ROBOSCREEN DIAGNOSTICS

Instructions for Use

RoboGene HBV DNA Quantification Kit 3.1

Rev. 0 _ 10 / 2025

CE 0483

IVD

Order No.:

847-0207720032	32 reactions
847-0207720096	96 reactions
847-0207720192	192 reactions

CE
0483

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IFU RoboGene HBV DNA Quantification Kit 3.1 Rev 0 10 / 2025

Contents

1 Introduction

1.1 Intended use

RoboGene HBV DNA Quantification Kit 3.1 is a **non-automated in vitro diagnostic test, based on real-time PCR technology,** for quantification of Hepatitis B Virus (HBV) DNA (genotypes A-H) in **human EDTA-plasma samples**.

RoboGene HBV DNA Quantification Kit 3.1 is intended for quantification of HBV DNA as an aid in the management of patients with HBV infection undergoing antiviral therapy.

All results must be interpreted within the context of all relevant clinical and laboratory findings.

RoboGene HBV DNA Quantification Kit 3.1 has not been approved as a screening test for the presence of HBV in blood or blood products.

RoboGene HBV DNA Quantification Kit 3.1 is intended for use by professional users trained in molecular biological techniques and in vitro diagnostic procedures.

1.2 Pathogen information

The human hepatitis B virus (HBV), which was discovered by Blumberg in 1965, is a small-enveloped DNA virus with a genome of approximately 3200 nucleotides causing acute and chronic hepatitis. Despite the introduction of a vaccine for HBV in 1981, HBV infection still represents a major global health burden. Many studies highlight chronic HBV infection as the main risk factor for hepatocellular carcinoma development. HBV transmission occurs vertically and horizontally via exchange of body fluids. The chronicity rate is about 5 % in adult-acquired, but it reaches 90 % in neonatally acquired infections [1]. Due to the lack of proofreading activity of the viral polymerase, the genetic variability of HBV is high. At present 9 HBV genotypes, A-I, are phylogenetically classified, based on an intergroup divergence of ≥8 % across the complete genome, with a

possible 10th genotype J that currently has been detected in only one patient [2]. Genotypes A-D, F, H and I are further differentiated into several subgenotypes with a sequence diversity of at least 4 %. The HBV genotypes, and certain subgenotypes, show distinct geographical predominancies, and vary in clinical manifestation of infection and response to antiviral therapy [2].

1.3 Technical assistance

CONSULT INSTRUCTIONS FOR USE

The Instructions for use must be read carefully prior to use. Given instructions must be followed accordingly. Reliability of results cannot be guaranteed if there are any deviations from the instructions for use.

If you have any questions or problems regarding any aspects of the RoboGene HBV DNA Quantification Kit 3.1 please do not hesitate to contact our technical support team which consists of experts with long-time experience in the field of molecular diagnostics. For technical assistance please contact us at the manufacturer site as shown inside the cover of the Instructions for use.

1.4 Reporting incidents

In case of any serious incidents that have occurred in relation to RoboGene HBV DNA Quantification Kit 3.1, please inform us and your competent authority.

1.5 Symbols and abbreviations

For easy reference and orientation, the IFU uses the following warning and information symbols:

Symbol	Information
	**REF** Catalogue number.
	**Content** Contains number of IVD-determinations as indicated.
	**Storage temperature** Store at temperatures between upper and lower limits as indicated.
	**Consult instructions for use** This information must be observed to avoid improper use of the kit.
	**Used by** Expiry date. The product is to be used by the indicated date.
	**Lot number** The lot number of the kit.
	**CE-IVD symbol** *In vitro* diagnostic medical device.
	**Manufactured by** Contact information of the manufacturer.
	**For single use only** Single use only. Do not use the product twice.
	**Note / Attention** Observe the notes marked in this way to ensure correct function of the device and to avoid operating errors for obtaining correct results.

The following abbreviations are used in the IFU:

Ct	Threshold cycle value
CV	Coefficient of variation
dNTP	2'-deoxynucleotide 5'-triphosphate
HBV	Hepatitis B Virus
IC	Internal Control
IFU	Instruction for use
IU	International Units
NA	Nucleic acid
NTC	Non-template control
PEI	Paul-Ehrlich-Institut, Langen, Germany
RM	Reagent Mix
rxn	Reactions
RT	Real-time
SD	Standard deviation
WHO	World Health Organization

1.6 Summary of safety and performance

The current Summary of Safety and Performance for professional users (SSP) for our product RoboGene HBV DNA Quantification Kit 3.1 is available at EUDAMED and can additionally be requested by email under the addresses given on the title page.

2 Test description and principle

2.1 Principle of the TaqMan™ assay

TaqMan™ real-time PCR is a highly sensitive assay that combines amplification with fluorescence-based online detection of the nucleic acid of interest (target, template). The assay is based on a conventional set of target and internal control-specific primers in combination with fluorescence-labelled oligonucleotide probes, complementary to the desired target sequences. In the presence of targets the probes hybridize with their target-complementary sequences. The Taq DNA polymerase from the RT PCR Enzyme possesses a 5`→ 3` exonuclease activity that hydrolyses the probes and displaces the fluorescent dye from the quencher. This event results in an increase of the fluorescence signal, which is directly proportional to the target amplification during each PCR cycle.

2.2 Explanation of the test

RoboGene HBV DNA Quantification Kit 3.1 is a real-time PCR test for the quantification of HBV DNA in human EDTA plasma samples. The kit contains primers and probes specific for the S-gene of the viral genome.

The test is validated for quantification of HBV genotypes A-H.

Quantification of specimen is performed by parallel amplification of the included quantification standard.

A synthetic internal control is integrated to control the whole procedure from NA extraction to the real-time PCR. Thus, the risk for false-negative results is drastically reduced, yielding an increase of diagnostic correctness. Amplification of HBV DNA in samples and standards and of IC-RNA is measured independently at different wavelengths due to probes labelled with different fluorescent reporter dyes. HBV DNA is detected in the FAM channel. For detection of IC-RNA the kit provides two options depending on the set up of the

real-time PCR system allowing detection in Yakima Yellow/VIC/JOE or Cy5 channel.

RoboGene HBV DNA Quantification Kit 3.1 is validated in combination with real-time PCR systems listed in **chapter 6** and viral nucleic acid extraction kits listed in **chapter 7** of this IFU. Nucleic acid extraction must be performed strictly according to manufacturer's instructions.

2.3 Restrictions

RoboGene HBV DNA Quantification Kit 3.1 is validated only for use of human EDTA-plasma as sample material.

Heparinized plasma and citrate plasma must be excluded from analysis. If other than the recommended sample types are used incorrect results may be obtained.

Lipaemic samples must be excluded from analysis. This is substantiated by the results of the performance evaluation revealing an inhibitory effect for highly lipaemic samples containing 1,000-2,000 mg/dl of lipids

RoboGene HBV DNA Quantification Kit 3.1 is to be used only by professionals specially instructed and trained in in vitro diagnostic procedures.

Safety and performance of RoboGene HBV DNA Quantification Kit 3.1 cannot be guaranteed if there are any deviations from the instructions in this IFU.

Do not use expired components or mix components from different kit lots.

3 Safety precautions

NOTE

Read this chapter carefully to guarantee your own safety and a trouble-free operation.

Follow all safety instructions explained in the IFU, as well as all given messages and information.

Human plasma samples must be considered as potentially infectious. Thus, always wear lab coat and gloves.

Always use clean and nuclease-free equipment.

Extraction of nucleic acids, PCR reagent assembly and amplification should be performed in different rooms.

NTC should be included in each real-time PCR to reveal absence of false positive results.

Be careful while pipetting specimen material to avoid carry-over contaminations.

Discard sample and assay waste according to your in-house safety regulations.

Maintain your laboratory equipment regularly, e.g., calibration of thermomixer temperature.

FOR SINGLE USE ONLY!

This kit is made for single use only!

4 Performance assessment

RoboGene HBV DNA Quantification Kit 3.1 was validated according to Regulation (EU) 2022/1107 of 4 July 2022 laying down common specifications for certain class D in vitro diagnostic medical devices [3].

Validation of the RoboGene HBV DNA Quantification Kit 3.1 was conducted with the equivalent product RoboGene HBV DNA Quantification Kit 3.0 (Roboscreen GmbH, 847-0207710032, 847-0207710096, 847-0207710192). Both IVD-products are technically identical and performance data are transferable to RoboGene HBV DNA Quantification Kit 3.1 without restriction.

In the performance assessment analyses nucleic acid extraction was carried out with the IVD-kit for viral nucleic acid extraction INSTANT Virus RNA/DNA Kit.

INFORMATION

Quantification standards of RoboGene HBV DNA Quantification Kit 3.1 are calibrated against the 4th WHO International Standard for HBV DNA (NIBSC-code: 10/266) and the PEI HBV DNA reference material (#3620/05) that itself was calibrated against the 1st WHO International Standard for HBV DNA (NIBSC-code: 97/746).

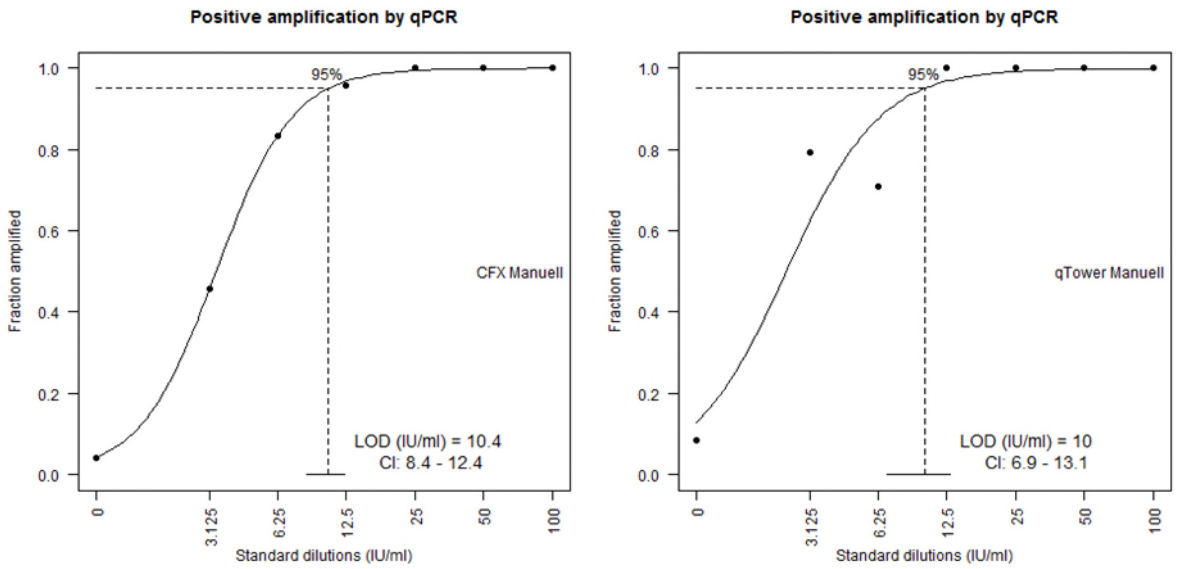
4.1 Analytical Sensitivity

The analytical sensitivity of RoboGene HBV DNA Quantification Kit 3.1 was determined by analyzing dilution series of PEI Reference Material HBV DNA (#3620/05, genotype D). At least 24 replicates of each dilution step of the PEI reference material were quantified with the test in combination with real-time PCR-systems CFX96 Touch, qTOWER^3, LightCycler 480II, 7500 Fast and Rotor-Gene 3000. The detection limits were calculated by PROBIT analysis. Analytical sensitivities of each of the NAT-systems are summarized in Table 1.

Table 1: Limits of detection and confidence intervals of RoboGene HBV DNA Quantification Kit 3.1 in combination with real-time PCR systems.

Real-time PCR system	Limit of detection (LOD) (IU/ml)	95 % confidence interval (IU/ml)	
qTOWER^3 (qT)	10.0	6.9	13.1
CFX96 (CFX)	10.4	8.4	12.4
LightCycler 480 II (LC)	8.0	6.4	9.6
7500 Fast (FS)	7.3	5.8	8.7
Rotor-Gene 3000 (RG)	10.4	8.4	12.4

The detection limit was calculated by PROBIT analysis of at least 24 replicates of each dilution of reference material on each real-time PCR-system with confidence of 95 % (see Figure 1).



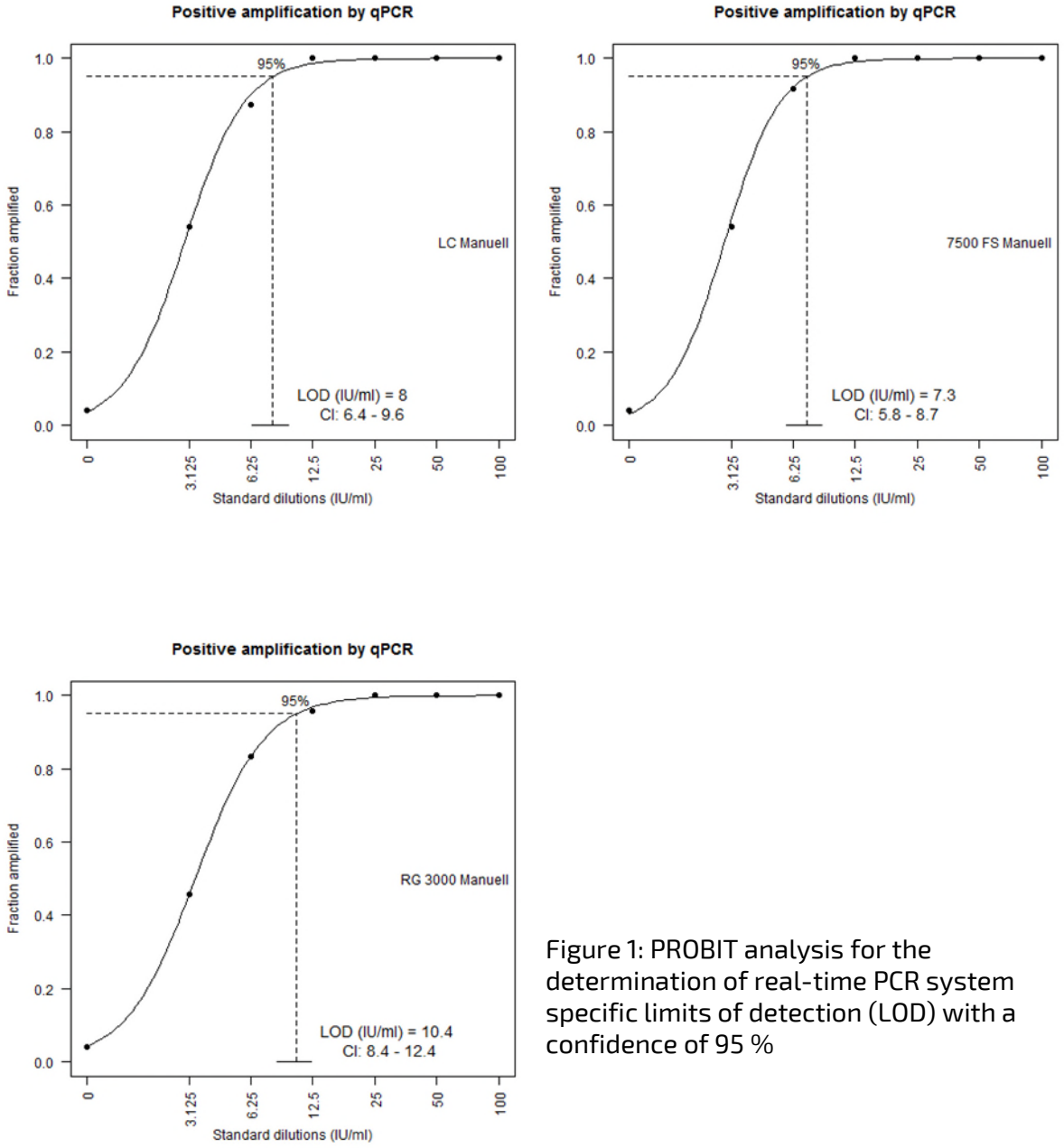


Figure 1: PROBIT analysis for the determination of real-time PCR system specific limits of detection (LOD) with a confidence of 95 %

4.2 Genotype detection and quantification

Specificity of RoboGene HBV DNA Quantification Kit 3.1 for detection and quantification of known HBV genotypes/subtypes was tested using the 1st WHO International Reference Panel for Hepatitis B Virus Genotypes for Nucleic Acid Amplification Techniques – Based Assays

PEI code 5086/08 (Version 2, 28th Nov 2011). The genotype panel included 15 samples of the most prevalent HBV genotypes (A-G).

Half-log dilution series of all specimens were prepared and viral nucleic acids were extracted from 400 µl sample volume using INSTANT Virus RNA/DNA Kit. Quantification of HBV DNA was carried out with the test and real-time PCR Systems CFX96 Touch, qTOWER^3, LightCycler 480II, 7500 Fast and Rotor-Gene 3000.

The quantification efficiency of the test for genotype A-G was proven by linear regression analysis with slopes from 0,8 to 1,08 and determination coefficients R^2 between 0,9734 and 0,9996.

Comparison of the obtained quantification results with the concentrations of the reference panel verified accurate genotype detection of the RoboGene HBV DNA Quantification Kit 3.1 within the ±0.6 $\log_{10}$ acceptance interval. Data shown in figure 2.

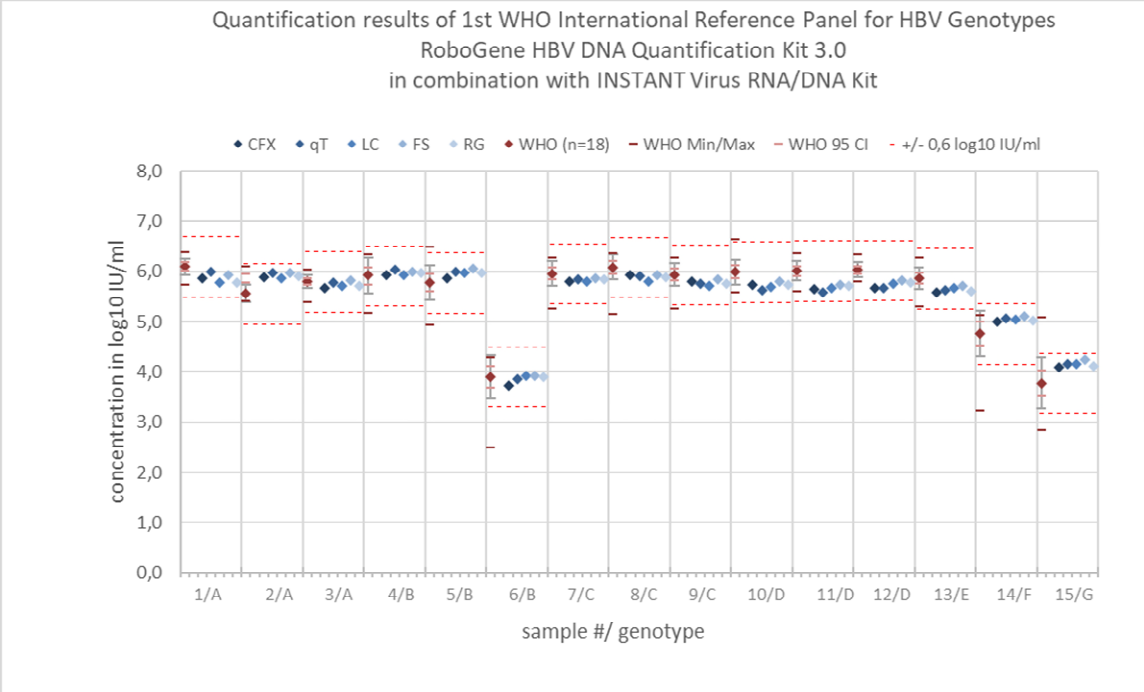


Figure 2: Shown are the quantification results obtained with the test compared to the reference concentrations of the WHO International Reference Panel for Hepatitis B Virus Genotypes (mean of n = 18 participants, standard deviation, minimum and maximum values). Quantification results are separately indicated for each combined real-time PCR-system.

HBV genotype H is comparably rare and prevalent only in South America (Mexico, El Salvador, Guatemala and Honduras). Quantification of genotype H by the RoboGene HBV DNA Quantification Kit 3.1 was evaluated using a synthetic desoxyribonucleic acid sequence specific for HBV genotype H.

A dilution series of the synthetic nucleic acid including 11 concentrations within an 8 log range was prepared and quantified with RoboGene HBV DNA Quantification Kit 3.1 (without NA-extraction) in combination with each of the real-time PCR systems CFX96 Touch, qTOWER^3, LightCycler 480II, 7500 Fast and Rotor-Gene 3000. The quantification efficiency of the test for genotype H was proven by linear regression analysis with slopes from 0,99 to 1,04 and determination coefficients R^2 between 0,9993 and 0,9999).

The detection capability of RoboGene HBV DNA Quantification Kit 3.1 for other confirmed HBV genotypes and subtypes not included in the WHO reference panel was verified by aligning the sequences of the test-specific primer and probe oligonucleotides against sequence data of the respective HBV-subtype reference strains (data not shown).

4.3 Linear Range

Linear range of the quantification of HBV DNA was determined by analyzing dilution series of synthetic HBV DNA ranging from $2.5x10^9$ to 8 IU/ml (n=8 for each dilution) and by quantification of native sample material (genotype E) containing HBV DNA concentrations ranging between $1x10^8$ and 10 IU/ml.

Viral nucleic acids were extracted from the sample material in triplicate, and HBV DNA was quantified with n=1 using real-time PCR Systems CFX96, qTOWER^3, LightCycler 480II, 7500 Fast and Rotor-Gene 3000. Data analysis was carried out according to CLSI guideline EP06-A [4].

Results verify linearity over 8 $\log_{10}$ steps from 8.0 to $2.5x10^9$ IU/ml. The assay specific lower limit of quantification (LLOQ) is equivalent

to the limit of detections (LOD) and was calculated between 7.3 and 10.4 IU/ml (see table 1). Data are shown in figures 3 and 4.

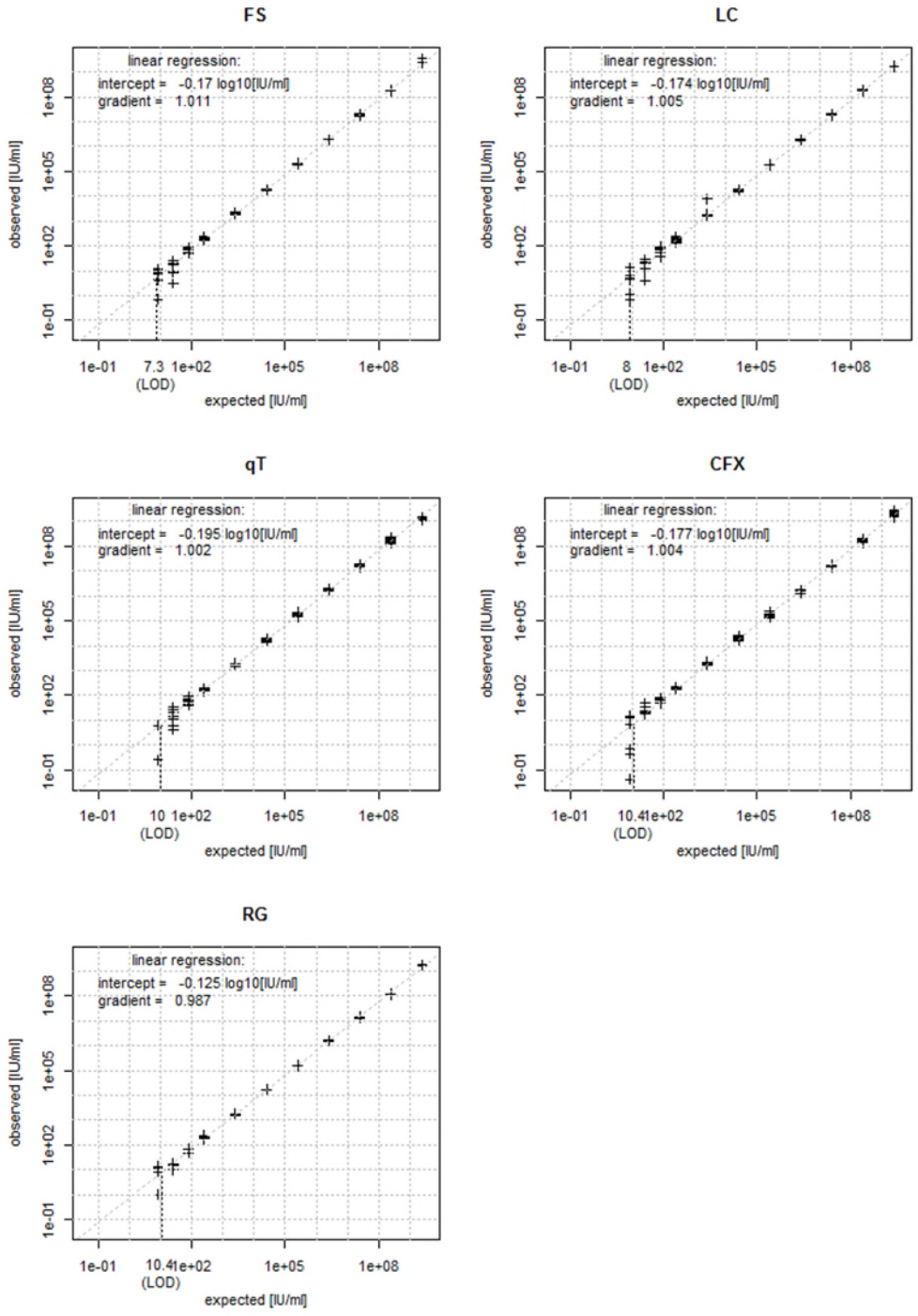


Figure 3: Linear range of quantification of the HBV DNA Quantification Kit 3.1. Data were obtained using a 8 log_{10} dilution series (8 IU/ml – 2.5x10^9 IU/ml) of synthetic HBV DNA. The assay performed linear over the 8 log_{10} steps tested

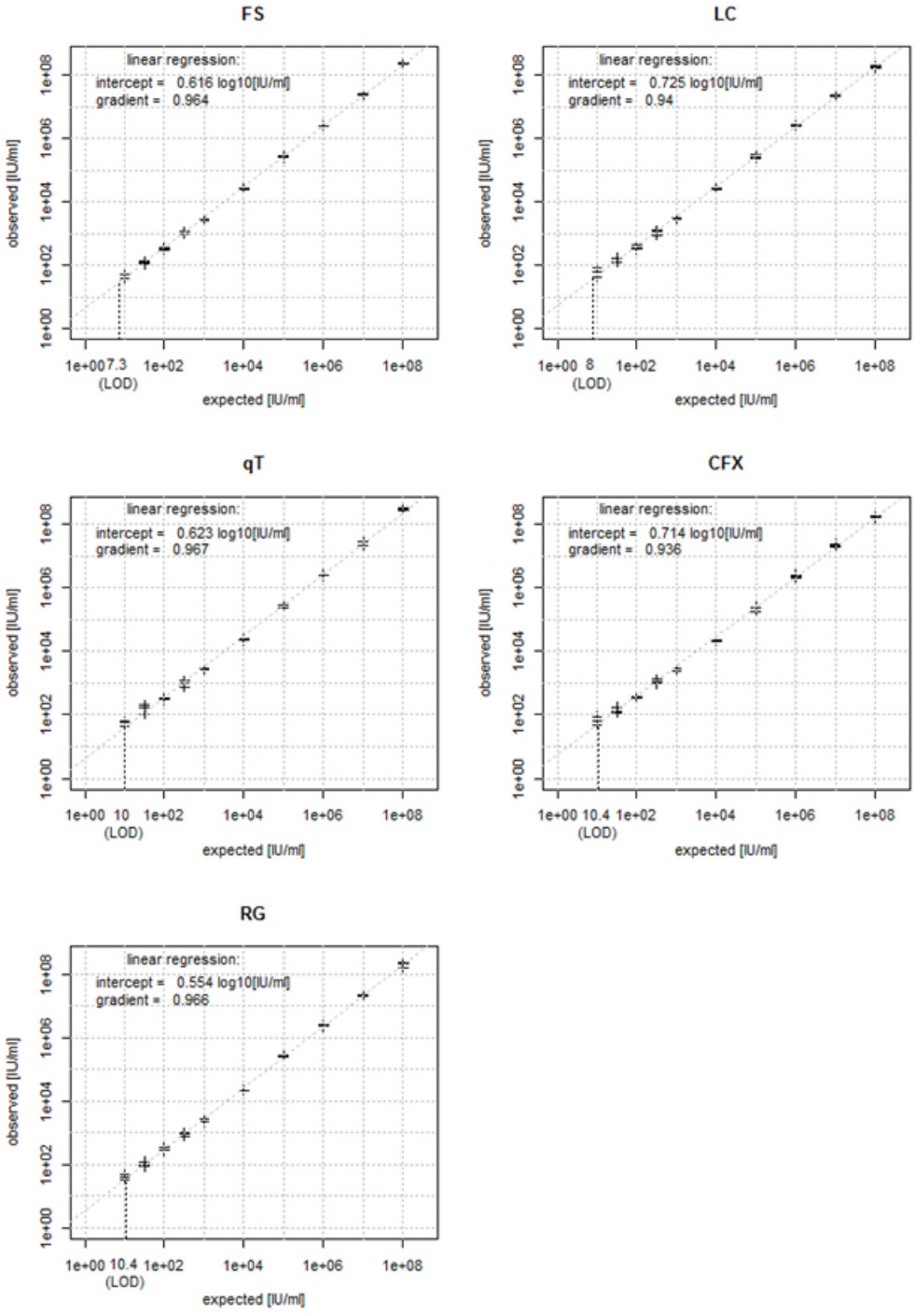


Figure 4: Linear range of quantification of the HBV DNA Quantification Kit 3.1. Data were obtained using a 7 log_{10} dilution series (10 IU/ml – $1x10^8$ IU/ml) of a high-titer HBV-patient specimen. The assay performed linear over the 7 log_{10} steps.

4.4 Analytical specificity

Analysis of 13 non-HBV positive human DNA-virus samples (HSV type 1 and 2; PVB19; EBV; HPV16; HPV18; HCMV) and two RNA-virus samples (HIV; HCV) confirmed 100 % analytical specificity of RoboGene HBV DNA Quantification Kit 3.1 (see Table 2).

Table 2: Results of analysis of 14 non-HBV positive human DNA/RNA-virus samples.

Sample	HBV detection	IC detection
HBV positive control (n = 1)	1/1	1/1
Virus negative control (n = 1)	0/1	1/1
HSV type 1 (n = 1)	0/1	1/1
HSV type 2 (n = 1)	0/1	1/1
PVB19 (n = 2)	0/2	2/2
EBV (n = 1)	0/1	1/1
HPV16 (n = 1)	0/1	1/1
HPV18 (n = 1)	0/1	1/1
HCMV (n = 4)	0/4	4/4
HIV (n = 1)	0/1	1/1
HCV (n = 1)	0/1	1/1

4.5 Accuracy (resulting from trueness and precision)

The analysis of accuracy of HBV DNA quantification was carried out with RoboGene HBV DNA Quantification Kit 3.1 in combination with the viral nucleic acid extraction kit INSTANT Virus RNA/DNA Kit and real-time PCR systems CFX96, qTOWER^3, LightCycler 480II, 7500 Fast and Rotor-Gene 3000.

5 step dilution series of an HBV DNA positive clinical sample covering 4 $\log_{10}$ steps were quantified using 3 different production lots of the test.

For each lot the quantifications were carried out on 3 different days with different operators each and real-time PCR systems mentioned above. A total of 900 quantification results were generated.

Results of 899 valid quantifications showed an accuracy within the ±0.6 log_{10} IU/ml acceptance interval for all tested concentrations (see Table 3).

Table 3: Results of accuracy analysis

Dilution [IU/ml]	**Number of valid quantifications**	**Number of accepted quantifications within ±0.6 log_{10} IU/ml**
Dil 1: 588,235 IU/ml	180	180
Dil 2: 58,824 IU/ml	180	180
Dil 3: 5,882 IU/ml	180	180
Dil 4: 588 IU/ml	180	180
Dil 5: 58.8 IU/ml	180	179

4.6 Trueness

The analysis of trueness of HBV DNA quantification was carried out with RoboGene HBV DNA Quantification Kit 3.1, the viral nucleic acid extraction kit INSTANT Virus RNA/DNA Kit and real-time PCR systems CFX96, qTOWER^3, LightCycler 480II, 7500 Fast and Rotor-Gene 3000.

A half-log dilution of reference materials (5th WHO International Standard (NIBSC Code 22/120), 4th WHO International Standard (NIBSC Code 10/266), 1st WHO International Reference Panel for Hepatitis B Virus Genotypes (PEI Code 5086/08) and PEI HBV DNA reference material (#3620/05) were extracted with n=6 and quantified with n=1 using real-time PCR Systems CFX96, qTOWER^3, LightCycler 480II, 7500 Fast and Rotor-Gene 3000.

Results showed reliable trueness within the ±0.5 log_{10} IU/ml acceptance interval for all tested reference materials and genotypes (see Table 4).

Table 4: Trueness of the test depending on reference material as indicated

Reference material	Trueness over all devices [log_{10} IU/ml]
1st -WHO GT: 1/A 1	0.06
1st -WHO GT: 2/A 1	0.06
1st -WHO GT: 3/A 1	0.04
1st -WHO GT: 4/B 1	-0.19
1st -WHO GT: 5/B 1	0.20
1st -WHO GT: 6/B 1	0.16
1st -WHO GT: 7/C 1	0.04
1st -WHO GT: 8/C 1	-0.02
1st -WHO GT: 9/C 1	-0.06
1st -WHO GT: 10/D 1	0.00
1st -WHO GT: 11/D 1	-0.16
1st -WHO GT: 12/D 1	-0.03
1st -WHO GT: 13/E 1	0.07
1st -WHO GT: 14/F 1	0.23
1st -WHO GT: 15/G 1	0.34
HBV PEI	0.02
4th WHO	0.05
5th WHO	-0.02

The following diagrams show results of trueness analysis of the test in combination with each of the real-time PCR systems. The error indicator represents the standard deviation of the n=6 replicate analyses (see figures 5 und 6).

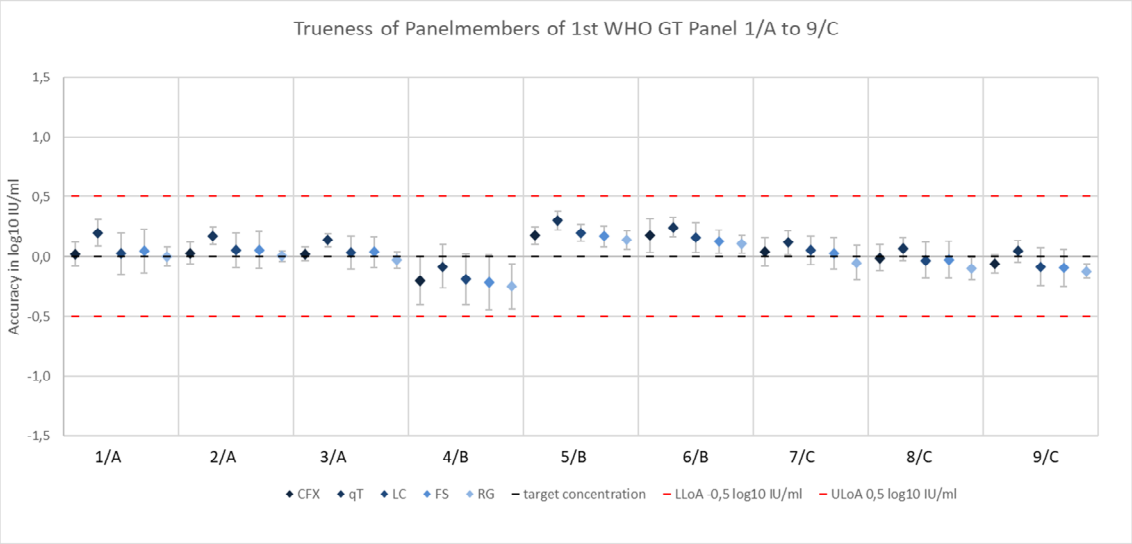


Figure 5: Shown are the quantification results obtained with the test compared to the reference concentrations of first 9 panel members of the 1st WHO International Reference Panel for Hepatitis B Virus Genotypes (PEI Code 5086/08). Quantification results are separately indicated for each of the real-time PCR systems.

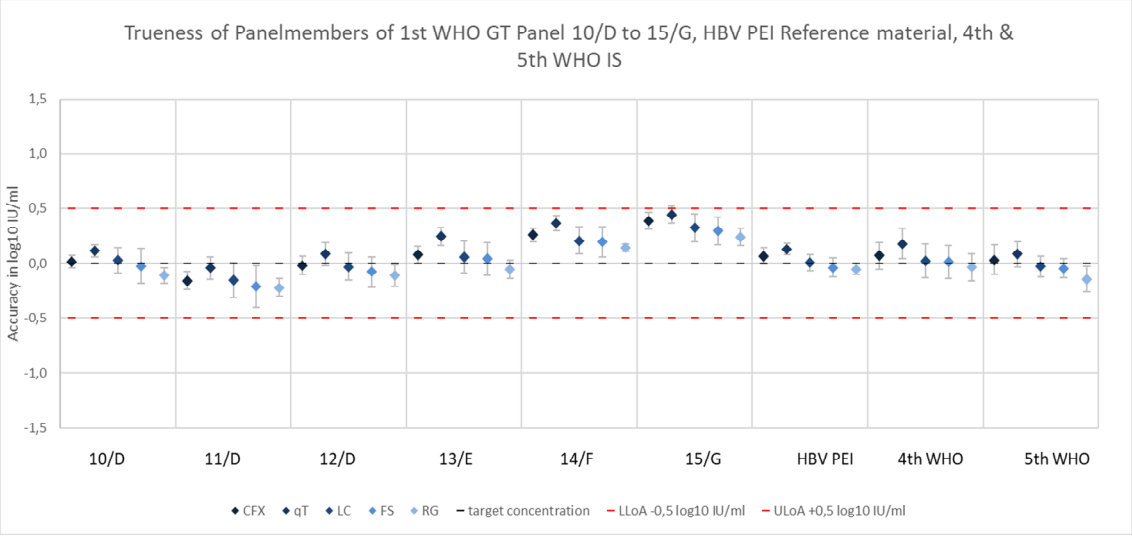


Figure 6: Shown are the quantification results obtained with the test compared to the reference concentrations of panel members 10 to 15 of the 1st WHO International Reference Panel for Hepatitis B Virus Genotypes (PEI Code 5086/08) as well as 5th WHO International Standard (NIBSC Code 22/120), 4th WHO International Standard (NIBSC Code 10/266), and PEI HBV DNA reference material (#3620/05). Quantification results are separately indicated for each of the real-time PCR systems.

4.7 Precision

The analysis of intra- and inter-assay precision of HBV DNA quantification was carried out with NAT-systems consisting of RoboGene HBV DNA Quantification Kit 3.1, the viral nucleic acid extraction kit INSTANT Virus RNA/DNA Kit and real-time PCR systems CFX96, qTOWER^3, LightCycler 480II, 7500 Fast and Rotor-Gene 3000.

5 step dilution series of an HBV DNA positive clinical sample covering 4 log_{10} steps were measured with 3 production lots of the test. The quantifications were carried out on 3 different days for each lot with different operators each and above mentioned real-time PCR systems. Results of the precision analyses are summarized below.

Intra-assay variability

Sample HBV DNA concentration >10^2 IU/ml

- Standard deviation: 0.03 – 0.06 log_{10} IU/ml
- Coefficient of variation: 0.50 – 1.80 %

Sample HBV DNA concentration ≤10^2 IU/ml

- Standard deviation: 0.08 – 0.11 log_{10} IU/ml
- Coefficient of variation: 4.09 – 5.22 %

Inter-assay variability

Sample HBV DNA concentration >10^2 IU/ml

- Standard deviation: 0.10 – 0.15 log_{10} IU/ml
- Coefficient of variation: 2.00 – 4.20 %

Sample HBV DNA concentration ≤10^2 IU/ml

- Standard deviation: 0.09 – 0.14 log_{10} IU/ml
- Coefficient of variation: 4.27 – 6.85 %

4.8 Whole-system failure rate

For analysis of the whole-system failure rate a total of 110 samples with an HBV DNA concentration of 25 IU/ml were generated using HBV DNA reference material (PEI code #3620/05) and EDTA-plasma tested negative for HBV DNA. All samples were analyzed in combination with INSTANT Virus RNA/DNA Kit and real-time PCR-systems CFX96, qTOWER^3, LightCycler 480II, 7500 Fast and Rotor-Gene® 3000. Indicated HBV DNA concentration represents the 3-fold 95 % cut-off value (LOD) of the test.

In the analysis whole-system failure rates of 0.0 % were determined in combination with each of the real-time PCR-systems. This demonstrates the test to comply with the requirements of Regulation (EU) 2022/1107 (failure rate ≤1 %). Results are shown in Table 5.

Table 5: Results of failure rate analysis

	(+) Results	Failure rate
CFX96		
HBV DNA (FAM)	110/110	0 %
IC (VIC/ Cy5)	110/110	
qTOWER^3		
HBV DNA (FAM)	110/110	0 %
IC (YY/ Cy5)	110/110	
LightCycler 480II		
HBV DNA (FAM)	110/110	0 %
IC (YY/ Cy5)	110/110	
7500 Fast		
HBV DNA (FAM)	110/110	0 %
IC (VIC/ Cy5)	110/110	
Rotor-Gene 3000		
HBV DNA (FAM)	110/110	0 %
IC (YY/ Cy5)	110/110	

4.9 Detection in relation to antibody status

Performance of RoboGene HBV DNA Quantification Kit 3.1 in relation to the patient antibody status was analyzed using pre-seroconversion panels PHM 937, PHM 939 and PHM 941 obtained from SeraCare Life Sciences, Inc. (USA). Seroconversion was characterized by increasing HBsAG-antibody levels (measured with Abbott ARCHITEKT HBsAG). HBV DNA quantification results obtained with the test were compared to the HBV DNA concentrations (quantified with IVD tests CAP/CTM v2.0 assay (Roche) and Abbot RealTime HBV m2000) of the respective seroconversion panel.

The analysis verifies the performance of RoboGene HBV DNA Quantification Kit 3.1 to be comparable to the performance of the reference IVD tests and therefore to be unaffected by the antibody status of the patient.

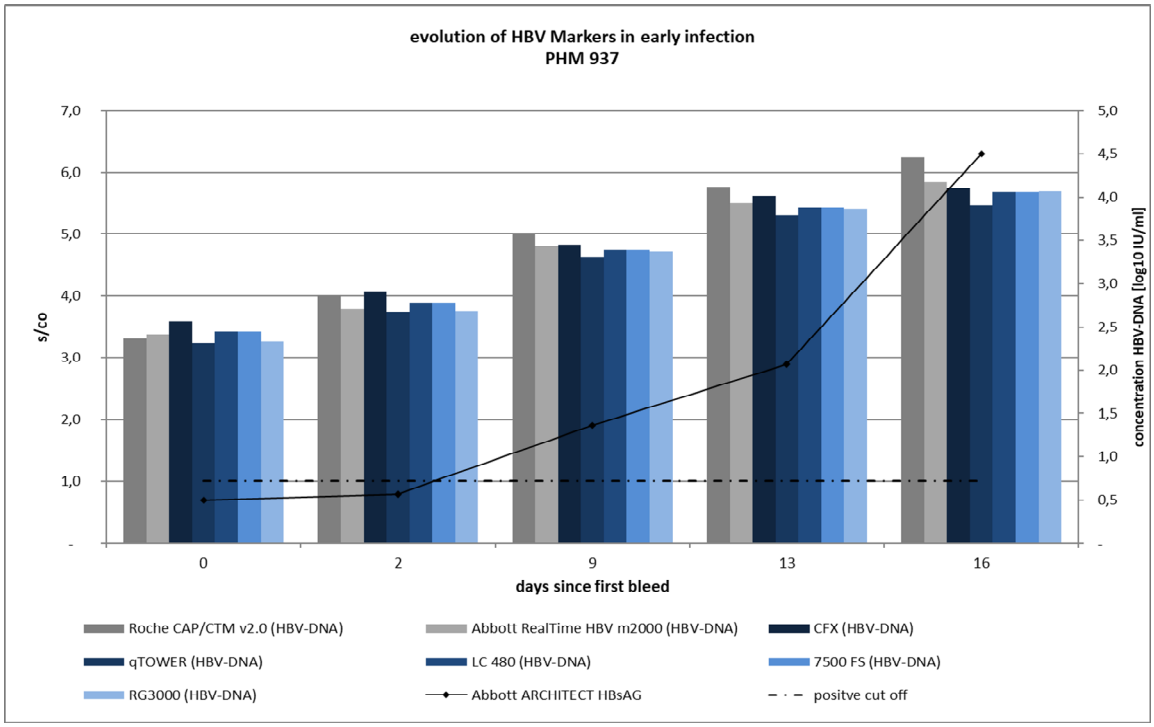


Figure 7: Performance of RoboGene HBV DNA Quantification Kit 3.1 during seroconversion. Seroconversion is characterized by increasing HBsAG-antibody levels (measured with Abbott ARCHITEKT HBsAG). Shown are HBV DNA quantification results measured with the test and real-time PCR devices as indicated in comparison to the HBV DNA quantification results of the reference IVD (CAP/CTM v2.0 assay (Roche) and Abbot RealTime HBV m2000). Results are shown for the seroconversion panel PHM937.

4.10 Diagnostic Sensitivity

Diagnostic sensitivity of RoboGene HBV DNA Quantification Kit 3.1 was analyzed with 104 clinical HBV DNA positive EDTA-plasma samples prequalified with state-of-the-art CE-IVD Tests for HBV DNA (COBAS® AmpliPrep/COBAS® TaqMan® HBV Test v2.0 (Roche) and Abbott RealTime HBV assay (Abbott)). Diagnostic sensitivity of RoboGene HBV DNA Quantification Kit 3.1 was determined in combination with INSTANT Virus RNA/DNA Kit and real-time PCR devices CFX96, qTOWER^3, LightCycler 480II, 7500 Fast and Rotor-Gene 3000.

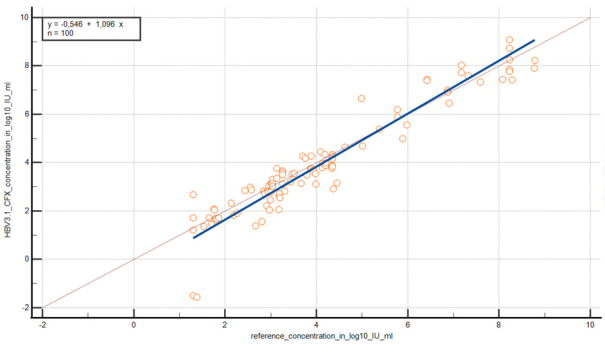
Quantitative data were analyzed by means of Deming regression analyses, Pearson correlation coefficient and Blant-Altman-plots. The results obtained for RoboGene HBV DNA Quantification Kit 3.1 showed a high degree of correlation to the results of the reference-IVD tests. Data of Pearson correlation coefficient are summarized in Table 6 and Plots for Deming regression and Bland-Altman are shown in figure 8.

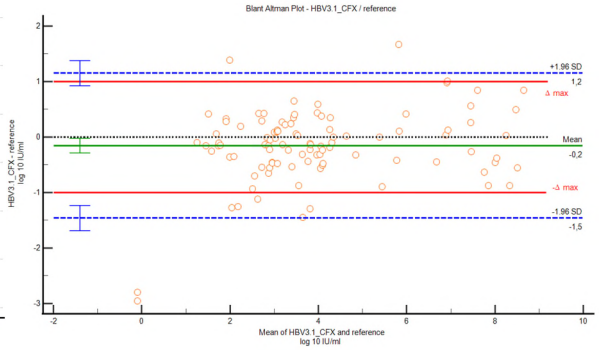
Table 6: Pearson Correlation Coefficient of RoboGene HBV DNA Quantification Kit 3.1

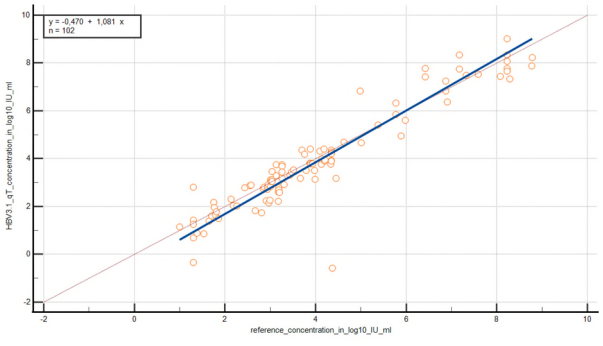
	CFX96	**qTOWER^3**	**7500 Fast**	**LightCycler 480II**	**Rotor-Gene 3000**
Pearson Correlation	0.9518	0.9402	0.9622	0.9507	0.966
Number of Samples	100	102	101	101	96

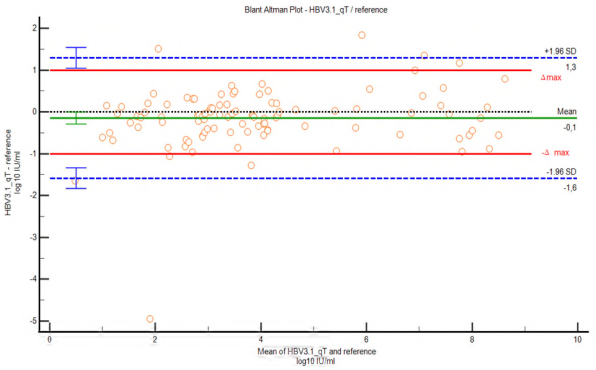
Deming - Regression

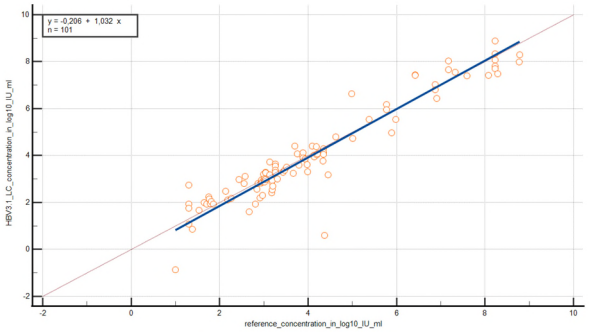
Bland-Altman-Plots

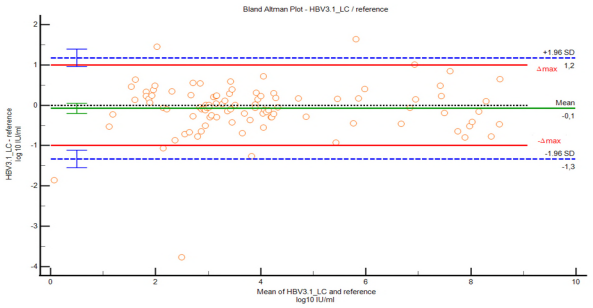


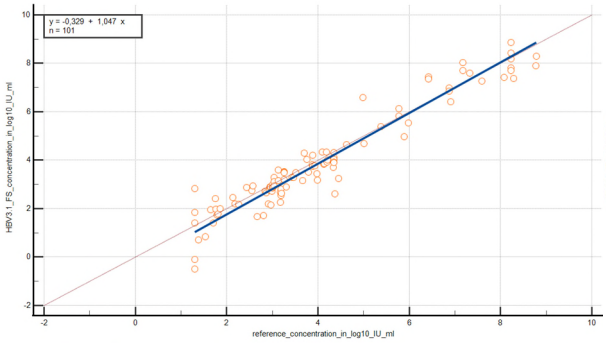


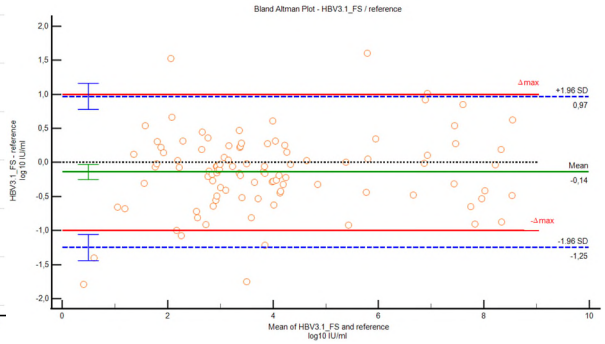












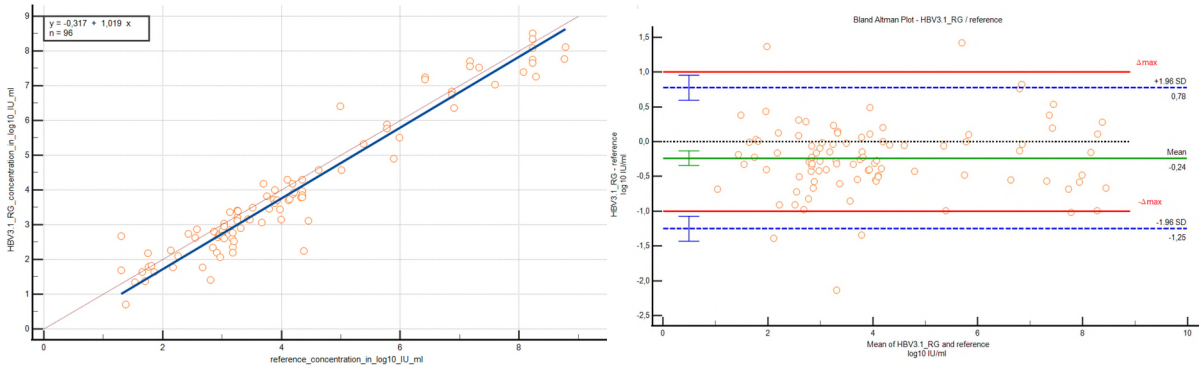


Figure 8: Diagnostic sensitivity: Deming Regression scatter plots (left) and Bland-Altman-Plots (right) comparing results of RoboGene HBV DNA Quantification Kit 3.1 and those generated with the reference-IVD CAP/CTM (Roche).

4.11 Diagnostic specificity

Diagnostic specificity is expressed as a true negative result in the absence of the HBV DNA target for at least 100 donor samples. In the study a total number of 105 donor samples acquired from HiSS Diagnostics and IKTM Jena were analyzed. All samples were pretested negative for HBV DNA using Grifols Procleix Ultrio Elite/ Plus or PoET HBV kits (both IVD CE).

All samples were extracted with INSTANT Virus RNA/DNA Kit and quantified with RoboGene HBV DNA Quantification Kit 3.1 in combination with real-time PCR devices CFX96, qTOWER^3, LightCycler 480II, 7500 Fast and Rotor-Gene 3000. All analyses showed valid (IC) and true negative results for HBV DNA. In summary the analysis verified a 100% diagnostic specificity of the test (see Table 7).

Table 7: Diagnostic Specificity

Sample	HBV detection	IC detection
HBV negative patient samples (n = 105)	0 of 105	105 of 105

4.12 Interfering substances

To assess the influence of endogenous substances (haemoglobin, lipids and bilirubin) and exogenous substances (EDTA and Heparin) on the NAT-system samples of the AcroMetrix™ Inhibition Panel were adjusted with HBV DNA reference material (PEI code #3620/05) to a concentration of 1,000 IU/ml. The panel included samples containing the following potentially interfering substances.

Inhibition panel member	Substance	Concentration range
Member 1, EDTA plasma	EDTA	25.5-34.5 mM
Member 2, haemolytic blood (Low)	Haemoglobin	0.9-1.2 g/dL
Member 3, haemolytic blood (Mid)	Haemoglobin	1.7-2.3 g/dL
Member 4, haemolytic blood (High)	Haemoglobin	3.4-4.6 g/dL
Member 5, heparinized plasma	Heparin	25-55 USP/mL
Member 6, lipemic plasma	Triglycerides	1,000-2,000 mg/dL
Member 7, icteric plasma	Bilirubin	25-35 mg/dL

The analysis reveals unimpaired performance of RoboGene HBV DNA Quantification Kit 3.1 in presence of EDTA, haemoglobin (low to high) and bilirubin.

Samples including lipids and heparin also gave valid results and were quantified ≤0.4 $\log_{10}$ below the target concentration. Although the results prove robustness of the kit, lipemic samples and heparin plasma samples are generally excluded from analysis using the kit.

4.13 Interference of anti-HBV therapy related medication

To assess the influence of therapy related exogenous substances (medication taken before blood donation) on the test, samples were prepared containing a calculated 1-fold and 10-fold systemic concentration of antiviral drugs PEG-IF Na-2a, ETV and TDF. The antiviral medication represents recommendations of the current european clinical practice guidelines on the management of hepatitis B virus infection [7]. The systemic drug concentration was calculated based on the therapeutic dosage of the drug and an assumed patient's body weight of 75 kg.

All prepared samples were spiked with HBV DNA reference material (PEI code #3620/05) and adjusted to a concentration of 1,000 IU/ml. Each sample was extracted with INSTANT Virus RNA/DNA Kit in triplicate and quantified with RoboGene HBV DNA Quantification Kit 3.1 (n=1). The following drugs and drug combinations were tested.

Therapeutic drug	**1-fold systemic drug concentration**	**10-fold systemic drug concentration**
Pegasys pegyliertes Interferon alfa (PEG-IF Na-2a)	17.8 ng/ml	177.8 ng/ml
Entecavir Amarox (ETV)	49.4 ng/ml	493.8 ng/ml
Tenofovirdisoproxil-fumarat (TDF)	24.2 µg/ml	241.9 µg/ml
Entecavir Amarox (ETV) in combination with Tenofovirdisoproxil-fumarat (TDF)	49.4 ng/ml (ETV) 24.2 µg/ml (TDF)	493.8 ng/ml (ETV) 241.9 µg/ml (TDF)

All analyses gave valid results with quantifications between 2.84 and 3.32 log_{10} IU/ml with a target value of 3.00 log_{10} IU/ml. The study verifies unimpaired quantification results of the test in presence of drugs and drug combinations examined in the analysis.

5 Kit components, storage and stability

RoboGene HBV DNA Quantification Kit 3.1 is shipped at ambient temperature, except for component RT PCR Enzyme which is shipped separately on dry ice.

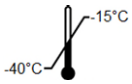
NOTE

It is recommended to store component RT PCR Enzyme together with the other components of the corresponding lot of the kit soon after arrival.

Table 8: Kit components and configurations

		Σ 32	Σ 96	Σ 192
REF		847-0207720032	847-0207720096	847-0207720192
IC [1]		Internal Control Lyophilized 1 Tube	Internal Control Lyophilized 3 Tubes	Internal Control Lyophilized 6 Tubes
HBV/IC STD 1 – 4[2]		Quantification standard Lyophilized 4 Strips	Quantification standard Lyophilized 4 Strips	Quantification standard Lyophilized 4 Strips
HBV/IC RM[3]		Reagent Mix Vacuum dryed 1 Tube	Reagent Mix Vacuum dryed 2 Tubes	Reagent Mix Vacuum dryed 4 Tubes
PCR grade H_2O[4]		Water 1.5 ml 1 Tube	Water 1.5 ml 2 Tubes	Water 1.5 ml 4 Tubes
RT PCR Enzyme[5]		Enzyme Mix 0.235 ml 1 Tube	Enzyme Mix 0.660 ml 1 Tube	Enzyme Mix 0.660 ml 2 Tubes

STORAGE CONDITIONS



After arrival store RoboGene HBV DNA Quantification Kit 3.1 including **RT PCR Enzyme** at -40 °C to -15 °C.

The kit is stable until the expiry date when stored under these conditions.

IMPORTANT

1 An appropriate amount of **IC** should be dissolved in **PCR grade H_2O** shortly before use.

Remaining dissolved **IC** can be stored at -40°C to -15°C up to 60 days.

Repeated freezing and thawing up to 5 times is possible.

2 The **HBV/IC STD 1 – 4** should be dissolved in **PCR grade H_2O** shortly before use.
Storage of dissolved **HBV/IC STD 1 – 4** is not possible.

3 An appropriate amount of Reagent Mix **HBV/IC RM** should be dissolved in **PCR grade H_2O** shortly before use.

Remaining dissolved **HBV/IC RM** can be stored at -40°C to -15°C up to 60 days.

Repeated freezing and thawing up to 5 times is possible.

4 Repeated freezing and thawing of **PCR grade H_2O** is possible.

5 **RT PCR Enzyme** should be stored at -40°C to -15°C.

Repeated freezing and thawing up to 5 times is possible.

RT PCR Enzyme should always be kept on ice-cold racks during usage.

6 Necessary laboratory equipment and kits

Table 9: Real-time PCR systems validated in combination with RoboGene HBV DNA Quantification Kit 3.1

Real-time PCR system	Software version	Manufacturer
qTOWER^3	qPCRsoft Version 4.1.3.0	Analytik Jena
CFX96 Touch Real-Time PCR Detection System	Maestro 1.1 V4.1.2433.1219	Bio-Rad
LightCycler 480 II	LightCycler® 480 Software Release 1.5.1.62	Roche
7500 Fast Real-Time PCR System	Version 2.0.4	Applied Biosystems
Rotor Gene 3000	Rotor-gene 6 Version 6.1 (Build 93)	Corbett Research

Table 10: Common laboratory equipment

Common equipment	
Centrifuges	Micro centrifuge
	Plate centrifuge
Mixer	Thermal mixer
	Vortex mixer
Pipettes	Adjustable pipettes suitable for a volume range of 1 to 1000 µl

7 Consumables not included in the kit

Table 11: Validated kits for nucleic acid extraction

Validated kits for nucleic acid extraction	Manufacturer	Order number
INSTANT Virus RNA/DNA Kit	Roboscreen GmbH	847-0259200602 / 847-0259200603

Table 12: Recommended PCR consumables and ordering information.

Real-time PCR system	PCR consumable	Manufacturer / Order number
qTOWER^3 CFX96 Touch Real-Time PCR Detection System	96 Well PCR Plate 0.2 ml, Full skirt, white	Sarstedt / 721980010
	Optical sealing foil	Azenta / 4ti-0565
LightCycler 480II	LightCycler 480 Multiwell Plate 96, white	Roche / 04729692001
	LightCycler 480 Sealing Foil	Roche / 04729757001
7500 Fast	MicroAmp Fast Optical 96-Well Reaction Plate 0.1 ml (clear)	ThermoFisher / 4346907
	LightCycler 480 Sealing Foil	Roche / 04729757001
Rotor-Gene 3000	Strip Tubes and Caps, 0.1 ml	Qiagen / 981103

Consumables not included in the kit

Table 13: Control material

Control material	Manufacturer	Order number	Intention
HBV-positive control (PEI Reference Preparation HBV DNA)	Paul-Ehrlich-Institute	PEI code #3620/05	Prove of recovery (evaluation not mandatory)
Human EDTA-plasma, positive for HBV DNA (high viral load)	e.g., Cerba Xpert or in-house sample stock	upon request	Positive control (dilute for higher aliquot number)
HBV-negative control (human EDTA-plasma free of HBV DNA)	e.g., in.vent Diagnostika GmbH or in-house sample stock	upon request	Negative control and diluent

Table 14: Consumables

Consumables
1.5 ml tubes
2.0 ml tubes
Sterile and PCR grade pipette aerosol-barrier tips, suitable for a volume range of 1 to 1000 µl

8 Procedure

8.1 Collection and handling of clinical samples

- Collect 5-10 ml of whole blood with standard specimen collection tubes.
- **Preparation of plasma**: EDTA anticoagulant must be used. Heparin is non-applicable due to its inhibitory effect on PCR.
- The cells must be removed from plasma by centrifugation at 1,000–2,000 x g for 10 minutes [5].

8.2 Storage conditions for EDTA plasma

- Store EDTA plasma at 2-8 °C. Test within 5 days [5].
- EDTA Plasma samples should be stored deeply frozen for up to 2 months at -20 °C or lower. Avoid repeated freezing and thawing [6].

8.3 HBV DNA purification from clinical samples

RoboGene HBV DNA Quantification Kit 3.1 has been validated in combination with a manual method for nucleic acid extraction.

For manual nucleic acid extraction from 400 µl sample volume use INSTANT Virus RNA/DNA Kit and follow the IFU of the extraction kit carefully using **'Protocol 2: Isolation using IC Spiking Tube'**.

8.4 Internal Control

RoboGene HBV DNA Quantification Kit 3.1 includes component **IC**, containing lyophilized internal control RNA and carrier nucleic acid.

Adding reconstituted **IC** to samples prior to nucleic acid extraction allows to control the whole diagnostic procedure and to detect false-negative results.

To judge the correctness of the diagnostic result, the Ct value obtained from the internal control should not exceed the limits summarized in chapter 11.3 (Criteria for run validation).

8.5 General procedure of quantitative analysis

Quantification standard **HBV/IC STD 1 – 4** is a 4-well strip stably coated with four defined amounts of synthetic HBV DNA. The standards are calibrated against the PEI reference material HBV DNA (#3620/05, calibrated against the 1st WHO International Standard for HBV DNA (NIBSC-code: 97/746)). The standard values are specified in [IU/ml], allowing the HBV DNA concentration of the analyzed sample to be directly calculated from the reference curve without the need for subsequent conversion by an equation.

NOTE

Quantification results obtained with RoboGene HBV DNA Quantification Kit 3.1 are only valid when the test is used in combination with the viral nucleic acid extraction kit INSTANT Virus RNA/DNA Kit and the real-time PCR systems and consumables indicated in chapters 6 and 7.

9 Protocol

9.1 Preparation of Internal Control

NOTE

RoboGene HBV DNA Quantification Kit 3.1 has been evaluated in combination with INSTANT Virus RNA/DNA Kit for viral nucleic acid extraction. The internal control IC is a component of RoboGene HBV DNA Quantification Kit 3.1. Prepare the IC according to the instructions below and introduce the IC into NA extraction following the instructions of the viral nucleic acid extraction kit.

1. Centrifuge the **IC** briefly at full speed to collect the lyophilized **IC** at the bottom of the tube.
2. Add **520 µl PCR grade H_2O** to the vial; close the tube, mix by vortexing briefly followed by brief centrifugation to collect solution at the bottom of the tube.
3. Incubate at 37° C for 5 min using a thermal mixer (800-1,000 rpm) mix by vortexing briefly followed by brief centrifugation to collect solution at the bottom of the tube.

9.2 Application of IC during manual viral NA extraction

1. Add **10 µl** of resuspended **IC** per **400 µl** sample volume to the Lysis Solution of the corresponding extraction kit.
2. Follow instructions given in the IFU of the extraction kit. When using INSTANT Virus RNA/DNA Kit apply '**Protocol 2: Isolation using IC Spiking Tube**'.
3. The elution volume used for NA extraction kit in combination with RoboGene HBV DNA Quantification Kit 3.1 is **60 µl**.

9.3 Preparation of 25x Reagent Mix

1. Centrifuge **HBV/IC RM** briefly at full speed to collect the dried reagent mix at the bottom of the tube.
2. Add **53 µl PCR grade H_2O** to **HBV/IC RM**; close the tube, mix by brief vortexing followed by brief centrifugation to collect the solution at the bottom of the tube.
3. Incubate at **37° C** for **5 min** using a thermal mixer (800-1000 rpm), mix by brief vortexing followed by brief centrifugation to collect the solution at the bottom of the tube.

9.4 Preparation of 1x Master Mix

1. Before setting up the Master Mix gently invert **RT PCR Enzyme** several times and centrifuge briefly.
2. Prepare the 1x Master Mix according to the following table in a 1.5 ml or 2.0 ml tube.
3. Mix by vortexing for at least 10 sec followed by brief centrifugation to collect the solution at the bottom of the tube.

Table 15: Composition of 1x Master Mix per reaction.

Component	Volume for 1x rxn (µl)	Final concentration
PCR grade H_2O	7.75	-
HBV/IC RM Reagent Mix, 25x	1.00	1x
RT PCR Enzyme Enzyme Mix, 4x	6.25	1x
Total	15.00	-

9.5 Preparation of Quantification Standards

1. Uncover standard strip **HBV/IC STD 1 - 4** and place the strip onto a suitable ice-cold rack.

2. Add **25 µl PCR grade H_2O** to each well of the quantification standard **HBV/IC STD 1 - 4**; mix by pipetting up and down several times.

NOTE

Use a new pipette tip for each standard to avoid carry over contamination.

For proper solving it is important to mix quantification standards **HBV/IC STD 1 - 4** by pipetting up and down several times. Do not vortex!

Store quantification standard on ice or an ice-cold rack until transfer into the master mix.

9.6 Preparation of reaction mixtures

1. Place real-time PCR device-specific consumables (see chapter 7) onto a suitable ice cold rack.
2. Add **15 µl** of 1x Master Mix to all wells intended for sample analysis, NTCs and quantification standards **HBV/IC STD 1-4**.
3. Add **10 µl** of **PCR grade H_2O** to wells that serve as NTC.
   Add **10 µl** of resuspended **HBV/IC STD 1 - 4** to all wells that serve as quantification standards. Add quantification standards in the right order. Make sure all reaction mixtures to be mixed properly. Mix by pipetting up and down several times.

NOTE

After use discard remaining solutions of **HBV/IC STD 1 - 4**. To avoid contamination, we recommend sealing the quantification standard with a suitable cover (e.g. parafilm).

4. Add **10 µl eluate** from the nucleic acid extraction to the respective sample wells pre-filled with 1x Master Mix. Make sure all reaction mixtures to be mixed properly. Mix by pipetting up and down several times.

NOTE

Do not exceed a final reaction volume of 25 µl.

Use a new pipette tip for each standard and sample for transfer into the master mix to avoid carry over contamination.

5. Seal the PCR consumables with recommended sealing foil. Centrifuge PCR plates for **1 min** at **1,000 rpm** (not necessary for Rotor-Gene® tubes).
6. Program the applied real-time PCR-system as indicated in chapter 10 and start the program.

10 PCR thermal profile and data acquisition

NOTE

The essential in-run standard curve provides run validation criteria slope and R2 value (see chapter 11.3).

Never use external standard curves for quantification.

Table 16: PCR program for qTOWER^3 and CFX96

Step	Cycle	Profile	Temperature	Time	Ramping
1	1	Reverse transcription	47° C	15 min	5° C/sec
2	1	Taq activation	95° C	2 min	5° C/sec
3	45	Denaturation	95° C	15 sec	2.5° C/sec
		Annealing/ Elongation*	57° C	1 min	5° C/sec
* Data acquisition via fluorescence detection					
For qTOWER^3 the following presetting is recommended: Open new project -> Scan -> Gain: for FAM = 4; for VIC/JOE = 3; for Cy5 = 5					

Table 17: PCR program for 7500 Fast and Rotor-Gene® 3000

Step	Cycle	Profile	Temperature	Time	Ramping
1	1	Reverse transcription	47° C	15 min	Not adjustable
2	1	Taq activation	95° C	2 min	
3	45	Denaturation	95° C	15 sec	
		Annealing/Elongation*	57° C	1 min	
* Data acquisition via fluorescence detection					

Table 18: PCR program for LightCycler 480II

Step	Cycle	Profile	Temperature	Time	Ramping
1	1	Reverse transcription	47° C	15 min	4.4° C/sec
2	1	Taq activation	95° C	2 min	4.4° C/sec
3	45	Denaturation	95° C	15 sec	2.5° C/sec
		Annealing/Elongation*	57° C	1 min	2.2° C/sec
4	1	Cooling	40° C	30 sec	Max

* Data acquisition via fluorescence detection

The setting of **detection channels** for data acquisition is as follows:

FAM (Target: HBV DNA)

Yakima Yellow/ VIC/ JOE (IC)

Cy5 (IC)

ATTENTION

Due to diversity of RT-PCR systems including configuration of detection channels, the kit contains an internal control allowing detection of the IC signal by detection channels YY/ VIC/ JOE or Cy5 according to real-time PCR-system available.

Crosstalk between FAM- and YY/ VIC/ JOE- signals and detection channels is a known phenomenon of some RT-PCR systems with older software versions not further defined.

To avoid crosstalk use Color Compensation for FAM and YY/ VIC/ JOE of corresponding software, if available (see chapter 11.2).

11 Data analysis

Each DNA amplification is associated with generation of a fluorescence signal measurable in FAM channel (for HBV DNA) and in YY/VIC/JOE and/ or Cy5 channel (for IC) resulting in a sigmoid growth curve (log scale).

The data analysis is performed according to manufacturer's instructions of the real-time PCR instrument using the respective software. Check all settings of the real-time PCR-system for analysis (see chapter 11.2).

Check the obtained data to ensure that the run is valid and to interpret results (see chapter 11.3).

11.1 Settings for data analysis

HBV DNA concentration of clinical specimens is expressed in IU/ml and is determined by means of a standard curve resulting from quantification of HBV STD 1-4.

The following table lists the corresponding HBV DNA concentrations of quantification standard HBV/IC STD 1-4.

Table 19: HBV DNA quantification standard concentrations.

HBV/IC STD 1 - 4	HBV DNA [IU/ml]
1	25,000,000
2	250,000
3	2,500
4	250

11.2 Threshold setting

The setting of thresholds may markedly influence Ct values. Nevertheless, the quantification is in the linear range of logarithmic scaled amplification curves only slightly influenced by the setting of the threshold.

In general, it is recommended to set the threshold in the lower linear range of logarithmic scaled amplification curves but above the baseline noise of NTC and negative control (see figure 9).

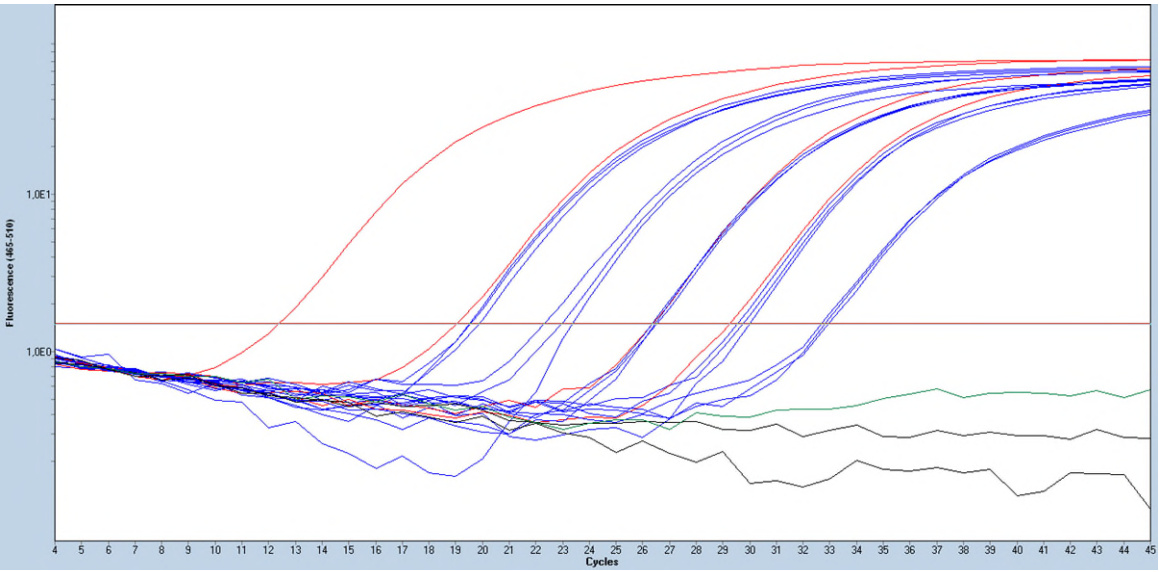


Figure 9: Logarithmic scaled amplification curves and threshold setting (red line)

Recommendations for threshold setting on validated RT-PCR systems are shown in table 20 below.

NOTE

It is important to maintain the real-time PCR-system so that the settings are effective.

Table 20: RT-PCR system specific threshold Settings

real-time PCR-system	FAM channel	YY/ VIC/ JOE channel	Cy5 channel
qTOWER^3	6 - 7	5	5
CFX96 Touch Real-Time PCR Detection System	400 - 700	150 - 250	100 - 150
7500 Fast	0.1 - 0.2	0.04 - 0.05	0.04 - 0.1
Rotor-Gene 3000	0.03 – 0.05	0.015 – 0,03	0.03 – 0.05
LightCycler 480II	1.5 - 5.9	1.4 - 2.6	1.0 - 2.4

Further settings for qTOWER^3:

- Analysis Type: Analysis -> Absolute quantification
- Color Compensation: Settings -> Scan -> Standard1

Further settings for Light Cycler® 480II:

- Analysis Type: Abs Quant/ Fit Points
- Color Compensation (In Database) for FAM and VIC channel
- Noiseband: FAM 0.7-1.6; YY/ VIC/ JOE 0.8-1.5; Cy5 0.5-0.9
- Fit points: 4

Further settings for Rotor-Gene 3000:

- Analysis -> Quantitation -> Dynamic Tube
- Analysis -> Quantitation -> Slope Correct for IC-detection channels, for FAM-channel optional, depending on confirmation of run validation criteria

If slope and/or R^2 are out of range (Table 21), one of the four quantification standards may be excluded (most outlying of regression line), as three quantification standards are sufficient for valid results.

11.3 Run validation and interpretation

Run validation: Criteria for run validation are the slope and R^2 value of the standard curve (see table 21).

Table 21: Criteria for run validation

Parameter	Acceptance range/ limit
Slope and R^2 of quantification standard curve	
Range of slope	-3.10 to -3.60
linear regression coefficient R^2	0.98 and 1.00 (Not applicable to LightCycler 480II analysis)
Expected Ct values for IC of the quantification standards, samples negative- and positive for HBV DNA (dependent on the set of threshold value, see above)	
YY/ VIC/ JOE	≤ 40
Cy5	≤ 38

The ranges of expected Ct values of the standards refer to own validation data and should be used as guidelines for setting threshold values (see tables 22 to 23).

Table 22: Guidance Ct values of the quantification standards for qTOWER^3, LightCycler 480II and CFX 96.

HBV/IC STD 1-4	Expected increment between Ct values	qTOWER^3		LightCycler 480II		CFX96	
		mean	from – to	mean	from – to	mean	from – to
1	-	14.8	14.0 – 15.6	15.4	13.7 – 17.2	15.2	14.2 – 16.1
2	1 to 2 + ~ 6.64	21.4	20.7 – 22.2	22.2	20.5 – 23.8	21.8	21.0- 22.7
3	2 to 3 + ~ 6.64	28.3	27.5 – 29.1	28.9	27.2 – 30.7	28.5	27.6- 29.5
4	3 to 4 + ~ 3.32	31.6	30.7 – 32.4	32.3	30.6 – 34.0	32.0	31.0- 33.0

Table 23: Guidance Ct values of the quantification standards for 7500 Fast and Rotor-Gene® 3000.

HBV/IC STD 1-4	Expected increment between Ct values	7500 Fast		Rotor-Gene 3000	
		mean	from – to	mean	from – to
1	-	14.7	13.7 - 15.6	12.5	11.4 - 13.6
2	1 to 2 + ~ 6.64	21.4	20.6 - 22.2	19.1	17.9 - 20.4
3	2 to 3 + ~ 6.64	28.1	27.3 - 29.0	25.6	24.6 - 26.7
4	3 to 4 + ~ 3.32	31.5	30.6 – 32.3	28.9	27.7 - 30.1

Interpretation of results

If the run is valid continue with the interpretation of measured samples as shown in Table 24, starting with interpretation of detection results followed by interpretation of quantification results.

Quantification results (FAM) are given in international units (IU/ml).

Table 24: Interpretation of results

FAM channel x = Ct value	YY/ VIC/ JOE or Cy5 channel x ≤ 40 Ct or x ≤ 38 Ct	Interpretation
Interpretation of detection results		
x	x	Valid - detection of HBV DNA and IC Sample positive for HBV DNA.
x	-	Not valid - detection of HBV DNA Quantification may be incorrect, repeat analysis.
-	x	Valid - detection of IC Sample negative for HBV DNA.
-	-	Not valid - no detection of HBV DNA and IC repeat analysis
Interpretation of quantification results		
-	x	No Ct-value, HBV DNA not detected. HBV DNA not present or below limit of detection. Indication: “HBV DNA not detected; < LOD”
< LLOQ (including < LOD)	x	Below lower limit of quantification of test, HBV DNA cannot be quantified accurately. Indication: “HBV DNA detected; result < LLOQ”
≥ LLOQ ≤ ULOQ $2.5x10^9$ IU/ml	x	Within linear range of quantification of test, HBV DNA can be quantified accurately. Indication: “HBV DNA detected; result”
> ULOQ $2.5x10^9$ IU/ml	x	Above upper limit of quantification of test, HBV DNA cannot be quantified accurate. Indication: “HBV DNA detected; result > ULOQ”

NOTE

For validated limits of detection and quantification of the test in combination with the various real-time PCR-systems that can be used please refer to chapter 4 (Performance Assessment).

12 Troubleshooting

Problem / probable cause	Comments and suggestions
No signal at all	
■ Fluorescence measurement not activated	Read the user guide of the real-time PCR device.
■ False channels selected	Select FAM channel for HBV DNA and YY/VIC/JOE or Cy5 channel for IC.
■ Incorrect cycling program	Check instrument settings, repeat run.
■ Incorrect application of the kit	Read instruction for use.
■ Storage conditions did not comply with instructions, expiry date of detection kit is exceeded	Check storage conditions and expiry date.
Low fluorescence signal recorded for both target and IC, target copy number underestimated	
■ Target DNA degraded	Use DNase and RNase free consumables and reagents, store DNA on ice. Read instruction for use of the extraction kit.
■ Optical lenses contaminated (Rotor-Gene®)	See maintenance provisions of the respective instrument. Alternatively clean lense once per month using absolute isopropanol and cotton swabs.
■ Thermal block and/or optics polluted (96-well block format)	See maintenance provisions of the respective instrument. Alternatively fill each well with isopropanol, incubate 10 min at 50 °C, remove isopropanol and rinse with H_2O.
No or weak signal for IC in HBV-negative sample	
■ Incorrect cycling program	Check instrument settings, repeat run.
■ Excess of inhibitors in the sample/ loss of DNA during extraction	Use the recommended extraction kit and follow exactly manufacturer's instructions.
■ Incorrect sample material (e.g. heparinized plasma)	Request for fresh EDTA plasma
■ Storage conditions did not comply with instructions, expiry date of detection kit is exceeded	Check storage conditions and expiry date.

Unexpectedly low Ct values for IC particularly with high standards or high viral load samples	
▪ Cross talk between target and IC recording channels (especially YY/VIC/JOE)	Calibrate instrument using pure fluorescence dyes or repeat run using Cy5 channel for IC detection.
Non-sigmoidal growth curves of quantification standards, unacceptable high deviation of Ct from expected values	
▪ Frequent freezing/thawing or incorrect storage of dissolved reagent mix	Read IFU, check storage conditions, prepare new reagent mix.
▪ Storage conditions did not comply with instructions, expiry date of detection kit is exceeded	Check storage conditions and expiry date.
▪ Ct-value of standards could not be aligned to the correct HBV DNA concentration	Check if standards contained in HBV/IC STD 1-4 are pipetted into the PCR consumable in the right order.
Different amplification behavior of sample HBV DNA and standards, non-parallel growth curves in exponential phase of reaction	
▪ Excess of inhibitors in the sample	Use the recommended extraction kit, follow exactly the manufacturer's instructions; consult attending doctor for patient medication.
▪ Incorrect sample material	Use recommended sample type.
FAM signal for HBV-negative samples / NTC recorded	
▪ Contamination with HBV DNA or DNA amplicons	Repeat extraction and/or PCR with new reagents; decontaminate instruments and work space.

If you have any further questions which are not answered, please contact our technical service.

13 References

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[6] Baleriola C. et al., Stability of Hepatitis C Virus, HIV, and Hepatitis B Virus Nucleic Acids inPlasma Samples after Long-Term Storage at -20°C and -70°C. Journal of Clinical Microbiology. 2011: 3163–3167.

[7] EASL 2017 Clinical Practice Guidelines on the management of hepatitis B virus infection (European Association for the Study of the Liver) Journal of Hepatology 2017 vol. 67 I 370–398

14 Document revision

IFU Revision Documentation		