RAS Lifesciences Pvt Ltd
Striving for better health

Amplisure® HBV Quantitative Kit

(Real Time PCR Kit)

QT-HBV-25	**: 25 rxns**
QT-HBV-50	**: 50 rxns**
QT-HBV-100	**:100 rxns**

IVD

Product Insert

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Introduction

Hepatitis B virus (HBV) is an enveloped DNA virus that belongs to the family Hepadnaviridae. Approximately 350 million people are chronically infected with HBV worldwide, placing them at high risk for developing cirrhosis, end-stage liver disease and hepatocellular carcinoma. Detection and quantification of circulating HBV in plasma or serum play an important role in diagnosing and monitoring HBV infection as well as assessing response to therapy. Clinically, HBV DNA titers vary greatly, from levels as high as 10^{10} copies/mL during acute HBV infection to very low levels in HBe antigen- negative chronic carriers, in patients undergoing antiviral therapy and in those with occult HBV infection. Among the methods most commonly used for HBV DNA quantification, assays based on real-time PCR technology offer the great sensitivity and broad linear dynamic range.

Intended Use

The Amplisure™ HBV quantitative kit is used to detect the genome of HBV genotypes(A-H) and quantify HBV genome in plasma , using Real-Time PCR after nucleic acid extraction. The viral load is measured using a range of four quantification standards provided in the kit. Combined with other methods of biological investigation (clinical picture and other laboratory markers), the results obtained with the Amplisure™ HBV quantitative kit are used to monitor HBV infection .

This kit cannot be used for screening blood from donors or blood products.

Product Description

Amplisure™ HBV Quantitative Kit is an *in-vitro* diagnostic kit for quantitation of Hepatitis B Virus in human plasma. The kit contains all the necessary reagents for performing HBV quantitation by Real time PCR.

Pathogen detection by Real Time polymerase chain reaction (PCR) is based on the amplification of specific region (S gene) of the pathogen genome. The assay principle is based on Taqman probes which allow higher specificity and sensitivity.

In addition, the Amplisure™ HBV Quantitative Kit contains a second amplification system to identify possible PCR inhibition by using an internal control (IC) without affecting the analytical sensitivity of the assay. External quantitation controls calibrated with WHO control are supplied, which allow the determination of the amount of Hepatitis B viral DNA.

Recommended Work areas

Molecular Diagnostics work area includes:

a) Sample preparation area/room – for extraction of nucleic acids from clinical samples
b) Pre-PCR area/room - for setting up PCR reaction
c) PCR area/room – for performing PCR using the thermocyclers/real time PCR

As part of Good Laboratory Practices (GLP), it is recommended to have dedicated areas to avoid cross contamination.

General Precautions

Precautions while extracting Nucleic acid

Always wear proper attire (powder free gloves, facemask and Head cap) before starting the nucleic acid extraction procedure. During preparation of samples, open one sample tube at a time and close it before opening another tube, follow it at every step of isolation. Compliance with good laboratory practices is essential to minimize the risk of cross-contamination between samples, and the inadvertent introduction of Nucleases into samples during and after the extraction procedure. Proper aseptic technique should always be used when working with Nucleic acid.

The Sample Preparation Area is dedicated to processing samples. All reagents used in the Sample Preparation Area should remain in this dedicated area. Sample Preparation Area must have dedicated laboratory coats, pipettes, pipette tips and cyclo-mixer and not to be moved to other areas. Discard the gloves before leaving this area. Do not bring amplified product into the Sample Preparation Area. Sample preparation should be performed in a Biosafety cabinet using aerosol free tips.

Precautions while setting up a PCR reaction

PCR assay is sensitive and any accidental introduction of product from previous amplification reactions can lead to incorrect results. Hence measures to reduce the risk of contamination in the laboratory should include physically separating the activities involved in performing PCR and complying with good laboratory practices. PCR hood should be used to avoid contamination.

It is recommended to have proper cleaning procedures to minimize the risk of cross contamination and carry over contamination (e.g. DNA OUT™, RNase OUT™, 0.1% Sodium Hypochlorite, Fumigation etc.).

It is recommended that areas should be defined in Pre-PCR room for preparation of mastermix and addition of templates. Laboratory coats and equipment used in the Pre-PCR Area must remain in this area and should not be moved to the Sample Preparation Area.

Precautions for post PCR or equipment area/room

The Real time PCR instrument/s should be kept in a separate segregated area away from Sample preparation area and Pre-PCR area.

Precautions after completion of Real time PCR assay

The reaction tubes or strips should be properly discarded without opening the caps, after the completion of run to avoid carry over contamination.

Usage Limitations

1. All the kit components are for *in-vitro* diagnostics only.
2. The product is to be used by personnel specially trained in the *in-vitro* diagnostics procedures only.
3. Follow the product insert strictly for optimal results.
4. Do not use the kit beyond the expiry date mentioned on the box.
5. Follow the guidelines provided in product insert for sample collection, storage and transport.
6. For ideal performance, store the kit under recommended conditions only.

Safety Precautions

1. All patient specimens should be considered as potentially infectious and handled in a BSL2 biosafety hood with BSL3 practices.
2. Wear personal protective equipment, including gloves, head cap, face mask and lab coats when handling kit reagents/sample extraction. Wash hands thoroughly using detergents before and after performing the test.
3. Do not smoke, drink or eat in areas where kit reagents and/or human specimens are being used.
4. Dispose of unused kit reagents and human specimens as per regulatory guidelines.

Storage Conditions and Product Stability

1. All the kit reagents should be stored at -20 °C. Replace all the kit components immediately at -20 °C after usage.
2. Repeated thawing and freezing (more than 6 x) of all kit reagents should be avoided, as it reduces assay sensitivity. If needed, make aliquots of the kit reagents according to the volume used in the protocol prior to freezing.
3. Allow reagents to be thawed completely on Ice/4 °C prior to use.
4. Kit reagents are stable through the end of the expiration date indicated on the box when stored at -20 °C.

Symbols

Description of Symbol	Denotation
	*in-vitro* Diagnostic medical device
	Consult Instruction manual (Product Insert) for use
	Lot Number of the kit or Kit contents
	Catalogue number of Kit
 <N>	Contains sufficient for <N> reactions (Pack Size)
	Manufacturer
	Temperature limitation (Storage Condition)
	Use by MMM-YYYY (Expiry Date)
	Biological risk (handle carefully)
	Important Note

Kit Components

Color Coding (Caps)	Contents	Description	25 rxns (QT-HBV-25)	50 rxns (QT-HBV-50)	100 rxns (QT-HBV-100) 2 x 50 rxns
Yellow	RAS qDNA PCR Mix	DNA Amplification Reagent	1 x 315 µL	1 x 625µL	2 x 625µL
Brown	RAS HBV PPM	HBV Primer-Probe Mix	1 x 50 µL	1 x 100 µL	2 x 100 µL
Lilac	RAS HBQS1 (2×10^4 IU/ µL)	HBV Quantitation Standards	1 x30 µL	1 x 60 µL	2 x 60 µL
Lilac	RAS HBQS2 (2×10^3 IU/ µL)		1 x30 µL	1 x 60 µL	2 x 60 µL
Lilac	RAS HBQS3 (2×10^2 IU/ µL)		1 x30 µL	1 x 60 µL	2 x 60 µL
Lilac	RAS HBQS4 (2×10^1 IU/ µL)		1 x30 µL	1 x 60 µL	2 x 60 µL
Natural	RAS IC PCR Mix	Internal Control	1 x 25 µL	1 x 50 µL	2 x 50 µL
Natural	RAS D-IC EX Mix		1 x 0.5 mL	1 x 1.00 mL	2 x 1.00 mL
White	MBGW	Molecular Biology Grade water	1 x 0.5 mL	1 x 0.5 mL	2 x 0.5 mL

Materials required but not supplied

The materials which are required but not supplied are listed below:

1. Viral DNA Extraction kit
2. Biosafety Cabinet
3. PCR Hood
4. Calibrated variable micropipettes
5. Sterile pipette filter tips (aerosol free)
6. Cyclo- mixer(Vortexer)
7. Dry Bath

8. Benchtop centrifuge with rotor for 1.5 mL reaction tubes
9. Real Time PCR machine
10. Strip Tubes and Caps (0.2 mL) or PCR Tubes (0.2 mL) or 96 well plate
11. Cooling block (96 x 0.2 mL tubes)
12. 1.5 mL centrifuge tubes
13. 1.5 mL centrifuge tube stand
14. Cooling block (2 mL tubes)
15. Sterile Nitrile gloves
16. Facemask
17. Head cap
18. Lab coats

Quality Systems

In accordance with ISO-certified Quality Management System (9001:2008 and 13485: 2003) of RAS Lifesciences, each lot of Amplisure ™ HBV Quantitative Kit is tested against predetermined specifications to ensure consistent product quality.

Sample Type/Collection, Storage and Transport

Sample Type

Plasma (K_2EDTA-Blood)
Heparinized Blood must not be used as they inhibit the PCR reaction

Sample Collection, Storage and Transport

3-5 mL of blood has to be drawn into a K_2EDTA vaccutainer. Cap and swirl the tubes for uniform mixing of blood and K_2EDTA. When K_2EDTA is used, whole blood can be collected in tubes with or without a gel separator. The K_2EDTA blood samples are centrifuged (20 minutes at 1000–1500 x *g)* to separate plasma from cellular material and in the case of a non gel separator tube, the plasma should be removed to a secondary sterile tube within 4 hours of phlebotomy.

Collection and storage of unstabilized whole blood is not recommended for PCR analysis, because Nucleic acid degradation occurs in blood stored *ex vivo*. The

sensitivity of the assay can be reduced if whole blood samples are frozen or stored for a longer period of time.

Plasma separated in a gel separator tube may be transported to the laboratory *in situ.* Plasma should be shipped at 2 to 8 °C and stored at -20 °C as it is stable for up to five days at 2 to 8 °C and longer if frozen at -20 °C or -70 °C or lower. Do not store plasma samples in a "frost -free" freezer as the temperature is cycled several times per day in this type of freezer, which may cause degradation of nucleic acid targets.

Sample material should be transported in a leak proof, unbreakable transport container to avoid leakage of sample. The samples should be transported following the local and national instructions for the transport of pathogen material.

Assay Procedure

DNA Extraction

Amplisure ™ HBV Quantitative Kit has been validated using the following Viral DNA extraction kits:

1- Roche High Pure Viral Nucleic Acid kit (Cat. No. 11858874001)
2- QIAamp DNA Blood Mini Kit (Cat. No. 51104)

Follow the manufacturer's instructions mentioned in the manual for Viral DNA extraction. Different pack sizes of the above mentioned kits can be used. However, the customer can also validate their own extraction process using other Viral DNA extraction Kits.

Recommended sample volume for extraction and elution are as follows:

Name of the DNA Isolation Kit	**Recommended Sample volume *(to be taken for DNA Extraction)***	**Recommended Final Elution volume**
Roche High Pure Viral Nucleic Acid kit (Cat. No. 11858874001)	200 µL	100 µL
QIAamp DNA Blood Mini Kit (Cat. No. 51104)	200 µL	100µL

Use of Internal Control (IC)

Internal controls are supplied (RAS D-IC Ex Mix and RAS IC PCR Mix) along with Amplisure™ HBV Quantitative Kit. This allows the user to control the Viral DNA isolation procedure as well as to check for possible PCR inhibition.

Internal control should only be used once, either at the Extraction step or at the PCR step

Usage of Internal Control at the DNA extraction step

If internal control (IC) is required to be added at the time of DNA extraction, add 20 µL of RAS *D-IC Ex mix* per isolation to the lysis buffer along with other components of kit used for lysis (as per kit instructions) and vortex for 5 seconds prior to usage.

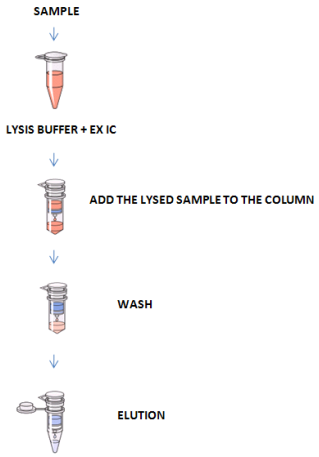

Fig1 Viral DNA Extraction Overview

Usage of Internal Control at the Real time PCR step

The internal control can optionally be used exclusively to check for possible PCR inhibition. For this application, add the internal control directly to the PCR master mix as described on Pg. No 13. Addition of Internal control should be done only once, either at the time of sample extraction or during PCR setup.

qPCR Protocol

Preparation of Reaction Master mix

Quantitation procedure with Amplisure™ HBV Quantitative Kit involves single step of amplification in Real time PCR machine. It is recommended that a minimum of three standards and a negative control (MBGW should be used as negative control) are required to be included in a single run for acquiring proper results.

Set up a real time single step qPCR reaction as follows:

1- qPCR reaction mix composition without Internal Control (When D-IC Ex Mix is added during DNA extraction)

Components	Volume per reaction (µL) (for final vol. of 25 µL)
RAS qDNA PCR Mix	12.5
RAS HBV PPM	2.0
DNA/HBQS/ MBGW	5.0
MBGW	5.5

2- qPCR reaction mix composition with Internal Control (When D-IC Ex Mix is not added during DNA extraction)

Components	Volume per reaction (µL) (for final vol. of 25 µL)
RAS qDNA PCR Mix	12.5
RAS HBV PPM	2.0
DNA/HBQS/ MBGW	5.0
RAS IC PCR Mix	1.0
MBGW	4.5

1. **Addition of IC PCR mix (if required) should be done at the time of preparation of master mix.**
2. **The results may be inconsistent, if the IC PCR mix is added individually.**

Place the PCR plate/tubes/strips in real time thermocycler.

PCR Programming

The Amplisure™ HBV Quantitative Kit is validated on the following instruments:

- Rotor-Gene™ 6000
- Rotor-Gene™ Q 5plex
- ABI 7500 DX Real-Time PCR System
- ABI 7300 Real-Time PCR System
- Eppendorf Realplex 4
- Bio-Rad™ CFX 96

Plate Setup

1. Program the plate setup by labeling the slots as per tube/strip/plate labels. The sequence of labeling of slots should be the same way as the tube/strip/plate is kept in the machine.
2. Select the type of sample (Unknown/Standard/NTC) for each slot.
3. When Standard is selected as sample type, mention the quantitation value in IU/µL (2E4, 2E3 etc)
4. Select the channel for acquisition (FAM/Yakima Yellow)

S. No.	Name of channel	Source wavelength (nm)	Detection wavelength (nm)
1.	FAM (Pathogen target)	470	510
2.	Yakima Yellow (Internal Control)	530	555

5. For background calibration in different instruments, follow the procedure described below:

   Rotor-Gene™ 6000 - Perform 'Gain optimization'
   Rotor-Gene™ Q 5plex - Perform 'Gain optimization'
   ABI 7500 DX Real-Time PCR System - Select Passive Reference dye 'ROX'
   ABI 7300 Real-Time PCR System - Select Passive Reference dye 'ROX'
   Eppendorf Realplex 4 - Select 'ROX' for background calibration
   Bio-Rad™ CFX 96 - Select 'ROX' for background calibration

Preparation of reaction mastermix and cycling conditions are same for all the instruments listed in the product insert. For instrument specific protocols, please contact our technical support team at amplisure@raslifesciences.com

Cycling conditions

1. Configure the following program in the machine.

Steps	No. of cycles	Temperature (°C)	Time
1 (Initial denaturation)	1	95	10 min.
2 (PCR cycling)	40	95	15 sec.
		60*	60 sec
***Plate Read/Data Acquisition in FAM and Yakima Yellow channel**			

2. Set the reaction volume as 25 µL.
3. Plate read/Data Acquisition for FAM and Yakima Yellow channel should be incorporated in the second stage of step 2 (60 °C/60 sec).
4. The ideal run time for the assay is 90 minutes. Note: *In case of Eppendorf Realplex 4, select RAMP rate as 35%.*

Preparation of reaction mastermix and cycling conditions are same for all the instruments listed in the product insert. For instrument specific protocols, please contact our technical support team at amplisure@raslifesciences.com

Data Analysis

Analyze the data after completion of the run. Check the R_n/*Cycle* amplification plot and ΔR_n/*Cycle* amplification plot to observe the amplification signal generated by different samples in the run. Compare both the plots for data analysis. Also look for noisy signals, if observed as it might not give you a proper result.

Setting the threshold for the qPCR Data analysis

The threshold should be set either automatically (by the machine itself)/ or manually just above the background signal of the negative controls and negative samples by referring to R_n/*Cycle* amplification plot. The mean threshold value calculated from these experiments will most likely work for the majority of future runs, but the user should nevertheless review the generated threshold value at regular intervals.

Result

The values for unknown samples would appear in the result column in *IU/µL* and the negative control should not show any value in the result column of FAM Channel.

Interpretation

Interpret the values for unknown samples, only if the Slope of Standards is between -3.1 to – 3.6 and PCR efficiency is between 90%-110% (0.9 - 1.1) and there should be no amplification in FAM channel in negative control.
If all the above mentioned specifications are matched, proceed with further analysis.

Observation	Interpretation	Conclusion
Amplification signal detected in HBV channel (FAM) and in Internal control channel (Yakima Yellow)	Quantify HBV DNA	Proceed for further Analysis
Amplification signal detected in HBV channel (FAM) but no signal in Internal Control channel (Yakima Yellow)	HBV DNA detected. For accurate quantitation repeat the quantitation with diluted(1:100) And undiluted sample	
Amplification signal not detected in HBV channel (FAM) but detected in Internal control channel (Yakima Yellow)	HBV DNA below detection limit	
No Amplification signal detected in HBV channel (FAM) as well as Internal control channel (Yakima Yellow)	Reaction can't be interpreted as Inhibition of PCR or incorrect extraction	Repeat the extraction and PCR

To convert the results from *IU/µL* to IU/mL use the following formula:

$$\text{Result (IU/mL)} = \frac{\text{Result (IU/µL) x Elution Volume (µL)}}{\text{Sample Volume (mL)}}$$

*** For calculating the result of diluted sample (1:100); multiply the observed IU/mL value by dilution factor, 100**

$$\text{Result of 1:100 diluted sample (IU/mL)} = \text{Dilution Factor (100)} \times \frac{\text{Result (IU/µL) x Elution Volume (µL)}}{\text{Sample Volume (mL)}}$$

Troubleshoot

Observation	Possible cause	Solution
No amplification signal for Standards in FAM channel	1. One of the components may not have been added. 2. Incorrect channel selection 3. Incorrect programming of the real time machine. 4. Instrument is not working properly	1. Repeat the assay by following the correct protocol and addition of reagents 2&3. Please recheck the PCR program 4. Contact manufacturer of thermocycler for technical support.
Weak amplification signal for standards (Signal below threshold) in FAM Channel	1. Improper PCR programming. 2. Inaccurate dispensing of reagents 3. Possible deterioration of kit components due to improper storage	1. Repeat the assay by following the correct protocol 2. Minimize Pipetting errors/Check for calibration status of pipettes
Amplification observed in FAM channel in negative control	1. Aerosols generated 2. Environmental Pipettes, /Work area. 3. Possible contamination of Kit reagents	1. Use fresh aliquots of Standards/Kit Reagents (if available) 2. Clean the PCR rack/Pipettes thoroughly as per GLP 3. Clean and Fumigate the work area overnight prior to use

For any other technical query; please contact amplisure@raslifesciences.com

Assay Characteristics

The analytical sensitivity of PCR assay alone and DNA Extraction and PCR assay) was determined for the Amplisure™ HBV Quantitative Kit.

The analytical sensitivity , using specific method, is determined using WHO standards/ HBV positive clinical samples.

Analytical Sensitivity of qPCR assay

The analytical sensitivity of qPCR assay is determined (independent of extraction method), using standard of known concentration i.e. 3rd WHO International Standard (NIBSC code: 10/264) for Hepatitis B Virus for Nucleic acid amplification techniques.

To determine the analytical sensitivity of the Amplisure™ HBV Quantitative Kit, a standard dilution series was set up from 0.02 IU/µL to 50 IU/µL and analyzed with Amplisure™ HBV Quantitative Kit.

Testing was carried out on 5 different days on 3 replicates and statistical analysis of the obtained data was carried out.

The analytical sensitivity of the Amplisure™ HBV Quantitative Kit is 0.04 IU/µL.

Analytical Sensitivity with Roche Extraction Method

Nucleic acid extraction of 3rd WHO International Standard(NIBSC code: 10/264) was carried of using Roche High Pure Viral Nucleic Acid kit (Cat. No .11858874001). Eluted nucleic acid is diluted from 850 IU/mL to 0.85 IU/mL using Nuclease free water.

Each of the dilution was analyzed with the Amplisure™ HBV Quantitative Kit on 5 different days with 3 replicates and the results obtained were statistically analyzed.

The analytical sensitivity in consideration of the purification (with Roche High Pure Viral Nucleic Acid kit) of the Amplisure™ HBV Quantitative Kit is determined to be as ***17 IU/mL****.*

Linear Range

The linear range (analytical measurement) of the Amplisure™ HBV Quantitative Kit was determined by analyzing a dilution series of HBV quantitation standards from 2 x 10^7 IU/µL to 0.02 IU/µL. The dilution series has been calibrated against the 3rd WHO International Standard for Hepatitis B Virus for Nucleic acid amplification techniques (NIBSC code: 10/264).

Each dilution was tested in replicates (n = 3) using the Amplisure™ HBV Quantitative Kit.

The linear range of the Amplisure™ HBV Quantitation Kit has been determined to cover concentrations from **0.04 IU/µL to 2 x 10^7 IU/µL.**

Conversion Factor (IU/µL to Copies/µL)

The 3rd WHO International Standard for Hepatitis B Virus for Nucleic acid amplification techniques (NIBSC code: 10/264) has been assigned an International Unit (IU) by the WHO as the number of genome equivalents.

The conversion factor value for Amplisure™ HBV Quantitative kit has been assigned after calibration with the 3rd WHO International Standard for Hepatitis B Virus for Nucleic acid amplification techniques (NIBSC code: 10/264).The Conversion Factor (IU/µL to Copies/µL) is entirely based on the current assay components of the kit. A Correlation and Regression analysis was performed to compare the two standard curves of HBV DNA values obtained by Amplisure® HBV Quantitative kit (copies/µL) and HBV NIBSC standards (IU/µL)The correlation coefficient between the expected and estimated values is very good.

r = 0.9992

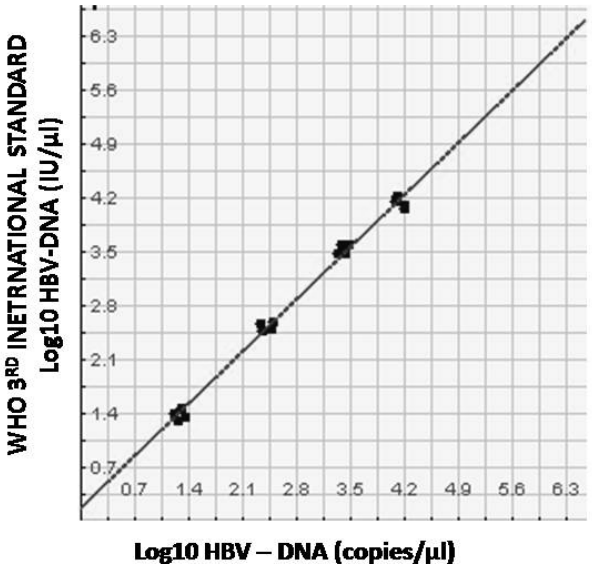

Fig. 2 Correlation and Regression plot of HBV NIBSC standards (IU/µL) and HBV DNA (copies/µL)

The conversion factor calculated for Amplisure® HBV PCR kit from IU/µL to Copies/ µL is 1.28, i.e. **1 IU/µL = 1.28 copies/µL**

To convert into copies/mL use the following formula:

$$\text{Result (copies/mL)} = \frac{\text{Calculated Value (copies/µL) x Elution Volume (µL)}}{\text{Sample Volume (mL)}}$$

Specificity

The specificity of the Amplisure™ HBV Quantitative Kit is 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 published sequences (Genbank) by BLAST analysis.

Detection of Different HBV genotypes (A-H) using International Standard

The detectability of all relevant genotypes (A-H) was ensured by testing defined HBV genotype samples with Amplisure™ HBV Quantitative kit.

S. No.	HBV Genotype Controls	Result obtained with Amplisure® HBV Quantitative Kit
1.	Geno A	Detected
2.	Geno B	Detected
3.	Geno C	Detected
4.	Geno D	Detected
5.	Geno E	Detected
6.	Geno F	Detected
7.	Geno G	Detected
8.	Geno H	Detected

Cross Reactivity Data

A potential cross-reactivity of the Amplisure™ HBV Quantitative Kit was tested using the control group listed below. None of the tested pathogens has been reactive. No cross-reactivity appeared with mixed infections. Moreover, the specificity was

validated with 50 different healthy plasma specimens/other various sample types. These did not generate any signals with the HBV specific primers and probes, which are included in the Amplisure™ HBV Quantitative Kit.

Pathogen Tested	Cross Reactivity with the HBV Primers/Probes
Adenovirus	-ve
Herpes Simplex Virus-1	-ve
Herpes Simplex Virus-2	-ve
Epstein Barr virus	-ve
Human Immunodeficiency Virus	-ve
Cytomegalo virus	-ve
Hepatitis C virus	-ve
Enterovirus	-ve
BK virus	-ve
MTB complex	-ve
Plasmodium sps.	-ve

Precision

The precision data of the Amplisure™ HBV Quantitative Kit have been generated for HBV positive clinical specimens and HBV quantitation standards.

Intra-assay variability

The precision data consists of the *Intra-assay variability* which is variability of multiple results of samples of the same concentration within one experiment.

The data obtained were used to determine the standard deviation and the coefficient of variation for the pathogen specific PCR. Precision data of the Amplisure™ HBV Quantitative Kit have been collected using all the quantitation standards (HBQS1-HBQS4). Also included were three different samples with different viral loads.

Inter-assay variability

The precision data consists of the *Inter-assay variability* which is variability of multiple results of samples of the same concentration within different experiments.

The data obtained were used to determine the standard deviation and the coefficient of variation for the pathogen specific PCR. Precision data of the Amplisure™ HBV Quantitative Kit have been collected using all the quantitation standards (HBQS1-HBQS4). Also included were three different samples with different viral loads. Testing was performed with 3 replicates for 10 days.

The precision data was calculated on basis of the CT values obtained.

Sample Type	Mean [Log Value (IU/µL)]	Variability Testing	Standard Deviation	Coefficient of Variation (%)
HBQS1	Log 4.3	Intra-assay variability	0.030	0.001
		Inter-assay variability	0.370	0.017
HBQS2	Log 3.3	Intra-assay variability	0.040	0.001
		Inter-assay variability	0.250	0.010
HBQS3	Log 2.3	Intra-assay variability	0.010	0.001
		Inter-assay variability	0.460	0.020
HBQS4	Log 1.3	Intra-assay variability	0.020	0.001
		Inter-assay variability	0.320	0.010
HBV Control 1	Log 9.15	Intra-assay variability	0.190	0.010
		Inter-assay variability	0.300	0.057
HBV Control 2	Log 7.30	Intra-assay variability	0.210	0.020
		Inter-assay variability	0.360	0.038
HBV Control 3	Log 4.48	Intra-assay variability	0.410	0.020
		Inter-assay variability	0.400	0.016

Performance study on samples

Biological performances of the Amplisure™ HBV Quantitative Kit on plasma samples have been evaluated in an Indian Microbiology Laboratory, using samples collected during the laboratory's routine activity.

The laboratory's routine technique is a commercial real-time quantitative PCR kit which was used to amplify a fragment of 134 bp of the Hepatitis B genome. The results were expressed directly in IU/mL.

The samples of plasma were extracted with the Roche High Pure Viral Nucleic Acid kit (Cat. No. 11858874001) using the protocol recommanded by the manufacturer on 200 µL with an elution volume of 100 µL. The extracted samples were amplified on Rotor-Gene Q using both kit reagents following manufacturer's instructions.

A total of 105 plasma samples was tested.

Results of the concordance obtained on the plasma samples

		COMMERCIAL PCR KIT		Total
		+	-	
Amplisure™ HBV Quantitative Kit	+	79	3*	82
	-	0	23	23
	Total	79	26	105

Global concordance: 102/105= 97.1% [91.9;99.4](95%exact CI)

Of 105 samples tested, 3 gave discordant results.

* Of these 3 samples negative for the commercial HBV PCR kit and positive with Amplisure™ HBV Quantitative Kit (viral load between 170 and 555 IU/mL), 3 were found to be positive by sequencing and identified as HBV variant, alpha-1.

Quantitative analysis of positive plasma samples in the two kits

The quantitative analysis of results for plasma between the two techniques was carried out on confirmed positive samples.

It showed good correlation between the quantifications of the two techniques for plasma. Indeed, the mean difference in quantification between the two PCR techniques was around +0.16 log_{10} IU/mL.

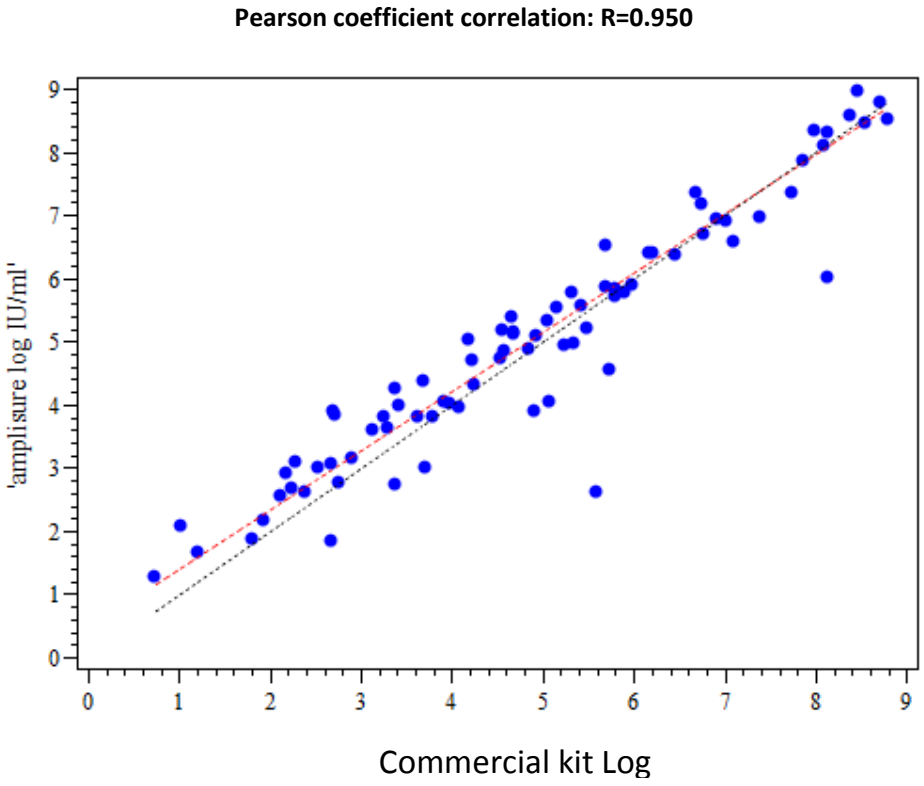

Fig. 3 : The quantification performance of the Amplisure HBV kit on blood plasma was therefore demonstrated.

Abbreviations

Abbreviation	*Expansion*
HBV	Hepatitis B Virus
DNA	Deoxy Ribonucleic Acid
IC	Internal Control
DNases	Deoxyribonucleases
PCR	Polymerase Chain Reaction
BSL2	Bio Safety Level 2
BSL3	Bio Safety Level 3
mL	Milli Liters
µL	Micro Liters
K_2EDTA	Potassium Ethylene Diamine Tetra Acetate
G	Relative Centrifugal Force
qPCR Protocol	Quantitative PCR protocol
MBGW	Molecular Biology Grade Water
NTC	No Template Control
FAM	Carboxyfluorescein
ROX	Carboxy-X-rhodamine
NIBSC	National Institute for Biological Standards and
IU	International Units
WHO	World Health Organization
BLAST	Basic Local Alignment Search Tool
Rxn	Reaction

References

1. Handbook "Sampling, Transportation, and Storage of Clinical Material for PCR Diagnostics", developed by Federal State Institution of Science Central Research Institute of Epidemology of Federal Service for Surveillance on Consumers' Rights Protection and Human Well-Being, Moscow, 2008.

2. Liu Y, Hussain M, Wong S, Fung SK, Yim HJ, Lok AS. A genotype- independent real – time PCR assay for quantification of hepatitis B virus DNA. J Clin Microbiol. 2007; 45(2):553-558.

Ordering Information

QT-HBV-25 : 25 rxns
QT-HBV-50 : 50 rxns
QT-HBV-100 : 100 rxns