Internal Control* was assessed by purification and analysis of 100 HBV negative plasma samples. The total failure rate was 0 %. Inhibitions were not observed. Thus, the robustness of the *artus* HBV LC PCR Kit is ≥ 99 %.

11.6 Reproducibility

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

11.7 Diagnostic Evaluation

The *artus* HBV LC PCR Kit was evaluated in a study (Stelzl et al., 2004). Comparing the *artus* HBV LC PCR Kit to the COBAS® AMPLICOR® HBV MONITOR® 117 serum specimens were analysed retrospectively. All serum specimens were pre-analysed positive or negative using the COBAS AMPLICOR HBV MONITOR for routine diagnostics.

HBV DNA for testing the *artus* HBV LC PCR Kit was isolated using an automated sample preparation with the COBAS AMPLIPREP Total Nucleic Acid Isolation kit on the COBAS AMPLIPREP Analyzer. For comparative testing with the COBAS AMPLICOR HBV MONITOR Test HBV DNA was isolated according to the instructions of the manufacturer provided in the package insert.

The results obtained by using the *artus* HBV LC PCR Kit were compared to those of the COBAS AMPLICOR HBV MONITOR Test. No discordant results could be observed (see Table 5).

All samples tested positive with the COBAS AMPLICOR HBV MONITOR Test were also tested positive with the *artus* HBV LC PCR Kit. Therefore, the diagnostic sensitivity is 100 %.

All samples tested negative with the COBAS AMPLICOR HBV MONITOR Test were also tested negative with the *artus* HBV LC PCR Kit. Therefore, the diagnostic specificity is 100 %.

Table 5: Results of the 117 analysed retrospective serum samples.

	Number of Samples	**Positive Samples** as determined with the COBAS AMPLICOR HBV MONITOR	**In comparison with COBAS AMPLICOR HBV MONITOR**	
			Sensitivity	**Specificity**
Serum	117	41.88 % (49/117)	100 % (49/49)	100 % (68/68)

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.
- Although rare, mutations within the highly conserved regions of the viral genome covered by the kit's primers and/or probe may result in underquantitation or failure to detect the presence of the virus in these cases. Validity and performance of the assay design are revised at regular intervals.

13. Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDSs). These are available online in convenient and compact PDF format at www.qiagen.com/support/MSDS.aspx where you can find, view, and print the MSDS 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

14. Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of *artus* HBV LC PCR Kit has been 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) Stelzl E, Muller Z, Marth E, Kessler HH. Rapid Quantification of Hepatitis B Virus DNA by Automated Sample Preparation and Real-Time PCR. J Clin Microbiol 2004; 42 (6): 2445 – 2449.

16. Explanation of Symbols

Use by

Batch code

Manufacturer

Catalogue number

Material number

Handbook

In vitro diagnostic medical device

Components

Contains

Number

<N>

Contains sufficient for <N> tests

Temperature limitation

Consult instructions for use

QS	*Quantitation Standard*
IC	*Internal Control*
Mg-Sol	*Magnesium Solution*

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Belgium ■ Orders 0800-79612 ■ Fax 0800-79611 ■ Technical 0800-79556

Brazil ■ Orders 0800-557779 ■ Fax 55-11-5079-4001 ■ Technical 0800-557779

Canada ■ Orders 800-572-9613 ■ Fax 800-713-5951 ■ Technical 800-DNA-PREP (800-362-7737)

China ■ Orders 86-21-3865-3865 ■ Fax 86-21-3865-3965 ■ Technical 800-988-0325

Denmark ■ Orders 80-885945 ■ Fax 80-885944 ■ Technical 80-885942

Finland ■ Orders 0800-914416 ■ Fax 0800-914415 ■ Technical 0800-914413

France ■ Orders 01-60-920-926 ■ Fax 01-60-920-925 ■ Technical 01-60-920-930 ■ Offers 01-60-920-928

Germany ■ Orders 02103-29-12000 ■ Fax 02103-29-22000 ■ Technical 02103-29-12400

Hong Kong ■ Orders 800 933 965 ■ Fax 800 930 439 ■ Technical 800 930 425

Ireland ■ Orders 1800 555 049 ■ Fax 1800 555 048 ■ Technical 1800 555 061

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Mexico ■ Orders 01-800-7742-639 ■ Fax 01-800-1122-330 ■ Technical 01-800-7742-436

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